HUMAN GENE THERAPY 24:906–913 (November 2013)

DOI: 10.1089/hum.2013.2515

An Emerging Adeno-Associated Viral Vector Pipeline for Cardiac Gene Therapy

Aravind Asokan[1,2] and R. Jude Samulski[1,3]

Abstract

The naturally occurring adeno-associated virus (AAV) isolates display diverse tissue tropisms in different hosts. Robust cardiac transduction in particular has been reported for certain AAV strains. Successful applications of these AAV strains in preclinical and clinical settings with a focus on treating cardiovascular disease continue to be reported. At the same time, these studies have highlighted challenges such as cross-species variability in AAV tropism, transduction efficiency, and immunity. Continued progress in our understanding of AAV capsid structure and biology has provided the rationale for designing improved vectors that can possibly address these concerns. The current report provides an overview of cardiotropic AAV, existing gaps in our knowledge, and newly engineered AAV strains that are viable candidates for the cardiac gene therapy clinic.

Recombinant Adeno-Associated Virus Vectors in Cardiac Gene Transfer: A Historical Perspective

Adeno-associated viruses (AAV) are nonpathogenic, helper-dependent parvoviruses with a 4.7 kb single-stranded DNA genome. The icosahedral ($T = 1$) virion shell is 25 nm in diameter and comprises 60 copies of viral capsid protein (VP) subunits. The biology of host cell entry, antigenicity, and tissue tropisms displayed in different hosts are determined by clusters of amino acid residues, interdigitating loops, surface topologies, and the three-dimensional structure observed for different AAV capsids (Bowles *et al*., 2006). Since the discovery of the first AAV strain (AAV2) in the 1960s, several other serotypes and new AAV variants have been isolated. A summary of current knowledge pertaining to the biology, preclinical studies, and clinical applications of the different AAV serotypes and pertinent literature has been reviewed elsewhere (Agbandje-McKenna and Kleinschmidt, 2011; Mingozzi and High, 2011). The focus of the current review is to provide a historical perspective of AAV as a reagent for cardiac gene transfer, outline the biology of various cardiotropic AAV strains, their evaluation in preclinical studies as well as clinical trials focused on cardiac gene therapy, and provide examples of newly engineered AAV strains available for clinical translation.

One of the earliest studies demonstrating that recombinant AAV vectors can be utilized for gene transfer in mammalian hearts was carried out using AAV serotype 2 (Kaplitt *et al*., 1996). Long-term expression of the lacZ reporter was observed after direct intramyocardial injections in the rat heart for 2 months and up to 6 months after intracoronary infusion in adult pigs. Later studies reported significantly higher transduction efficiencies after intracoronary artery infusions of recombinant AAV2 vectors in adult mice (Svensson *et al*., 1999). These pilots were rapidly followed by preclinical studies evaluating AAV2-mediated cardiac delivery of therapeutic transgenes such as vascular endothelial growth factor (VEGF) in mice (Su *et al*., 2000, 2002, 2004); delta-sarcoglycan in a hamster model of dilated cardiomyopathy (Kawada *et al*., 2002; Li *et al*., 2003); heme oxygenase-1 and superoxide dismutase in a rat model of ischemia-induced myocardial injury (Melo *et al*., 2002; Agrawal *et al*., 2004); human growth hormone under the myosin heavy chain promoter in mice (Aikawa *et al*., 2002); acid alpha-1,4 glucosidase in a mouse model of Pompe disease (Fraites *et al*., 2002); phospholamban in mouse, rat, and hamster models of heart failure (Hoshijima *et al*., 2002; Champion *et al*., 2003; Iwanaga *et al*., 2004); dominant-negative suppressor of cytokine signaling-1 in a model of enterovirus-mediated cardiac injury (Yasukawa *et al*., 2003); and microdystrophin delivery in the *mdx* mouse heart (Yue *et al*., 2003). In addition to these studies, continued validation of AAV2 as a tool for cardiac gene transfer in large animal models, including pigs (Kaspar *et al*., 2005), dogs (Ferrarini *et al*., 2006), and baboons (McTiernan *et al*., 2007), was carried out by several other groups.

[1]Gene Therapy Center, [2]Department of Genetics, and [3]Department of Pharmacology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27516.

Against this backdrop of these promising preclinical studies with AAV2, evaluation of new AAV serotypes/isolates for cardiac gene transfer began about a decade ago. In these early comparative studies, AAV1, which was isolated as a contaminant in primate adenovirus stocks, emerged as the lead candidate for cardiac gene transfer (Du *et al*., 2004). However, at the wake of these studies, robust cardiac transduction was also reported after direct myocardial injections of AAV6, AAV7, and AAV8, all nonhuman primate isolates (Kawamoto *et al*., 2005; Palomeque *et al*., 2007); intravenous administration of AAV8 (Wang *et al*., 2005; Zhu *et al*., 2005; Palomeque *et al*., 2007); and intravenous administration of AAV9, a human tissue isolate (Inagaki *et al*., 2006; Pacak *et al*., 2006) in various small animal models. Efficacy testing of these new AAV serotypes in different animal models of cardiac disease/heart failure was carried out over the next few years (Su *et al*., 2006, 2008; Pleger *et al*., 2007; Townsend *et al*., 2007; Andino *et al*., 2008; Bish *et al*., 2008b,c; Bostick *et al*., 2008; Byrne *et al*., 2008; Kawase *et al*., 2008; Odom *et al*., 2008; Raake *et al*., 2008).

After over a decade of evaluating recombinant AAV vectors as prospective tools for cardiac gene transfer, the first phase I/II clinical trial for treatment of heart failure using AAV1 vectors was designed. This study involved intracoronary infusion of AAV1 vectors packaging the *SERCA2a* gene, which encodes a major cardiac calcium cycling protein (Hajjar *et al*., 2008). Progress and updates pertaining to this clinical trial have been reported in detail (Hajjar, 2013). In the meantime, the validation of cardiotropic AAV serotypes in animal models of cardiac disease has continued over the past 5 years. These specific applications of recombinant AAV vectors in cardiac gene therapy have been reviewed elsewhere (Pacak and Byrne, 2011; Lai and Duan, 2012; Tang *et al*., 2012; Hajjar, 2013; Scimia *et al*., 2013).

Cardiotropic AAV Serotypes: Vector Biology and Species Differences

Recombinant AAV vectors utilize a diverse array of receptors for host cell binding and entry. These early events in the infectious pathway of AAV capsids have a profound impact on the blood circulation profile, biodistribution, tissue uptake and clearance, as well as subsequent downstream events such as intracellular trafficking. Further, variations in capsid structure have been shown to affect AAV tissue tropism, antigenicity, host receptor recognition, and efficiencies of capsid uncoating. When combined with genetic variation in different preclinical animal models and human beings, AAV biology presents a complex challenge toward achieving effective clinical translation. Thus, a thorough understanding of the molecular and cellular factors that dictate AAV cardiotropism in different hosts is essential.

AAV1

Alpha-2,3 and alpha-2,6-linked sialic acids (SA) have been identified as glycans essential for cell surface binding by AAV1 (Wu *et al*., 2006b). Coreceptors for AAV1 capsids are currently not known. Cross-species variability in SA linkage is well documented with human beings preferentially expressing alpha-2,6-linked SA, while other preclinical animal models generally overexpress alpha-2,3-linked SA (Altheide *et al*., 2006; Varki and Schauer, 2009; Cohen and Varki, 2010). In addition, humans possess an inactivating mutation in the CMP N-Acetyl neuraminic acid hydroxylase gene, which results in the loss of synthesis of glycolylated SA or Neu5Gc and the overexpression of acetylated SA or Neu5Ac (Altheide *et al*., 2006; Varki and Schauer, 2009; Cohen and Varki, 2010). Since AAV1 was isolated as a contaminant from primate adenovirus stocks, it is conceivable that AAV1 might have evolved to recognize Neu5Gc and SA linkage commonly found in primates. Despite these potential caveats and although no direct comparisons have been made to date, cardiac transduction by AAV1 in different large animal models appears to be quite robust. For instance, AAV1 has been proven effective for cardiac gene transfer through intracoronary infusion in a sheep model of ischemic heart failure (Fargnoli *et al*., 2013). Another study carried out in a porcine model demonstrated that coadministration of nitroglycerin, a vasodilator, increases AAV1 uptake and mRNA expression in cardiac tissue (Karakikes *et al*., 2012). These results support the notion that AAV1 does not readily traverse the vascular wall and there is room for improving cardiac gene transfer efficiency with AAV1 vectors. It is also interesting to note that such treatment with vasodilators did not result in increased uptake of AAV1 in lung or liver tissue. These observations are consistent with the notion that AAV1 only appears to transduce tissues in the immediate vicinity of the site of administration. Therefore, both intramyocardial (intramuscular) and intracoronary routes appear optimal for AAV1 administration. In contrast, intravenous injections of AAV1 vectors appear to largely result in liver sequestration. This phenomenon can possibly be attributed to binding of AAV1 capsids by certain serum factors (see the next section on AAV6) that could alter the biodistribution and consequently tissue tropism. These factors should be taken into account while evaluating AAV1 as a lead candidate for cardiac gene therapy trials.

AAV6

Similar to AAV1, AAV6 is known to utilize alpha2,3- or alpha2,6-linked SA as receptors for cell surface binding (Wu *et al*., 2006b). However, AAV6 has the distinction of being the only known naturally occurring strain capable of recognizing two glycan receptors—SA and heparan sulfate (HS). Further, epidermal growth factor receptor is a known coreceptor for AAV6 host cell entry (Weller *et al*., 2010). Of the six amino acid residues that differ between AAV6 and AAV1, a single lysine (K531) has been shown to be impart HS binding ability to AAV6 (Wu *et al*., 2006a; Ng *et al*., 2010; Xie *et al*., 2011). Three other surface-exposed residues are thought to play a critical role in other interactions between the AAV6 capsid and host cells. Whether the ability of AAV6 capsids to bind dual glycan receptors (SA and HS) confers the ability to mediate robust and long-term myocardial gene expression has not been directly examined. In one comparative study, intracoronary injections in mice revealed higher transgene expression levels in the heart with AAV6 vectors in comparison with AAV1, 7, 8, or 9 (Zincarelli *et al*., 2010). In another study comparing different AAV serotypes 1–8 after intramyocardial injection in rats, AAV1 and AAV6 were found be equally robust, but less efficient than AAV8 in transducing the heart (Palomeque *et al*., 2007). Further, both AAV1 and AAV6 displayed identical transduction profiles after intrapericardial administration in murine hearts (Bish *et al*., 2008b). In general, AAV6 appears to

have emerged as a superior vector that can achieve highly effective cardiac gene transfer after transendocardial or intracoronary injections in large animal models such as rhesus macaques, dogs, pigs, and sheep (White *et al*., 2011; Gao *et al*., 2011b; Bish *et al*., 2012; Raake *et al*., 2013). However, it should also be noted that intravenous administration of AAV6 vectors results in significant levels of transgene expression in the liver and skeletal muscle. Thus, similar to AAV1, intravenous administration of AAV6 does not appear to be optimal for cardiac-specific gene transfer applications. Further, coadministration of vasodilators such as VEGF can improve the transduction efficiency of AAV6, corroborating the potential for generating mutants with an improved cardiac transduction profile (Gregorevic *et al*., 2004).

More recently, both AAV1 and AAV6 have been shown to interact with canine and human galectin 3 binding protein (G3BP), a soluble scavenger receptor (Denard *et al*., 2012). This interaction is potentiated by the K531 residue unique to AAV6. Interestingly, both AAV1 and AAV6 do not interact with murine or macaque G3BP. Further, both AAV strains have been shown to bind murine C-reactive protein, but not the human counterpart (Denard *et al*., 2013). These findings highlight the potential for variability in species-specific interactions between AAV capsids and host proteins in general. Whether these interactions (or lack thereof) could affect the biodistribution and transduction profile of AAV6 in patients remains to be determined. Further, it should be noted that preexisting humoral immunity to AAV6 (and AAV1) is higher than that observed for AAV8 or AAV9 in both human beings and different animal models [reviewed recently by Rapti *et al*. (2012) and Louis Jeune *et al*. (2013)]. These studies demonstrate that immunoglobulin and nonimmunoglobulin neutralizing factors affecting AAV6 transduction were particularly high in canine models.

AAV9

Some of the biology of AAV9, a human isolate, has been resolved over the past two years. Terminal galactose is the primary cell surface receptor for AAV9 (Bell *et al*., 2011; Shen *et al*., 2011). Coreceptors for AAV9 are currently unknown. Comparative studies evaluating the transduction efficiencies of different AAV serotypes after intrapericardial or intramyocardial injections have revealed higher transduction by AAV9 in the mouse and rat hearts compared with AAV1, AAV5, AAV6, AAV7, or AAV8 after direct intramyocardial administration (Bish *et al*., 2008a; Qi *et al*., 2010; Prasad *et al*., 2011a,b; Fang *et al*., 2012). In contrast, transendocardial administration of AAV6, AAV8, and AAV9 in canines revealed that AAV9 is less efficient than AAV6 (Bish *et al*., 2008c). These results have since been corroborated by another comprehensive study in adult rhesus macaques, wherein AAV9 vectors were found to mediate less efficient cardiac gene transfer when compared with AAV6 and AAV8 after transendocardial injection (Gao *et al*., 2011a). Despite these findings, recent preclinical studies have demonstrated effective cardiac gene transfer after intracoronary administration of AAV9 vectors in porcine models of heart failure (Pleger *et al*., 2011; Fish *et al*., 2013). However, it should be noted that unlike AAV6, AAV9 vectors are less cardiac-specific and display broad biodistribution in off-target organs after administration. Taken together, there appears to be a lack of correlation in the cardiac transduction profile of AAV9 observed in rodents and large animal models. Such cross-species variability can likely be attributed in part to the propensity for AAV9 to rapidly spread to multiple organs after injection into any tissue type. Studies in our lab and others have demonstrated that the prolonged blood circulation profile of AAV9 vectors (Kotchey *et al*., 2011; Shen *et al*., 2012) and low glycan binding avidity of this serotype profoundly affect the cardiac transduction profile (Shen *et al*., 2012). Whether galactose or glycan expression levels in different organs of human and animal origin can serve as an indicator of the cardiac gene transfer efficiency of AAV serotypes in general remains to be determined. It is important to note that despite these drawbacks, AAV9 is more effective than AAV1 or AAV6 for achieving cardiac gene transfer when administered through the intravenous route. Further, preexisting humoral immunity to AAV9 is lower than that observed for AAV1 or AAV6 in both human beings and different animal models [reviewed recently by Rapti *et al*. (2012) and Louis Jeune *et al*. (2013)]. It is critical to consider these factors before advancing AAV9 into the cardiac gene transfer clinic.

Engineered AAV Strains: New Vectors for the Cardiac Gene Transfer Clinic

As discussed above, several naturally occurring AAV serotypes show promise for cardiac gene transfer. AAV1 and AAV6 appear more suitable for cardiac gene transfer through intramyocardial, intrapericardial, or intracoronary routes, while AAV8 and AAV9 can achieve efficient cardiac transduction when administered intravenously. However, these preclinical and clinical studies have also highlighted critical challenges for each vector: (a) variability among different species, (b) transduction of off-target organs such as the liver, and (c) neutralization caused by preexisting humoral immunity and non-immunoglobulin-based serum factors. Novel mutant and chimeric AAV strains displaying improved transduction profiles have since been engineered to address some of these challenges. Several examples are reviewed in detail below, and a comparative analysis of natural and engineered cardiotropic AAV strains is outlined in Table 1.

Synthetic AAV2-derived strains

The earliest examples of mutant AAV vectors with an improved cardiotropic profile were engineered using AAV2 capsids as a template (Kern *et al*., 2003; Muller *et al*., 2006; Raake *et al*., 2008). These modified AAV2 strains were generated by ablating binding of AAV2 capsids to HS through site-directed mutagenesis of critical arginine residues (R484 and/or R585). The resulting heparin binding-deficient mutants displayed decreased liver tropism and continued cardiac transduction after intravenous administration. The cardiac transduction efficiency of liver-detargeted AAV2 mutants could be potentiated by coadministration of vasodilators such as VEGF (Raake *et al*., 2008). Subsequent studies in our labs engineered a hybrid liver-detargeted strain, AAV2i8, which is capable of traversing the blood vessel barrier and transducing cardiac and skeletal muscle tissue with high efficiency (Asokan *et al*., 2010). Since then, we have successfully carried out systemic studies resulting in robust gene expression in primates (Asokan, Samulski and Tarantal, unpublished). More recently, we have engineered novel

Table 1. Comparative Analysis of Natural and Engineered Cardiotropic Adeno-Associated Virus Strains, Their Biology, Tissue Tropisms, and Antigenicity

AAV strain	*Reference*	*Origin*	*Glycan receptor usage*	*Optimal routes of administration*	*Liver-detargeted?*	*Transduction in noncardiac tissues*	*Human NAb prevalence*
AAV1	See main text	Primate isolate	SA	Intramyocardial/ intracoronary	No	Low liver, vasculature	High
AAV6	See main text	Primate isolate	SA and HS	Intramyocardial/ intracoronary	No	Liver, skeletal muscle	High
AAV8	See main text	Primate isolate	Unknown	Intravenous	No	Liver, skeletal muscle, pancreas	Moderate
AAV9	See main text	Human isolate	Gal	Intravenous	No	Liver, skeletal muscle, brain	Moderate
AAVM41	Yang *et al.* (2009)	DNA shuffling/ directed evolution	Unknown	Intravenous	Yes	Skeletal muscle	Unknown
AAV2-PSVSVRP	Ying *et al.* (2010)	Peptide display/ combinatorial screening	Unknown; HS-deficient	Intravenous	Yes	Lung, pancreas	Unknown
AAV2-VNSTRLP	Ying *et al.* (2010)	Peptide display/ combinatorial screening	Unknown; HS-deficient	Intravenous	Yes	Lung, pancreas	Unknown
AAV2/R585E	Kern *et al.* (2003)	Rational mutagenesis	HS-deficient	Intravenous	Yes	Skeletal muscle	High
AAV2i8	Asokan *et al.* (2010)	Receptor footprint engineering	HS-deficient	Intravenous	Yes	Skeletal muscle	Unknown
AAV2i8G9	Shen *et al.* (2013)	Receptor footprint engineering	HS-deficient; Gal	Intravenous	Yes	Skeletal muscle	Unknown
AAV2.5	Bowles *et al.* (2012)	Rational mutagenesis	HS	Intramyocardial/ intracoronary	No	Liver	Low, NAb escape mutant
AAV2/265	Li *et al.* (2012)	Rational mutagenesis	HS	Intramyocardial/ intracoronary	No	Liver	Low, NAb escape mutant
AAV3/SASTG	Piacentino *et al.* (2012)	Rational mutagenesis	HS	Intramyocardial/ intracoronary	Unknown	Not reported	Unknown
AAV1.9–6	Kotchey *et al.* (2011)	Domain swapping	Gal	Intravenous	No	Liver	Unknown
AAV9.24/ AAV9.45	Pulicherla *et al.* (2011)	Random mutagenesis/ combinatorial screening	Gal-deficient	Intravenous	Yes	Skeletal muscle, brain	Unknown

AAV, adeno-associated virus; Gal, galactose; HS, heparan sulfate; NAb, neutralizing antibody; SA, sialic acid.

chimeric AAV strains by engrafting the galactose receptor footprint from AAV9 onto AAV2i8. The resulting AAV2i8G9 strain can mediate robust cardiac gene expression comparable to AAV9 after intravenous injection (Shen *et al*., 2013). More importantly, AAV2i8G9 shows 50–1000-fold higher and preferential transduction of cardiac tissue over other major off-target organs such as liver, kidney, and spleen.

Another AAV2 mutant, dubbed AAV2.5, was the first example of a hybrid AAV vector to proceed to clinical trials. The AAV2.5 vector is a rationally engineered AAV strain designed to graft the muscle tropism determinants of AAV1 onto parental AAV2 (Bowles *et al*., 2012). As shown in preclinical studies and in a phase I clinical trial of Duchenne muscular dystrophy, AAV2.5 is capable of robust gene transfer in skeletal muscle. More recently, AAV2.5 and related mutants thereof were shown to display significantly different humoral immune profiles when compared with the AAV2 parent backbone. The ability to confer altered antigenicity has since been narrowed down to a single amino acid at position 265 and appears to mediate decreased recognition by antisera when administered in mice (Li *et al*., 2012). In an effort to test the importance of this domain in alternative AAV backbones, a series of capsid variants have been generated (AAV 1–9) and determined to mediate robust transgene expression in numerous murine tissues. Interestingly, a subclass of these mutants appears to preferentially transduce the murine heart after intramyocardial injection (Warischalk and Samulski, unpublished observations). Another related chimera, dubbed AAV3-SASTG, demonstrated robust cardiac transduction in mice higher than AAV2.5 and other related mutants, AAV1 as well as AAV9 vectors, after intramyocardial administration (Piacentino *et al*., 2012). Evaluation of these novel AAV1/AAV2/AAV3-derived mutant strains in large animal models is forthcoming and likely to provide further insight into the biology of AAV-cardiac tissue interactions in different hosts and improved reagents for cardiac gene transfer.

Synthetic AAV9-derived strains

Nakai and others generated a chimeric AAV1 strain capable of robust cardiac transduction in mice after intravenous administration (Kotchey *et al*., 2011). The AAV1.9–6 mutant was generated by swapping a 37 amino acid domain (of which 11 residues differ) from the C-terminal region of the AAV9 VP subunit onto AAV1. The mechanisms underlying the enhanced cardiac and liver transduction of this vector remain to be determined. Using a random mutagenesis approach, our lab discovered several novel liver-detargeted AAV9 mutants capable of robust cardiac transduction at levels similar to the parental AAV9 strain after intravenous administration (Pulicherla *et al*., 2011). The AAV9.24, AAV9.45, and 9.61 mutants harbored 1–2 point mutations in residues within or adjacent to the galactose-binding region and displayed 10–25-fold higher and preferential cardiac transduction over liver and other off-target organs. Since then, we have successfully carried out systemic studies resulting in robust cardiac gene expression in a porcine model of heart failure (Hammond and Asokan, unpublished). Zhong, Srivastava, and others have recently developed a series of tyrosine-mutant AAV vectors that display improved gene transfer efficiency in a wide variety of organs, including liver, muscle, and eye (Zhong *et al*., 2008; Petrs-Silva *et al*., 2011a,b; Qiao *et al*., 2012). However, it appears that single tyrosine mutations in the context of AAV9 capsids were unable to enhance cardiac transduction efficiency in two different strains of mice (Qiao *et al*., 2012).

Synthetic AAV strains from combinatorial libraries

Synthetic cardiotropic AAV strains have also been obtained from combinatorial AAV-based peptide display libraries (Ying *et al*., 2010). These libraries were generated by insertion of random peptide sequences at the N587/R588 position on the AAV2 VP template. After intravenous injection of the AAV peptide library in mice, hearts were excised at 3 days after injection and subjected to super-infection with human adenovirus 5 as organotypic cultures. Replicating AAV strains were subjected to further rounds of PCR amplification, and screening yielded two new cardiotropic AAV strains. Both AAV2-PSVSPRP and AAV2-VNSTRLP yielded 50–100-fold higher and preferential cardiac transduction over off-target organs such as the liver and kidney. In addition, these strains were found more effective than wild-type AAV2, the liver-detargeted AV2/R585E mutant, and AAV9 vectors after intravenous administration. Additional work in preclinical large animal models will be needed to determine whether these targeting strategies can translate across multiple species.

A myocardium-tropic AAV strain, AAVM41, was recently obtained by subjected DNA-shuffled libraries to directed evolution in mice (Yang *et al*., 2009). The resulting chimeric AAV capsid was derived from largely from AAV1 and 6 interrupted by an ~20 amino acid residue stretch from AAV8 and ~200 amino acid domain derived from AAV7. AAVM41 was shown to be more efficient than AAV6 in transducing the murine heart and as efficient as AAV9 after intravenous administration. In addition, the mutant also demonstrated >10-fold attenuated tropism for liver, skeletal muscle, and other off-target organs. Efficient rescue of cardiac function was also demonstrated after intravenous administration of AAVM41 vectors delivering delta-sarcoglycan in a hamster cardiomyopathy model. Further evaluation of this chimeric AAV portfolio in large animal models would unequivocally establish their position in the clinical pipeline.

Similar directed evolution and combinatorial engineering strategies are likely to provide new and improved vector candidates that can evade preexisting humoral immunity. Early examples of such efforts include the AAV2-derived mutants, AAV2.15 and AAV2.4, both of which contain mutations at critical antigenic sites, thereby capable of evading neutralizing antibodies in human serum (Maheshri *et al*., 2006). Another approach is to mutate previously mapped immunogenic epitopes on AAV capsids to engineer neutralizing antibody escape mutants (Maersch *et al*., 2010). The recent mapping of surface-exposed antigenic epitopes using cryo-EM (Gurda *et al*., 2012, 2013; Harbison *et al*., 2012; Lerch *et al*., 2012) is likely to enable structure-driven genetic manipulation of different cardiotropic AAV serotypes to generate novel, neutralizing antibody escape variants suitable for administration in the presence of preexisting humoral immunity. When combined with mutations that enhance cardiotropism and/or cardiac gene transfer efficiency, these next-generation AAV strains are likely to provide the cardiovascular community with improved tools for clinical gene transfer.

Acknowledgments

We would like to dedicate this review to the memory of Dr. Sonia Skarlatos. We would also like to acknowledge the National Institutes of Health for research support (R.J.S.—P01HL112761, R01AR064369, U54AR056953, and R01AI072176; A.A.—R01HL089221 and P01HL112761)

Author Disclosure Statement

No competing financial interests exist.

References

Agbandje-Mckenna, M., and Kleinschmidt, J. (2011). AAV capsid structure and cell interactions. Methods Mol. Biol. 807, 47–92.

Agrawal, R.S., Muangman, S., Layne, M.D., *et al*. (2004). Preemptive gene therapy using recombinant adeno-associated virus delivery of extracellular superoxide dismutase protects heart against ischemic reperfusion injury, improves ventricular function and prolongs survival. Gene Ther. 11, 962–969.

Aikawa, R., Huggins, G.S., and Snyder, R.O. (2002). Cardiomyocyte-specific gene expression following recombinant adeno-associated viral vector transduction. J. Biol. Chem. 277, 18979–18985.

Altheide, T.K., Hayakawa, T., Mikkelsen, T.S., *et al*. (2006). System-wide genomic and biochemical comparisons of sialic acid biology among primates and rodents: evidence for two modes of rapid evolution. J. Biol. Chem. 281, 25689–25702.

Andino, L.M., Takeda, M., Kasahara, H., *et al*. (2008). AAV-mediated knockdown of phospholamban leads to improved contractility and calcium handling in cardiomyocytes. J. Gene Med. 10, 132–142.

Asokan, A., Conway, J.C., Phillips, J.L., *et al*. (2010). Re-engineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle. Nat. Biotechnol. 28, 79–82.

Bell, C.L., Vandenberghe, L.H., Bell, P., *et al*. (2011). The AAV9 receptor and its modification to improve *in vivo* lung gene transfer in mice. J. Clin. Invest. 121, 2427–2435.

Bish, L.T., Morine, K., Sleeper, M.M., *et al*. (2008a). Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. Hum. Gene Ther. 19, 1359–1368.

Bish, L.T., Morine, K., Sleeper, M.M., *et al*. (2008b). Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. Hum. Gene Ther. 19, 1359–1368.

Bish, L.T., Sleeper, M.M., Brainard, B., *et al*. (2008c). Percutaneous transendocardial delivery of self-complementary adeno-associated virus 6 achieves global cardiac gene transfer in canines. Mol. Ther. 16, 1953–1959.

Bish, L.T., Sleeper, M.M., Forbes, S.C., *et al*. (2012). Long-term restoration of cardiac dystrophin expression in golden retriever muscular dystrophy following rAAV6-mediated exon skipping. Mol. Ther. 20, 580–589.

Bostick, B., Yue, Y., Lai, Y., *et al*. (2008). Adeno-associated virus serotype-9 microdystrophin gene therapy ameliorates electrocardiographic abnormalities in mdx mice. Hum. Gene Ther. 19, 851–856.

Bowles, D.E., Rabinowitz, J.E., and Samulski, R.J. (2006). The genus *Dependovirus*. In *Parvoviruses*. J.R. Kerr, S.F. Cotmore, M.E. Bloom, *et al*., eds. (Edward Arnold Ltd., New York, NY), pp. 15–24.

Bowles, D.E., McPhee, S.W., Li, C., *et al*. (2012). Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. Mol. Ther. 20, 443–455.

Byrne, M.J., Power, J.M., Preovolos, A., *et al*. (2008). Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. Gene Ther. 15, 1550–1557.

Champion, H.C., Georgakopoulos, D., Haldar, S., *et al*. (2003). Robust adenoviral and adeno-associated viral gene transfer to the *in vivo* murine heart: application to study of phospholamban physiology. Circulation 108, 2790–2797.

Cohen, M., and Varki, A. (2010). The sialome—far more than the sum of its parts. Omics 14, 455–464.

Denard, J., Beley, C., Kotin, R., *et al*. (2012). Human galectin 3 binding protein interacts with recombinant adeno-associated virus type 6. J. Virol. 86, 6620–6631.

Denard, J., Marolleau, B., Jenny, C., *et al*. (2013). C-reactive protein (CRP) is essential for efficient systemic transduction of recombinant adeno-associated virus vector 1 (rAAV-1) and rAAV-6 in mice. J. Virol. 87, 10784–10791.

Du, L., Kido, M., Lee, D.V., *et al*. (2004). Differential myocardial gene delivery by recombinant serotype-specific adeno-associated viral vectors. Mol. Ther. 10, 604–608.

Fang, H., Lai, N.C., Gao, M.H., *et al*. (2012). Comparison of adeno-associated virus serotypes and delivery methods for cardiac gene transfer. Hum. Gene Ther. Methods 23, 234–241.

Fargnoli, A.S., Katz, M.G., Yarnall, C., *et al*. (2013). Cardiac surgical delivery of the sarcoplasmic reticulum calcium ATPase rescues myocytes in ischemic heart failure. Ann. Thorac. Surg. 96, 586–595.

Ferrarini, M., Arsic, N., Recchia, F.A., *et al*. (2006). Adeno-associated virus-mediated transduction of VEGF165 improves cardiac tissue viability and functional recovery after permanent coronary occlusion in conscious dogs. Circ. Res. 98, 954–961.

Fish, K.M., Ladage, D., Kawase, Y., *et al*. (2013). AAV9.I-1c delivered via direct coronary infusion in a porcine model of heart failure improves contractility and mitigates adverse remodeling. Circ. Heart Fail. 6, 310–317.

Fraites, T.J., Jr., Schleissing, M.R., Shanely, R.A., *et al*. (2002). Correction of the enzymatic and functional deficits in a model of Pompe disease using adeno-associated virus vectors. Mol. Ther. 5, 571–578.

Gao, G., Bish, L.T., Sleeper, M.M., *et al*. (2011a). Transendocardial delivery of AAV6 results in highly efficient and global cardiac gene transfer in Rhesus macaques. Hum. Gene Ther. 22, 979–984.

Gao, G., Bish, L.T., Sleeper, M.M., *et al*. (2011b). Transendocardial delivery of AAV6 results in highly efficient and global cardiac gene transfer in rhesus macaques. Hum. Gene Ther. 22, 979–984.

Gregorevic, P., Blankinship, M.J., Allen, J.M., *et al*. (2004). Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat. Med. 10, 828–834.

Gurda, B.L., Raupp, C., Popa-Wagner, R., *et al*. (2012). Mapping a neutralizing epitope onto the capsid of adeno-associated virus serotype 8. J. Virol. 86, 7739–7751.

Gurda, B.L., Dimattia, M.A., Miller, E.B., *et al*. (2013). Capsid antibodies to different adeno-associated virus serotypes bind common regions. J. Virol. 87, 9111–9124.

Hajjar, R.J. (2013). Potential of gene therapy as a treatment for heart failure. J. Clin. Invest. 123, 53–61.

Hajjar, R.J., Zsebo, K., Deckelbaum, L., *et al*. (2008). Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. J. Card. Fail. 14, 355–367.

Harbison, C.E., Weichert, W.S., Gurda, B.L., *et al*. (2012). Examining the cross-reactivity and neutralization mechanisms of a panel of mAbs against adeno-associated virus serotypes 1 and 5. J. Gen. Virol. 93, 347–355.

Hoshijima, M., Ikeda, Y., Iwanaga, Y., *et al*. (2002). Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of phospholamban via *in vivo* cardiac rAAV gene delivery. Nat. Med. 8, 864–871.

Inagaki, K., Fuess, S., Storm, T.A., *et al*. (2006). Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. Mol. Ther. 14, 45–53.

Iwanaga, Y., Hoshijima, M., Gu, Y., *et al*. (2004). Chronic phospholamban inhibition prevents progressive cardiac dysfunction and pathological remodeling after infarction in rats. J. Clin. Invest. 113, 727–736.

Kaplitt, M.G., Xiao, X., Samulski, R.J., *et al*. (1996). Long-term gene transfer in porcine myocardium after coronary infusion of an adeno-associated virus vector. Ann. Thorac. Surg. 62, 1669–1676.

Karakikes, I., Hadri, L., Rapti, K., *et al*. (2012). Concomitant intravenous nitroglycerin with intracoronary delivery of AAV1.SERCA2a enhances gene transfer in porcine hearts. Mol. Ther. 20, 565–571.

Kaspar, B.K., Roth, D.M., Lai, N.C., *et al*. (2005). Myocardial gene transfer and long-term expression following intracoronary delivery of adeno-associated virus. J. Gene Med. 7, 316–324.

Kawada, T., Nakazawa, M., Nakauchi, S., *et al*. (2002). Rescue of hereditary form of dilated cardiomyopathy by rAAV-mediated somatic gene therapy: amelioration of morphological findings, sarcolemmal permeability, cardiac performances, and the prognosis of TO-2 hamsters. Proc. Natl. Acad. Sci. USA 99, 901–906.

Kawamoto, S., Shi, Q., Nitta, Y., *et al*. (2005). Widespread and early myocardial gene expression by adeno-associated virus vector type 6 with a beta-actin hybrid promoter. Mol. Ther. 11, 980–985.

Kawase, Y., Ly, H.Q., Prunier, F., *et al*. (2008). Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. J. Am. Coll. Cardiol. 51, 1112–1119.

Kern, A., Schmidt, K., Leder, C., *et al*. (2003). Identification of a heparin-binding motif on adeno-associated virus type 2 capsids. J. Virol. 77, 11072–11081.

Kotchey, N.M., Adachi, K., Zahid, M., *et al*. (2011). A potential role of distinctively delayed blood clearance of recombinant adeno-associated virus serotype 9 in robust cardiac transduction. Mol. Ther. 19, 1079–1089.

Lai, Y., and Duan, D. (2012). Progress in gene therapy of dystrophic heart disease. Gene Ther. 19, 678–685.

Lerch, T.F., O'Donnell, J.K., Meyer, N.L., *et al*. (2012). Structure of AAV-DJ, a retargeted gene therapy vector: cryo-electron microscopy at 4.5 A resolution. Structure 20, 1310–1320.

Li, J., Wang, D., Qian, S., *et al*. (2003). Efficient and long-term intracardiac gene transfer in delta-sarcoglycan-deficiency hamster by adeno-associated virus-2 vectors. Gene Ther. 10, 1807–1813.

Li, C., Diprimio, N., Bowles, D.E., *et al*. (2012). Single amino acid modification of adeno-associated virus capsid changes transduction and humoral immune profiles. J. Virol. 86, 7752–7759.

Louis Jeune, V., Joergensen, J.A., Hajjar, R.J., and Weber, T. (2013). Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum. Gene Ther. Methods. 24, 59–67.

Maersch, S., Huber, A., Buning, H., *et al*. (2010). Optimization of stealth adeno-associated virus vectors by randomization of immunogenic epitopes. Virology 397, 167–175.

Maheshri, N., Koerber, J.T., Kaspar, B.K., and Schaffer, D.V. (2006). Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. Nat. Biotechnol. 24, 198–204.

McTiernan, C.F., Mathier, M.A., Zhu, X., *et al*. (2007). Myocarditis following adeno-associated viral gene expression of human soluble TNF receptor (TNFRII-Fc) in baboon hearts. Gene Ther. 14, 1613–1622.

Melo, L.G., Agrawal, R., Zhang, L., *et al*. (2002). Gene therapy strategy for long-term myocardial protection using adeno-associated virus-mediated delivery of heme oxygenase gene. Circulation 105, 602–607.

Mingozzi, F., and High, K.A. (2011). Therapeutic *in vivo* gene transfer for genetic disease using AAV: progress and challenges. Nat. Rev. Genet. 12, 341–355.

Muller, O.J., Leuchs, B., Pleger, S.T., *et al*. (2006). Improved cardiac gene transfer by transcriptional and transductional targeting of adeno-associated viral vectors. Cardiovasc. Res. 70, 70–78.

Ng, R., Govindasamy, L., Gurda, B.L., *et al*. (2010). Structural characterization of the dual glycan binding adeno-associated virus serotype 6. J. Virol. 84, 12945–12957.

Odom, G.L., Gregorevic, P., Allen, J.M., *et al*. (2008). Microutrophin delivery through rAAV6 increases lifespan and improves muscle function in dystrophic dystrophin/utrophin-deficient mice. Mol. Ther. 16, 1539–1545.

Pacak, C.A., and Byrne, B.J. (2011). AAV vectors for cardiac gene transfer: experimental tools and clinical opportunities. Mol. Ther. 19, 1582–1590.

Pacak, C.A., Mah, C.S., Thattaliyath, B.D., *et al*. (2006). Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction *in vivo*. Circ. Res. 99, e3–e9.

Palomeque, J., Chemaly, E.R., Colosi, P., *et al*. (2007). Efficiency of eight different AAV serotypes in transducing rat myocardium *in vivo*. Gene Ther. 14, 989–997.

Petrs-Silva, H., Dinculescu, A., Li, Q., *et al*. (2011a). Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. Mol. Ther. 19, 293–301.

Petrs-Silva, H., Dinculescu, A., Li, Q., *et al*. (2011b). Novel properties of tyrosine-mutant AAV2 vectors in the mouse retina. Mol. Ther. 19, 293–301.

Piacentino, V., 3rd, Milano, C.A., Bolanos, M., *et al*. (2012). X-linked inhibitor of apoptosis protein-mediated attenuation of apoptosis, using a novel cardiac-enhanced adeno-associated viral vector. Hum. Gene Ther. 23, 635–646.

Pleger, S.T., Most, P., Boucher, M., *et al*. (2007). Stable myocardial-specific AAV6-S100A1 gene therapy results in chronic functional heart failure rescue. Circulation 115, 2506–2515.

Pleger, S.T., Shan, C., Ksienzyk, J., *et al*. (2011). Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. Sci. Transl. Med. 3, 92ra64.

Prasad, K.M., Smith, R.S., Xu, Y., and French, B.A. (2011a). A single direct injection into the left ventricular wall of an adeno-associated virus 9 (AAV9) vector expressing extracellular superoxide dismutase from the cardiac troponin-T promoter protects mice against myocardial infarction. J. Gene Med. 13, 333–341.

Prasad, K.M., Xu, Y., Yang, Z., *et al*. (2011b). Robust cardiomyocyte-specific gene expression following systemic injection of AAV: *in vivo* gene delivery follows a Poisson distribution. Gene Ther. 18, 43–52.

Pulicherla, N., Shen, S., Yadav, S., *et al*. (2011). Engineering liver-detargeted AAV9 vectors for cardiac and musculoskeletal gene transfer. Mol. Ther. 19, 1070–1078.

Qi, Y., Liu, X., Li, H., *et al*. (2010). Selective tropism of the recombinant adeno-associated virus 9 serotype for rat cardiac tissue. J. Gene Med. 12, 22–34.

Qiao, C., Yuan, Z., Li, J., *et al*. (2012). Single tyrosine mutation in AAV8 and AAV9 capsids is insufficient to enhance gene delivery to skeletal muscle and heart. Hum. Gene Ther. Methods 23, 29–37.

Raake, P.W., Hinkel, R., Muller, S., *et al*. (2008). Cardio-specific long-term gene expression in a porcine model after selective pressure-regulated retroinfusion of adeno-associated viral (AAV) vectors. Gene Ther. 15, 12–17.

Raake, P.W., Schlegel, P., Ksienzyk, J., *et al*. (2013). AAV6.betaARKct cardiac gene therapy ameliorates cardiac function and normalizes the catecholaminergic axis in a clinically relevant large animal heart failure model. Eur. Heart J. 34, 1437–1447.

Rapti, K., Louis-Jeune, V., Kohlbrenner, E., *et al*. (2012). Neutralizing antibodies against AAV serotypes 1, 2, 6, and 9 in sera of commonly used animal models. Mol. Ther. 20, 73–83.

Scimia, M.C., Cannavo, A., and Koch, W.J. (2013). Gene therapy for heart disease: molecular targets, vectors and modes of delivery to myocardium. Expert Rev. Cardiovasc. Ther. 11, 999–1013.

Shen, S., Byrant, K.D., Brown, S.M., *et al*. (2011). Terminal N-linked galactose is the primary receptor for adeno-associated virus 9. J. Biol. Chem. 286, 13532–13540.

Shen, S., Bryant, K., Sun, J., *et al*. (2012). Glycan binding avidity determines the systemic fate of adeno-associated virus 9. J. Virol. 86, 10408–10417.

Shen, S., Horowitz, E.D., Troupes, A.N., *et al*. (2013). Engraftment of a galactose receptor footprint onto adeno-associated viral capsids improves transduction efficiency. J. Biol. Chem. 288, 28814–28823.

Su, H., Lu, R., and Kan, Y.W. (2000). Adeno-associated viral vector-mediated vascular endothelial growth factor gene transfer induces neovascular formation in ischemic heart. Proc. Natl. Acad. Sci. USA 97, 13801–13806.

Su, H., Arakawa-Hoyt, J., and Kan, Y.W. (2002). Adeno-associated viral vector-mediated hypoxia response element-regulated gene expression in mouse ischemic heart model. Proc. Natl. Acad. Sci. USA 99, 9480–9485.

Su, H., Joho, S., Huang, Y., *et al*. (2004). Adeno-associated viral vector delivers cardiac-specific and hypoxia-inducible VEGF expression in ischemic mouse hearts. Proc. Natl. Acad. Sci. USA 101, 16280–16285.

Su, H., Huang, Y., Takagawa, J., *et al*. (2006). AAV serotype-1 mediates early onset of gene expression in mouse hearts and results in better therapeutic effect. Gene Ther. 13, 1495–1502.

Su, H., Yeghiazarians, Y., Lee, A., *et al*. (2008). AAV serotype 1 mediates more efficient gene transfer to pig myocardium than AAV serotype 2 and plasmid. J. Gene Med. 10, 33–41.

Svensson, E.C., Marshall, D.J., Woodard, K., *et al*. (1999). Efficient and stable transduction of cardiomyocytes after intramyocardial injection or intracoronary perfusion with recombinant adeno-associated virus vectors. Circulation 99, 201–205.

Tang, T., Gao, M.H., and Hammond, H.K. (2012). Prospects for gene transfer for clinical heart failure. Gene Ther. 19, 606–612.

Townsend, D., Blankinship, M.J., Allen, J.M., *et al*. (2007). Systemic administration of micro-dystrophin restores cardiac geometry and prevents dobutamine-induced cardiac pump failure. Mol. Ther. 15, 1086–1092.

Varki, A., and Schauer, R. (2009). Sialic acids. In *Essentials of Glycobiology*. A. Varki, R.D. Cummings, J.D. Esko, *et al*., eds. (The Consortium of Glycobiology Editors, La Jolla, CA). pp. 199–218.

Wang, Z., Zhu, T., Qiao, C., *et al*. (2005). Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. Nat. Biotechnol. 23, 321–328.

Weller, M.L., Amornphimoltham, P., Schmidt, M., *et al*. (2010). Epidermal growth factor receptor is a co-receptor for adeno-associated virus serotype 6. Nat. Med. 16, 662–664.

White, J.D., Thesier, D.M., Swain, J.B., *et al*. (2011). Myocardial gene delivery using molecular cardiac surgery with recombinant adeno-associated virus vectors *in vivo*. Gene Ther. 18, 546–552.

Wu, Z., Asokan, A., Grieger, J.C., *et al*. (2006a). Single amino acid changes can influence titer, heparin binding, and tissue tropism in different adeno-associated virus serotypes. J. Virol. 80, 11393–11397.

Wu, Z., Miller, E., Agbandje-Mckenna, M., and Samulski, R.J. (2006b). Alpha2,3 and alpha2,6 N-linked sialic acids facilitate efficient binding and transduction by adeno-associated virus types 1 and 6. J. Virol. 80, 9093–9103.

Xie, Q., Lerch, T.F., Meyer, N.L., and Chapman, M.S. (2011). Structure-function analysis of receptor-binding in adeno-associated virus serotype 6 (AAV-6). Virology 420, 10–19.

Yang, L., Jiang, J., Drouin, L.M., *et al*. (2009). A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and *in vivo* selection. Proc. Natl. Acad. Sci. USA 106, 3946–3951.

Yasukawa, H., Yajima, T., Duplain, H., *et al*. (2003). The suppressor of cytokine signaling-1 (SOCS1) is a novel therapeutic target for enterovirus-induced cardiac injury. J. Clin. Invest. 111, 469–478.

Ying, Y., Muller, O.J., Goehringer, C., *et al*. (2010). Heart-targeted adeno-associated viral vectors selected by *in vivo* biopanning of a random viral display peptide library. Gene Ther. 17, 980–990.

Yue, Y., Li, Z., Harper, S.Q., *et al*. (2003). Microdystrophin gene therapy of cardiomyopathy restores dystrophin-glycoprotein complex and improves sarcolemma integrity in the mdx mouse heart. Circulation 108, 1626–1632.

Zhong, L., Li, B., Mah, C.S., *et al*. (2008). Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses. Proc. Natl. Acad. Sci. USA 105, 7827–7832.

Zhu, T., Zhou, L., Mori, S., *et al*. (2005). Sustained whole-body functional rescue in congestive heart failure and muscular dystrophy hamsters by systemic gene transfer. Circulation 112, 2650–2659.

Zincarelli, C., Soltys, S., Rengo, G., *et al*. (2010). Comparative cardiac gene delivery of adeno-associated virus serotypes 1–9 reveals that AAV6 mediates the most efficient transduction in mouse heart. Clin. Transl. Sci. 3, 81–89.

Address correspondence to:
Dr. Aravind Asokan
Gene Therapy Center
CB# 7352, Thurston Bldg.
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27516

E-mail: aravind@med.unc.edu

Dr. R. Jude Samulski
Department of Pharmacology
CB# 7352, Thurston Bldg.
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27516

E-mail: rjs@med.unc.edu