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TITLE OF THE INVENTION *(280 characters maximum)*

CHIMERIC VECTORS

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CHIMERIC VECTORS

FIELD OF THE INVENTION

The present invention relates to novel chimeric viral vectors and methods of making and administering the same.

BACKGROUND OF THE INVENTION

It is becoming clear that vectors based upon adeno-associated virus (AAV) are the vectors of choice for certain gene therapy applications such as muscle delivery. The utilization of AAV vectors in such protocols is based on the advantageous properties of AAV. These properties include lack of pathogenicity and pathology, ease of preparation and purification, long term expression in many tissues including the muscle, and lack of a detrimental cell-mediated immune response.

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.

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). Further, in particular embodiments, the chimeric virus has a different immunological profile than the parent virus (*i.e.*, is only weakly or not at all recognized by neutralizing antisera or antibodies against the parent virus), thereby allowing for administration to subjects that have antibodies directed against the parent virus.

These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

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**.

Figure 1B. Alignment of AAV1 and AAV2 using the Align program in Vector NTI software package. The amino acids tested in the triage approach are shown with arrows. The circled amino acids correspond to those amino acids previously identified in Hauck et al., (2003) *J. Virology* 77:2768-2774).

Figure 2A. Changing the amino acid of AAV2 at the 324, 328 positions did not enhance muscle transduction. Amino acid positions 324, 328 of AAV2 were changed to that of AAV1. 1×10^{10} viral genome-containing particles were injected into each gastrocnemius of male Balb/c mice. Each mouse was imaged at 7, 14, 28,

and 42 days post injection. The virus used in this experiment was purified using cesium chloride gradients.

Figure 2B. Quantitation of light emission from muscle of AAV2 versus 324, 328 variant. The amount of light emitted from the experiment depicted in Figure 2A was calculated using CMIR_image software. The regions of interest (ROI) from each leg were defined and used to calculate total photons emitted. Data is represented as an average of all 6 limbs.

Figure 3A. Changing the amino acids of AAV2 to create the 2.5 variant greatly enhanced the muscle tropism of this variant. 1×10^{10} viral genome-containing particles were injected into each gastrocnemius of male Balb/c mice. Each mouse was imaged at 7, 14, 28, 42, and 95 days post injection. The virus used in this experiment was purified using heparin HPLC. The amount of light emitted from each animal was calculated using CMIR_image software. The regions of interest (ROI) from each leg were defined and used to calculate total photons emitted. Data is represented as an average of all 6 limbs.

Figure 3B. Locations of the amino acid positions on the AAV2 capsid monomer. Depicted is a 3D ribbon structure of the AAV2 capsid monomer. The locations of the changes made in the 2.5 variant are indicated by arrows. The top arrow points to the 263, 265 regions, and the 3 bottom arrows point to the 709, 712, 720 amino acids.

Figure 3C. The position of the 2.5 amino acids on the AAV2 pentamer. The location of the 2.5 variant amino acids is indicated by the circle. Although 2 of the amino acids lie on one portion of the individual subunit and 3 other amino acids lie on another portion of the same subunit, when 2 subunits come together to form the AAV capsid, these 5 amino acids lie in very close proximity at the 2-fold axis of symmetry.

Figure 4. 263, 265 amino acids confer enhanced muscle tropism. Subsets (263, 265; 709, 712, 720) of the 2.5 variant were generated. 1×10^{10} viral genome-containing particles were injected into each gastrocnemius of male Balb/c mice. Each mouse was imaged at 7, 14, 28, and 42 days post injection. The virus used in this experiment was purified using cesium chloride gradients. The 263, 265 variant exhibit enhanced muscle tropism similar to the 2.5 variant, whereas the 709, 712, 720 variant exhibited a muscle tropism profile similar to AAV2.

Figure 5. The threonine insertion at position 265 is responsible for the majority of the enhanced muscle tropism of 2.5. Subsets (263 or 265) of the 263, 265 variant were generated. 1×10^{10} viral genome-containing particles were injected into each gastrocnemius of male Balb/c mice. Each mouse was imaged at 7, 14, 28, 42, and 100 days post injection. The virus used in this experiment was purified using cesium chloride gradients. The 265 variant exhibited enhanced muscle tropism similar to the 263, 265 variant whereas the 263 variant did not exhibit enhanced muscle tropism.

Figure 6. The 454, 461 variant exhibited enhanced muscle tropism at later time points post injection. 1×10^{10} viral genome-containing particles of AAV2 or 454, 461 were injected into each gastrocnemius of male Balb/c mice. Each mouse was imaged at 7, 14, 21, 28, and 42 days post injection. The virus used in this experiment was purified using cesium chloride gradients. 454, 461 muscle transduction was similar to AAV2 at early time points (day 7 and 14) but expression was better than AAV2 at later time points (days 21-42).

Figure 7. Identification of the 263, 265 region of other AAV serotypes as an area of natural insertions. The capsid sequences of AAV serotypes 1-9 were subjected to multiple sequence alignment using the Align program in the Vector NTI software package. Shown in the box are the amino acids of the other serotypes corresponding to the amino acids identified in AAV1 as responsible for enhanced muscle tropism.

Figure 8. Heparin binding profile of AAV variants. Equivalent particles of each AAV variant were applied to heparin agarose type 1 and allowed to bind. The columns were washed with PBS, followed by elution in sodium chloride. The number of particles present in the flow thru, washes and elutions were determined via dot blot hybridization. Data is depicted as percentage of unbound particles (wash and flow thru) and bound (elution).

Figure 9. shows an amino acid alignment of the capsid sequence of the 263, 265 mutant in an AAV2 background with the capsid sequence of AAV2. Amino acid 1 is numbered with respect to the VP1 sequence.

Figure 10. shows an amino acid alignment of the capsid sequence of the 263, 265 mutant in an AAV3b background with the capsid sequence of AAV3b. Amino acid 1 is numbered with respect to the VP1 sequence.

Figure 11. shows an amino acid alignment of the capsid sequence of the AAV 2.5 mutant in an AAV2 background with the capsid sequence of AAV2. Amino acid 1 is numbered with respect to the VP1 sequence.

DETAILED DESCRIPTION OF THE INVENTION

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.

The present invention will now be described with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

The designation of all amino acid positions in the AAV capsid subunits in the description of the invention and the appended claims is with respect to VP1 capsid subunit numbering.

Except as otherwise indicated, standard methods known to those skilled in the art may be used for the construction of rAAV constructs, modified capsid proteins, packaging vectors expressing the parvovirus rep and/or cap sequences, and transiently and stably transfected packaging cells. Such techniques are known to those skilled in the art. *See, e.g.*, SAMBROOK *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

Definitions.

The following terms are used in the description herein and the appended claims:

As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

The term "parvovirus" as used herein encompasses the family *Parvoviridae*, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.*, BERNARD

N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV. *See, e.g.*, BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). Recently, a number of new AAV serotypes and clades have been identified (*see, e.g.*, Gao et al., (2004) *J. Virology* 78:6381-6388 and **Table 1**).

The genomic sequences of the various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as GenBank. *See*, *e.g.*, GenBank Accession Numbers NC 002077, NC 001401, NC 001729, NC 001863, NC 001829, NC 001862, NC 000883, NC 001701,
NC 001510, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, NC 001358, NC 001540, AF513851, AF513852, AY530579; the disclosures of which are incorporated herein in their entirety. *See also, e.g.*, Srivistava et al., (1983) *J. Virology* **45**:555; Chiorini et al., (1998) *J. Virology* **71**:6823; Chiorini *et al.*, (1999) *J. Virology* **73**:1309; Bantel-Schaal et al., (1999) *J. Virology* **73**:939; Xiao et al., (1999) *J. Virology* **73**:3994; Muramatsu et al., (1996)
Virology **221**:208; Shade et al., (1986) *J. Virol.* **58**:921; Gao et al., (2002) *Proc. Nat. Acad. Sci. USA* **99**:11854; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; U.S. Patent No. 6,156,303; the disclosures of which are incorporated herein in their entirety. *See also* **Table 1**. An early description of the AAV1, AAV2 and AAV3 terminal repeat sequences is provided by Xiao, X., (1996), "Characterization of Adeno- associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein it its entirety).

Table 1

Complete Genomes	Gene Bank Accession Number		Gene Bank Accession Number		Gene Bank Accession Number
		Hu T88	AY695375	Clade E	
Adeno-associated virus 1	NC_002077,AF063497	Hu T71	AY695374	Rh38	AY530558
Adeno-associated virus 2	NC_001401	Hu T70	AY695373	Hu66	AY530626
Adeno-associated virus 3	NC_001729	Hu T40	AY695372	Hu42	AY530605
Adeno-associated virus 3B	NC_001863	Hu T32	AY695371	Hu67	AY530627
Adeno-associated virus 4	NC_001829	Hu T17	AY695370	Hu40	AY530603
Adeno-associated virus 5	Y18065, AF085716	Hu LG15	AY695377	Hu41	AY530604
Adeno-associated virus 6	NC_001862			Hu37	AY530600
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828	Clade C		Rh40	AY530559
Avian AAV strain DA-1	NC_006263, AY629583	Hu9	AY530629	Rh2	AY243007
Bovine AAV	NC_005889, AY388617	Hu10	AY530576	Bb1	AY243023
		Hu11	AY530577	Bb2	AY243022
Clade A		Hu53	AY530615	Rh10	AY243015
AAV1	NC_002077,AF063497	Hu55	AY530617	Hu17	AY530582
AAV6	NC_001862	Hu54	AY530616	Hu6	AY530621
Hu.48	AY530611	Hu7	AY530628	Rh25	AY530557
Hu 43	AY530606	Hu18	AY530583	Pi2	AY530554
Hu 44	AY530607	Hu15	AY530580	Pi1	AY530553
Hu 46	AY530609	Hu16	AY530581	Pi3	AY530555
		Hu25	AY530591	Rh57	AY530569
Clade B		Hu60	AY530622	Rh50	AY530563
Hu. 19	AY530584	Ch5	AY243021	Rh49	AY530562
Hu. 20	AY530586	Hu3	AY530595	Hu39	AY530601
Hu 23	AY530589	Hu1	AY530575	Rh58	AY530570
Hu22	AY530588	Hu4	AY530602	Rh61	AY530572
Hu24	AY530590	Hu2	AY530585	Rh52	AY530565
Hu21	AY530587	Hu61	AY530623	Rh53	AY530566
Hu27	AY530592			Rh51	AY530564
Hu28	AY530593	Clade D		Rh64	AY530574
Hu 29	AY530594	Rh62	AY530573	Rh43	AY530560
Hu63	AY530624	Rh48	AY530561	AAV8	AF513852
Hu64	AY530625	Rh54	AY530567	Rh8	AY242997
Hu13	AY530578	Rh55	AY530568	Rh1	AY530556
Hu56	AY530618	Cy2	AY243020		
Hu57	AY530619	AAV7	AF513851	Clade F	
Hu49	AY530612	Rh35	AY243000	Hu14 (AAV9)	AY530579
Hu58	AY530620	Rh37	AY242998	Hu31	AY530596
Hu34	AY530598	Rh36	AY242999	Hu32	AY530597
Hu35	AY530599	Cy6	AY243016		
AAV2	NC_001401	Cy4	AY243018	Clonal Isolate	
Hu45	AY530608	Cy3	AY243019	AAV5	Y18065, AF085716
Hu47	AY530610	Cy5	AY243017	AAV 3	NC_001729
Hu51	AY530613	Rh13	AY243013	AAV 3B	NC_001863
Hu52	AY530614			AAV4	NC_001829
Hu T41	AY695378			Rh34	AY243001
Hu S17	AY695376			Rh33	AY243002
				Rh32	AY243003

The term "tropism" as used herein refers to preferred entry of the virus into the cell, optionally and preferably followed by expression (*e.g.*, transcription and, optionally, translation) of sequences carried by the viral genome in the cell, *e.g.*, for a recombinant virus, expression of the heterologous nucleotide sequence(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.*, for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of a rAAV genome, gene expression from the viral genome may be from a stably integrated provirus, from a non-integrated episome, as well as any other form in which the virus may take within the cell.

As used herein, "infection" of a cell by a parvovirus, AAV or recombinant virus vector of the invention means that the parvovirus/AAV enters the cell to establish an active (*i.e.*, lytic) infection. As used herein, "transduction" of a cell by AAV or a chimeric virus particle means that the AAV enters the cell to establish a persistent infection. Those skilled in the art will appreciate that unlike wildtype AAV vectors, rAAV vectors and the chimeric virus vectors of the invention do not generally integrate into human chromosome 19 (*see, e.g.*, McCarty et al., (2004) *Annu. Rev. Genet.* 38:819-845).

As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

A "polynucleotide" is a sequence of nucleotide bases, and may be RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotide), but are preferably either single or double stranded DNA sequences.

As used herein, an "isolated" polynucleotide (*e.g.*, an "isolated DNA" or an "isolated RNA") means a polynucleotide separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide.

Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

A "therapeutic polypeptide" is a polypeptide that can alleviate or reduce symptoms that result from an absence or defect in a protein in a cell or subject. Alternatively, a "therapeutic polypeptide" is one that otherwise confers a benefit to a subject, *e.g.*, anti-cancer effects or improvement in transplant survivability.

A "heterologous nucleotide sequence" or "heterologous nucleic acid" is typically a sequence that is not naturally occurring in the virus. Generally, the heterologous nucleic acid comprises an open reading frame that encodes a polypeptide or nontranslated RNA.

As used herein, the term "vector" or "gene delivery vector" may refer to a parvovirus (*e.g.*, AAV) particle that functions as a nucleic acid delivery vehicle, and which comprises viral DNA (*i.e.*, the vector genome) packaged within a parvovirus (*e.g.*, AAV) capsid. Alternatively, in some contexts, the term "vector" may be used to refer to the vector genome/vDNA alone.

As used herein, a "recombinant parvovirus vector genome" is a parvovirus genome (*i.e.*, vDNA) into which a heterologous nucleotide sequence has been inserted. A "recombinant parvovirus particle" comprises a recombinant parvovirus vector genome packaged within a parvovirus capsid.

Likewise, a "rAAV vector genome" or "rAAV genome" is an AAV genome (*i.e.*, vDNA) that comprises one or more heterologous nucleotide sequences. rAAV vectors generally require only the 145 base terminal repeat(s) in *cis* to generate virus. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). Typically, the rAAV vector genome will only retain the minimal terminal repeat (TR) sequence(s) so as to maximize the size of the transgene that can be efficiently packaged by the vector. The structural and non-structural protein coding sequences may be provided in *trans* (*e.g.*, from a vector, such as a plasmid, or by stably integrating the sequences into a packaging cell). The rAAV vector genome comprises at least one AAV terminal repeat, more typically two AAV terminal repeats, which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s). The TRs can be the same or different from each other.

A "rAAV particle" comprises a rAAV vector genome packaged within an AAV capsid.

A "parvovirus terminal repeat" may be from any parvovirus, including autonomous parvoviruses and AAV (all as defined above). An "AAV terminal

repeat" may be from any AAV, *e.g.*, serotypes 1, 2, 3, 4, 5, 6, 7, 8 and 9. The term "terminal repeat" includes synthetic sequences that function as an AAV inverted terminal repeat, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.*, the disclosure of which is incorporated in its entirety herein by reference. The AAV terminal repeats need not have a wild-type terminal repeat sequence (*e.g.*, a wild-type sequence may be altered by insertion, deletion, truncation or missense mutations), as long as the terminal repeat mediates the desired functions, *e.g.*, replication, virus packaging, integration, and/or provirus rescue, and the like.

The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

The chimeric virus vector of the invention can further be a "targeted" virus vector (*e.g.*, having a directed tropism) and/or a "hybrid" parvovirus (*i.e.*, in which the rAAV genome and viral capsid are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao et al., (2000) *Molecular Therapy* 2:619 (the disclosures of which are incorporated herein by reference in their entireties). In particular embodiments, the rAAV genome and virus capsid are from different AAV.

The chimeric viruses of the invention can further be duplexed parvovirus particles as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety).

Accordingly, as used herein, the terms "parvovirus" and "AAV" encompass hybrid, chimeric, targeted and duplexed virus particles, as well as other modified forms of parvoviruses and AAV.

Chimeric Virus Vectors.

The inventors have identified selected regions within the AAV1/6/7 serotypes that confer desirable properties including, but not limited to, enhanced transduction ability (*e.g.*, enhanced transduction of skeletal muscle cells), higher expression of a transgene delivered by the recombinant genome and/or earlier onset of transgene expression. These selected regions can be transferred or "engineered" into other AAV capsids to confer the property(ies) of interest to the resulting chimeric particle. To illustrate, in the case of AAV2, the inventors have discovered that with a single

amino acid insertion from AAV1, AAV2 can acquire the enhanced transduction capability of AAV1, while retaining the ease of purification (*i.e.*, retains ability to bind heparin) and the known safety features of AAV2. Further, in particular embodiments, the resulting chimeric virus has a different immunological profile than the parent virus (*e.g.*, is weakly or not at all recognized by neutralizing antiserum to the parent virus), thereby allowing for repeat administration to subjects that have developed antibodies against the parent virus. For example, the AAV2.5 vector described herein is only weakly recognized by neutralizing antisera against AAV2.

In the present studies, the inventors have compared the linear amino acid sequences of two serotypes with high levels of skeletal muscle transduction (AAV1 and AAV7) with two that are less effective in transducing skeletal muscle (AAV2 and AAV8). Although these four serotypes overall exhibit a high degree of sequence similarity, there are regions of divergence and the characteristics of these four serotypes as delivery vectors are distinct. Using sequence analysis, a number of candidate amino acids in AAV 1 and/or AAV7 were identified for incorporation into AAV2 to determine if they would confer the enhanced skeletal muscle transduction of AAV1/7 to AAV2. This initial pool of candidate positions was narrowed considerably based on analysis of the known crystal structure of AAV2, *e.g.*, to identify amino acid positions that are located in regions that are likely to affect the biology of the virus. This smaller group of amino acids was then substituted and/or inserted into AAV2, either individually or in combination and several of the resulting mutants demonstrated the enhanced skeletal muscle transduction of AAV1. Corresponding modifications can readily be introduced into other AAV based on the present disclosure in view of the high level of skill in the art. A "corresponding" modification can be an insertion and/or a substitution and/or a deletion. For example, a modification that is an insertion in AAV2 may be a substitution mutation in another AAV. Further, in another AAV, a deletion mutation may bring an amino acid into the desired position within the capsid subunits.

In representative embodiments, the invention provides a chimeric virus vector comprising: (a) a recombinant AAV (rAAV) genome; and (b) a chimeric AAV capsid comprising a selective amino acid insertion and/or substitution that results in an amino acid insertion following any of amino acid position 260, 261, 262, 263, 264, 265, 266, 267 or 268 (*e.g.*, following amino acid position 264) in the AAV2 capsid subunits (VP1 numbering) or a corresponding change in other AAV capsids; wherein

said chimeric virus vector has enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector. By "following amino acid position X" it is intended that the insertion immediately follows the indicated amino acid (for example, "following amino acid position 264" indicates a point insertion at position 265 or a larger insertion, *e.g.*, from positions 265 to 268, *etc.*).

The designation of all amino acid positions in the description of the invention and the appended claims is with respect to VP1 numbering. It will be understood by those skilled in the art that the modifications described herein will result in modifications in the VP1, VP2 and VP3 capsid subunits.

Those skilled in the art will appreciate that for some AAV the corresponding modification will be an insertion and/or a substitution, depending on whether the corresponding amino acid positions are partially or completely present in the virus or, alternatively, are completely absent. Likewise, when modifying AAV other than AAV2, the specific amino acid position(s) may be different than the position in AAV2. However, the corresponding amino acid position(s) will be readily apparent to those skilled in the art using well-known sequence alignment techniques (*see, e.g.*, **Figure 7**).

In particular embodiments, the modifications described herein can be introduced into the capsid subunits at the position that corresponds to the position of the amino acid(s) of interest in the AAV1, AAV6 and/or AAV7 subunits. Based on crystal structure analysis, it will be clear that in some instances the insertion/substitution can be moved 1, 2, 3, 4 or even 5 amino acids in either direction and still confer the desired characteristic. Because many of the modifications are made in loop structures, those skilled in the art will understand that the result of the modification can be driven more by physical presentation on the surface of the virion than the specific amino acid position.

According to the present invention, a "selective" amino acid change(s) is introduced into the virus capsid. This approach is in contrast to previous work with whole subunit or large domain swaps between AAV serotypes (*see, e.g.*, international patent publication WO 00/28004 and Hauck et al., (2003) *J. Virology* 77:2768-2774). A "selective" amino acid change results in the insertion and/or substitution and/or deletion of less than about 20, 18, 15, 12, 10, 9, 8, 7, 6, 5, 4 or 3 contiguous amino acids. In particular embodiments, only two contiguous amino

acids or even point mutations (*i.e.*, one amino acid) are inserted and/or substituted into and/or deleted from the capsid subunits.

One or more selective amino acid changes can be introduced at different positions within the capsid subunits. For example, in particular embodiments, two, three, four, five, six, seven, eight, nine, ten or more selective amino acid changes can be introduced into the AAV capsid subunits.

It will be understood that the term "chimeric virus vector" or "chimeric capsid" excludes those virus vectors or capsids that have the indicated amino acids at the specified positions in their native state.

The invention contemplates that the chimeric viruses of the invention can be produced by modifying the capsids of any AAV now known or later discovered. Further, the starting AAV that is to be modified can be one of the characterized AAV serotypes or clades, *e.g.*, AAV2, AAV3a or 3b, AAV4, AAV5, AAV8 or AAV9 (*see also* **Table 1**). Alternatively, the starting virus may already have modifications/alterations as compared with the naturally occurring viruses. Such viruses are also within the scope of the present invention. For example, the starting or parent virus can be an AAV that is derived from AAV8, but has a heparan sulfate-binding domain (*e.g.*, from the AAV2 capsid) incorporated therein. As another illustrative example, the AAV can be derived from any of the known serotypes or clades, but have a peptide targeting sequence incorporated therein. As yet another possibility, the AAV capsid can comprise capsid subunits from different serotypes. Thus, in particular embodiments, the parent virus comprises a capsid from an AAV serotype or clade that has been modified to comprise sequences that are not from that serotype or clade (*e.g.*, are exogenous).

In representative embodiments, the insertion and/or substitution and/or deletion in the capsid subunits results in the insertion or substitution of an amino acid that maintains the hydrophilic loop structure in that region and/or an amino acid that alters the configuration of the loop structure, a charged amino acid, or an amino acid that can be phosphorylated or sulfated or otherwise acquire a charge by post-translational modification (*e.g.*, glycosylation) following any of amino acid position 260, 261, 262, 263, 264, 265, 266, 267 or 268 (*e.g.*, following position 264) in the AAV2 capsid subunits or a corresponding change in other AAV capsids; wherein said chimeric virus vector has enhanced transduction as compared with the parent rAAV vector. Suitable amino acids include aspartic acid, glutamic acid, lysine,

arginine, threonine, serine, tyrosine, glycine, alanine, proline, asparagine or glutamine. In particular embodiments, a threonine is inserted or substituted into the capsid subunit.

According to this aspect of the invention, in particular embodiments the chimeric virus vector comprises a chimeric AAV capsid comprising an amino acid insertion following amino acid position 264 in the AAV2 or AAV3b capsid subunits or in the corresponding position in an AAV2 or AAV3b capsid that has been modified to comprise non-AAV2 or AAV3b sequences, respectively. The amino acid corresponding to position 264 in the AAV2 (or AAV3b) capsid subunits will be readily identifiable in the starting virus that has been derived from AAV2 (or AAV3b), which can then be further modified according to the present invention.

Alternatively, the chimeric virus vectors can comprise a chimeric AAV2 or AAV3b capsid comprising an amino acid insertion following amino acid position 264 in the AAV2 or AAV3b capsid subunits.

Alternatively, the invention provides other modifications based on the AAV1, AAV6 and/or AAV7 serotypes that confer one or more desirable properties associated with these serotypes and/or alters the immunological profile of the chimeric virus as compared with the parent virus.

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

(a) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(b) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(c) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

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

(f) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 454 to 460 (*e.g.*, position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

These modifications can be used in any combination with each other and in any combination with the modification following any of positions 260 to 268 (*e.g.*, following position 264) described above.

In particular embodiments, the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or in the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences. Alternatively, the chimeric virus vector can comprise a chimeric AAV3b capsid comprising an amino acid substitution at amino acid position 263 in the AAV3b capsid subunits or in the corresponding position in an AAV3b capsid that has been modified to comprise non-AAV3b sequences.

In still other embodiments, the substitution is at amino acid position 263 of AAV1, a substitution at amino acid position 263 in AAV3a, substitutions and/or insertions and/or deletions at amino acid positions 264 and 265 in AAV7, substitutions and/or insertions and/or deletions at amino acid positions 263 and 264 of AAV8, or substitutions and/or insertions and/or deletions at amino acid positions 262 and 263 of AAV9; or at the corresponding position(s) in an AAV capsid from any of the foregoing serotypes that has been modified to comprise exogenous sequences.

Suitable amino acid substitutions include but are not limited to substitutions of small nonpolar amino acids such as alanine, glycine, valine, leucine or isoleucine or even proline, asparagine, serine or threonine into the capsid subunits (*e.g.*, substitution of these amino acids for the glutamine found at this position in the AAV2

or AAV3b capsid subunits). In particular embodiments, an alanine is substituted into the capsid subunits.

Another chimeric virus vector of the invention comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences.

In other embodiments, the invention provides for a chimeric virus vector having an amino acid substitution at amino acid position 706 of the AAV1 capsid subunits, a substitution at amino acid position 706 of the AAV3a capsid subunits, a substitution at amino acid position 706 of the AAV3b capsid subunits, a substitution at amino acid position 707 of the AAV7 capsid subunits, a substitution at amino acid position 708 of the AAV8 capsid subunits, or a substitution at amino acid position 706 of the AAV9 capsid subunits capsid subunits; or at the corresponding position(s) in an AAV capsid from any of the foregoing serotypes that has been modified to comprise exogenous sequences.

In representative examples of the embodiments of the invention described in the previous two paragraphs, suitable amino acids include but are not limited to serine, threonine, tyrosine, proline, glutamine, alanine, glycine, valine, leucine or isoleucine (*e.g.*, substituted for the asparagine at this position in the AAV2 capsid subunits). In particular embodiments, alanine is substituted into the capsid subunits.

A further chimeric virus vector according to the invention comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences.

In other embodiments, the invention provides for a chimeric virus vector having an amino acid substitution at amino acid position 709 of the AAV1 capsid subunits, an amino acid substitution at amino acid position 709 of the AAV3a capsid subunits, an amino acid substitution at amino acid position 709 of the AAV3b capsid subunits, an amino acid substitution at amino acid position 710 of the AAV7 capsid subunits, an amino acid substitution at amino acid position 711 of the AAV8 capsid subunits, or an amino acid substitution at amino acid position 709 of the AAV9 capsid subunits; or at the corresponding position in an AAV capsid from any of the foregoing serotypes that has been modified to comprise exogenous sequences.

In representative examples of the embodiments of the invention described in the previous two paragraphs, the substitution results in a substitution of serine, threonine, tyrosine, proline, asparagine, glutamine, alanine, glycine, leucine or isoleucine into the capsid subunits (*e.g.*, for the valine at this position in the AAV2 capsid subunits). One exemplary substitution is a substitution of alanine into the capsid subunits.

The invention also provides a chimeric virus vector comprising a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 716 in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences.

In other embodiments, the invention provides for a chimeric virus vector having an amino acid substitution at amino acid position 717 of the AAV1 capsid subunits, an amino acid substitution at amino acid position 717 of the AAV3a capsid subunits, an amino acid substitution at amino acid position 717 of the AAV3b capsid subunits, an amino acid substitution at amino acid position 718 of the AAV7 capsid subunits, an amino acid substitution at amino acid position 719 of the AAV8 capsid subunits, or an amino acid substitution at amino acid position 717 of the AAV9 capsid subunits; or at the corresponding position in an AAV capsid from any of the foregoing serotypes that has been modified to comprise exogenous sequences.

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.

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

Alternatively, the substitution can be a substitution of a serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine into the capsid subunit (*e.g.*, substitution of the threonine at position 450 in AAV2). One particular chimeric virus comprises an asparagine substituted at this position into the capsid subunits.

Also provided is a chimeric virus vector comprising a chimeric AAV2 capsid comprising an amino acid substitution at any of amino acid position 454, 455, 456, 457, 458, 459 or 460 (*e.g.*, at position 457) in the AAV2 capsid subunits or at the corresponding position in an AAV2 capsid that has been modified to comprise non-AAV2 sequences. Suitable amino acid substitutions include substitution of a glycine, alanine, valine, lysine, isoleucine, proline, serine, threonine or asparagine into the capsid subunits. In representative embodiments, an asparagine is substituted into the capsid subunit (*e.g.*, an asparagine is substituted for the glutamine found at position 457 in the AAV2 capsid subunits).

One example of a particular chimeric virus vector of the invention comprises (a) a rAAV genome; and (b) a chimeric AAV capsid (*e.g.*, AAV2 capsid) comprising: (i) a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; and (ii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; wherein said chimeric virus has enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector. For example, the chimeric virus vector can comprise a chimeric AAV2 capsid in which a threonine has been inserted following amino acid position 264 in the AAV2 capsid subunits and an alanine has been substituted for glutamine at amino acid position 263 in the AAV2 capsid subunits; or these changes can be made at the corresponding positions in an AAV2 capsid that has been modified to comprise non-AAV2 sequences. The sequence of the 263, 265 variant described herein is shown in AAV2 (**Figure 9**) and AAV3b (**Figure 10**) backgrounds and compared with the sequence of the parent virus.

Another exemplary chimeric virus vector comprises (a) a rAAV genome; (b) a chimeric AAV capsid *e.g.*, (AAV2 capsid) comprising: (i) a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino

acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; and (ii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; (iii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; (iv) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; and (v) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 716 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; wherein said chimeric virus has enhanced transduction and/or an altered immunological profile as compared with the parent rAAV vector.

According to this embodiment, the chimeric virus vector can comprise a chimeric AAV capsid (*e.g.*, AAV2 capsid) comprising:

(a) a threonine insertion following amino acid position 264 in the AAV2 capsid subunits;

(b) an alanine for glutamine substitution at amino acid position 263 in the AAV2 capsid subunits;

(c) an alanine for asparagine substitution at amino acid position 705 in the AAV2 capsid subunits;

(d) an alanine for valine substitution at amino acid position 708 in the AAV2 capsid subunits; and

(e) an asparagine for threonine substitution at amino acid position 716 in the AAV2 capsid subunits;

or these changes can be made at the corresponding positions in an AAV2 capsid that has been modified to comprise non-AAV2 sequences.

Further, the chimeric virus vector can be the AAV 2.5 variant disclosed herein. The sequence of the AAV 2.5 mutant as compared with AAV2 is shown in **Figure 11**.

Another exemplary chimeric virus vector comprises (a) a rAAV genome; and (b) a chimeric AAV capsid (*e.g.*, AAV2 capsid) comprising:

(i) a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

(iii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

According to particular aspects of this embodiment of the invention, the chimeric virus vector comprises a chimeric AAV2 capsid comprising:

(a) a threonine insertion following amino acid position 264 in the AAV2 capsid subunits;

(b) an asparagine for threonine substitution at amino acid position 450 in the AAV2 capsid subunits; and

(c) an asparagine for glutamine substitution at amino acid position 457 in the AAV2 capsid subunits;

or these changes can be made at the corresponding positions in an AAV2 capsid that has been modified to comprise non-AAV2 sequences.

The invention further provides a chimeric virus vector comprising (a) a rAAV genome; and (b) a chimeric AAV capsid (*e.g.*, AAV2 capsid) comprising:

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

(ii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

According to particular embodiments, a chimeric AAV2 capsid comprises (i) an asparagine for threonine substitution at amino acid position 450 in the AAV2 capsid subunits; and (ii) an asparagine for glutamine substitution at amino acid position 457 in the AAV2 capsid subunits; or these changes can be made at the corresponding positions in an AAV2 vector that has been modified to comprise non-AAV2 sequences.

The chimeric virus vectors of the invention comprise a rAAV genome, *i.e.*, the AAV genome comprises one or more heterologous nucleic acids that encode a polypeptide or a non-translated RNA. Heterologous nucleic acids are discussed in more detail hereinbelow.

Chimeric Capsids.

The present invention further encompasses chimeric virus capsids essentially as described above, *i.e.*, in the absence of a rAAV genome.

The chimeric virus capsids can be used as "capsid vehicles," as has been described in U.S. Patent No. 5,863,541 (the disclosure of which is incorporated by reference herein in its entirety). Molecules that can be packaged by the chimeric virus capsids and transferred into a cell include heterologous DNA, RNA, polypeptides, small organic molecules, or combinations of the same. Heterologous molecules are defined as those that are not naturally found in an AAV infection, *i.e.*, those not encoded by a wild-type AAV genome. Further, therapeutically useful molecules can be associated with the outside of the chimeric virus capsid for transfer of the molecules into host target cells. Such associated molecules can include DNA, RNA, small organic molecules, carbohydrates, lipids and/or polypeptides. In one embodiment of the invention the therapeutically useful molecule is covalently linked (*i.e.*, conjugated or chemically coupled) to the capsid proteins. Methods of covalently linking molecules are known by those skilled in the art.

The chimeric virus capsids of the invention also find use in raising antibodies against the novel capsid structures. As a further alternative, an exogenous amino acid sequence may be inserted into the parvovirus capsid for antigen presentation to a cell, *e.g.*, for administration to a subject to produce an immune response to the exogenous amino acid sequence.

According to some embodiments, chimeric virus capsids can be administered to a subject concurrently (*e.g.*, within minutes or hours of each other) with an AAV vector or chimeric virus vector according to the invention. Further, the invention provides compositions and pharmaceutical formulations comprising the inventive chimeric virus capsids and an AAV vector or chimeric virus vector of the invention.

The invention also provides nucleic acids (optionally, isolated nucleic acids) encoding the chimeric virus capsids and capsid subunits of the invention. Further provided are vectors comprising the nucleic acids, and cells (*in vivo* or in culture)

comprising the nucleic acids and/or vectors of the invention. Such nucleic acids, vectors and cells can be used, for example, as reagents (*e.g.*, helper packaging constructs or packaging cells) for the production of chimeric virus capsids or vectors as described herein.

Methods of Producing Chimeric Virus Vectors.

The present invention further provides methods of producing the inventive chimeric virus vector.

In one particular embodiment, the present invention provides a method of producing a recombinant chimeric virus vector, comprising providing to a cell, (a) a rAAV template comprising (i) one or more heterologous nucleotide sequences, and (ii) packaging signal sequences sufficient for the encapsidation of the AAV template into chimeric virus particles, and (b) AAV sequences sufficient for replication and encapsidation of the rAAV template into chimeric viral particles (*e.g.*, the AAV rep and chimeric cap sequences comprising one or more selective insertions or substitutions from another AAV therein). The rAAV template and AAV replication and capsid sequences are provided under conditions such that recombinant chimeric virus particles comprising the rAAV template packed within the chimeric capsid are produced in the cell. The method can further comprise the step of collecting the virus particles from the cell. Virus particles may be collected from the medium and/or by lysing the cells.

The cell is typically a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed. Mammalian cells are preferred. Also preferred are trans-complementing packaging cell lines that provide functions deleted from a replication-defective helper virus, *e.g.*, 293 cells or other E1a trans-complementing cells.

The AAV replication and capsid sequences may be provided by any method known in the art. Current protocols typically express the AAV rep/cap genes on a single plasmid. The AAV replication and packaging sequences need not be provided together, although it may be convenient to do so. The AAV rep and/or cap sequences may be provided by any viral or non-viral vector. For example, the rep/cap sequences may be provided by a hybrid adenovirus or herpesvirus vector (*e.g.*, inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV cap and rep genes. One

advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (*i.e.*, are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," *see* Margolski, (1992) *Curr. Top. Microbiol. Immun.* **158**:67).

As a further alternative, the rep/cap sequences may be stably embedded within a cell.

Typically, and preferably, the AAV rep/cap sequences will not be flanked by the AAV packaging sequences (*e.g.*, AAV ITRs), to prevent rescue and/or packaging of these sequences.

The rAAV template can be provided to the cell using any method known in the art. For example, the rAAV template may be supplied by a non-viral (*e.g.*, plasmid) or viral vector. In particular embodiments, the rAAV template is supplied by a herpesvirus or adenovirus vector (*e.g.*, inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo et al., (1998) *J. Virology* **72**:5025, describe a baculovirus vector carrying a reporter gene flanked by the AAV ITRs. EBV vectors may also be employed to deliver the rAAV template, as described above with respect to the rep/cap genes.

In another representative embodiment, the rAAV template is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus is stably integrated into the chromosome of the cell.

To obtain maximal virus titers, helper virus functions (*e.g.*, adenovirus or herpesvirus) essential for a productive AAV infection will be provided to the cell. Helper virus sequences necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, *e.g.*, as a non-infectious adenovirus miniplasmid that carries all of the helper genes required for efficient AAV production as described by Ferrari et al., (1997) *Nature Med.* **3**:1295, and U.S. Patent Nos. 6,040,183 and 6,093,570.

Further, the helper virus functions may be provided by a packaging cell with the helper genes embedded in the chromosome or maintained as a stable extrachromosomal element. It is preferred that these helper virus sequences cannot be packaged into AAV virions, *e.g.*, are not flanked by AAV ITRs.

Those skilled in the art will appreciate that it may be advantageous to provide the AAV replication and capsid sequences and the helper virus sequences (*e.g.*, adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct, but is preferably a hybrid adenovirus or hybrid herpesvirus comprising the AAV rep/cap genes.

In one particular preferred embodiment, the AAV rep/cap sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector further contains the rAAV template. The AAV rep/cap sequences and/or the rAAV template may be inserted into a deleted region (*e.g.*, the E1a or E3 regions) of the adenovirus.

In a further embodiment, the AAV rep/cap sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. The rAAV template is provided as a plasmid template.

In another illustrative embodiment, the AAV rep/cap sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the rAAV template is integrated into the cell as a provirus. Alternatively, the rAAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (*e.g.*, as an EBV based nuclear episome).

In a further exemplary embodiment, the AAV rep/cap sequences and adenovirus helper sequences are provided by a single adenovirus helper. The rAAV template is provided as a separate replicating viral vector. For example, the rAAV template may be provided by a rAAV particle or a second recombinant adenovirus particle.

According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' *cis* sequences sufficient for adenovirus replication and packaging (*i.e.*, the adenovirus terminal repeats and PAC sequence). The AAV rep/cap sequences and, if present, the rAAV template are embedded in the adenovirus backbone and are flanked by the 5' and 3' *cis* sequences, so that these sequences may be packaged into adenovirus capsids. As described above, it is preferred that the adenovirus helper sequences and the AAV rep/cap sequences are not flanked by the AAV packaging sequences (*e.g.*, the AAV ITRs), so that these sequences are not packaged into the AAV virions.

Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously

facilitate for more scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway et al., (1999) *Gene Therapy* **6**:986 and WO 00/17377, the disclosures of which are incorporated herein in their entireties).

As a further alternative, the virus vectors of the invention can be produced in insect cells using baculovirus vectors to deliver the *rep/cap* genes and rAAV template as described by Urabe et al., (2002) *Human Gene Therapy* 13:1935-43.

AAV vector stocks free of contaminating helper virus may be obtained by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin et al. (1999) *Gene Therapy* **6**:973). Preferably, deleted replication-defective helper viruses are used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV virus. Adenovirus mutants defective for late gene expression are known in the art (*e.g.*, ts100K and ts149 adenovirus mutants).

The inventive packaging methods may be employed to produce high titer stocks of chimeric virus particles. Preferably, the virus stock has a titer of at least about 10^5 transducing units (tu)/ml, more preferably at least about 10^6 tu/ml, more preferably at least about 10^7 tu/ml, yet more preferably at least about 10^8 tu/ml, yet more preferably at least about 10^9 tu/ml, still yet more preferably at least about 10^{10} tu/ml.

Recombinant Parvovirus Vectors.

The parvovirus vectors of the present invention are useful for the delivery of nucleic acids to cells *in vitro*, *ex vivo*, and *in vivo*. In particular, the parvovirus vectors can be advantageously employed to deliver or transfer nucleic acids to animal, more preferably mammalian, cells.

Any heterologous nucleotide sequence(s) (as defined above) may be delivered in the chimeric virus vectors of the present invention. Nucleic acids of interest include nucleic acids encoding polypeptides, preferably therapeutic (*e.g.*, for medical or veterinary uses) or immunogenic (*e.g.*, for vaccines) polypeptides.

Therapeutic polypeptides include, but are not limited to, cystic fibrosis transmembrane regulator protein (CFTR), dystrophin (including the protein product

of dystrophin mini-genes, *see, e.g*, Vincent *et al.*, (1993) *Nature Genetics* **5**:130; U.S. Patent Publication No. 2003017131, the disclosures of which are incorporated herein in their entireties by reference), utrophin (Tinsley *et al.*, (1996) *Nature* 384:349), clotting factors (*e.g.*, Factor VIII, Factor IX, Factor X, *etc.),* erythropoietin, angiostatin, endostatin, catalase, tyrosine hydroxylase, superoxide dismutase, leptin, the LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, α_1-antitrypsin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase, β-glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase A, branched-chain keto acid dehydrogenase, RP65 protein, cytokines (*e.g.*, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, granulocyte-macrophage colony stimulating factor, lymphotoxin, and the like), peptide growth factors, neurotrophic factors and hormones (*e.g.*, somatotropin, insulin, insulin-like growth factors 1 and 2, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, nerve growth factor, neurotrophic factor –3 and –4, brain-derived neurotrophic factor, glial derived growth factor, transforming growth factor –α and –β, and the like), lysosomal acid α-glucosidase, α-galactosidase A, receptors (*e.g.*, the tumor necrosis growth factorα soluble receptor), anti-inflammatory factors such as IRAP, anti-myostatin proteins, monoclonal antibodies (including single chain monoclonal antibodies; an exemplary Mab is the herceptin Mab). Other illustrative heterologous nucleotide sequences encode suicide gene products (*e.g.*, thymidine kinase, cytosine deaminase, diphtheria toxin, and tumor necrosis factor), proteins conferring resistance to a drug used in cancer therapy, tumor suppressor gene products (*e.g.*, p53, Rb, Wt-1), TRAIL, FAS-ligand, and any other polypeptide that has a therapeutic effect in a subject in need thereof.

Heterologous nucleotide sequences encoding polypeptides include those encoding reporter polypeptides (*e.g.*, an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, Green Fluorescent Protein, β-galactosidase, alkaline phosphatase, luciferase, and chloramphenicol acetyltransferase gene.

Alternatively, the heterologous nucleic acid may encode an antisense nucleic acid, a ribozyme (*e.g.*, as described in U.S. Patent No. 5,877,022), RNAs that effect spliceosome-mediated *trans*-splicing (*see*, Puttaraju *et al.*, (1999) *Nature Biotech.* **17**:246; U.S. Patent No. 6,013,487; U.S. Patent No. 6,083,702), interfering RNAs

(RNAi) that mediate gene silencing (*see*, Sharp et al., (2000) *Science* **287**:2431) or other non-translated RNAs, such as "guide" RNAs (Gorman *et al.*, (1998) *Proc. Nat. Acad. Sci. USA* **95**:4929; U.S. Patent No. 5,869,248 to Yuan *et al.*), and the like.

The parvovirus vector may also comprise a heterologous nucleotide sequence that shares homology with and recombines with a locus on the host chromosome. This approach may be utilized to correct a genetic defect in the host cell.

The present invention also provides parvovirus vectors that express an immunogenic polypeptide, *e.g.*, for vaccination. The nucleic acid may encode any immunogen of interest known in the art including, but are not limited to, immunogens from human immunodeficiency virus, influenza virus, gag proteins, tumor antigens, cancer antigens, bacterial antigens, viral antigens, and the like.

The use of parvoviruses as vaccines is known in the art (*see*, *e.g.*, Miyamura *et al.*, (1994) *Proc. Nat. Acad. Sci USA* **91**:8507; U.S. Patent No. 5,916,563 to Young *et al.*, 5,905,040 to Mazzara *et al.*, U.S. Patent No. 5,882,652, U.S. Patent No. 5,863,541 to Samulski *et al.*; the disclosures of which are incorporated herein in their entireties by reference). The antigen may be presented in the parvovirus capsid. Alternatively, the antigen may be expressed from a heterologous nucleic acid introduced into a recombinant vector genome.

An immunogenic polypeptide, or immunogen, may be any polypeptide suitable for protecting the subject against a disease, including but not limited to microbial, bacterial, protozoal, parasitic, fungal and viral diseases. For example, the immunogen may be an orthomyxovirus immunogen (*e.g.*, an influenza virus immunogen, such as the influenza virus hemagglutinin (HA) surface protein or the influenza virus nucleoprotein gene, or an equine influenza virus immunogen), or a lentivirus immunogen (*e.g.*, an equine infectious anemia virus immunogen, a Simian Immunodeficiency Virus (SIV) immunogen, or a Human Immunodeficiency Virus (HIV) immunogen, such as the HIV or SIV envelope GP160 protein, the HIV or SIV matrix/capsid proteins, and the HIV or SIV *gag*, *pol* and *env* genes products). The immunogen may also be an arenavirus immunogen (*e.g.,* Lassa fever virus immunogen, such as the Lassa fever virus nucleocapsid protein gene and the Lassa fever envelope glycoprotein gene), a poxvirus immunogen (*e.g.*, vaccinia, such as the vaccinia L1 or L8 genes), a flavivirus immunogen (*e.g.,* a yellow fever virus immunogen or a Japanese encephalitis virus immunogen), a filovirus immunogen

(*e.g.*, an Ebola virus immunogen, or a Marburg virus immunogen, such as NP and GP genes), a bunyavirus immunogen (*e.g.*, RVFV, CCHF, and SFS viruses), or a coronavirus immunogen (*e.g.*, an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein gene, or a porcine transmissible gastroenteritis virus immunogen, or an avian infectious bronchitis virus immunogen). The immunogen may further be a polio immunogen, herpes immunogen (*e.g.*, CMV, EBV, HSV immunogens) mumps immunogen, measles immunogen, rubella immunogen, diphtheria toxin or other diptheria immunogen, pertussis antigen, hepatitis (*e.g.*, hepatitis A, hepatitis B or hepatitis C) immunogen, or any other vaccine immunogen known in the art.

Alternatively, the immunogen may be any tumor or cancer cell antigen. Preferably, the tumor or cancer antigen is expressed on the surface of the cancer cell. Exemplary cancer and tumor cell antigens are described in S.A. Rosenberg, (1999) *Immunity* **10**:281). Other illustrative cancer and tumor antigens include, but are not limited to: BRCA1 gene product, BRCA2 gene product, gp100, tyrosinase, GAGE-1/2, BAGE, RAGE, NY-ESO-1, CDK-4, β-catenin, MUM-1, Caspase-8, KIAA0205, HPVE, SART-1, PRAME, p15, melanoma tumor antigens (Kawakami et al., (1994) *Proc. Natl. Acad. Sci. USA* **91**:3515; Kawakami et al., (1994) *J. Exp. Med.*, **180**:347; Kawakami et al., (1994) *Cancer Res.* **54**:3124) including MART-1 (Coulie et al., (1991) *J. Exp. Med.* **180**:35), gp100 (Wick et al., (1988) *J. Cutan. Pathol.* **4**:201) and MAGE antigen, MAGE-1, MAGE-2 and MAGE-3 (Van der Bruggen et al., (1991) *Science*, **254**:1643); CEA, TRP-1, TRP-2, P-15 and tyrosinase (Brichard et al., (1993) *J. Exp. Med.* **178**:489); HER-2/neu gene product (U.S. Pat. No. 4,968,603), CA 125, LK26, FB5 (endosialin), TAG 72, AFP, CA19-9, NSE, DU-PAN-2, CA50, SPan-1, CA72-4, HCG, STN (sialyl Tn antigen), c-erbB-2 proteins, PSA, L-CanAg, estrogen receptor, milk fat globulin, p53 tumor suppressor protein (Levine, (1993) *Ann. Rev. Biochem.* **62**:623); mucin antigens (international patent publication WO 90/05142); telomerases; nuclear matrix proteins; prostatic acid phosphatase; papilloma virus antigens; and antigens associated with the following cancers: melanomas, adenocarcinoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, colon cancer, non-Hodgkin's lymphoma, Hodgkin's lymphoma, leukemias, uterine cancer, breast cancer, prostate cancer, ovarian cancer, cervical

cancer, bladder cancer, kidney cancer, pancreatic cancer and others (*see, e.g.*, Rosenberg, (1996) *Ann. Rev. Med.* **47**:481-91).

Alternatively, the heterologous nucleotide sequence may encode any polypeptide that is desirably produced in a cell *in vitro*, *ex vivo*, or *in vivo*. For example, the parvovirus vectors may be introduced into cultured cells and the expressed gene product isolated therefrom.

It will be understood by those skilled in the art that the heterologous nucleotide sequence(s) of interest may be operably associated with appropriate control sequences. For example, the heterologous nucleic acid may be operably associated with expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), promoters, enhancers, and the like.

Those skilled in the art will appreciate that a variety of promoter/enhancer elements may be used depending on the level and tissue-specific expression desired. The promoter/enhancer may be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer may be native or foreign and can be a natural or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

Promoter/enhancer elements that are native to the target cell or subject to be treated are most preferred. Also preferred are promoters/enhancer elements that are native to the heterologous nucleic acid sequence. The promoter/enhancer element is chosen so that it will function in the target cell(s) of interest. Mammalian promoter/enhancer elements are also preferred. The promoter/enhance element may be constitutive or inducible.

Inducible expression control elements are preferred in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoters/enhancer elements for gene delivery are preferably tissue-specific promoter/enhancer elements, and include muscle specific (including cardiac, skeletal and/or smooth muscle), neural tissue specific (including brain-specific), eye (including retina-specific and cornea-specific), liver specific, bone marrow specific, pancreatic specific, spleen specific, and lung specific promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible

promoters/enhancer elements include, but are not limited to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

In embodiments wherein which the heterologous nucleic acid sequence(s) will be transcribed and then translated in the target cells, specific initiation signals are generally required for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

Gene Transfer Technology.

The parvovirus vectors according to the present invention also provide a means for delivering heterologous nucleotide sequences into a broad range of cells, including dividing and non-dividing cells. The parvovirus vectors may be employed to deliver a nucleotide sequence of interest to a cell *in vitro*, *e.g.*, to produce a polypeptide *in vitro* or for *ex vivo* gene therapy. The vectors are additionally useful in a method of delivering a nucleotide sequence to a subject in need thereof, *e.g.*, to express an immunogenic or therapeutic polypeptide. In this manner, the polypeptide may thus be produced *in vivo* in the subject. The subject may be in need of the polypeptide because the subject has a deficiency of the polypeptide, or because the production of the polypeptide in the subject may impart some therapeutic effect, as a method of treatment or otherwise, and as explained further below.

In general, the parvovirus vectors of the present invention may be employed to deliver any foreign nucleic acid with a biological effect to treat or ameliorate the symptoms associated with any disorder related to gene expression. Alternatively, the invention can be used to treat any disease state for which it is beneficial to deliver a therapeutic polypeptide. Illustrative disease states include, but are not limited to: cystic fibrosis (cystic fibrosis transmembrane regulator protein) and other diseases of the lung, hemophilia A (Factor VIII), hemophilia B (Factor IX), thalassemia (ß-globin), anemia (erythropoietin) and other blood disorders, AIDS, Alzheimer's disease (myostatin, also known as GDF), multiple sclerosis (ß-interferon), Parkinson's disease (glial-cell line derived neurotrophic factor [GDNF]), Huntington's disease (RNAi to remove repeats), amyotrophic lateral sclerosis, epilepsy (galanin, neurotrophic factors), and other neurological disorders, cancer (endostatin,

angiostatin, TRAIL, FAS-ligand, cytokines including interferons; RNAi), diabetes mellitus (insulin), muscular dystrophies including Duchenne (dystrophin, mini-dystrophin, insulin-like growth factor I) and Becker, Gaucher disease (glucocerebrosidase), Hurler's disease (α-L-iduronidase), adenosine deaminase deficiency (adenosine deaminase), glycogen storage diseases (*e.g.*, Fabry disease [α-galactosidase] and Pompe disease [lysosomal acid α-glucosidase]) and other metabolic defects, congenital emphysema (α1 antitrypsin), Lesch-Nyhan Syndrome (hypoxanthine guanine phosphoribosyl transferase), Niemann-Pick disease (sphingomyelinase), Tays Sachs disease (lysosomal hexosaminidase A), Maple Syrup Urine Disease (branched-chain keto acid dehydrogenase), retinal degenerative diseases (and other diseases of the eye and retina; *e.g.*, PDGF for macular degeneration), diseases of solid organs (*e.g.*, brain, liver, kidney, heart including congestive heart failure [*e.g.*, by delivering PI3kinase, BART, phospholamban, sirca, *etc.*), muscle wasting (insulin-like growth factor I), kidney deficiency (erythropoietin), anemia (erythropoietin), arthritis (anti-inflammatory factors such as IRAP and TNFα soluble receptor), autoimmune diseases, and the like. The invention can further be used following organ transplantation to increase the success of the transplant and/or to reduce the negative side effects of organ transplantation or adjunct therapies (*e.g.*, by administering immunosuppressant agents or inhibitory nucleic acids to block cytokine production).

Alternatively, a gene transfer vector may be administered that encodes any therapeutic polypeptide.

Gene transfer has substantial potential use in understanding and providing therapy for disease states. There are a number of inherited diseases in which defective genes are known and have been cloned. In general, the above disease states fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and unbalanced states, which may involve regulatory or structural proteins, and which are typically inherited in a dominant manner. For deficiency state diseases, gene transfer could be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced disease states, gene transfer could be used to create a disease state in a model system, which could then be used in efforts to counteract the disease state. Thus parvovirus vectors produced according to the methods of the present invention permit the

treatment of genetic diseases. As used herein, a disease state is treated by partially or wholly remedying the deficiency or imbalance that causes the disease or makes it more severe. The use of site-specific recombination of nucleic sequences to cause mutations or to correct defects is also possible.

The parvovirus vectors according to the present invention may also be employed to provide an antisense nucleic acid or RNAi to a cell *in vitro* or *in vivo*. Expression of the antisense nucleic acid or RNAi in the target cell diminishes expression of a particular protein by the cell. Accordingly, antisense nucleic acids or RNAi may be administered to decrease expression of a particular protein in a subject in need thereof. Antisense nucleic acids or RNAi may also be administered to cells *in vitro* to regulate cell physiology, *e.g.*, to optimize cell or tissue culture systems.

Finally, the parvovirus vectors according to the instant invention find further use in diagnostic and screening methods, whereby a gene of interest is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

Delivery of Immunogenic Polypeptides.

As a further aspect, parvovirus vectors of the present invention may be used to produce an immune response in a subject. According to this embodiment, a parvovirus vector comprising a nucleotide sequence encoding an immunogen may be administered to a subject, and an active immune response is mounted by the subject against the immunogen. Immunogens are as described hereinabove. Preferably, a protective immune response is elicited.

Alternatively, the parvovirus vector may be administered to a cell *ex vivo* and the altered cell is administered to the subject. The heterologous nucleotide sequence is permitted to be introduced into the cell, and the cell is administered to the subject, where the heterologous nucleotide sequence encoding the immunogen is preferably expressed and induces an immune response in the subject against the immunogen. In particular embodiments, the cell is an antigen presenting cell (*e.g.*, a dendritic cell).

An "active immune response" or "active immunity" is characterized by "participation of host tissues and cells after an encounter with the immunogen. It involves differentiation and proliferation of immunocompetent cells in lymphoreticular tissues, which lead to synthesis of antibody or the development of cell-mediated reactivity, or both." Herbert B. Herscowitz, *Immunophysiology: Cell Function and*

Cellular Interactions in Antibody Formation, in IMMUNOLOGY: BASIC PROCESSES 117 (Joseph A. Bellanti ed., 1985). Alternatively stated, an active immune response is mounted by the host after exposure to immunogens by infection or by vaccination. Active immunity can be contrasted with passive immunity, which is acquired through the "transfer of preformed substances (antibody, transfer factor, thymic graft, interleukin-2) from an actively immunized host to a non-immune host." *Id.*

A "protective" immune response or "protective" immunity as used herein indicates that the immune response confers some benefit to the subject in that it prevents or reduces the incidence of disease. Alternatively, a protective immune response or protective immunity may be useful in the treatment of disease, in particular cancer or tumors (*e.g.*, by causing regression of a cancer or tumor and/or by preventing metastasis and/or by preventing growth of metastatic nodules). The protective effects may be complete or partial, as long as the benefits of the treatment outweigh any disadvantages thereof.

According to the foregoing methods of inducing an immune response in a subject, it is preferred that the parvovirus vector carrying the heterologous nucleotide sequence is administered in an immunogenically effective amount, as described below.

The parvovirus vectors of the present invention may also be administered for cancer immunotherapy by administration of a parvovirus vector expressing cancer cell antigens (or an immunologically similar molecule) or any other immunogen that produces an immune response against a cancer cell. To illustrate, an immune response may be produced against a cancer cell antigen in a subject by administering a parvovirus vector comprising a heterologous nucleotide sequence encoding the cancer cell antigen, for example to treat a patient with cancer. The parvovirus vector may be administered to a subject *in vivo* or by using *ex vivo* methods, as described herein.

As used herein, the term "cancer" encompasses tumor-forming cancers. Likewise, the term "cancerous tissue" encompasses tumors. A "cancer cell antigen" encompasses tumor antigens.

The term "cancer" has its understood meaning in the art, for example, an uncontrolled growth of tissue that has the potential to spread to distant sites of the body (*i.e.*, metastasize). Exemplary cancers include, but are not limited to, leukemias, lymphomas, colon cancer, renal cancer, liver cancer, breast cancer, lung

cancer, prostate cancer, ovarian cancer, melanoma, and the like. Preferred are methods of treating and preventing tumor-forming cancers.

The term "tumor" is also understood in the art, for example, as an abnormal mass of undifferentiated cells within a multicellular organism. Tumors can be malignant or benign. Preferably, the methods disclosed herein are used to prevent and treat malignant tumors.

Cancer cell antigens according to the present invention have been described hereinabove. By the terms "treating cancer" or "treatment of cancer," it is intended that the severity of the cancer is reduced or the cancer is prevented or at least partially eliminated. Preferably, these terms indicate that metastasis of the cancer is prevented or reduced or at least partially eliminated. It is further preferred that these terms indicate that growth of metastatic nodules (*e.g.*, after surgical removal of a primary tumor) is prevented or reduced or at least partially eliminated. By the terms "prevention of cancer" or "preventing cancer" it is intended that the methods at least partially eliminate or reduce the incidence or onset of cancer. Alternatively stated, the onset of cancer in the subject may be slowed, controlled, decreased in likelihood or probability, or delayed.

In particular embodiments, cells may be removed from a subject with cancer and contacted with parvovirus particles according to the instant invention. The modified cell is then administered to the subject, whereby an immune response against the cancer cell antigen is elicited. This method is particularly advantageously employed with immunocompromised subjects that cannot mount a sufficient immune response *in vivo* (*i.e.*, cannot produce enhancing antibodies in sufficient quantities).

It is known in the art that immune responses may be enhanced by immunomodulatory cytokines (*e.g.*, α-interferon, β-interferon, γ-interferon, ω-interferon, τ-interferon, interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin 5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin 12, interleukin-13, interleukin-14, interleukin-18, B cell Growth factor, CD40 Ligand, tumor necrosis factor-α, tumor necrosis factor-β, monocyte chemoattractant protein-1, granulocyte-macrophage colony stimulating factor, and lymphotoxin). Accordingly, immunomodulatory cytokines (preferably, CTL inductive cytokines) may be administered to a subject in conjunction with the parvovirus vectors.

Cytokines may be administered by any method known in the art. Exogenous cytokines may be administered to the subject, or alternatively, a nucleotide sequence encoding a cytokine may be delivered to the subject using a suitable vector, and the cytokine produced *in vivo*.

Subjects, Pharmaceutical Formulations, and Modes of Administration.

Parvovirus vectors according to the present invention find use in both veterinary and medical applications. Suitable subjects include both avians and mammals. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys, pheasant, parrots, parakeets. The term "mammal" as used herein includes, but is not limited to, humans, non-human primates bovines, ovines, caprines, equines, felines, canines, lagomorphs, *etc.* Human subjects include neonates, infants, juveniles, and adults.

In particular embodiments, the present invention provides a pharmaceutical composition comprising a virus particle of the invention in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, *etc.* For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and will preferably be in solid or liquid particulate form.

By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects.

One aspect of the present invention is a method of transferring a nucleotide sequence to a cell *in vitro*. The virus particles may be introduced to the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of virus to administer can vary, depending upon the target cell type and number, and the particular virus vector, and can be determined by those of skill in the art without undue experimentation. Preferably, at least about 10^3 infectious units, more preferably at least about 10^5 infectious units are introduced to the cell.

The cell(s) to be introduced the parvovirus vector may be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons and oligodendricytes),

lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), epithelial cells (*e.g.*, gut and respiratory epithelial cells), muscle cells, dendritic cells, pancreatic cells (including islet cells), hepatic cells, myocardial cells, bone cells (*e.g.*, bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. Alternatively, the cell may be any progenitor cell. As a further alternative, the cell can be a stem cell (*e.g.*, neural stem cell, liver stem cell). As still a further alternative, the cell may be a cancer or tumor cell. Moreover, the cells can be from any species of origin, as indicated above.

The parvovirus vectors may be introduced to cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, the parvovirus vector is introduced therein, and the cells are then replaced back into the subject. Methods of removing cells from subject for treatment *ex vivo*, followed by introduction back into the subject are known in the art (*see, e.g.*, U.S. patent No. 5,399,346; the disclosure of which is incorporated herein in its entirety). Alternatively, the recombinant parvovirus vector is introduced into cells from another subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof.

Suitable cells for *ex vivo* gene therapy are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 or about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In particular embodiments, the cells transduced with the parvovirus vector are administered to the subject in a therapeutically effective amount in combination with a pharmaceutical carrier.

A "therapeutically effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a "therapeutically effective" amount is an amount that will provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

In some embodiments, cells that have been transduced with a parvovirus vector may be administered to elicit an immunogenic response against the delivered

polypeptide (*e.g.*, expressed as a transgene or in the capsid). Typically, a quantity of cells expressing an immunogenically effective amount of the polypeptide in combination with a pharmaceutically acceptable carrier is administered. An "immunogenically effective amount" is an amount of the expressed polypeptide that is sufficient to evoke an active immune response in the subject to which the pharmaceutical formulation is administered. Preferably, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof.

A further aspect of the invention is a method of administering the parvovirus particles to subjects. Administration of the parvovirus particles produced according to the present invention to a human subject or an animal in need thereof can be by any means known in the art for administering virus vectors. Preferably, the parvovirus vector is delivered in a therapeutically effective dose in a pharmaceutically acceptable carrier.

The parvovirus vectors of the invention can further be administered to elicit an immunogenic response (*e.g.*, as a vaccine). Typically, vaccines of the present invention comprise an immunogenically effective amount of virus in combination with a pharmaceutically acceptable carrier. Preferably, the dosage is sufficient to produce a protective immune response (as defined above). The degree of protection conferred need not be complete or permanent, as long as the benefits of administering the immunogenic polypeptide outweigh any disadvantages thereof. Subjects and immunogens are as described above.

Dosages of the parvovirus particles to be administered to a subject will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular virus vector, and the nucleic acid to be delivered, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are virus titers of at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^3, 10^{14}, 10^{15} transducing units or more, preferably about $10^8 - 10^{13}$ transducing units, yet more preferably 10^{12} transducing units.

In particular embodiments, more than one administration (*e.g.*, two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, *e.g.*, daily, weekly, monthly, yearly, *etc.*

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Exemplary modes of administration include oral, rectal, transmucosal, topical, intranasal, intrathecal, intraocular, transdermal, *in utero* (or *in ovo*), inhalation, parenteral (*e.g.*, intravenous, subcutaneous, intradermal, intramuscular, and intraarticular) administration, and the like, as well as direct tissue or organ injection.

Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the virus particle can be delivered adhered to a surgically implantable matrix (*e.g.*, as described in U.S. Patent Publication No. US-2004-0013645-A1).

The parvovirus vectors disclosed herein may be administered to the lungs of a subject by any suitable means, but are preferably administered by administering an aerosol suspension of respirable particles comprised of the parvovirus vectors, which the subject inhales. The respirable particles may be liquid or solid. Aerosols of liquid particles comprising the parvovirus vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. *See, e.g.*, U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the parvovirus vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Materials and Methods

Plasmid and DNA Mutagenesis

Starting plasmids for these experiments were pxr1, pxr2, prx3 (Rabinowitz et al, (2001) *J. Virology* 76:781-801). All plasmid mutagenesis was performed using either the Quik Change Multi Site Mutagenesis or the Quik Change Site Directed Mutagenesis kits (both from Stratagene). Accuracy of the nucleotide changes was verified by DNA sequence analyses followed by DNA subcloning to eliminate any

unwanted artifacts generated by the mutagenesis approach. In certain instances, subcloning was performed using the newly generated mutant to generate another mutant.

Recombinant Virus Production

All recombinant AAV (rAAV) viruses were generated using the standard triple transfection method (Haberman et al. 1999. Production of recombinant adeno-associated viral vectors and use in in vitro and in vivo administration, p. 4.17.1-4.17.25. *In* J. Crawley, et al. (eds.), Current protocols in neuroscience, vol. 1. John Wiley & Sons, Inc., New York, NY). rAAV was purified in this study using standard cesium chloride gradients or using heparin column chromatography (*Id.*). The method used for virus purification is indicated in the corresponding figure. The physical titer of the different viral preparations was evaluated using dot blot hybridization. The preparations being compared in one experiment were evaluated on the same dot blots to control for variation between different dot blots.

Animal Imaging

1×10^{10} viral genome containing particles (vg) were injected into the gastrocnemius of 6-week-old male BALB/c mice. A total of 6 limbs were injected for each virus type using 25 µl of virus. Animals were imaged at different days post injection using the Roper Scientific Imaging (Princeton Instruments). Briefly, animals were anesthetized and injected IP with luciferin substrate. 10 minutes post-injection the animals were placed in the chamber and light emission was determined. The average number of total pixels per region of interest was determined using the CMIR_Image software (Center for Molecular Imaging Research, Mass. General) and plotted over time.

Heparin Binding Experiments

Batch binding of rAAV to heparin agarose was performed as described previously (Rabinowitz, (2004) *J. Virology* 78:4421-4432). Briefly, equivalent particles of rAAV virions were applied to heparin agarose type 1 (H-6508, Sigma, St. Louis, MO) in 1x PBS, allowed to bind for one hour at room temperature, centrifuged at low speed for 2 minutes, and supernatant (flow through) was removed. Six washes of five bed-volumes of PBS 1 mM $MgCl_2$ were performed, followed by a

three-step elution of five bed-volumes of PBS 1 mM $MgCl_2$ containing 0.5 M NaCl (step 1), 1.0 M NaCl (step 2), or 1.5 M NaCl (step 3). The number of rAAV particles present in the washes and the 3-step elution was determined by dot blot hybridization.

Results

Rationale of the Triage Approach

It is clear in the literature that different serotypes of AAV exhibit differing abilities to transduce different tissues. One well-documented example is the ability of AAV1 to preferentially transduce skeletal muscle. The amino acid sequences of AAV1 and AAV2 are 83% identical. One previous study exchanged large regions of AAV2 capsid with the corresponding amino acids of AAV1 and examined the ability of the resultant vectors to transduce skeletal muscle (Hauck et al., (2003) *J. Virology* 77:2768-2774). They were able to identify 2 amino acids that appeared to confer enhanced skeletal muscle transduction by AAV2, albeit not as well as AAV1. We wished to examine the phenomenon in a more detailed manner. Employing bio-informatic and structure-function analysis, we were able to identify key amino acids residues that account for enhanced AAV1 transduction to test in the context of majority AAV2 capsid. This approach would avoid the need to make all the different changes of amino acids between the 2 serotypes and all of the different permutations. We took advantage of the crystal structure of AAV2, amino acid sequence alignments, and known properties of the various AAV serotypes. 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**).

Some AAV variants exhibited no difference between AAV2 while others are much better than AAV2.

The different AAV variants were evaluated for their ability to transduce skeletal muscle following injection of 1 x10^{10} genome containing viral particles into the gastrocnemius muscle of BALB/c mice. Some virus variants did not exhibit any differences in the capability to transduce skeletal muscle (**Figure 2a and b**).

Although the 324, 328 positions are located on loops predicted to lie on the capsid surface, changing of these amino acids to the corresponding amino acids in AAV1 did nothing to improve the capability of this virus to transduce skeletal muscle (**Figure 2A and 2B**).

One variant (2.5) exhibited much higher muscle transduction than AAV2 (**Figure 3A**). This variant had 5 amino acid changes that were located in the 2-fold axis of symmetry (**Figure 3B**). Although it appears that groups of these 5 amino acids lie on different parts of the same subunit (2 on one side and 3 on another), they are in very close proximity when 2 of the subunits come together (**Figure 3C**).

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.

The same insertion has been made at position 264 in the AAV3b backbone (**Figure 10**).

Another variant that exhibits higher muscle transduction than AAV2 is the 454, 461 variant (**Figure 6**). Different combinations of 265 with the 454, 461 changes are assessed to determine whether further enhancement can be observed by combining these variants.

Interestingly, the 263, 265 region is divergent amongst the serotypes (**Figure 7** boxed region). There are additional amino acid insertions in this area compared to AAV2.

Finally, we examined the ability of the different variants to be purified by heparin (**Figure 8**). All of the variants tested exhibited heparin binding profiles similar to AAV2.

Immunological Profile

Similar to other non-enveloped viruses, high doses of AAV generate neutralizing antibody that prevents repeated dosing. With the advent of new serotypes, repeat administration is theoretically possible. Although a limited number of serotypes are available, transduction efficiency and cross reactivity of neutralizing antibodies to these reagents at high doses in animal model and human studies suggest otherwise, but further analysis is required (Grimm et al., (2003) Blood 102:2412-9). To explore the ability to avoid a pre-existing immune response to AAV2, we tested the chimeric AAV 2.5 vectors for transgene expression *in vitro* after exposure to serum from animals pre-exposed to different AAV serotypes (1, 2, and 2.5 respectively).

To generate animals with a robust immune response to AAV virion shell, $4x10^{10}$ particles of AAV serotype 1, 2, and 2.5 vector were independently injected intramuscularly in C57blk6 mice. Four weeks post-injection blood was isolated and serum collected. Serum from these animals was than used in a neutralizing antibody assay using 293 cells and AAV specific serotype vectors expressing GFP as a reporter gene. In this assay, serum is sequentially diluted and than mixed with a known amount of serotype specific vector ($1x10^8$ particles) at 4 °C for 2 hr. This mixture of serum and vector is then added to 293 cells in 24-well plates in the presence of adenovirus helper virus at a multiplicity of infection of 5. Under these conditions, green fluorescent protein (GFP) expression is a measure of serotype-specific vector ability to infect cells in the presence of neutralizing antibodies. The neutralizing antibody titer is then calculated as the highest dilution where GFP expression is 50% or less than control vector (without pre-mixture with serotype specific serum).

As seen in **Table 2**, animals pre-exposed to AAV1 could neutralize AAV 1 GFP transduction with dilutions as high as 1:1000. However this serotype 1 specific neutralizing antibody required more mouse serum to neutralize AAV chimeric 2.5 (1:100 dilution). More importantly, this observation was true for mouse sera obtained from animals pre-exposed to AAV serotype 2 virion shells. In this assay, only after sera were diluted 1:10,000 did we observe 50% GFP transduction when compared to AAV2 control. However, for chimeric 2.5, 50% GFP transduction was observed with only 1:100 dilution of this mouse serum. Since only 0.6% of the amino acid changes differ from AAV2 in this chimeric vector, these alterations had profound

effects on the ability of pre-existing AAV2 neutralizing antibody to recognize the AAV 2.5 capsid shell. As expected, animals pre-exposed to AAV 2.5 and then assayed for neutralizing activity against AAV 1, 2, and 2.5 yielded expected results (see **Table 2**) with highest dilution required for AAV 2.5 (1:8000) followed by 1:1000 for AAV 2 and 1:100 for AAV serotype 1 respectively. The conclusion from these studies is that the 5 amino acid alteration in chimeric AAV 2.5 although small in number (0.6% total amino acids) was sufficient to significantly affect the immune profile for this virion when challenged with neutralizing antibodies specific for AAV2.

Based on these studies, AAV 2.5 would be suited when transducing individuals pre-exposed to AAV1, AAV2 or both, thereby providing greater versatility in available vectors. For example, this chimeric vector would allow for re-administration in animals and patients pre-exposed to AAV2. In addition, this chimeric vector demonstrates for the first time that selected amino acids can be changed in the AAV2 amino acid capsid sequence that significantly alter immune response. Based on these results, similar alterations at similar capsid coding regions for other AAV serotypes would also result in unique immune profiles allowing one to easily generate a large number of alternative AAV variants for repeat administration without having to change to a new serotype capsid each time.

Table 2

The Neutralizing Antibody Assay for AAV2.5 virus

Sera	Target virus	NA titer
AAV1	AAV1	1:1000
	AAV2.5	1:100
AAV2	AAV2	1:10000
	AAV2.5	1:100
AAV2.5	AAV1	1:100
	AAV2	1:1000
	AAV2.5	1:8000

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

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That which is claimed is:

1. A chimeric virus vector comprising:

(a) a recombinant adeno-associated virus (rAAV) genome; and

(b) a chimeric AAV capsid comprising a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

2. The chimeric virus vector of claim 1, wherein the insertion and/or substitution is an insertion and/or substitution of a charged amino acid, an amino acid that can be phosphorylated or sulfated, or an amino acid that preserves the hydrophilic loop structure into the capsid subunits.

3. The chimeric virus vector of claim 2, wherein the insertion and/or substitution is an insertion and/or substitution of threonine, serine or tyrosine into the capsid subunits.

4. The chimeric virus vector of claim 3, wherein the insertion and/or substitution is an insertion and/or substitution of threonine into the capsid subunits.

5. The chimeric virus vector of claim 2, wherein the insertion and/or substitution is an insertion and/or substitution of aspartic acid, glutamic acid, lysine or arginine into the capsid subunits.

6. The chimeric virus vector of claim 2, wherein the insertion and/or substitution is an insertion or substitution of glycine, alanine, proline, asparagine or glutamine.

7. The chimeric virus vector of claim 1, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid insertion following the amino acid corresponding to amino acid position 264 in the AAV2 capsid subunits.

8. The chimeric virus vector of claim 1, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits.

9. The chimeric virus vector of claim 7 or claim 8, wherein the insertion is an insertion of a charged amino acid, an amino acid that can be phosphorylated or sulfated, or an amino acid that preserves the hydrophilic loop structure.

10. The chimeric virus vector of claim 8, wherein the insertion is an insertion of threonine, serine or tyrosine.

11. The chimeric virus vector of claim 10, wherein the insertion is an insertion of threonine.

12. The chimeric virus vector of claim 9, wherein the insertion is an insertion of aspartic acid, glutamic acid, lysine or arginine.

13. The chimeric virus vector of claim 9, wherein the insertion or substitution is an insertion or substitution of glycine, alanine, proline, asparagine or glutamine.

14. The chimeric virus vector of claim 1, wherein the chimeric virus vector comprises a chimeric AAV3b capsid that has been modified to comprise non-AAV3b sequences and further comprises an amino acid insertion following the amino acid corresponding to amino acid position 264 in the AAV3b capsid subunits.

15. The chimeric virus vector of claim 1, wherein the chimeric virus vector comprises a chimeric AAV3b capsid comprising an amino acid insertion following amino acid position 264 in the AAV3b capsid subunits.

16. The chimeric virus vector of claim 14 or claim 15, wherein the insertion is an insertion of a charged amino acid, an amino acid that can be phosphorylated or sulfated, or an amino acid that preserves the hydrophilic loop structure.

17. The chimeric virus vector of claim 1, wherein the chimeric virus vector comprises a chimeric AAV capsid further comprising a selective amino acid insertion and/or substitution selected from the group consisting of:

(a) an amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(b) an amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(c) an amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

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

(f) an amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; and

(g) any combination of (a) to (f) above.

18. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 263 in the AAV2 capsid subunits.

19. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits.

20. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV3b capsid that has been modified to comprise non-AAV3b sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 263 in the AAV3b capsid subunits.

21. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV3b capsid comprising an amino acid substitution at amino acid position 263 in the AAV3b capsid subunits.

22. The chimeric virus vector of any one of claims 18-21, wherein the substitution is a substitution of alanine, glycine, valine, leucine, isoleucine, proline, asparagine, serine or threonine for glutamine.

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

24. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 705 in the AAV2 capsid subunits.

25. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits.

26. The chimeric virus vector of claim 24 or claim 25, wherein the substitution is a substitution of serine, threonine, tyrosine, proline, glutamine, alanine, glycine, valine, leucine or isoleucine for asparagine.

27. The chimeric virus vector of claim 26, wherein the substitution is a substitution of alanine for asparagine.

28. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2

sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 708 in the AAV2 capsid subunits.

29. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits.

30. The chimeric virus vector of claim 28 or claim 29, wherein the substitution is a substitution of serine, threonine, tyrosine, proline, asparagine, glutamine, alanine, glycine, leucine or isoleucine for valine.

31. The chimeric virus vector of claim 30, wherein the substitution is a substitution of alanine for valine.

32. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 716 in the AAV2 capsid subunits.

33. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 716 in the AAV2 capsid subunits.

34. The chimeric virus vector of claim 32 or claim 33, wherein the substitution is a substitution of an amino acid that cannot be phosphorylated.

35. The chimeric virus vector of claim 32 or claim 33, wherein the substitution is a substitution of serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine for threonine.

36. The chimeric virus vector of claim 35, wherein the substitution is a substitution of asparagine for threonine.

37. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 450 in the AAV2 capsid subunits.

38. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits.

39. The chimeric virus vector of claim 37 or claim 38, wherein the substitution is a substitution of an amino acid that cannot be phosphorylated.

40. The chimeric virus vector of claim 37 or claim 38, wherein the substitution is a substitution of serine, tyrosine, glycine, alanine, proline, valine, leucine, isoleucine, asparagine or glutamine for threonine.

41. The chimeric virus vector of claim 40, wherein the substitution is a substitution of asparagine for threonine.

42. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 457 in the AAV2 capsid subunits.

43. The chimeric virus vector of claim 17, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits.

44. The chimeric virus vector of claim 42 or claim 43, wherein the substitution is a substitution of glycine, alanine, valine, leucine, isoleucine, proline, serine, threonine or asparagine for glutamine.

45. The chimeric virus vector of claim 44, wherein the substitution is a substitution of asparagine for glutamine.

46. A chimeric virus vector comprising:

(a) a recombinant adeno-associated virus (rAAV) genome; and

(b) a chimeric AAV capsid comprising:

(i) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(ii) a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(iii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

(iv) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits or a corresponding change in other AAV capsids; and

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

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

47. The chimeric virus vector of claim 46, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises:

(a) an amino acid substitution at the amino acid corresponding to amino acid position 263 in the AAV2 capsid subunits;

(b) an amino acid insertion following the amino acid corresponding to amino acid position 264 in the AAV2 capsid subunits;

(c) an amino acid substitution at the amino acid corresponding to amino acid position 705 in the AAV2 capsid subunits;

(d) an amino acid substitution at the amino acid corresponding to amino acid position 708 in the AAV2 capsid subunits; and

(e) an amino acid substitution at the amino acid corresponding to amino acid position 716 in the AAV2 capsid subunits.

48. The chimeric virus vector of claim 46, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising:

(a) an amino acid substitution at amino acid position 263 in the AAV2 capsid subunits;

(b) an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits;

(c) an amino acid substitution at amino acid position 705 in the AAV2 capsid subunits;

(d) an amino acid substitution at amino acid position 708 in the AAV2 capsid subunits; and

(e) an amino acid substitution at amino acid position 716 in the AAV2 capsid subunits.

49. The chimeric virus vector of claim 47, wherein the chimeric AAV2 capsid comprises:

(a) an alanine for glutamine substitution at the amino acid that corresponds to amino acid position 263 in the AAV2 capsid subunits;

(b) a threonine insertion following the amino acid that corresponds to amino acid position 264 in the AAV2 capsid subunits;

(c) an alanine for asparagine substitution at the amino acid that corresponds to amino acid position 705 in the AAV2 capsid subunits;

(d) an alanine for valine substitution at the amino acid that corresponds to amino acid position 708 in the AAV2 capsid subunits; and

(e) an asparagine for threonine substitution at the amino acid that corresponds to amino acid position 716 in the AAV2 capsid subunits.

50. The chimeric virus vector of claim 48, wherein the chimeric virus vector comprises:

(a) an alanine for glutamine substitution at amino acid position 263 in the AAV2 capsid subunits;

(b) a threonine insertion following amino acid position 264 in the AAV2 capsid subunits;

(c) an alanine for asparagine substitution at amino acid position 705 in the AAV2 capsid subunits;

(d) an alanine for valine substitution at amino acid position 708 in the AAV2 capsid subunits; and

(e) an asparagine for threonine substitution at amino acid position 716 in the AAV2 capsid subunits.

51. The chimeric virus vector of claim 50, wherein the chimeric virus vector is the AAV 2.5 vector disclosed herein.

52. A chimeric virus vector comprising:

(a) a recombinant adeno-associated virus (rAAV) genome; and

(b) a chimeric AAV capsid comprising:

(i) a selective amino acid insertion and/or substitution that results in an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

(iii) a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

53. The chimeric virus vector of claim 52, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises:

(a) an amino acid insertion following the amino acid that corresponds to amino acid position 264 in the AAV2 capsid subunits;

(b) an amino acid substitution at the amino acid that corresponds to amino acid position 450 in the AAV2 capsid subunits; and

(c) an amino acid substitution at the amino acid that corresponds to amino acid position 457 in the AAV2 capsid subunits.

54. The chimeric virus vector of claim 52, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising:

(a) an amino acid insertion following amino acid position 264 in the AAV2 capsid subunits;

(b) an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits; and

(c) an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits.

55. The chimeric virus vector of claim 53, wherein the chimeric virus vector comprises:

(a) a threonine insertion following the amino acid that corresponds to amino acid position 264 in the AAV2 capsid subunits;

(b) an asparagine for threonine substitution at the amino acid that corresponds to amino acid position 450 in the AAV2 capsid subunits; and

(c) an asparagine for glutamine substation at the amino acid that corresponds to amino acid position 457 in the AAV2 capsid subunits.

56. The chimeric virus vector of claim 54, wherein the chimeric virus vector comprises:

(a) a threonine insertion following amino acid position 264 in the AAV2 capsid subunits;

(b) an asparagine for threonine substitution at amino acid position 450 in the AAV2 capsid subunits; and

(c) an asparagine for glutamine substitution at amino acid position 457 in the AAV2 capsid subunits.

57. A chimeric virus vector comprising:

(a) a recombinant adeno-associated virus (rAAV) genome; and

(b) a chimeric AAV capsid comprising a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

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

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

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

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

62. A chimeric virus vector comprising:

(a) a recombinant adeno-associated virus (rAAV) genome; and

(b) a chimeric AAV capsid comprising a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino

acid position 457 in the AAV2 capsid subunits or a corresponding change in other AAV capsids;

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

63. The chimeric virus vector of claim 62, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises an amino acid substitution at the amino acid corresponding to amino acid position 457 in the AAV2 capsid subunits.

64. The chimeric viral vector of claim 62, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising an amino acid substitution at amino acid position 457 in the AAV2 capsid subunits.

65. The chimeric virus vector of claim 63 or claim 64, wherein the substitution is a substitution of glycine, alanine, valine, leucine, isoleucine, praline, serine, asparagine for glutamine substitution.

66. The chimeric virus vector of claim 62, wherein the chimeric AAV capsid further comprises a selective amino acid insertion and/or substitution that results in an amino acid substitution at amino acid position 450 in the AAV2 capsid subunits or a corresponding change in other AAV capsids.

67. The chimeric virus vector of claim 66, wherein the chimeric virus vector comprises a chimeric AAV2 capsid that has been modified to comprise non-AAV2 sequences and further comprises:

(a) a substitution of asparagine for threonine at the amino acid that corresponds to amino acid position 450 in the AAV2 capsid subunits;

(b) a substitution of asparagine for glutamine at the amino acid that corresponds to amino acid position 457 in the AAV2 capsid subunits.

68. The chimeric virus vector of claim 66, wherein the chimeric virus vector comprises a chimeric AAV2 capsid comprising:

(a) a substitution of asparagine for threonine at position 450 in the AAV2 capsid subunits;

(b) a substitution of asparagine for glutamine at position 457 in the AAV2 capsid subunits.

69. The chimeric virus vector of any of claims 1-68, wherein the rAAV genome comprises a heterologous sequence encoding a polypeptide.

70. The chimeric virus vector of claim 69, wherein the polypeptide is a therapeutic polypeptide.

71. The chimeric virus vector of claim 70, wherein the therapeutic polypeptide is selected from the group consisting of: dystrophin, mini-dystrophin, utrophin, a clotting factor including Factor IX or Factor VIII, a growth factor including insulin-like growth factor I, a neurotrophic factor, TGF-alpha soluble receptor, IRAP, alpha1-antitrypsin, lysosomal acid-alpha glucosidase, beta-glucocerebrosidase, alpha-galactosidase A, a cytokine, an interferon including beta-interferon, TRAIL, FAS-ligand, endostatin, angiostatin, cystic fibrosis transmembrane regulator protein, erythropoietin, LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, adenosine deaminase, hypoxanthine guanine phosphoribosyl transferase, sphingomyelinase, lysosomal hexosaminidase, branched-chain keto acid dehydrogenase, a hormone, a suicide gene product, a tumor suppressor gene product, and any combination thereof.

72. The chimeric virus vector of any of claims 1-71, wherein the rAAV genome comprises a heterologous sequence encoding an untranslated RNA.

73. The chimeric virus vector of claim 72, wherein the untranslated RNA in an antisense RNA or an interfering RNA (RNAi).

74. The chimeric virus vector of any of claims 1-73, wherein the chimeric virus vector has enhanced transduction for skeletal muscle.

75. The chimeric virus vector of any of claims 1-74, wherein the chimeric virus vector has a different immunological profile than the parent rAAV vector.

76. A pharmaceutical formulation comprising the chimeric virus vector according to any of claims 1-75 in a pharmaceutically acceptable carrier.

77. A method of administering a nucleic acid to a cell comprising contacting the cell with the chimeric virus vector of any of claims 1-75 or the pharmaceutical formulation of claim 76.

78. A method of delivering a nucleic acid to a subject comprising administering to the subject the chimeric virus vector of any of claims 1-77 or the pharmaceutical formulation of claim 76.

79. The method of claim 78, wherein the subject is a human subject.

80. The method of claim 78, wherein the subject has a condition selected from the group consisting of a muscular dystrophy including Duchenne or Becker muscular dystrophies, hemophilia A, hemophilia B, multiple sclerosis, diabetes mellitus, Gaucher disease, Fabry disease, Pompe disease, cancer, arthritis, muscle wasting, heart disease, a neurological disorder including epilepsy, Huntington's disease, Parkinson's disease or Alzheimer's disease, an autoimmune disease, cystic fibrosis, thalassemia, Hurler's disease, anemia, arthritis, a retinal degenerative disorder including macular degeneration, and adenosine deaminase deficiency.

81. The method of claim 78, wherein the subject has antibodies against one or more of the AAV serotypes.

82. The method of claim 81, wherein the subject has antibodies against AAV1 or AAV2.

83. The method of claim 81, wherein the subject has previously been administered an AAV vector from the one more AAV serotypes.

CHIMERIC VECTORS

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.

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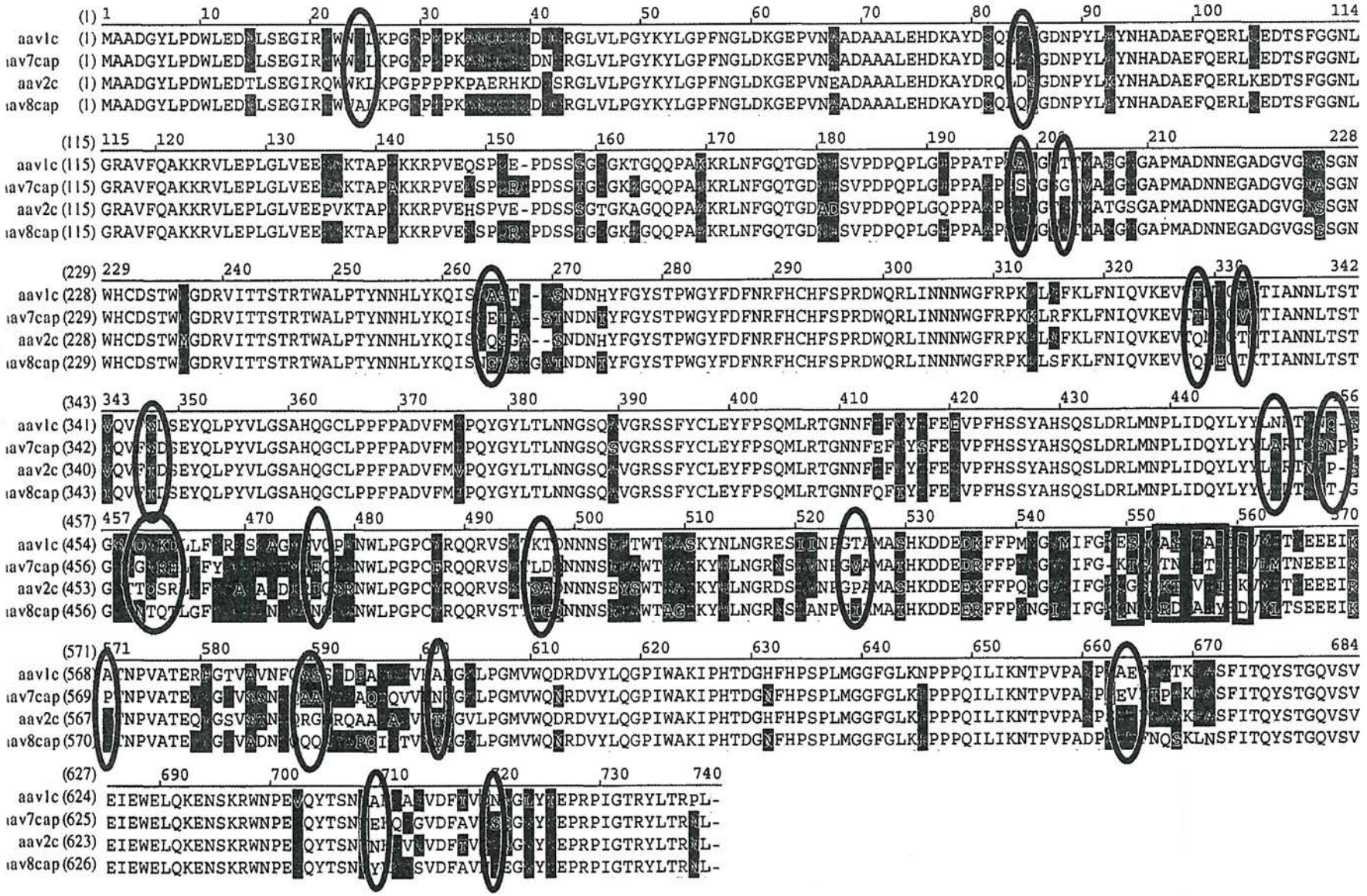

FIGURE 1A

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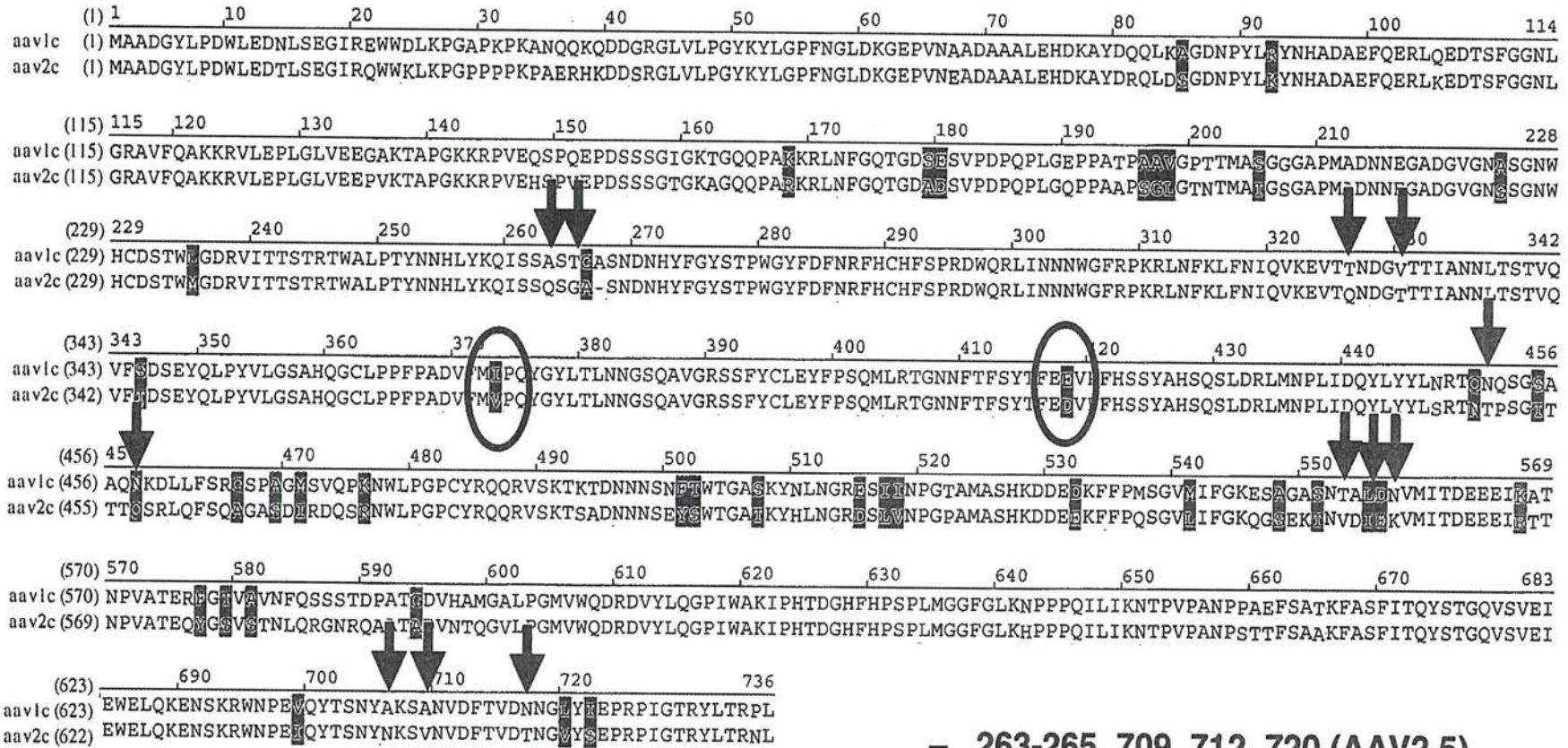
12 candidates that tested based on amino acid and crystal structure analyses

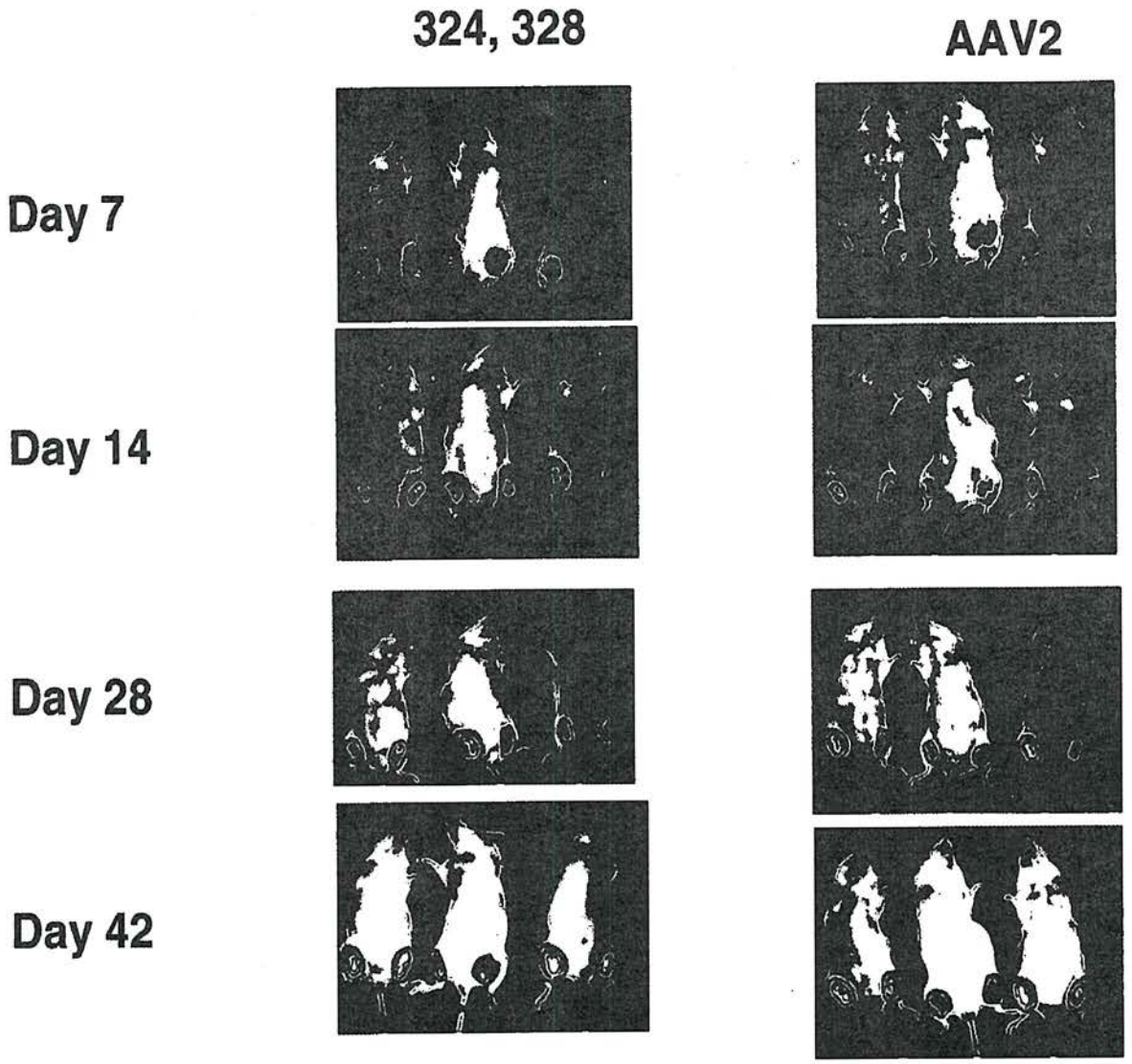


- **263-265, 709, 712, 720 (AAV2.5)**
- **556, 558, 560**
- **324, 328**
- **454, 461**
- **371, 416**

FIGURE 1B

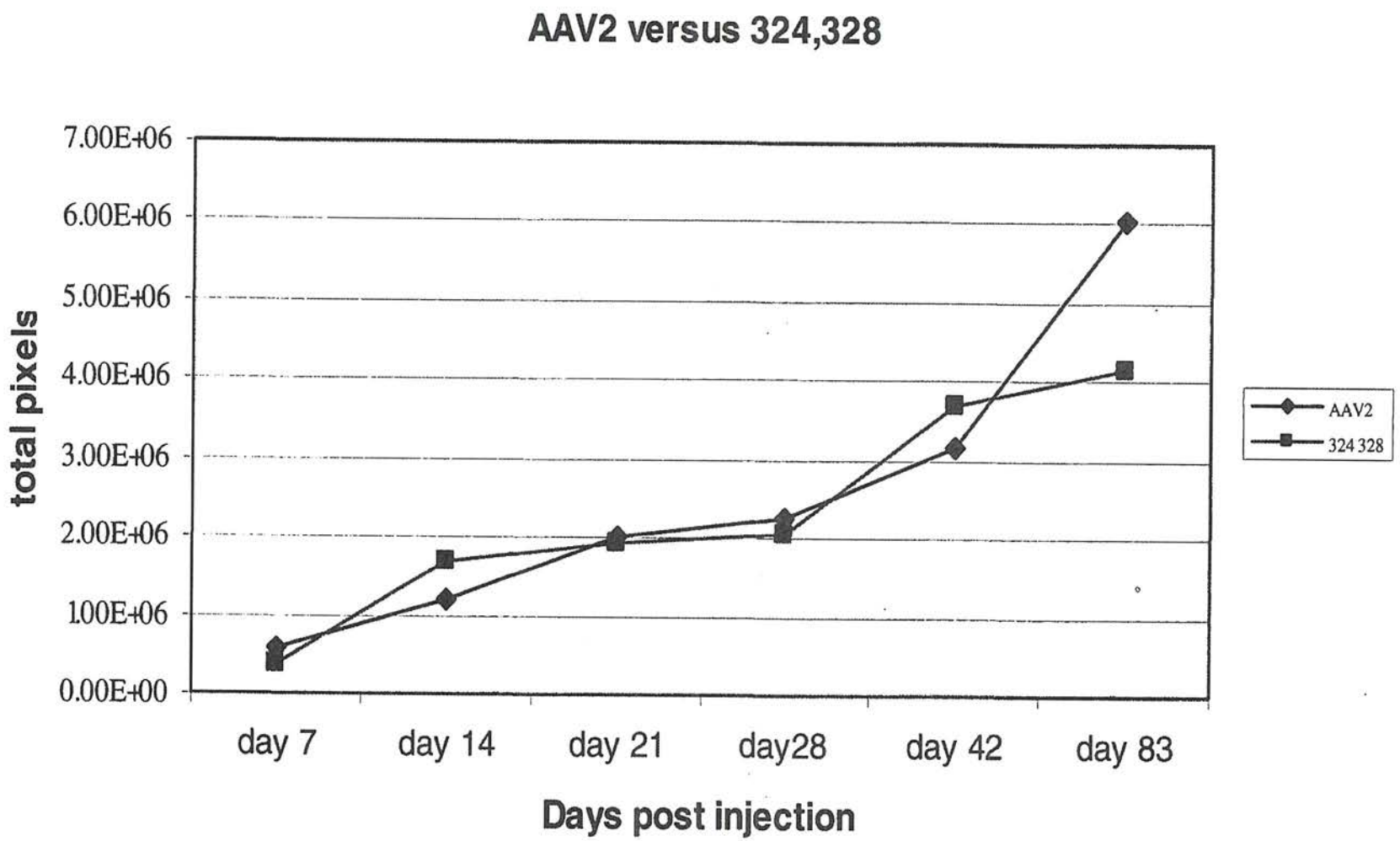
Sarepta Exhibit 1059, page 65

FIGURE 2A



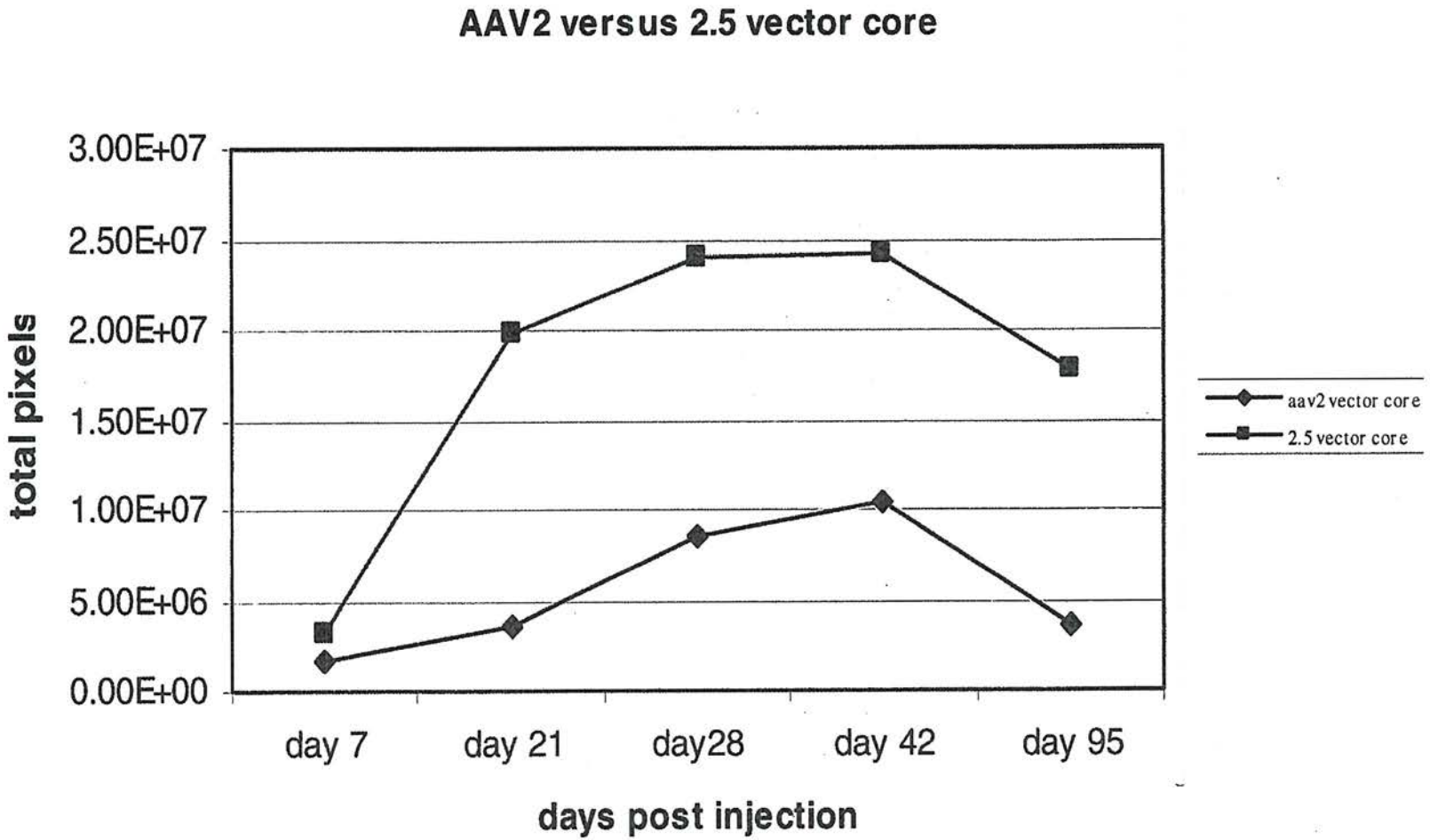
Sarepta Exhibit 1059, page 66

FIGURE 2B



Sarepta Exhibit 1059, page 67

FIGURE 3A



Sarepta Exhibit 1059, page 68

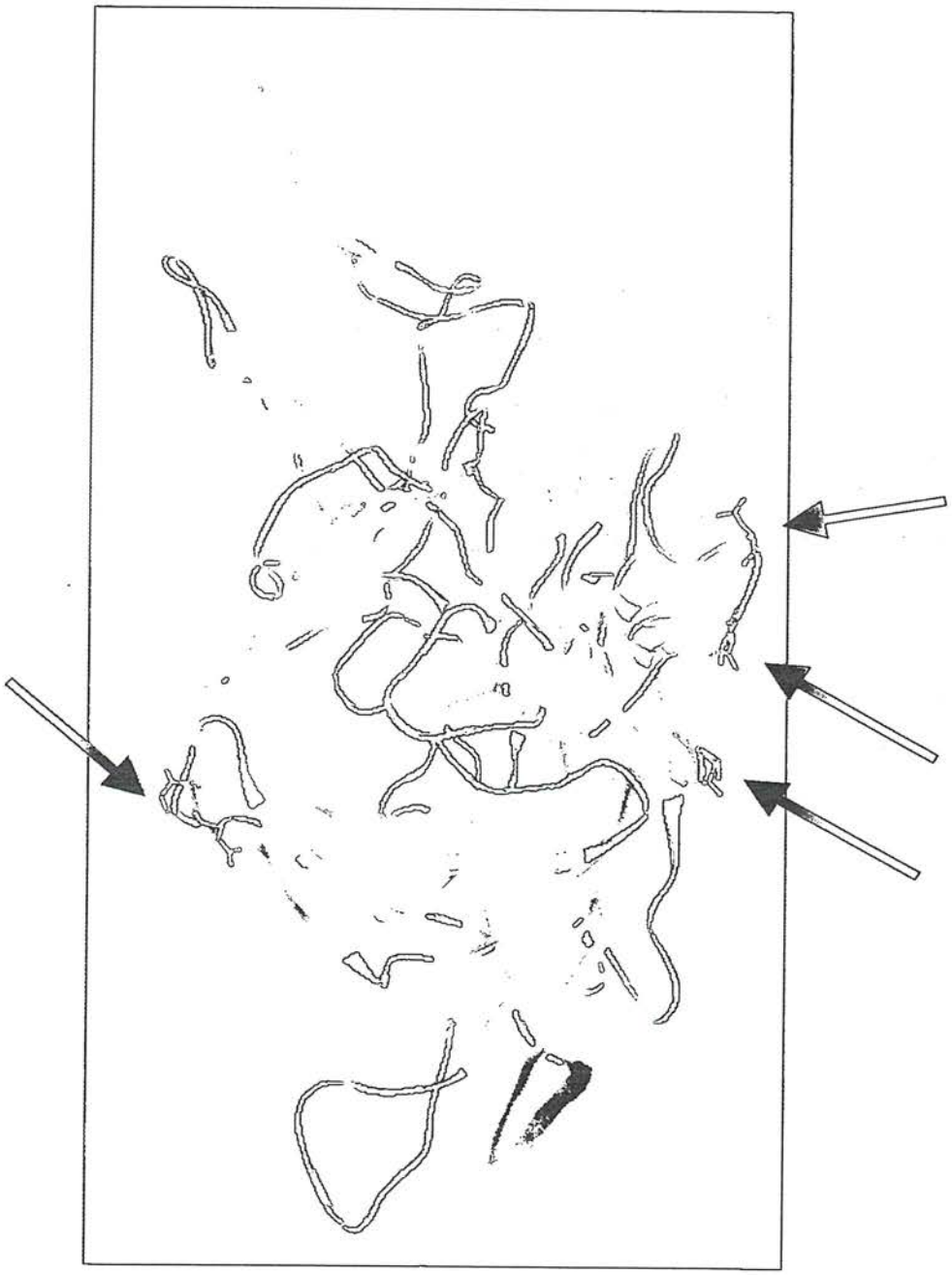
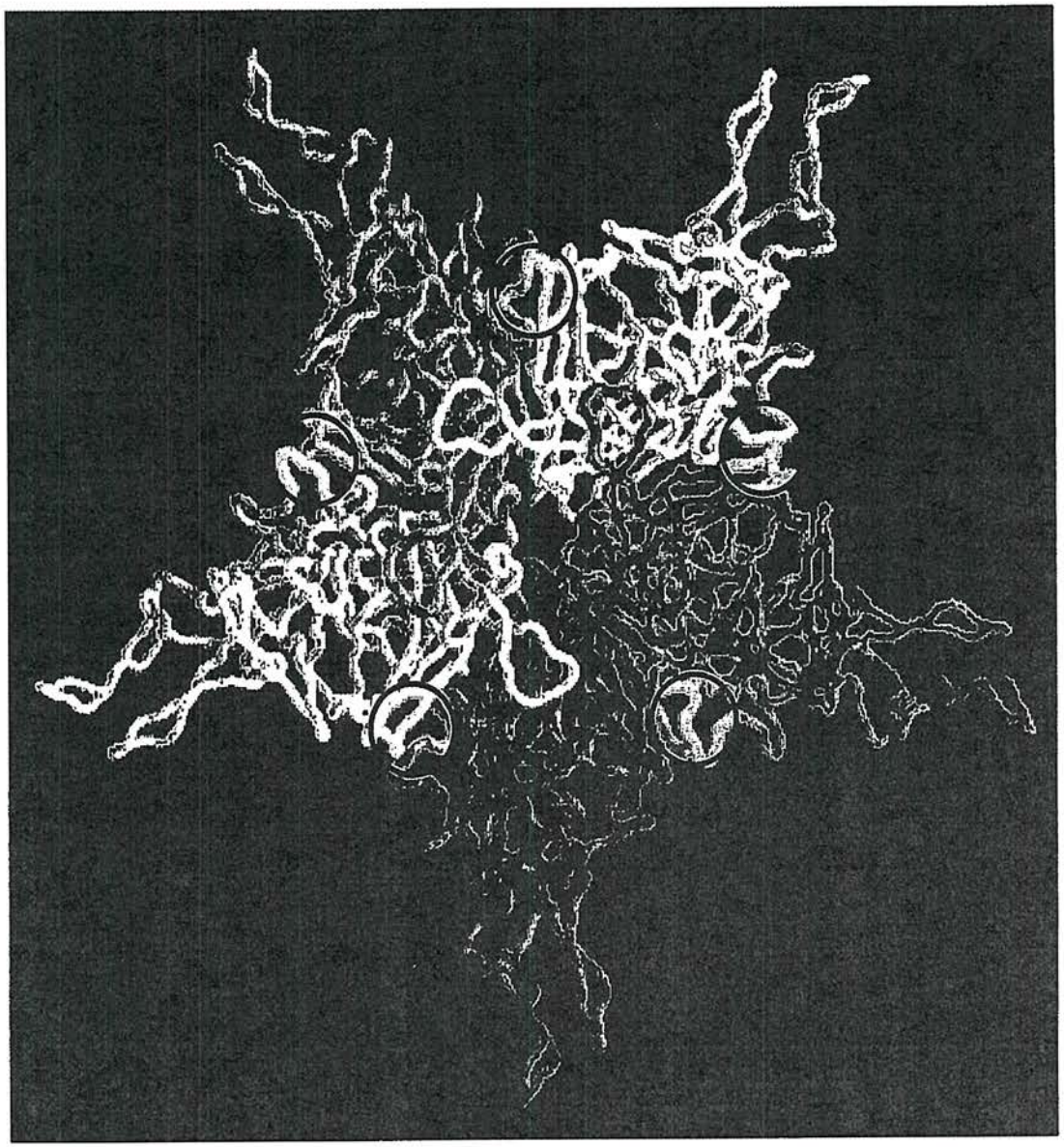


FIGURE 3B

FIGURE 3C

Position on Pentamer



Sarepta Exhibit 1059, page 70

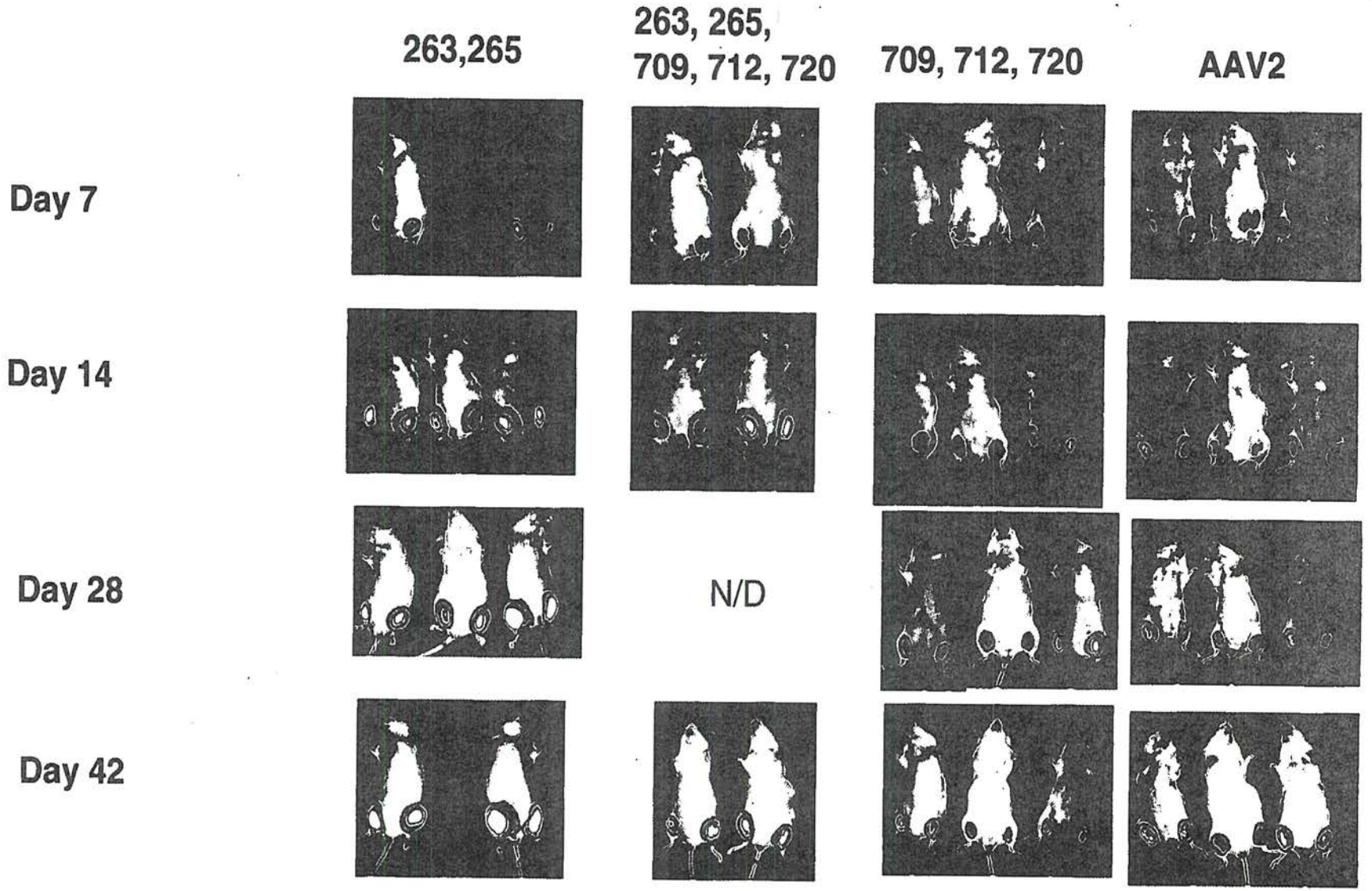
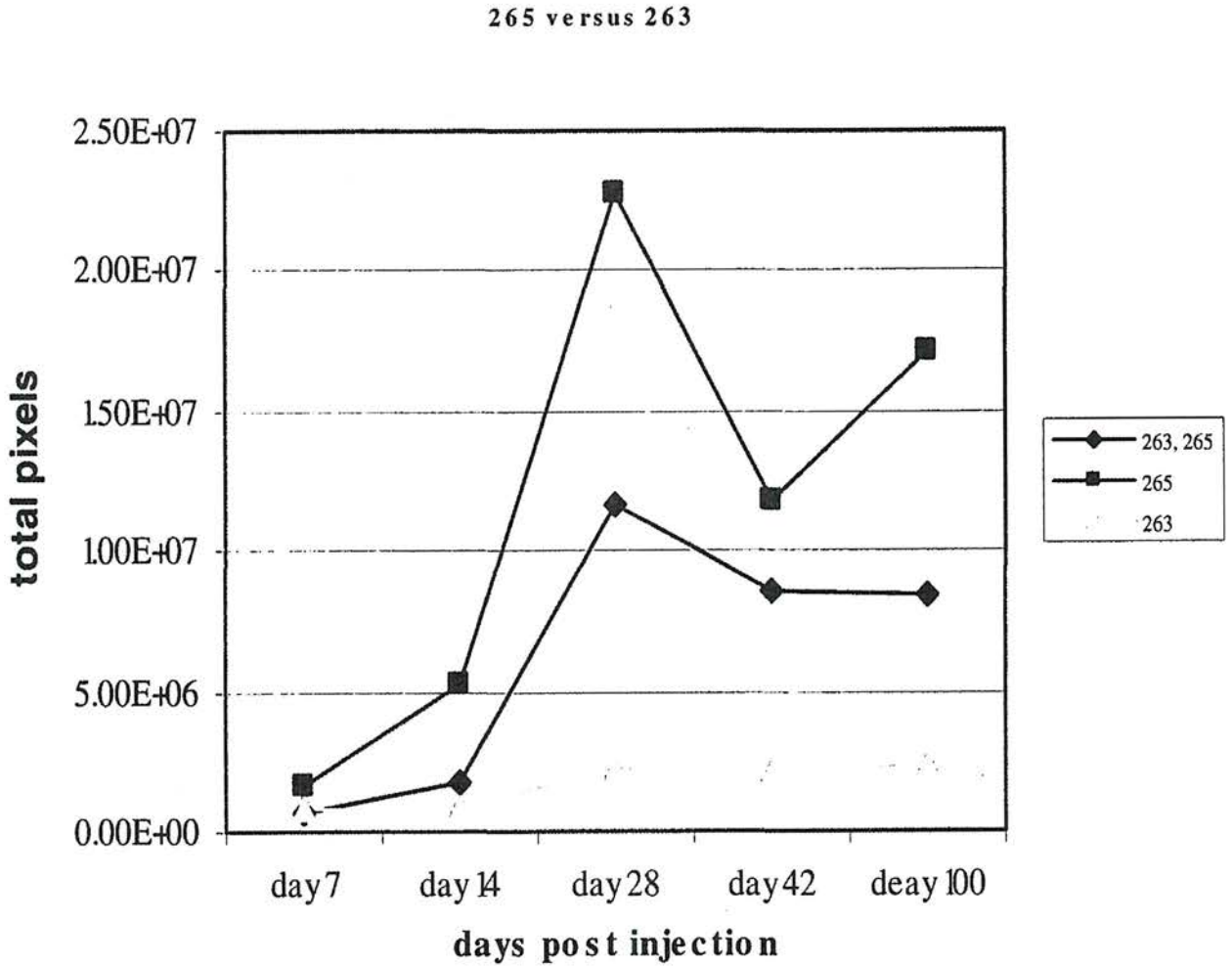


FIGURE 4

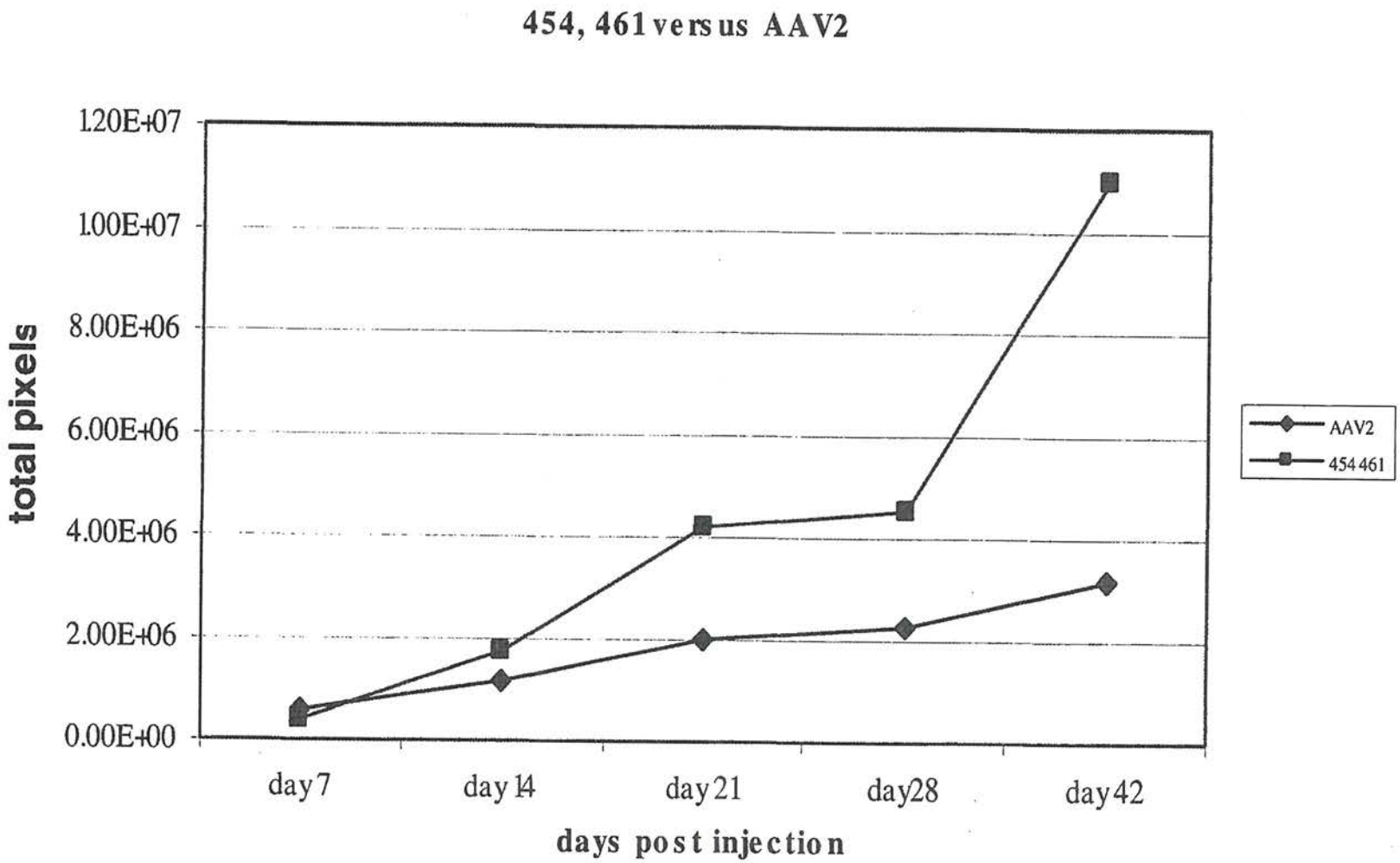
Sarepta Exhibit 1059, page 71

FIGURE 5



Sarepta Exhibit 1059, page 72

FIGURE 6


454, 461 versus AAV2
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
total pixels
day 7
day 14
day 21
day28
day 42
days post injection
AAV2
454 461

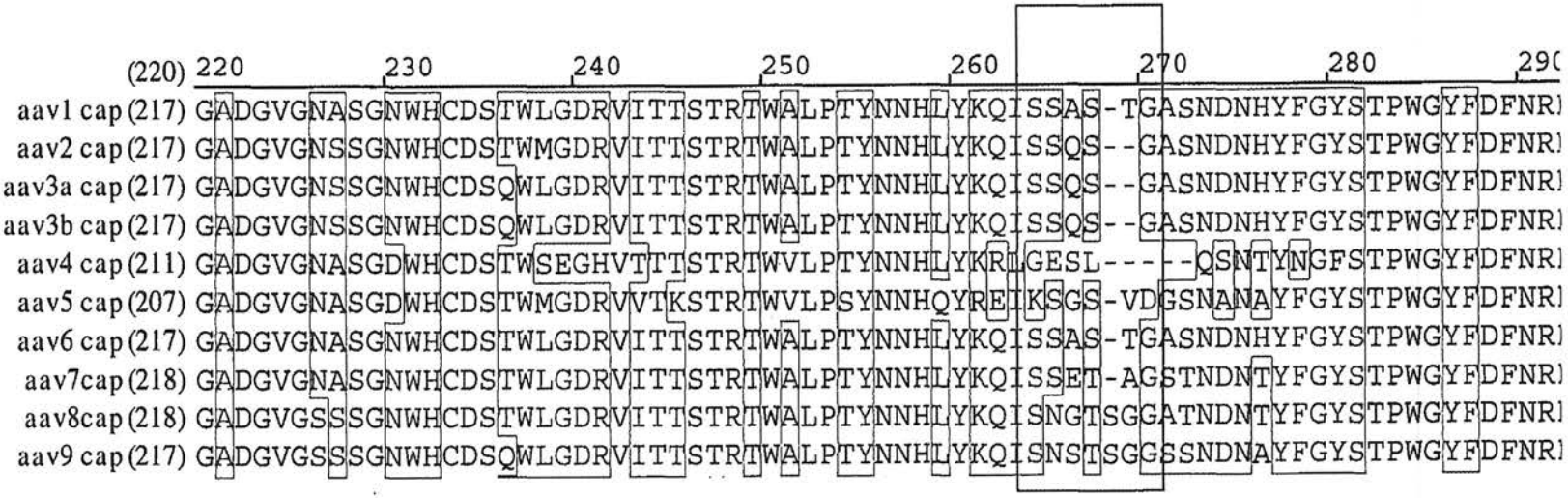
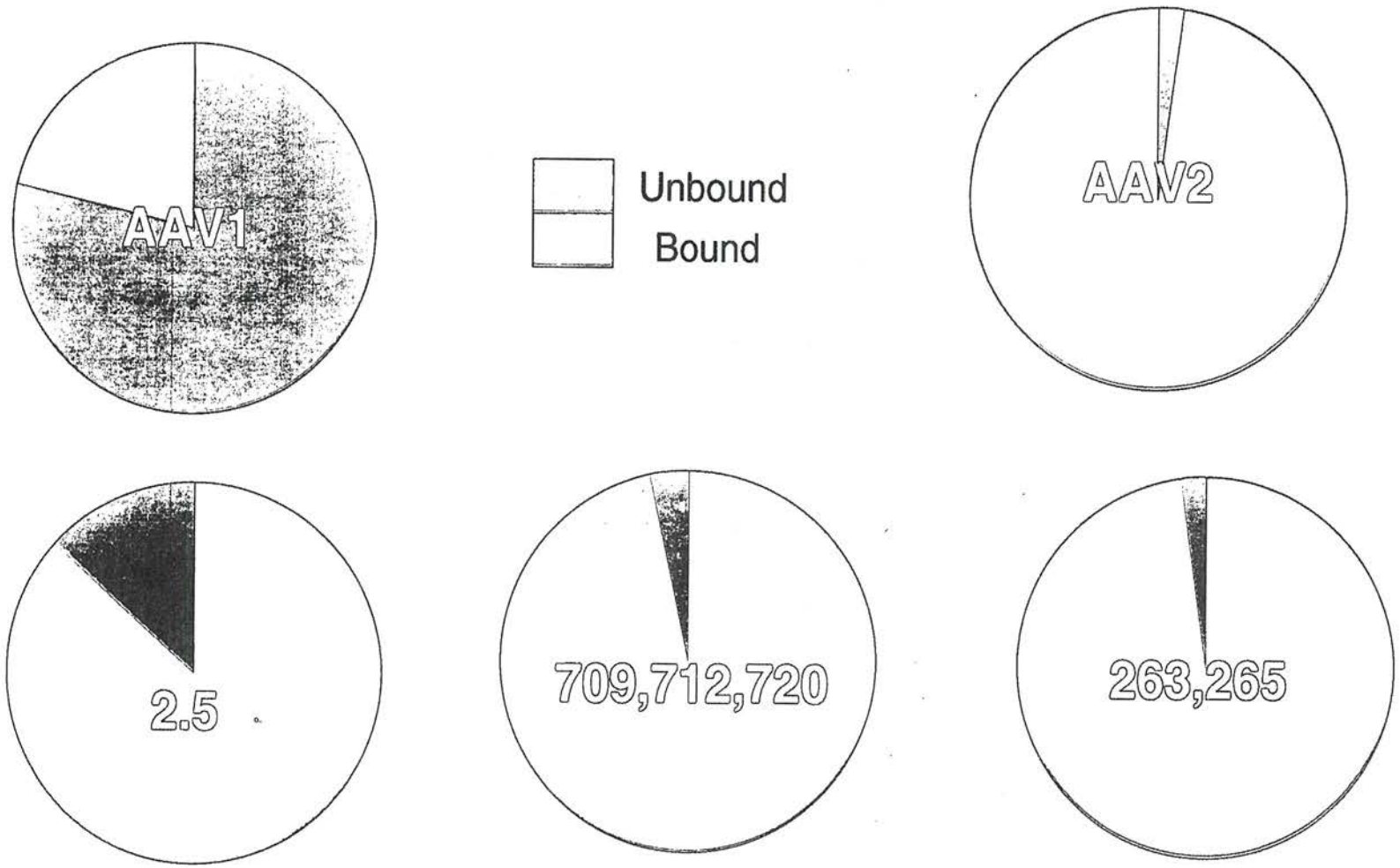

(220) 220 230 240 250 260 270 280 290
aav1 cap (217) GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAS-TGASNDNHYFGYSTPWGYFDFNR
aav2 cap (217) GADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQS--GASNDNHYFGYSTPWGYFDFNR
aav3a cap (217) GADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQS--GASNDNHYFGYSTPWGYFDFNR
aav3b cap (217) GADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQS--GASNDNHYFGYSTPWGYFDFNR
aav4 cap (211) GADGVGNASGDWHCDSTWSEGHVTTTSTRTWVLPTYNNHLYKRLGESL----QSNTYNGFSTPWGYFDFNR
aav5 cap (207) GADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYNNHQYREIKSGS-VDGSNANAYFGYSTPWGYFDFNR
aav6 cap (217) GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSAS-TGASNDNHYFGYSTPWGYFDFNR
aav7cap (218) GADGVGNASGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISSET-AGSTNDNTYFGYSTPWGYFDFNR
aav8cap (218) GADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDFNR
aav9 cap (217) GADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNR

FIGURE 7

FIGURE 8

Heparin Binding Profiles



Sarepta Exhibit 1059, page 75

```
                    1                                                  50
263, 265      (1)   MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
aav2 cap      (1)   MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
                    51                                                 100
263, 265     (51)   KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
aav2 cap     (51)   KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
                    101                                                150
263, 265    (101)   QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
aav2 cap    (101)   QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
                    151                                                200
263, 265    (151)   VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
aav2 cap    (151)   VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
                    201                                                250
263, 265    (201)   NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP
aav2 cap    (201)   NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP
                    251                                                300
263, 265    (251)   TYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
aav2 cap    (251)   TYNNHLYKQISSQS-GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
                    301                                                350
263, 265    (301)   INNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
aav2 cap    (300)   INNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
                    351                                                400
263, 265    (351)   LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
aav2 cap    (350)   LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
                    401                                                450
263, 265    (401)   SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN
aav2 cap    (400)   SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN
                    451                                                500
263, 265    (451)   TPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSE
aav2 cap    (450)   TPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSE
                    501                                                550
263, 265    (501)   YSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEK
aav2 cap    (500)   YSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEK
                    551                                                600
263, 265    (551)   TNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG
aav2 cap    (550)   TNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG
                    601                                                650
263, 265    (601)   VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIK
aav2 cap    (600)   VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIK
                    651                                                700
263, 265    (651)   NTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
aav2 cap    (650)   NTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
                    701                                736
263, 265    (701)   YTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
aav2 cap    (700)   YTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
```

FIGURE 9

```
                    1                                                  50
  aav3b cap     (1) MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGY
aav3b sastg     (1) MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGY
                   51                                                 100
  aav3b cap    (51) KYLGPGNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
aav3b sastg    (51) KYLGPGNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
                  101                                                 150
  aav3b cap   (101) QERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEAAKTAPGKKRPVDQSP
aav3b sastg   (101) QERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEAAKTAPGKKRPVDQSP
                  151                                                 200
  aav3b cap   (151) QEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGS
aav3b sastg   (151) QEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGS
                  201                                                 250
  aav3b cap   (201) NTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALP
aav3b sastg   (201) NTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALP
                  251                                                 300
  aav3b cap   (251) TYNNHLYKQISSQS-GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
aav3b sastg   (251) TYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
                  301                                                 350
  aav3b cap   (300) INNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
aav3b sastg   (301) INNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
                  351                                                 400
  aav3b cap   (350) LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
aav3b sastg   (351) LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
                  401                                                 450
  aav3b cap   (400) SQMLRTGNNFQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ
aav3b sastg   (401) SQMLRTGNNFQFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQ
                  451                                                 500
  aav3b cap   (450) GTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYRQQRLSKTANDNNNS
aav3b sastg   (451) GTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYRQQRLSKTANDNNNS
                  501                                                 550
  aav3b cap   (500) NFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTT
aav3b sastg   (501) NFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTT
                  551                                                 600
  aav3b cap   (550) ASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQ
aav3b sastg   (551) ASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQ
                  601                                                 650
  aav3b cap   (600) GALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMI
aav3b sastg   (601) GALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMI
                  651                                                 700
  aav3b cap   (650) KNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEI
aav3b sastg   (651) KNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEI
                  701                 737
  aav3b cap   (700) QYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
aav3b sastg   (701) QYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
```

FIGURE 10

```
                    1                                                  50
2.5 capsid     (1)  MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
  aav2 cap     (1)  MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGY
                    51                                                 100
2.5 capsid    (51)  KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
  aav2 cap    (51)  KYLGPFNGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF
                    101                                                150
2.5 capsid   (101)  QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
  aav2 cap   (101)  QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
                    151                                                200
2.5 capsid   (151)  VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
  aav2 cap   (151)  VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGT
                    201                                                250
2.5 capsid   (201)  NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP
  aav2 cap   (201)  NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALP
                    251                                                300
2.5 capsid   (251)  TYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
  aav2 cap   (251)  TYNNHLYKQISSQS-GASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRL
                    301                                                350
2.5 capsid   (301)  INNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
  aav2 cap   (300)  INNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQ
                    351                                                400
2.5 capsid   (351)  LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
  aav2 cap   (350)  LPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFP
                    401                                                450
2.5 capsid   (401)  SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN
  aav2 cap   (400)  SQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTN
                    451                                                500
2.5 capsid   (451)  TPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSE
  aav2 cap   (450)  TPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSE
                    501                                                550
2.5 capsid   (501)  YSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEK
  aav2 cap   (500)  YSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEK
                    551                                                600
2.5 capsid   (551)  TNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG
  aav2 cap   (550)  TNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQG
                    601                                                650
2.5 capsid   (601)  VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIK
  aav2 cap   (600)  VLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIK
                    651                                                700
2.5 capsid   (651)  NTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
  aav2 cap   (650)  NTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
                    701                                736
2.5 capsid   (701)  YTSNYAKSANVDFTVDNNGVYSEPRPIGTRYLTRNL
  aav2 cap   (700)  YTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL
```

FIGURE 11

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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
60/636,126	12/15/2004	Dawn E. Bowles	5470-427PR

CONFIRMATION NO. 6188

20792
MYERS BIGEL SIBLEY & SAJOVEC
PO BOX 37428
RALEIGH, NC 27627

FORMALITIES LETTER

OC000000014944026

Date Mailed: 01/13/2005

NOTICE TO FILE MISSING PARTS OF PROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(c)

Filing Date Granted

An application number and filing date have been accorded to this provisional application. The items indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(l) of $25 for a small entity in compliance with 37 CFR 1.27, must be submitted with the missing items identified in this letter.
- The provisional application cover sheet under 37 CFR 1.51(c)(1), which may be an application data sheet (37 CFR 1.76), is required identifying:
  - either city and state or city and foreign country of the residence of each inventor.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$25** for a Small Entity

- **$25** Late oath or declaration Surcharge.

Replies should be mailed to: Mail Stop Missing Parts
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MAR 14 2005
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Attorney Docket No. 5470-427PR

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Bowles et al.
Serial No.: 60/636,126
Filing Date: December 15, 2004
For: *CHIMERIC VECTORS*

Group Art Unit:
Examiner:
Confirmation No. 6188

Date: March 14, 2005

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO NOTICE TO FILE MISSING PARTS

Sir:

In response to the Notice to File Missing Parts of Non-Provisional Application mailed January 13, 2005, enclosed please find a copy of the Notice to File Missing Parts and a check in the amount of $25.00 to cover the surcharge.

The amount enclosed is believed to be correct. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,

Karen A. Magri
Registration No. 41,965

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"Express Mail" mailing label number: EV675780828US
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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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PO BOX 37428
RALEIGH, NC 27627

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FORMALITIES LETTER

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Date Mailed: 01/13/2005

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FILED UNDER 37 CFR 1.53(c)

Filing Date Granted

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  - either city and state or city and foreign country of the residence of each inventor.

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Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

03/16/2005 ZJUHAR1 00000005 60636126
01 FC:2052 25.00 OP

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
60/636,126	12/15/2004	Dawn E. Bowles	5470-427PR

CONFIRMATION NO. 6188

20792
MYERS BIGEL SIBLEY & SAJOVEC
PO BOX 37428
RALEIGH, NC 27627

FORMALITIES LETTER

OC000000015636406

Date Mailed: 04/01/2005

NOTICE OF INCOMPLETE REPLY (PROVISIONAL)

Filing Date Granted

The U.S. Patent and Trademark Office has received your reply on 03/14/2005 to the Notice to File Missing Parts (Notice) mailed 01/13/2005 and it has been entered into the provisional application. The reply, however, does not include the following items required in the Notice.

The period of reply remains as set forth in the Notice. You may, however, obtain EXTENSIONS OF TIME under the provisions of 37 CFR 1.136 (a) accompanied by the appropriate fee (37 CFR 1.17(a)).

A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application.

- The provisional application cover sheet under 37 CFR 1.51(c)(1), which may be an application data sheet (37 CFR 1.76), is required identifying:
  - either city and state or city and foreign country of the residence of each inventor.

Replies should be mailed to: Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

A copy of this notice MUST be returned with the reply.

M. Tran

Office of Initial Patent Examination (703) 308-1202

PART 3 - OFFICE COPY

OIPE
APR 11 2005
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APR 12 2005

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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60/636,126	12/15/2004	Dawn E. Bowles	5470-427PR

CONFIRMATION NO. 6188

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MYERS BIGEL SIBLEY & SAJOVEC
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FORMALITIES LETTER

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  - either city and state or city and foreign country of the residence of each inventor.

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

A copy of this notice MUST be returned with the reply.

M. Tran

Office of Initial Patent Examination (703) 308-1202

PART 2 - COPY TO BE RETURNED WITH RESPONSE

OIPE JC85 APR 11 2005 PATENT & TRADEMARK OFFICE

Attorney Docket No. 5470-427PR PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Bowles et al.
Serial No.: 60/636,126
Filing Date: December 15, 2004
For: *CHIMERIC VECTORS*

Group Art Unit:
Examiner:
Confirmation No. 6188

Date: April 11, 2005

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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

Sir:

Applicant herewith submits a Substitute Cover Sheet for Filing Provisional Patent Application for the above-identified provisional patent application in response to the Notice of Incomplete Reply (Provisional) Filing Date Granted mailed April 1, 2005. This substitute transmittal provides the city and state of residence for all the inventors, as required under 37 CFR §1.51(c)(1).

A copy of the notice and a check in the amount of $60.00 is also enclosed to cover the one month Extension of Time fee . The amount enclosed is believed to be correct. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,

Karen Magri

Karen A. Magri
Registration No. 41,965

CERTIFICATE OF EXPRESS MAILING
"Express Mail" mailing label number: EV674758908US
Date of Deposit: April 11, 2005
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Sarah Brunmeier

Sarah Brunmeier

Customer No. 20792
Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428
Raleigh, North Carolina 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401

Attorney Docket No. **5470-427PR** PATENT

SUBSTITUTE

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: April 11, 2005

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 □	+

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
Address: Philadelphia, PA

Name: Richard Jude Samulski
Address: Chapel Hill, NC

TITLE OF THE INVENTION *(280 characters maximum)*

CHIMERIC VECTORS

SUBSTITUTE
Attorney Docket No.: 5470-427PR
Filed: Concurrently Herewith
Page 2

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification (Number of Pages 60)

☒ Drawing(s) (Number of Sheets 15)

☒ Claims (Number of Claims 83)
(A complete provisional application does not require claims 37 C.F.R. §1.51(c)).

☐ Application Data Sheet. See 37 CFR §1.76

☒ Other: Return Postcard

CORRESPONDENCE ADDRESS

Customer No. 20792

Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428
Raleigh, North Carolina 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401

METHOD OF PAYMENT

☒ Applicant claims small entity status. See 37 CFR §1.27.

	(Total Sheets - 100) / 50 =	Extra Sheets (round up)	**Small Entity** Rate Fee	Large Entity Rate Fee
APPLICATION SIZE FEE	(- 100) /50 = **60 sheets total**	**0**	x125 = **$ 0.00**	x250 = $.00
FILING FEE			**$100.00**	$200.00
☐ NON-ENGLISH SPECIFICATION			+ 130 = $	+ 130 = $
If the difference in Col. 1 is less than zero, Enter "0" in Col. 2			Total ~~$ 100.00~~ **(Previously Paid on Dec. 15, 2004)**	Total $.00

☐ Check or money order is enclosed to cover the filing fee of $ 0.00.
☐ Payment by credit card. Form PTO-2038 is attached.
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account No. 50-0220.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Karen Magri

Karen A. Magri
Registration No. 41,965

CERTIFICATE OF EXPRESS MAILING

"Express Mail" mailing label number: EV674758908US Date of Deposit: April 11, 2005
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Missing PartsCommissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Sarah Brunmeier

Sarah Brunmeier

Customer No. 20792
Myers Bigel Sibley & Sajovec, P.A.
P. O. Box 37428
Raleigh, North Carolina 27627
Telephone: (919) 854-1400
Facsimile: (919) 854-1401

OIPE
APR 11 2005
PATENT & TRADEMARK OFFICE

Attorney Docket No. 5470-427PR PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Bowles et al.
Serial No.: 60/636,126
Filing Date: December 15, 2004
For: *CHIMERIC VECTORS*

Group Art Unit:
Examiner:
Confirmation No. 6188

Date: April 11, 2005

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION AND FEE FOR EXTENSION OF TIME
(37 C.F.R. § 1.136(a))

1. This is a petition for an extension of time for a total period of one (1) month to respond to the Official Action dated April 1, 2005.

2. A response in connection with the matter for which this extension is requested:

☒ is filed herewith.

☐ has been filed.

3. Applicant:

☒ Claims small entity status. See 37 CFR 1.27.

☐ Does not claim small entity status.

4. Calculation of extension fee (37 C.F.R. § 1.17(a)-(d)):

	Total Months Requested	Fee For Other Than Small Entity	Fee for Small Entity
☒	one month	$120.00	**$60.00**
☐	two months	$450.00	$225.00
☐	three months	$1,020.00	$510.00
☐	four months	$1,590.00	$795.00
☐	five months	$2,160.00	$1,080.00

Fee Enclosed **$60.00**

04/13/2005 FFANAIA2 00000008 60636126
01 FC:2251 60.00 OP

☒ A check in the amount of the Extension of Time fee, **$60.00**, is enclosed.

☐ Payment by credit card (Form PTO-2038) is enclosed.

☒ Charge Deposit Account No. 50-0220 for any additional extension and/or fee required or credit for any excess fee paid.

Respectfully submitted,

Karen Magri

Karen A. Magri
Registration No. 41,965

CERTIFICATE OF EXPRESS MAILING UNDER 37 CFR 1.10

"Express Mail" mailing label number: EV674758908US
Date of Deposit: April 11, 2005
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Sarah Brunmeier

Sarah Brunmeier

Customer No. 20792
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Raleigh, North Carolina 27627
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MYERS BIGEL SIBLEY & SAJOVEC, P.A.
Patent Attorneys
4140 Parklake Avenue, Suite 600, Raleigh, NC 27612
or
P.O. Box 37428
Raleigh, NC 27627
919-854-1400
Facsimile 919-854-1401

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TELECOPIER TRANSMISSION COVER SHEET

Date: May 3, 2005

Application No. 60/636,126
Attorney Docket: 5470-427PR

Telecopier No.: 703-746-9195

To: Office of Initial Patent Examination, Customer Service Center
Commissioner for Patents

Company: United States Patent and Trademark Office

From: Karen A. Magri, Esq.

Number of Pages (Including Fax Cover): 2 Return fax to: Sarah B.

If there is a problem with this transmission, please call (919) 854-1400.
Our fax number is (919) 854-1401.

CERTIFICATION OF FACSIMILE TRANSMISSION
UNDER 37 CFR § 1.8

I hereby certify that this correspondence is being facsimile transmitted to the OIPE at the U.S. Patent and Trademark Office via facsimile number 703-746-9195 on May 3, 2005

Sarah Brunmeier

Sarah Brunmeier

Confidentiality Note

The information contained in this facsimile message is legally privileged and confidential information intended only for the use of the individual or entity named above. If the reader of this message is not the intended recipient, you are hereby prohibited. If you have received this telecopy in error, please immediately notify us by telephone and return the original message to us at the address above via the United States Postal Service. THANK YOU.

Attorney Docket No. 5470-427PR

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Bowles et al.
Serial No. 60/636,126
Filed: December 15, 2004
For: *CHIMERIC VIRUSES*

Confirmation No. 6188

RECEIVED
CENTRAL FAX CENTER
MAY 03 2005

Date: May 3, 2005

VIA FACSIMILE ONLY 703-746-9195
Attn: Office of Initial Patent Examination
Customer Service Center
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTED FILING RECEIPT

Sir:

In reviewing the official Filing Receipt for the above-referenced application, Applicant notes that one of the Inventor's name has been spelled incorrectly. Please correct the spelling of the name listed under "Applicant(s)" from Josh Greiger to:

Josh Grieger

Applicant respectfully requests that the spelling of the Inventor's name be corrected in the records of the U.S. Patent and Trademark Office and that a corrected Filing Receipt be issued.

No fee is believed due. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,

Karen Magri

Karen A. Magri
Registration No. 41,965

CERTIFICATION OF FACSIMILE TRANSMISSION UNDER 37 CFR § 1.8

I hereby certify that this correspondence is being facsimile transmitted to the OIPE at the U.S. Patent and Trademark Office via facsimile number 703-746-9195 on May 3, 2005.

Sarah Brunmeier

Sarah Brunmeier

Customer No. 20792
Myers Bigel Sibley & Sajovec, P.A.
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Telephone: (919) 854-1400
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