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Next Generation of AAV Vectors for Gene Therapy of Human Liver Diseases

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Introduction

Adeno-associated virus (AAV) is a small single-stranded DNA-containing non-pathogenic human parvovirus, which has gained attention as an efficient and safe vector for gene transfer [1-6]. Recombinant AAV vectors have been, or are currently being, used in 165 Phase I/II/III clinical trials, and thus far, no serious adverse events have ever been observed or reported. AAV serotype 2 (AAV2) vectors have shown clinical efficacy in 3 human diseases: Leber's congenital amaurosis (LCA) [7-10], aromatic L-amino acid decarboxylase deficiency (AADC) [11], and choroideremia [12]. In the past decade, at least 12 additional AAV serotype vectors, some derived from non-human primates, have also become available [13-21]. AAV1 vectors have successfully been used in the gene therapy of lipoprotein lipase deficiency [22], and AAV8 vectors have shown clinical efficacy in the potential gene therapy of hemophilia B [23-25]. More recently, AAV5 vectors have been to be effective in hemophilia A [26,27]. AAV9 vectors have successfully been used in gene therapy for Pompe

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DISCLOSURE STATEMENT

Dr. Berns is a board member of Lacerta Therapeutics, a recently launched AAV gene therapy company. Dr. Srivastava is a co-founder of, and holds equity in, Lacerta Therapeutics, aaVective, and Nirvana Therapeutics, and is an inventor on several issued patents on recombinant AAV vectors that have been licensed to various gene therapy companies.

disease [28] and in spinal muscular atrophy [29]. The AAV1-LPL vector was approved as a drug, designated as Alipogene tiparvovec, and marketed under the trade name Glybera®, in Europe in 2012. In 2017, an AAV2 vector expressing retinal pigment epithelium-specific 65 kDa protein (RPE65) was approved by the Food and Drug Administration as a drug, Voretigene Neparvovec (Luxturna), in the USA. A number of additional Phase I/II clinical trials have been, or are currently being pursued with AAV1, AAV2, AAV3, AAV5, AAV6, AAV8, AAV9, and AAV10 vectors for the potential gene therapy of a wide variety of human diseases [30].

Despite these remarkable achievements, it has become increasingly clear that the full potential of the first generation of AAV vectors will only be realized after these vectors have been modified to evade the host immune response [31]. A brief account of the use of AAV vectors in targeting the liver in general, and gene therapy of hemophilia in particular, follows.

Liver-tropic AAV vectors

Liver has long been considered among the ideal targets for the potential gene therapy of a wide variety of human diseases. In 1997, Ponnazhagan et al. [32] first reported the liver-tropism of rAAV2 vectors, following intra-venous administration of *rAAV2-lacZ* vectors, in a murine model *in vivo*. Koeberl et al. [33] and Snyder et al. [34] documented persistent expression of human clotting factor IX (F.IX) following intravenous injection in mice as well. Subsequently, persistent expression of canine factor IX in hemophilia B canines was also reported by Chao et al. [35]. Based on those pre-clinical studies, a Phase I clinical trial for hemophilia B was carried out with AAV2 vectors expressing h.FIX [35]. Even though in pre-clinical studies with both hemophilic murine and canine models, rAAV2-F.IX vectors provided complete phenotypic correction of the disease for the entire life-spans of these animals, the predicted dose of these vectors in humans did not express therapeutic levels of F.IX in humans. The results of the first Phase I clinical trial for the potential gene therapy of hemophilia B with the first generation of rAAV2 vectors were reported in 2006 [36]. At low (8×10^{10} vgs/kg), and medium (4×10^{11} vgs/kg) vector doses, rAAV2 vectors failed to express F.IX in two patients. At the high dose (2×10^{12} vgs/kg), rAAV2 vectors did lead to expression of therapeutic levels of F.IX in one patient, but it was short-lived due to the host immune response to AAV2 capsid proteins. Uptake of AAV2 vectors by dendritic cells, followed by proteasomal degradation of capsid proteins, led to activation of AAV2 capsid-specific CD8⁺ memory T cells, which in turn, led to the destruction of transduced hepatocytes, and consequently, the loss of F.IX levels in this patient [37]. The lesson learned from this first liver-directed gene therapy trial was that AAV2 serotype vectors, although extremely effective in mice and dogs, were not optimal for humans.

In the ensuing years a number of additional AAV serotype vectors became available, and their tissue/organ tropisms were determined [13-21,38]. The 10 most commonly used AAV serotype vectors are depicted schematically in Figure 1A. Several investigators reported remarkably high efficiency of transduction of murine liver with AAV8 serotype vectors [18,39,40]. A subsequent clinical trial with AAV8 vectors, performed by Nathwani et al. [23] proved to be a significant step forward, but a similar immune response was also

observed in the high-dose cohort of patients [24]. Although the choice of AAV8 vector was undoubtedly based on its superior performance over AAV2 vectors in transducing murine hepatocytes, it was not readily apparent whether AAV8 vectors would be ideal for transducing human hepatocytes. This question proved to be particularly difficult to address since AAV8 failed to transduce any cell type, including hepatocytes *in vitro*.

Selective human liver-tropism of AAV3 vectors

In the quest to identify an alternative AAV serotype for efficient transduction of human hepatocytes, Glushakova et al. [41] made an unexpected observation nearly a decade ago that AAV3 vectors, shown schematically in Figure 1B, could efficiently transduce human liver cancer cell lines as well as primary human hepatocytes efficiently *in vitro*. Cheng et al. [42] and Ling et al. [43] documented that human liver tumors could also be transduced remarkably well in xenograft mouse model *in vivo*. Ling et al. [44] provided the underlying basis of the remarkable tropism of AAV3 vectors for human hepatic cells because AAV3 uses human hepatocyte growth factor receptor (huHGFR) as a cellular co-receptor. Although murine HGFR (muHGFR) shares 88% identity with huHGFR, AAV3 fails to bind to muHGFR because of the location of amino acid differences along the interaction interface, including amino residues that make contact with hepatocyte growth factor (HGF). Lisowski et al. [45] reported that a shuffled rAAV vector, designated LK-03, which shares 97.7 and 98.9% homology with rAAV3B at the DNA and amino acids level, respectively, transduced human primary hepatocytes ~20-fold more efficiently than AAV8.

AAV3 and non-human primate studies

Although murine xenograft mouse models, used by Cheng et al. [42] and Ling et al. [43] provided a useful system to evaluate the efficacy of AAV3 vectors in human hepatocyte transduction, the safety of these vectors could not be assessed in such models since AAV3 vectors do not transduce any tissues or organs in mice [46]. Because of the close phylogenetic and physiologic similarities between non-human primates (NHPs) and humans]. The safety and efficacy of rAAV3 vectors was evaluated by Li et al. in a NHP model *in vivo* [47]. Interestingly, the human and the non-human primate HGFRs share 99% identity. High-efficiency transduction of NHP livers, both short-term [7-days] and long-term [91-days], following intravenous delivery of rAAV3 vectors, was indeed documented, with no apparent toxicity at a relatively high dose of 1×10^{13} vgs/kg. These findings revealed the efficient liver-targeted gene transfer by rAAV3 vectors following systematic administration. Remarkably, the vector-mediated EGFP expression and vector genome accumulation were largely restricted to the NHP liver. Although the use of the EGFP reporter gene allowed us to address both vector tropism and gene transfer efficiency simultaneously, it was less quantitative and likely immunogenic leading to transient expression. The use of a secreted self-antigen, rhCG, allowed us to study not only the efficiency, but also the onset and kinetics of transgene expression and long-term stability. In addition, since the rhCG used in this study can limit the potential host humoral and cellular immune responses to the transgene product, we were also able to evaluate the potential host immune responses against the vector capsid proteins for up to 3 months. The vector genome biodistribution study clearly revealed that the persisting vector genomes of rAAV3 vectors were

predominantly harbored in the monkey liver. Furthermore, the rhCG mRNA expression pattern in six selected organs displayed that the transgene expression was largely restricted to the liver, which also did not lead to any overt cytotoxicity. In addition, no obvious vector-related pathological changes in the liver, spleen and other organs were observed in any of the treated animals. These studies documented the remarkable specificity, efficacy, and safety of rAAV3 vectors in NHP livers following systemic administration. These studies were further corroborated by Wang et al. [48], which further established the remarkable specificity, efficacy, and safety of AAV3 vectors [49].

Next generation of AAV vectors

Hansen et al. [50] made the original observation that following infection of cells, only ~20% of the input AAV2 vectors gain entry into the nucleus, whereas ~80% fail to escape the endosome in the cytoplasm. It was subsequently reported by Duan et al. [51] that AAV2 capsids become ubiquitinated in the cytoplasm, where they are targeted for degradation by the host cell proteasomal machinery, thereby negatively impacting the transduction efficiency of the first generation of AAV vectors. One of the major obstacles that limit the transduction efficiency of AAV vectors in general is ubiquitination, followed by proteasomal-mediated degradation. Previously, Mah et al. [52] had observed that inhibition of the host cell epidermal growth factor receptor protein tyrosine kinase (EGFR-PTK) resulted in a significant increase in the transduction efficiency of AAV2 vectors. Thus, it was hypothesized that following infection, the AAV2 capsid protein becomes phosphorylated at surface-exposed tyrosine residues by EGFR-PTK, and that tyrosine phosphorylation leads to ubiquitination, followed by proteasomal degradation of AAV2 vectors in the cytoplasm [51,53]. Indeed, Zhong et al. [54] provided experimental evidence in 2007 to support this hypothesis. These studies provided the impetus to mutagenize the surface- exposed tyrosine residues in the AAV2 capsid to circumvent this barrier.

There are 7 tyrosine (Y) residues in the AAV2 capsid that are surface-exposed (Y252, Y272, Y444, Y500, Y700, Y704, Y730). Each of these Y residues was mutagenized by Zhong et al. [55] to phenylalanine (F) residues to generate 7 single-mutants (Y252F, Y272F, Y444F, Y500F, Y700F, Y704F, Y730F), the transduction efficiency of 3 of which (Y444F, Y500F, Y730F) was significantly higher than their WT counterpart [56]. The Y730F single-mutant AAV2 vector was the most efficient, the use of which resulted in the expression of therapeutic levels of h.FIX in 3 different strains of mice following intravenous or portal vein administration at 10-fold reduced vector doses [55]. When the 3 most efficient mutations were combined into one capsid, the resulting triple-mutant (Y444+500+730F) vectors showed a further 3-fold increase in F.IX expression in hemophilia B mice [56]. Furthermore, this triple-mutant AAV2 vector was shown to minimize *in vivo* targeting of transduced hepatocytes by capsid-specific CD8+ T cells [57].

In the quest to develop more efficient and potentially less immunogenic AAV vectors, these studies were extended to include two additional amino acid residues in the AAV capsid that are surface-exposed, and can also be phosphorylated by cellular serine/threonine protein kinases. For example, in addition to 7 tyrosine (Y) residues, the AAV2 capsid also contains 17 surface- exposed serine (S), and 15 surface-exposed threonine (T) residues, each of which

were mutagenized, and AAV2 vectors containing various permutations and combinations thereof, depicted schematically in Figure 2A, were generated [58,59]. In addition, since ubiquitination occurs on lysine (K) residues, all 7 surface-exposed residues in the AAV2 capsid were mutagenized, and a limited numbers of Y+S+T+K-mutant AAV2 vectors were generated [60].

Interestingly, however, most, if not all, of the surface-exposed Y, S, T, and K residues are highly conserved among all 10 commonly used AAV serotype vectors, and most of these residues have also been mutagenized in each of 10 AAV serotype vectors. The corresponding Y-, S-, T-, and K-mutants of AAV3 vectors are also shown in Figure 2A. A quadruple-mutant (Y444+500+730F+T491V) for AAV2, and a double-mutant (S663V+T492V) for AAV3, shown schematically in Figure 2B, have been identified to be the most efficient in transducing the normal and murine xenograft models, respectively [61].

In addition to the WT AAV3 vectors, Li et al. [47] also evaluated the safety and efficacy of the S663V+T492V double-mutant AAV3 (AAV3.ST) vectors in NHPs. The results revealed that the transduction efficiency of this capsid-modified vector was ~5-fold higher compared with its WT counterpart, with no apparent vector-related toxicity. Thus, the use of capsid-modified next generation of AAV vectors is likely to overcome some of the limitations associated with the first generation of AAV vectors.

AAV3 vectors and “humanized” mouse models

In 2014, Lisowski et al. [45] reported that a shuffled rAAV vector, designated LK-03, which shares 97.7 and 98.9% homology with rAAV3 at the DNA and amino acids level, respectively, transduced human primary hepatocytes in a humanized mouse model *in vivo*, and it was ~20-fold more efficient than AAV8. In studies performed by Li et al. [47] with a different humanized liver xenograft mouse model [62], it was observed that AAV3 was ~12-fold more efficient than AAV8 in transducing human hepatocytes *in vivo* [14]. In a more recent study, Vercauteren et al. [63] compared the transduction efficiencies of AAV3-ST vectors with AAV8 and AAV5 vectors, that are currently being used in gene therapy of hemophilia [Table 1]. In this humanized mouse model, it was reported that rAAV3-ST vectors were ~8-times more efficient than rAAV8 vectors, and ~82-times more efficient than rAAV5 vectors in transducing primary human hepatocytes [63]. Although the ultimate efficacy of AAV3 vectors will only be revealed by clinical trials in humans, it appears likely that these vectors will prove to be safer and more efficient than AAV8 and AAV5 vectors in targeting human liver diseases in general, and gene therapy of hemophilia in particular.

Gene therapy of hemophilia with AAV vectors

Table 1 lists all clinical trials in which various AAV serotypes and their variants that have been used, or are currently being used, in the potential gene therapy of both hemophilia B and hemophilia A. As stated above, following the short-lived expression h.FIX mediated by the first generation AAV2 vectors [37], the use of AAV8 vectors proved to be successful [23]. However, the h.FIX levels declined from ~8-12% to ~5% over a 3-year follow-up period [24]. Although the superior performance of AAV8 vectors in humans appeared to

parallel that observed in murine models, the vector genome used was double-stranded self-complementary (sc), in contrast to that used in the first clinical trial with AAV2 vectors, which was single-stranded (ss), and ssAAV DNA is known to transcriptionally-inactive, and viral second strand-DNA synthesis is known to be a rate-limiting step during AAV vector-mediated transgene expression. [64]. Thus, it appears that the use of scAAV genome, rather than the AAV8 serotype, contributed to the successful outcome since AAV8 vectors transduce human hepatocytes less efficiently than mouse hepatocytes [47,48,63]. The next trial, sponsored by Baxalta, also with scAAV8 vectors, yielded inconsistent results, and as a consequence, this trial was halted [65]. Interestingly, however, the trial sponsored by Spark Therapeutics, using a capsid-modified ssAAV vector, has led to therapeutic levels of h.FIX at relatively low doses [25]. The trial sponsored by Dimension Therapeutics using ssAAVrh10 vectors failed to lead to expression therapeutic levels of h.FIX, even at significantly high doses and a consequence, this trial was also halted [66]. Using scAAV5 vectors, uniQure recently reported modest levels of expression of h.FIX, however, enormously high vector doses were required [26]. (Table 1)

The results of two Phase I clinical trials for gene therapy of hemophilia A have also been reported. In the Biomarin-sponsored trial with ssAAV5 vectors, h.FVTVII levels ranging from ~50- 200% were achieved [27]. However, there has been a steady decline over time, ranging between ~32-59% [67]. More recently, Spark Therapeutics reported that with the use of ssLK-03, a shuffled ssAAV vector with significant homology to AAV3, h.FVTVIII levels ranging from ~16- 49% were achieved, but at the highest vector dose, two patients experienced severe adverse events [68].

Taken together, the following conclusions can be drawn: (i) The tropism, safety, and efficacy of AAV vectors in animal models do not necessarily translate well in humans; (ii) The use of AAV vectors composed of naturally occurring capsids are likely to induce immune responses, especially at high doses since the host immune system cannot distinguish between AAV as a virus versus AAV as a vector; (iii) The host immune response is directly correlated with the AAV vector dose; and (iv) Since the wild-type AAV did not evolve for the purposes of delivery of therapeutic genes, recombinant AAV vectors composed of naturally occurring capsid are unlikely to be optimal in human clinical trials [31]. Overall, our prediction is that, in contrast to the high vector doses that are currently being used with AAV8 and AAV5 vectors for hemophilia B and hemophilia A, respectively, the optimized AAV3 serotype vectors, in addition to being far more efficacious, will also offer the potential advantages of being less immunogenic, and more cost-effective, for their use in human liver diseases in general, and hemophilia in particular [4].

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KEY POINTS

- The first generation of recombinant AAV vectors have shown efficacy in a number of Phase I/II/III clinical trials targeting various human diseases, but the host immune response at high vector doses remains a challenge.
- The tissue-tropism of AAV vectors observed in animal models does not always correlate well with that in humans.
- A specific AAV serotype, AAV3, has been identified with which targeted delivery as well as high-efficiency transduction of human hepatocytes can be achieved.
- Next generation of AAV3 vectors have also been developed that are likely to circumvent most, if not all, of the problems associated with the first generation of AAV vectors in general.
- Further optimized AAV3 vectors should prove safe and effective for the potential gene therapy of human liver diseases in general, and hemophilia in particular.

SYNOPSIS

Recombinant vectors based on a non-pathogenic parvovirus, the adeno-associated virus (AAV) have taken center stage in the past decade. The safety of AAV vectors in 165 Phase I/II/III clinical trials in humans to date, and clinical efficacy in several human diseases, are now well documented. Despite these remarkable achievements, it is becoming increasingly clear that the full potential of AAV vectors composed of the naturally occurring capsids is unlikely to be realized. In this article, we describe the advances that have been made, and the challenges that remain, in the optimal use of AAV vectors in human gene therapy applications. More specifically, we provide an account of the progress that has been made in the development of AAV vectors to ensure both safety and efficacy of these vectors in targeting human liver diseases in general, and hemophilia in particular.

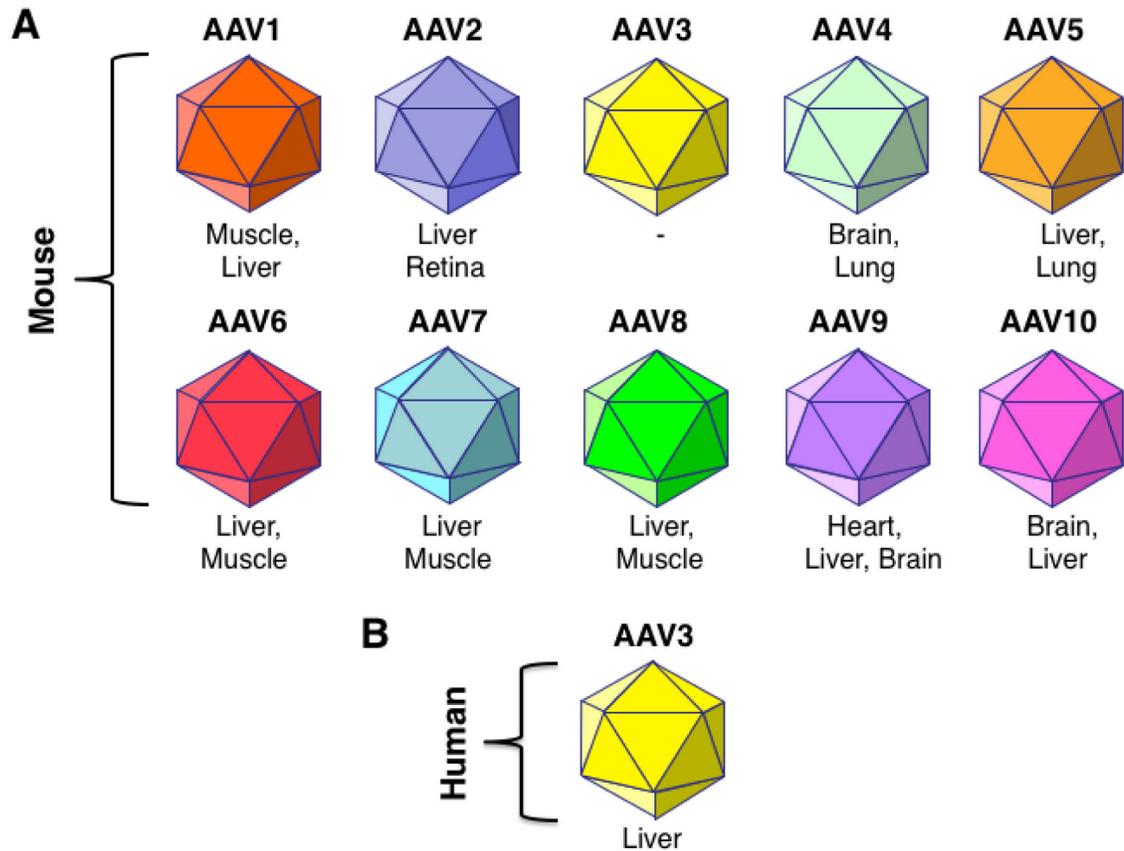


FIGURE 1: Schematic representation of the most commonly used recombinant AAV serotype vectors and their tissue-tropism.

Various *murine* tissues and organs that have been reported to be transduced efficiently with various AAV serotype vectors are indicated. AAV3 serotype vectors in particular, have been shown to transduce *human* hepatocytes well. Data from Glushakova LG, Lisankie MJ, Eruslanov EB, et al.. AAV3-mediated transfer and expression of the pyruvate dehydrogenase E1 alpha subunit gene causes metabolic remodeling and apoptosis of human liver cancer cells. *Mol Genet Metab* 2009;98:289-99, Cheng B, Ling C, Dai Y, et al.. Development of optimized AAV3 serotype vectors: Mechanism of high-efficiency transduction of human liver cancer cells. *Gene Ther* 2012; 19:375-84, Ling C, Wang Y, Zhang Y, Ejjigani A, Yin Z, Lu Y, Wang L, Wang M, Li J, Hu Z, Aslanidi GV et al.: Selective in vivo targeting of human liver tumors by optimized AAV3 vectors in a murine xenograft model. *Hum Gene Ther* (2014) 25(12):1023-1034, Ling C, Lu Y, Kalsi JK, Jayandharan GR, Li B, Ma W, Cheng B, Gee SW, McGoogan KE, Govindasamy L, Zhong L et al.: Human hepatocyte growth factor receptor is a cellular coreceptor for adeno-associated virus serotype 3. *Hum Gene Ther* (2010) 21 (12):1741- 1747, Li S, Ling C, Zhong L, Li M, Su Q, He R, Tang Q, Greiner DL, Shultz LD, Brehm MA, Flotte TR et al.: Efficient and targeted transduction of nonhuman primate liver with systemically delivered optimized AAV3b vectors. *Mol Ther* (2015) 23(12):1867-1876, Wang L, Bell P, Somanathan S, Wang Q, He Z, Yu H, McMenamin D, Goode T, Calcedo R, Wilson JM: Comparative study of liver gene transfer with AAV vectors based on natural and engineered aav capsids. *Mol Ther* (2015) 23(12):1877-1887, and

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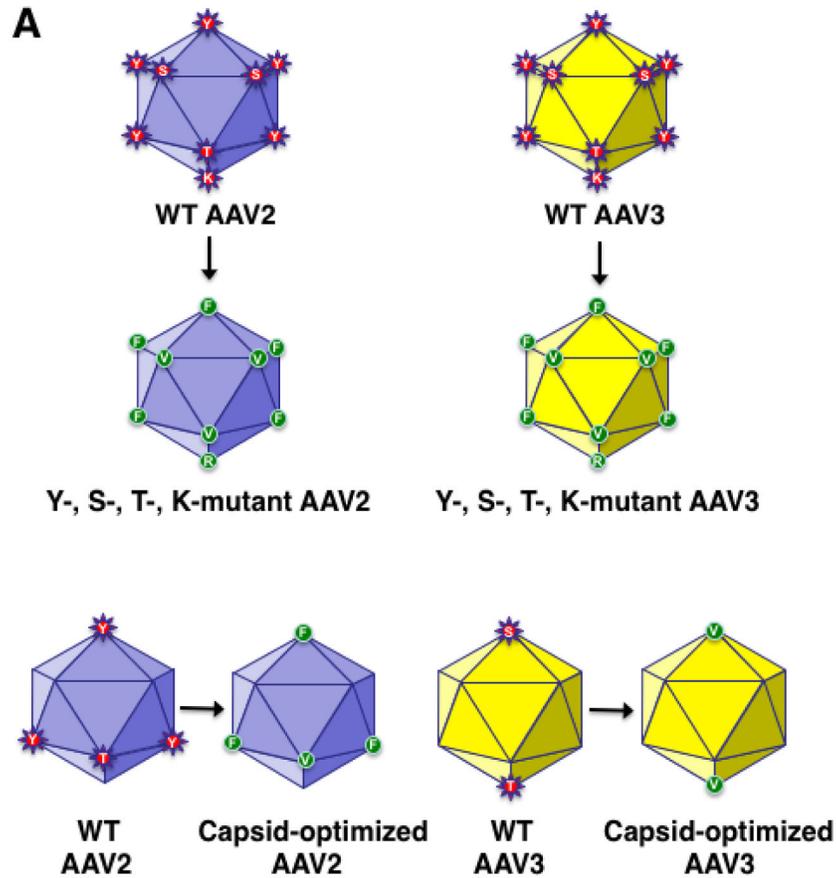


FIGURE 2: Schematic representation of capsid-modified next generation of recombinant AAV vectors.

Surface-exposed, specific tyrosine (Y), serine (S), and threonine (T) residues on AAV capsids can be phosphorylated, which is a signal for ubiquitination. Surface-exposed, specific lysine (K) residues on AAV capsids can be ubiquitinated, and subsequently degraded by the host cell proteasome machinery. Site-directed mutagenesis of these residues leads to the generation of AAV vectors that are more efficient at reduced vector doses, and consequently, less immunogenic. Specific examples of the most efficient AAV2 [55-57,59,61], and AAV3 [42,43,47,61,63], serotype vectors generated thus far, are also depicted. AAV2 data from Zhong L, Li B, Mah CS, et al.. 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* 2008;105:7827-32, Markusic DM, Herzog RW, Aslanidi GV, et al.. High-efficiency transduction and correction of murine hemophilia B using AAV2 vectors devoid of multiple surface-exposed tyrosines. *Mol Ther* 2010;18:2048-56, Martino AT, Basner-Tschakarjan E, Markusic DM, et al.. Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8+ T cells. *Blood* 2013;121:2224-33, Aslanidi GV, Rivers AE, Ortiz L, et al.. Optimization of the capsid of recombinant adeno-associated virus 2 (AAV2) vectors: The final threshold? *PLoS One* 2013;8:e59142, and Ling C, Li B, Ma W, et al.. Development of optimized AAV serotype vectors for high-efficiency transduction at further reduced doses. *Hum Gene Ther Meth* 2016;27:143-9, with permission. AAV3 data from Cheng B, Ling C, Dai Y, et al..

Development of optimized AAV3 serotype vectors: Mechanism of high-efficiency transduction of human liver cancer cells. *Gene Ther* 2012; 19:375-84, Ling C, Wang Y, Zhang Y, Ejjigani A, Yin Z, Lu Y, Wang L, Wang M, Li J, Hu Z, Aslanidi GV et al.: Selective in vivo targeting of human liver tumors by optimized AAV3 vectors in a murine xenograft model. *Hum Gene Ther* (2014) 25(12):1023-1034, Li S, Ling C, Zhong L, Li M, Su Q, He R, Tang Q, Greiner DL, Shultz LD, Brehm MA, Flotte TR et al.: Efficient and targeted transduction of nonhuman primate liver with systemically delivered optimized AAV3b vectors. *Mol Ther* (2015) 23(12):1867-1876, Ling C, Li B, Ma W, et al.. Development of optimized AAV serotype vectors for high-efficiency transduction at further reduced doses. *Hum Gene Ther Meth* 2016;27:143-9, and Vercauteren K, Hoffman BE, Zolotukhin I, et al.. Superior in vivo transduction of human hepatocytes using engineered AAV3 capsid. *Mol Ther* 2016;24:1042-49 with permission.

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Table 1:

First generation of recombinant AAV serotype vectors used/being used for the potential gene therapy of hemophilia.

	Investigators/ Sponsors	Vector	Dose	Expression level	Total dose*	Ref.
Hemophilia B	High/Kay	ssAAV2	8×10 ¹⁰ vgs/kg	0%	5.6 Trillion	[36]
			4×10 ¹¹ vgs/kg	0%	28 Trillion	
			2×10 ¹² vgs/kg	11%→0%	140 Trillion	
	Nathwani/ Davidoff	scAAV8	2×10 ¹¹ vgs/kg	2%	14 Trillion	[24,25]
			6×10 ¹¹ vgs/kg	2-4%	42 Trillion	
			2×10 ¹² vgs/kg	8-12%→5%	140 Trillion	
Baxalta**	scAAV8	2×10 ¹¹ vgs/kg	2-5%	14 Trillion	[65,69]	
		1×10 ¹² vgs/kg	20-25%	70 Trillion		
		3×10 ¹² vgs/kg	50%→?%	210 Trillion		
Spark Therapeutics	Modified ssAAV8	5×10 ¹¹ vgs/kg	28-41 %→26-33%	35 Trillion	[25]	
Dimension Therapeutics***	ssAAVrh10	1.6×10 ¹² vgs/kg	<2%	112 Trillion	[66]	
		3×10 ¹² vgs/kg	<2%	210 Trillion		
uniQure	scAAV5	5×10 ¹² vgs/kg	3-6.8%	350 Trillion	[26]	
		2×10 ¹³ vgs/kg	3-12.7%	1.4 Quadrillion		
Hemophilia A	BioMarin	ssAAV5	2×10 ¹³ vgs/kg	2-5%	1.4 Quadrillion	[27,67]
			6×10 ¹³ vgs/kg	50-200%→32-59%	4.2 Quadrillion	
	Spark Therapeutics	ssLK-03	5×10 ¹¹ vgs/kg	6-37%	35 Trillion	[68]
1×10 ¹² vgs/kg	7-24%	70 Trillion				
2×10 ¹² vgs/kg	16-49%****	140 Trillion				

* Based on an average patient's weight of 70 kg (Estimated number of cells in a 70 kg "reference man" = 3 x 10¹³, or 30 Trillion) Data from Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016;14:e1002533 with permission.

** Acquired by Shire.

*** Acquired by Ultragenyx.

**** Serious adverse events in 2 patients.