

Review

Various AAV Serotypes and Their Applications in Gene Therapy: An Overview

Shaza S. Issa ¹, Alisa A. Shaimardanova ², Valeriya V. Solovyeva ² and Albert A. Rizvanov ^{2,*}

¹ Department of Genetics and Biotechnology, St. Petersburg State University, 199034 St. Petersburg, Russia

² Institute of Fundamental Medicine and Biology, Kazan Federal University, 420008 Kazan, Russia

* Correspondence: rizvanov@gmail.com; Tel.: +7-(905)-3167599

Abstract: Despite scientific discoveries in the field of gene and cell therapy, some diseases still have no effective treatment. Advances in genetic engineering methods have enabled the development of effective gene therapy methods for various diseases based on adeno-associated viruses (AAVs). Today, many AAV-based gene therapy medications are being investigated in preclinical and clinical trials, and new ones are appearing on the market. In this article, we present a review of AAV discovery, properties, different serotypes, and tropism, and a following detailed explanation of their uses in gene therapy for disease of different organs and systems.

Keywords: AAV; gene therapy approach; serotype; tropism; gene therapy medication

1. Introduction

Thanks to the rapid progress in biomedical technologies, large developments of novel high-tech treatment approaches have become possible today for several human diseases. However, despite scientific discoveries in the field of gene and cell therapy, some diseases still have no effective treatment. Advances in genetic engineering methods have enabled the development of effective gene therapy methods for various diseases based on adeno-associated viruses (AAVs). AAVs are effectively used to treat a number of genetic and acquired human diseases, more specifically, monogenic hereditary ones, i.e., diseases caused by mutations in one gene. Today, many AAV-based gene therapy medications are being investigated in preclinical and clinical trials, and new ones are appearing on the market for the treatment of numerous human diseases, including those that were previously considered incurable.

To date, there are only a few approved AAV-based gene therapy medications. For example, the US Food and Drug Administration (FDA) recently approved “Hemgenix”, a drug for the treatment of hemophilia type B, which is caused by congenital deficiency of factor IX. The drug is an AAV serotype 5 (AAV5), carrying the gene of deficient factor IX. As for hemophilia type A, Roctavian has also recently been approved by the FDA as an AAV5-based gene therapy vector, carrying the deficient factor VIII gene, driven by a liver-selective promoter [1]. Another breakthrough gene therapy medication is “Zolgensma”, which was intended for spinal muscular atrophy (SMA) treatment, a disease characterized by degeneration of the anterior horns motor neurons in the spinal cord resulting in a loss of their functions. Zolgensma is an AAV-serotype-9 (AAV9)-based vector encoding complementary DNA (cDNA) of the survival motor neuron 1 gene (survival motor neuron, SMN1). The drug was approved by the FDA in May 2019 for the treatment of type 1 SMA. Following intravenous administration, AAV9 crosses the blood–brain barrier (BBB) and provides targeted delivery of the SMN gene to neurons. It has been shown that intravenous administration of Zolgensma leads to an improvement in patients’ motor skills, along with a decrease in the clinical manifestations of SMA [2]. Another FDA-approved AAV-based gene therapy medication is Luxturna, which is intended for the treatment of retinal



Citation: Issa, S.S.; Shaimardanova, A.A.; Solovyeva, V.V.; Rizvanov, A.A. Various AAV Serotypes and Their Applications in Gene Therapy: An Overview. *Cells* **2023**, *12*, 785. <https://doi.org/10.3390/cells12050785>

Academic Editors: Mauro Pistello and Shih-Heng (David) Chen

Received: 29 December 2022

Revised: 22 February 2023

Accepted: 26 February 2023

Published: 1 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

dystrophy caused by biallelic mutations in the *RPE65* gene (an enzyme of retinal cells involved in light-sensitive pigment regeneration). Drug delivery to the retinal cells leads to synthesis restoration of normal RPE65 protein, which is necessary for the regeneration of the photosensitive pigment [3]. The very first AAV-based gene therapy medication to be registered is Glybera, which was approved by the European Commission for the treatment of a rare hereditary disease, lipoprotein lipase deficiency [4].

Nevertheless, despite the successful development of AAV-based gene therapy medications, many preclinical and clinical studies still get suspended or terminated, without resulting in a drug registration, and therefore, many diseases, especially the rare ones, remain untreated. One explanation for that could be the lack of knowledge in gene therapeutics, in particular, features of AAV vectors. To tackle this issue, further research is needed to achieve better optimization and higher effectiveness of AAV-based gene therapy approaches. This review discusses various AAV serotypes, some features of infection, tissue and organ tropism, efficacy in many human diseases, etc.

2. Brief History of AAVs' Discovery

AAVs are small non-enveloped DNA viruses belonging to the *Parvoviridae* family, that were first isolated in 1965, as a contaminant in preparations of a simian adenovirus (Ad) [5,6]. The viruses were found incompetent to productively infect cells without a co-infection by a helper virus, usually an Ad or any type of herpesviruses, and thereby they were named as “adeno-associated”, and classified into the Dependovirus genus [7,8]. After being regarded as defective viruses for a long time due to their codependency, later studies on AAVs disproved this theory and showed that they rather launch a latent infection in the host cell, that could convert to productive infection under stress [8,9]. Although AAVs have a high seroprevalence in humans (it has been estimated that 50% to 96% of the human population is seropositive for the second serotype of AAV (AAV2) depending on age and ethnic group) [10–12], they, however, were not linked to any disease neither in humans, nor in any other species [8]. Different AAVs have not only been detected in primates isolates [13], but also from avian [14–16], caprine [17,18], bovine [19,20], and equine stocks [21].

3. Properties, Structure, and Genome Organization of AAVs

Aside from AAV5 being the most divergent, all AAVs share a similar structure and properties [22]. AAVs are easy to manipulate, as their particles can maintain biological stability in extreme conditions of pH and temperature [23,24]. They share a genome of approximately 4.7 kb single-stranded DNA packed into an icosahedral, non-enveloped capsid with a diameter of 20–25 nm [6,25]. The AAV genome consists mainly of two viral genes: rep (replication) and cap (capsid), flanked by inverted terminal repeats (ITRs) [12,25]. As the ITRs have a palindromic nucleotide sequence, they create characteristic T-shaped hairpin structures, providing essential structural elements for viral genome replication and packaging [26]. ITRs also play a regulatory role in viral gene expression and host genome integration [10,27]. The open reading frame (ORF) of rep encodes several nonstructural proteins that are required for gene regulation, replication, transcription, and encapsidation [12,28,29], while the ORF of cap encodes three structural proteins: virion protein 1 (VP1), VP2, and VP3, with a molar ratio of 1:1:10 in AAV particles [10,30,31]. Distinct tissue tropism of different AAV serotypes results from variations in the processing of this cap ORF, leading to variant immune and transduction profiles [12,32].

4. AAV Serotypes and Tropism

Depending on their serotype, AAVs can have specific tropism for specific organs and tissues of the body. There are different AAV serotypes that vary in many aspects. Next, each serotype will be separately discussed. Table 1 summarizes the characteristics and properties of AAV serotypes, and Figure 1 demonstrates their variant tropisms.

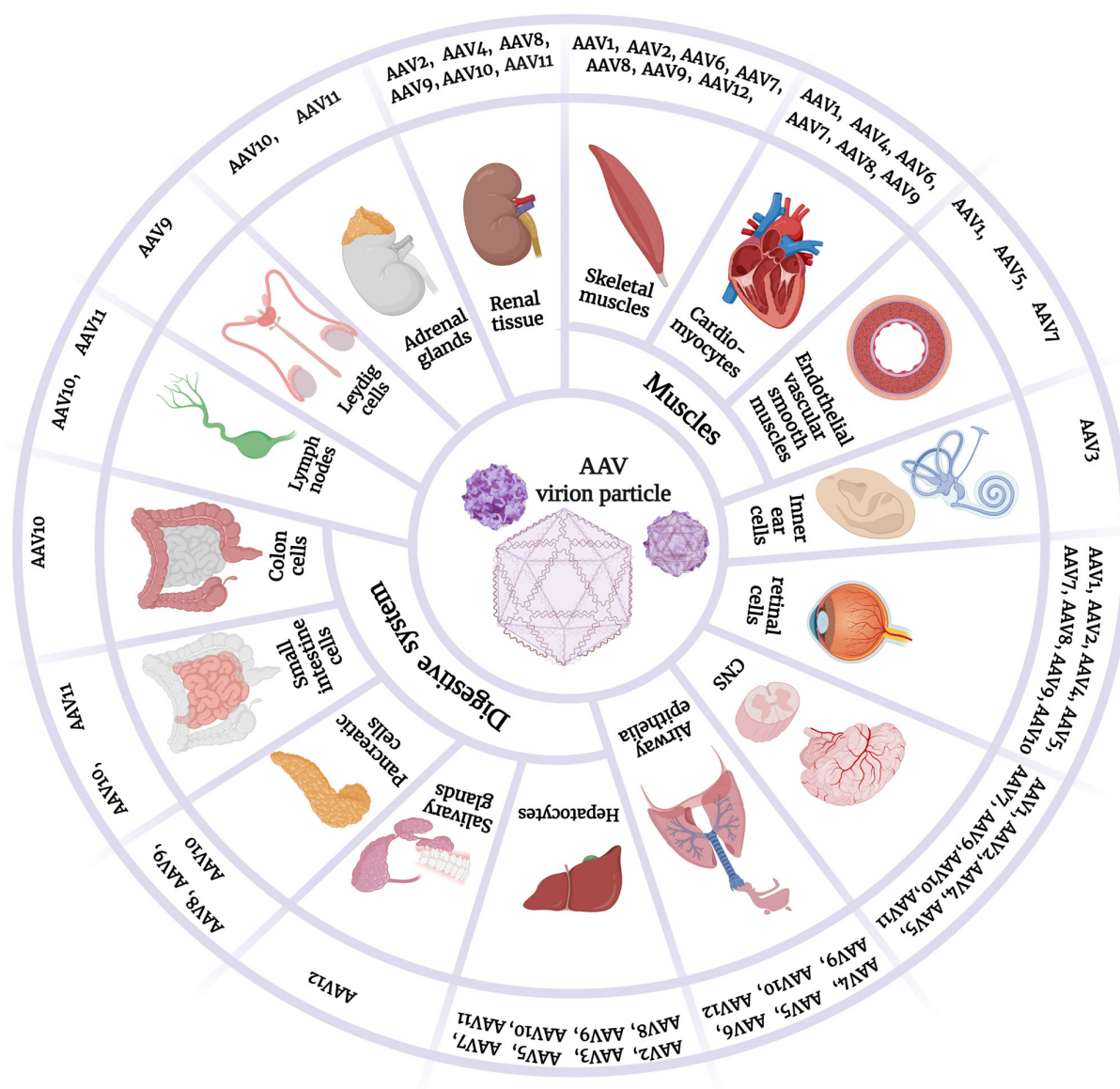


Figure 1. Variant tropisms of AAV serotypes.

4.1. AAV1

The exact origin of the first serotype of AAV (AAV1) is still unknown, as it was not initially isolated from tissues, but as a contaminant of Ad stocks [33,34], and its antibodies were found both in humans and non-human primates (NHPs) [35]. This serotype uses sialic acid as its primary cellular surface receptor [36], and AAV receptor (AAVR) as a coreceptor [37]. According to Rabinowitz J.E. et al., AAV1 does not bind heparin as it lacks R585 and R588, the amino acid residues required for such binding, and thereby it cannot be purified using heparin [33,38]. Zolotukhin S. et al., developed a protocol for AAV1 chromatographic purification by iodixanol gradient centrifugation and anion-exchange chromatography [39]. It can also be purified using mucin columns, as it can bind the sialic acid residues in mucin [40]. Moreover, recombinant AAV1 (rAAV1) was not found to contain any detectable post-translational modifications (PTMs), according to a systematic analysis conducted on ten AAV serotypes by Mary B. et al. [41], and it was the first viral vector to be approved for use in gene therapy [42]. In 1999, Xiao W. et al., conducted a study to investigate viral vectors for gene therapy, as a result of which, AAV1 was found to be the most efficient serotype for skeletal muscles' transduction [35]. Many studies have since confirmed AAV1 high tropism to skeletal muscles of murine, canine, and NHP

origins, compared to other serotypes [43–46]. AAV1 was also found to achieve an efficient transduction of neurons and glial and ependymal cells in the murine brain [47]. Moreover, it was found to be able to effectively transduce the heart [48,49], endothelial and vascular smooth muscles [50], and retina [51].

4.2. AAV2

AAV2 is considered the most studied serotype among all AAVs [45]. It was first discovered in 1965 as a contaminant of simian Ad preparations [52]. Later, in 1998, its primary cellular receptor, heparan sulfate proteoglycan (HSPG), was identified by Summerford C. and Samulski R.J. [53], and the amino acid residues providing its affinity to HSPG were suggested, afterwards, as R585 and R588 [54]. Accordingly, rAAV2 can be purified using heparin column affinity chromatography [55]. Nevertheless, the binding of AAV2 to its primary receptor was found to be insufficient for cell entry, so several coreceptors were later identified for it [52], including the human fibroblast growth factor receptor 1 (FGFR1) [56], $\alpha V\beta 5$ [57] and $\alpha 5\beta 1$ integrins [58], hepatocyte growth factor receptor (HGFR) [59], laminin receptor (LR) [60], and CD9 [61]. The capsid of rAAV2 is reported to acquire multiple PTMs, including ubiquitination, phosphorylation, SUMOylation, and multiple-site-glycosylation [41]. As the most studied serotype, AAV2, in fact, has shown various tropisms for several tissues in NHP, murine, canine, avian, and human cell types, including renal tissue [62–64], hepatocytes [65,66], retina [67–69], non-mitotic cells of the central nervous system (CNS) [70,71], and skeletal muscles [45,71]. Nonetheless, broader tissue tropisms of AAV2 were enabled by the further innovation of mosaicism and cross-packaging (or cross-typing) of AAVs (explained in detail later in this article), where the viral genome of one serotype could be packaged into a capsid of another type, providing a wider transduction spectrum [38,72,73].

4.3. AAV3

The third serotype of AAV (AAV3) was originally isolated from humans [34,45]. Similar to AAV2, this serotype uses HSPG, FGFR1, and LR receptors [60,74], along with the human HGFR (hHGFR) receptor [60]. Iodixanol gradient ultracentrifugation along with ion exchange chromatography have been used for AAV3 purification [75]. PTMs of rAAV3 capsid include acetylation, phosphorylation, and glycosylation [41]. Due to its inadequate transduction efficiency *in vitro* and in murine cell lines, AAV3 was mostly overlooked as a choice for gene therapy [76]. However, as it has been later found to use hHGFR as a coreceptor, it showed extremely efficient transduction of human liver cancer cells as well as human and NHP hepatocytes [76,77]. Since this selective tropism of AAV3 has been discovered, various studies have aimed at optimizing strategies to generate rAAV3 vectors with a higher transduction efficiency [76,78–81]. The developed strategies suggested different approaches, mainly, capsid modification of AAV3 vectors [76,80,81] and modification of hHGFR expression levels, along with the tyrosine kinase activity associated with it [78]. AAV3 was also found to have specific tropism to cochlear inner hair cells, showing high *in vivo* transduction efficiency in a murine model [82].

4.4. AAV4

The fourth serotype of AAV (AAV4) is considered one of the most antigenically distinct serotypes [83]. It has reportedly originated in NHPs [84], mainly in green African monkeys [85], as antibodies to its viral particles have been detected in their sera [86,87]. A study of the AAV4 structure showed that its capsid surface topology shares a significant similarity with that of human parvovirus B19 and Aleutian mink disease virus [83]. AAV4 uses α -2,3-O-linked sialic acid for cell binding and infection [88]; accordingly, mucin columns can be used for AAV4 purification, based on its ability to bind sialic acid residues in mucin [33,40]. In addition, as this serotype lacks heparin-binding activity, it cannot be purified using heparin column affinity chromatography like AAV2, however, ion-exchange chromatography procedures have been developed and proven a high purification effi-

ciency [89]. The only reported PTM of rAAV4 is ubiquitination of its capsid proteins [41]. AAV4 is suggested to be able to transduce human/NHP cells, as well as cells of murine and canine origins [45,87]. The specific tropism of AAV4 results in a transduction efficacy of specific cell types in the mammalian central nervous system (CNS), mainly the ependymal cells [90]. Moreover, following subretinal delivery, AAV4 has showed stable transduction of retinal pigmented epithelium (RPE) cells of rodent, canine, and nonhuman primate models, a distinctive feature enabled by the specificity of its capsid [91]. In a murine model, AAV4 has also shown a significant transduction rate of kidney, lung, and heart cells [92].

4.5. AAV5

As it was first isolated in 1983 from male genital lesions, AAV5 became the only AAV serotype to be isolated directly from a human tissue [93]. This serotype is considered the most genetically divergent of all AAVs [6,94–96], with a variety of unique characteristics, such as the distinct size and function of its ITR regions [22], utilizing herpes simplex virus (HSV) as its helper virus for human infections [94], and using an atypical endocytic route as a pathway for viral entry [33]. Another distinctive feature of AAV5 is its ability to transduce cells that cannot be transduced with AAV2, an exclusive advantage for gene therapy uses [97–99]. AAV5 was also found to use sialic acid as its primary receptor [88,100,101], along with platelet-derived growth factor receptors (PDGFR) α and β as coreceptors [102,103]. Similar to AAV1 and AAV4, mucin columns can be used for AAV5 purification [104,105], and ion-exchange chromatography procedures have also been developed for that aim [89]. Capsid proteins of rAAV5 are reported to undergo multiple PTMs, including ubiquitination, phosphorylation, SUMOylation, and glycosylation [41]. AAV5 proved to have a significant transduction efficiency for murine retinal cells [38,51], mainly for photoreceptor cells [106]. In addition, AAV5 tropism has been investigated in the murine brain, and proved, as a result, a transduction competence for multiple neural cell types, including Purkinje cells, stellate, basket, and Golgi neurons, and it was able to reach to the inferior colliculus and ventricular epithelium [107,108]. AAV5 is also known for its efficient transduction of murine airway epithelia by apical infection [98,109–111], vascular endothelial cells, and smooth muscles [50]. It is also reported to have tropism for liver cells in mice [92,112].

4.6. AAV6

The classification of the sixth serotype of AAV (AAV6) is still a matter of controversy, as it presents high genomic similarity with both AAV1 and AAV2 serotypes, however, it has still been assigned its own serotype numbering [33,35]. AAV6 has a serological profile almost identical to that of AAV1, and shares its sequence of coding region with a homology percentage of 99%, along with multiple regions identical to those of AAV2 [113]. Accordingly, it was suggested to be a naturally occurring hybrid resulting from homologous RECOMation between AAV1 and AAV2 [35,45,113,114]. AAV6 was first isolated from a human Ad preparation [45,113,115], and similar to AAV1, was found to bind sialylated proteoglycans, mainly α 2,3-/ α 2,6-linked sialic acid, as its primary receptor, as well as binding heparan sulfate [36,116,117]. As for its coreceptor, it binds epidermal growth factor receptor (EGFR) [118]. The only reported PTM of rAAV6 is acetylation of its capsid proteins [41]. Similar to the previously described serotypes, AAV6 can be purified by either heparin or mucin column affinity chromatography, as it can bind both [40]. AAV6 is reported to have tropism for a variety of tissues, including airway epithelia of murine and canine models [119,120], murine liver cells [92,121], and skeletal muscles of murine and canine models, with a transduction efficiency even higher than that of AAV2 [92,122–124], cardiomyocytes in murine [92,125], porcine [126], canine [127], and in sheep [128] models.

4.7. AAV7

The seventh serotype of AAV (AAV7) was first isolated in 2002 from NHP tissues, specifically, from Rhesus macaque monkeys [35,129]. Its mechanisms of cell binding and

cell entry are still unknown [52,130], but it is established that this serotype does not bind heparin, or any other glycan in general [131]. Capsid proteins of rAAV7 undergo multiple PTMs, including glycosylation, primarily, along with phosphorylation, SUMOylation, and acetylation [41]. A study by Calcedo R et al., investigated the epidemiology of AAV-neutralizing antibodies in the worldwide population, and found that the seroprevalence of AAV7 antibodies is relatively low in humans, an advantage of this serotype to be used in clinical applications [132]. Viral vectors based on AAV7 proved a high efficiency of transduction for skeletal muscle cells in murine models, similar to that achieved by AAV1, and higher than AAV2 [133]. This serotype also proved a strong tropism to hepatocytes in murine [92] and human [92] tissues. In CNS of NHPs, AAV7 viral vectors were found to achieve a robust transduction mainly in cortical and spinal tissues [134]. Moreover, AAV7-based viral vectors can, reportedly, achieve a significantly high transduction rate of murine neurons [135] and photoreceptor cells in the retina both in vivo and ex vivo [136]. AAV7 vectors also appear to have a limited tropism to vascular endothelial cells, that could be relatively enhanced through proteasome inhibition [130], and an in vivo transduction preference for epicardium cells in murine cardiac tissue [49].

4.8. AAV8

Similar to AAV7, the eighth serotype of AAV (AAV8) was first isolated in 2002 from Rhesus macaque monkeys [35,129]. As a primary receptor, AAV8 binds LR, the same receptor used by AAV2 and AAV3 [45,60]. Various procedures for rapid and scalable purification of AAV8 have been developed since its discovery, including, for example, dual-ion-exchange chromatography [137,138], or iodixanol gradient centrifugation [139,140]. Phosphorylation, glycosylation, and acetylation are the three PTMs reported for rAAV8 capsid proteins [41]. AAV8 is best known for its strong tropism to liver cells, and accordingly, its transduction efficiency of hepatocytes, which is far stronger and faster than those of all other AAV serotypes in different models, including murine, canine, and NHP [92,112,141–147]. Following systemic delivery in murine models, AAV8 was proven to be the most efficient serotype for transduction of both skeletal and cardiac muscles, owing to its ability to cross the blood vessel barrier, a feature that both AAV1 and AAV6 lack, limiting their efficiency of muscle transduction to local delivery only [148]. AAV8 could also achieve a successful in vivo transduction of murine pancreatic cells, following localized delivery [149,150]. Moreover, a high-rate transduction of murine renal cells could be reached by local direct delivery of AAV8 viral vectors into the kidney tissue [151]. AAV8 was also found to achieve an efficient transduction of different cells in the murine retina, including amacrine, Müller, and putative bipolar cells, along with some horizontal cells and cells in the ganglion cell layer (GCL) [152–154]. AAV8 transduction efficiency appears to be susceptible to proteasome levels in some tissues, and accordingly, could be increased using proteasome inhibitors [130].

4.9. AAV9

The ninth serotype of AAV (AAV9) was first identified in a human isolate in 2004, and was named a new serotype as it had a serological profile distinct from the previously known AAVs, however, it was suggested to be closely related to clades containing AAV7 and AAV8 [129,155]. As a primary receptor, AAV9 uses terminal N-linked galactose [156,157], and it is also suggested to bind a putative integrin, along with LR as coreceptors [60,156]. Scalable simple purification protocols have been developed for AAV9 purification, including ion-exchange chromatography [24] and sucrose gradient centrifugation [158]. Capsid of rAAV9 has one of the highest totals of PTMs, including multiple ubiquitination, phosphorylation, SUMOylation, and glycosylation modifications, along with acetylation [41]. In most tissues, AAV9 seems to achieve cell transduction with efficiency superior to other AAVs [33,129]. For example, in a study aimed to investigate AAV1–9 distribution following systemic delivery in a murine model, AAV9 has shown rapid-onset, the best genome distribution, and the highest protein levels, in comparison with all other AAVs [92]. Moreover,

in the CNS of murine, NHP, and feline models, it has a unique feature compared to other serotypes, as it can cross the BBB and transduce not only neuronal but also non-neuronal cells, including astrocytes, that cannot usually be transduced by other AAVs [27,159–162], also showing tropism to photoreceptor cells in the retina [163]. Viral vectors based on AAV9 have also proven to be more efficient than those of AAV1 and AAV8 (in some cases 5–10-fold higher than AAV8), for murine, NHP, and porcine cardiac muscle transduction [164–169], enabled with another distinctive feature of AAV9—its ability to traverse the physical barrier of vascular system endothelia [168]. Another example of AAV9 superiority over other AAVs was presented in a study by Inagaki K et al., where the serotype achieved robust transduction of murine hepatocytes, skeletal muscles, and pancreatic cells [81,169]. AAV9-based viral vectors also appear to be tropic to murine photoreceptor cells [170], renal tubular epithelium cells [171–173], Leydig cells in the testicular interstitial tissue [174], and alveolar and nasal epithelia [175,176].

4.10. AAV10 and AAV11

The ninth and tenth serotypes of AAV (AAV10 and AAV11) were first found and described in 2004 in NHP isolates, namely from cynomolgus monkeys, with capsid proteins of great resemblance to AAV8 and AAV4, respectively [34,177], resulting in serological cross-reactivity with those two serotypes [178]. However, antisera against AAV10 and AAV11 were not found to have any cross-reactivity against those of AAV2, which recommended them as good viral vector candidates for gene therapy in individuals having antibodies against the latter [33]. It remains unknown what cellular receptors and coreceptors AAV10 and AAV11 use for cell binding and entry [37,115]; therefore, procedures describing their purification protocols are generally based on iodixanol gradient centrifugation [179]. Similar to AAV9, capsid of rAAV10 has one of the highest totals of PTMs, including mainly multiple glycosylation and phosphorylation modifications, along with ubiquitination, SUMOylation, and acetylation [41]. A study investigating biological distribution of both AAV10 and AAV11 in monkeys suggested them to be tropic to NHP intestinal cells, hepatocytes, lymph nodes, and less frequently, to renal cells and adrenal glands [180]. AAV10 was also suggested to have tropism to murine small intestine and colon cells [181]. Compared to AAV8 and AAV9, AAV10 appears to have the largest tropism range to murine retinal cells, as it can reportedly transduce a variety of cell types, including RPE, cells in the ganglion cell layer, several cell types in the inner nuclear layer, photoreceptors, and a highly efficient transduction of horizontal cells [152]. Following intravenous delivery, AAV10 was found to target murine liver and lung cells [182], however, upon localized delivery, it transduced murine renal and pancreatic cells [183]. As for AAV11, it was found to have mild tropism to NHP CNS, mainly to the cerebrum and spinal cord [180]. A recent study of neural gene therapy using rAAV11 found that murine projection neurons and astrocytes could also be targeted using this serotype [184].

4.11. AAV12

The twelfth serotype of AAV (AAV12) was first isolated from a simian Ad stock, and then characterized as a novel serotype, as it exhibited distinctive biological and serological properties [185]. Although it was proven that AAV12 does not use heparan sulfate proteoglycans or sialic acids for attachment and cell entry, it remains unknown how exactly it binds target cells [177,185]. However, according to a study investigating components of a potential receptor complex for AAV12, mannose and mannosamine were suggested as components of such complex, as they inhibited AAV12 cell transduction [186]. Moreover, being resistant to neutralization by human antibodies, AAV12 represents a good candidate for human gene therapy applications [185,187]. AVB Sepharose affinity chromatography has been used for purification of AAV12 [188]. In murine models, it has shown tropism to salivary glands and muscles [185]. It has also shown strong, localized *in vivo* tropism to murine nasal epithelia following intranasal administration [187].

4.12. AAV13

The thirteenth serotype of AAV (AAV13) is another simian Ad that appears to bind HSPG, although its primary cell receptor remains unknown [189–191]. It was also found to share structural similarity with AAV2 and AAV3, making it the closest related AAV to those two serotypes, with a capsid conserving all AAV capsids' structural features [188], but there are only limited data on this serotype tropism and transduction efficiency [191].

4.13. Novel Hybrid AAV Vectors

In addition to the natural AAV serotypes described above, novel AAV vectors have been developed during the last two decades, and are still being developed [192]. Using different engineering strategies, novel hybrid vectors have been generated in order to enhance their transduction, modulate their immunogenicity, or limit their tropism to specific cells [193–197]. There has been different types of such engineered novel vectors, including mosaic, chimeric, and combinatorial vector libraries [33]. Mosaic vectors have multiple subunits of various serotypes in their capsid, chosen according to their properties of receptor binding and intracellular trafficking [33,198]. In chimeric virions, the capsid usually has modified protein generated by domain swapping and DNA shuffling strategies to alter specific amino acids [33,194]. Combinatorial vector libraries also use DNA shuffling and error-prone PCR methods to generate AAV libraries of novel serotypes with mixed genomes [199,200].

5. AAV as Viral Vectors for Gene Therapy Applications

A variety of AAV features have made it an appealing viral vector candidate to be used in gene therapy applications. To begin with, AAVs are non-pathogenic viruses, since no diseases have been linked to them [201]. AAV vectors have also proven their ability to provide stable integration into the target genome [202–205], and such feature is highly needed for gene therapeutic uses considering the high level of expression that could be maintained as a result [206], without affecting the target cell functions in the long term [207–209]. Moreover, having only ITRs, AAV vectors cannot interfere with the inserted gene regulation either [201]. More specifically, a unique feature of AAV vectors, contrary to all currently available gene-editing platforms, is utilizing the homologous recombination pathway, which does not involve exogenous nucleases, providing, therefore, a highly accurate editing process that preserves genome integrity without adding to the mutational burden of the target site [210]. Thanks to such unique gene-editing properties, AAV vectors are currently the leading platform for in vivo gene therapy delivery [210,211]. Another attractive feature is the very broad range of cells, tissues, and hosts that AAV can efficiently transduce in vivo and in vitro [87,201], including dividing and non-dividing cells [212,213] in humans [99], and NHP [214,215], murine [216–218], canine [219–221], feline [222,223], and a variety of other models. In addition to the genetic properties, AAVs' distinct physical properties represent more reasons why they should be used for gene therapy, such as easy manipulation, resistance to pH changes, heat changes, and detergents [87]. Next, we present various studies that investigated AAV vectors for a wide range of gene therapy applications (reviewed in Table 2).

5.1. AAV Viral Vectors for Gene Therapy of the CNS

AAV-mediated CNS gene therapy was first believed to predominantly target neurons, with lesser chance to affect other cells and tissues in the CNS [224], a fact that was later proven to be inaccurate in many studies [134,160,214,225]. For a variety of neurodevelopmental and neurodegenerative diseases, AAV-mediated gene therapy has been tested using different administration routes, including intraparenchymal, intrathecal, intracerebroventricular, and intracisternal injection, many of which have shown promising results [226].

Parkinson's disease (PD) has been one of the most studied neurological disorders as a target for AAV-mediated gene therapy [224,227,228]. In a phase I clinical trial, Kaplitt et al., investigated an AAV-mediated gene therapy approach for advanced PD patients, where

serotype 2 was used as a vector for unilateral subthalamic delivery of the glutamic acid decarboxylase (*GAD*) gene [229]. Providing a significant improvement in motor function scores up to 12 months after surgery, the approach was found to be safe and well-tolerated in patients. A similar double-blind, controlled, randomized clinical trial conducted later by LeWitt et al., investigated the same AAV2-*GAD* vector for bilateral subthalamic delivery in advanced PD patients, and showed similar results of safety and improvement of motor function [230]. Bartus et al., also tested the bilateral stereotactic delivery of AAV2-neurturin in PD patients of an open-label clinical trial, the initial obtained data of which supported the feasibility, safety, and good tolerance of the approach as a potential treatment for PD [231]. However, bilateral intra-striatal infusion of an AAV2 vector containing the aromatic L-amino acid decarboxylase (*AADC*) gene in moderately advanced PD patients led to an improvement in PD rating scales that was associated with a risk of intracranial hemorrhage in patients, along with headaches [232]. As for sustainable transgene expression, a clinical trial conducted by Mittermeyer G. et al., investigated the potentials of rAAV2 carrying the aromatic L-amino acid decarboxylase gene (rAAV2-*AADC*) [233]. In the trial, 10 patients with moderately advanced PD received bilateral infusions of recombinant vector into the putamen, showing good tolerance and a stable expression of transgene that lasted for the following 4 years, although higher vector doses were suggested for further studies. In children with *AADC* deficiency, the same delivery route for the same vector (rAAV2-*AADC*) was also assessed in an open-label phase I/II clinical trial [234]. The therapy was well-tolerated in general, providing evidence for potential improvement of motor function.

Similarly, spinal muscular atrophy (SMA) has always represented an attractive candidate for gene therapy, being caused by a single gene defect that affects the survival motor neuron (SMN) protein [235]. Therefore, SMA has been suggested as a target for AAV-mediated therapeutic approaches, mainly using AAV9 [161]. An open-label, phase I clinical trial was designed by AveXis, Inc. in 2014 to assess the safety and efficacy of AAV9-SMN as a treatment for SMA [236]. Following single-dose intravenous administration of the vector, a significant improvement of the motor function in all 15 patients was observed, reflected by their ability to perform different activities, such as unassisted sitting and walking, oral feeding, and speaking, with no reported motor function regression at the two-year follow-up [236]. A long-term safety assessment, however, was recommended.

Lysosomal storage diseases (LSDs), another group of neurodegenerative diseases, have also been extensively targeted by AAV-mediated gene therapy [224]. One example of which is mucopolysaccharidosis type VII (MPS VII or Sly disease), a disease that results from genetic deficiency of beta-glucuronidase (*GUS*) [237]. In the murine model, AAV-mediated gene therapy has been shown to provide stable expression of the deficient enzyme upon single administration, that was adequate for phenotype correction in the liver and most of the neuraxis [238,239]. As for neonatal murine model, intravenous delivery of rAAV-*GUS* proved to yield therapeutic levels of the deficient enzyme in multiple organs, including ones of the CNS, with the gene expression not being affected by rapid growth and differentiation of tissues [240]. The canine model of MPS VII has also shown promising therapy results following rAAV-*GUS* intrathecal injection of serotypes 9 and the NHP-derivate AAVrh10 [221], as high expression levels of *GUS* were detected in the CNS tissues, with the enzyme in brain tissue homogenates showing over 100% normal activity. Another type of MPS, MPS IIIA, has also been assessed for gene therapy in the canine model using AAV9 carrying the deficient enzyme's gene [241]. Following intra-cerebrospinal fluid (CSF) delivery, the administered vector provided sustained and widely distributed transgene expression with no toxicity for a duration of seven years after therapy. Metachromatic leukodystrophy (MLD) is also one of the LSDs that results from arylsulfatase A (*ARSA*) enzyme deficiency and has been targeted by AAV-mediated therapeutic approaches [242]. In the murine model of MLD, as well as in NHPs, serotypes 1, 5, 9, and rh10 carrying the *ARSA* gene have provided supporting results of their therapeutic potentials as gene therapy viral vectors [243–246]. GM2 gangliosidosis is another example of lysosomal storage diseases that have been assessed for AAV-mediated gene therapy, including Tay-Sachs disease

(TSD) and Sandhoff disease (SD) [247–249]. Preclinical data from many *in vivo* studies have provided evidence of AAVs' efficacy for GM2 gangliosidosis in murine [250], ovine [251], and feline [252] models. Clinically, there have been trials applying AAV-mediated gene therapy for different LSDs throughout the last decades with variant outcomes [242,248,253]. In 2018, four children with asymptomatic or early-stage MLD underwent a phase I/II trial for gene therapy using AAVrh.10-hARSA [253]. Although there was a significant elevation in ARSA levels recorded in the CSF following intracerebral vector delivery, no clinical improvement was noticed compared to the control group. As for gangliosidosis, the first clinical trial for gene therapy of Tay-Sachs disease using an AAV vector was conducted by Taghian et al., in 2020 [248]. The treatment proved to be safe for the two treated children and resulted in a broad distribution of the transgene in CNS.

Canavan disease (CD) is another rare inherited leukodystrophy belonging to LSDs, that has been targeted by AAV-mediated gene therapy [254]. CD results from aspartoacylase (ASPA) enzyme deficiency, that leads to N-acetylaspartic acid (NAA) accumulation in the brain, causing white matter degeneration [255]. In a murine model, rAAV2-ASPA delivery into the striatum and thalamus of the brain resulted in an increased ASPA activity and, thereby, decreased NAA accumulation and white matter degeneration. However, areas remote from the injection site, such as the cerebellum, were not affected [255,256]. In 2002, Janson et al., suggested a clinical protocol using rAAV2-ASPA for gene therapy in CD patients [257], and currently there are several ongoing clinical trials testing different serotypes of rAAV (including 2, 9, and olig001), carrying the ASPA gene for CD gene therapy [258–260].

Krabbe disease, also known as globoid cell leukodystrophy (GLD), causes demyelination in a similar way. The disease results from deficiency of the lysosomal enzyme galactocerebrosidase (GALC), leading to accumulation of its substrate, psychosine, which affects both the central and peripheral nervous systems [261]. During the last two decades, a large number of *in vivo* studies have been conducted to investigate different AAV serotypes as viral vectors for gene therapy of GLD in different animal models, including murine and canine. Intracerebral delivery of rAAV1-GALC has been tested in the murine model of GLD in an *in vivo* study conducted by Rafi et al. [262]. The approach resulted in sustained expression of the deficient enzyme, and thereby positively affected myelination status, and animals' lifespan. However, both treated and untreated mice died with similar symptoms, suggesting that the used approach should be initiated prior to symptoms' onset. rAAV2/5-GALC has also shown therapeutic efficacy in the murine model of GLD following intracranial delivery [263,264]. In the study conducted by Lin et al., in 2011, mice treated with rAAV2/5-GALC showed wide dispersion of the GALC transgene across the CNS, reaching areas remote from the injection site [263]. Moreover, rAAV2/5-GALC delivery resulted in a reduced loss of oligodendrocytes and Purkinje cells, along with a significant improvement of neuromotor function and a prolonged lifespan of treated mice. Karumuthil-Meethil et al., conducted another preclinical study using recombinant vectors AAV9, AAVrh10, and AAV-Olig001, carrying the GALC gene, for GLD gene therapy in the murine model, following lumbar intrathecal delivery [265]. All three serotypes provided wide distribution of the transgene across the CNS and liver, resulting in a significant improvement of myelination, and a prolonged lifespan, with AAV9 being the most effective when combined with bone marrow transplantation. AAV9 and AAVrh10 have also been tested for GLD therapy in the canine model [266,267]. Combined intravenous and intracerebroventricular delivery of AAVrh10 encoding canine GALC (AAVrh10-cGALC) resulted in delayed symptoms onset, prolonged lifespan, correction of biochemical defects, and also positively affected neuropathology in treated animals [266]. Similarly, AAV9-cGALC has shown promising therapeutic potency in the GLD canine model [267]. Reportedly, intrathecal delivery of AAV9-cGALC resulted in increased activity of the deficient enzyme and, therefore, normal levels of its substrate. It also improved myelination, and decreased inflammation both in the CNS and PNS, which, along with prevention of clinical neurological dysfunction, resulted in a significantly prolonged lifespan of treated dogs, compared

to the control group. However, sufficient dosing was found to be critical, as high doses significantly extended the lifespan even for post-symptomatic subjects, and a 5-fold lower dose of the vector resulted in an attenuated form of disease [267].

For ocular diseases, AAV viral vectors have also provided great therapeutic opportunities both in vivo and in human clinical trials [136,170,268–271]. In a study by Petrs-Silva et al., the efficacy of intraocular transduction of rAAV serotypes 2, 8, and 9 was investigated in mice for targeting ocular neurovascular and retinal diseases [170]. Besides transduction of retinal cells, both serotypes 8 and 9 efficiently transduced the ganglion cell layer, providing evidence for their potentials in therapeutic approaches of ocular diseases. Moreover, AAV2/7 and AAV2/8 hybrid serotypes have achieved high transduction rates of the murine photoreceptors, showing promising potentials for treatment of inherited photoreceptor diseases [136]. AAV2/6 has also shown an efficient transduction of murine cone photoreceptors following subretinal injection [268]. In humans, AAV2/4 has been used in a clinical trial for treatment of childhood blindness caused by Leber Congenital Amaurosis (LCA) disease, mainly by a mutation in the retinal pigment epithelium-specific protein (RPE65) [269,270]. Over a follow-up period between 1 and 3 years, subretinal injection of AAV2/4-RPE65 showed good tolerance both systemically and locally, with no adverse effects, and resulted in an improvement of visual function presented by different parameters in different patients, including improvement of visual acuity and color vision, as well as a reduction of visual fatigue or photophobia [269]. Another example of AAVs' applications in ocular disease therapy is a phase I/II clinical trial by MacLaren R et al., that has aimed to treat blindness caused by choroideremia, an X-linked recessive disease that results from a mutation affecting retinal escort protein 1 (REP1) [272,273]. In the trial, AAV2-REP1 resulted in improvement of retinal sensitivity in all six patients, following subretinal injection, with two of them having significant increases in visual acuity, supporting further consideration of the tested therapeutic approach [272]. Various other clinical trials have applied AAV-mediated gene therapy for ocular diseases, and many of which have presented promising results, using mainly AAV2 and AAV8 [274–281] (detailed in Table 2).

Similar to ocular diseases, hearing disorders have also been suggested for AAV-mediated gene therapy [282]. In a porcine model, Lalwani A. et al., tested AAV9 intracochlear delivery to assess its efficacy as a vector for gene therapy of hearing disorders, and demonstrated, as a result, transgene expression in the inner ear of animals following administration [282].

5.2. AAV Viral Vectors for Gene Therapy of Respiratory Diseases

For over 20 years, AAV vectors have been vastly investigated for gene therapeutics of respiratory diseases, both in preclinical experiments and human clinical trials [283,284]. However, a key limitation was that many AAV serotypes cannot efficiently transduce airway epithelial cells through the apical surface, suggesting molecular modifications of such serotypes to enhance their transduction efficiency [196]. Cystic fibrosis (CF) represents one of the most studied diseases as a target for AAV-mediated gene therapy [285]. In rabbits, AAV has proven to promote an efficient and stable gene transfer of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene into the airway epithelium, indicating, as a result, the vector potential to be used for gene therapy [286]. Subsequently, since 1998, clinical trials have started using recombinant AAV viral vectors to target different sites of the airway epithelium of CF patients, using variant administration routes [285,287,288]. The very first clinical trial to do so used the maxillary sinuses for delivery of recombinant AAV2 containing the *CFTR* gene (AAV2-*CFTR*) in ten CF patients, demonstrating a safe, successful transduction of targeted cells and a detected function restoration of the sinuses [288]. Soon after, a following phase II double-blind clinical trial tested unilateral administration of the same vector into the sinus for 23 CF patients, with an in-patient control, achieved by administering a placebo drug into the other sinus [289]. The approach again showed safety and good tolerance, although it did not confirm clinical effectiveness of the treatment. Aitken et al., also tested AAV2-*CFTR* in a phase I clinical trial for twelve

mild CF patients using aerolization by nebulation for delivery, confirming the approaches safety, but failing to yield an effective clinical treatment [290]. Further trials on AAV2-CFTR with single or repeated dosing have yielded similar results of safety and good tolerance, providing little evidence of clinical treatment after intranasal and endobronchial delivery [291–293]. In order to optimize such therapeutic approaches and produce a functional CFTR in CF patients, further modifications of recombinant AAV vectors have since been developed [294,295]. Alpha-1 antitrypsin (α 1AT or AAT) deficiency is another lung and liver disease that has been extensively studied as a target for AAV-mediated gene therapy both in vivo and in humans [284,296]. Reportedly, intravenous delivery of recombinant AAV vector carrying the human alpha-1 antitrypsin gene (AAV-hAAT) in the murine model resulted in potentially therapeutic serum levels of AAT [297,298]. Further research found that intrapleural delivery of rAAV2-hAAT or rAAV5-hAAT could achieve higher AAT levels both in lungs and serum compared to intramuscular delivery in C57BL/6 mice, with the rAAV5 showing 10-fold higher effectiveness than rAAV2 [299,300]. However, rAAV6/2-hAAT has been shown to transduce murine lung cells even more efficiently than rAAV5 both in vivo (in murine lung cells) and in vitro (in human airway epithelial cell culture) [301]. Similarly, rAAV8-hAAT has also been shown to provide murine lung cells' transduction superior to that of rAAV5 following intratracheal delivery, as it resulted in 6-fold and 2.5-fold AAT levels in serum and broncho-alveolar fluid, respectively [302], and a high transduction rate of murine hepatocytes [298]. Chiuchiolo M et al., also proved the safety of rhAAV10-hAAT as a viral vector for treatment of AAT in wild-type murine and NHP models [303]. Besides, the delivered vector resulted in persistent expression of the transgene in chest cavity cells of both models, suggesting the efficacy of the tested therapeutic approach [303,304]. Examples of other rAAV vectors' therapeutic potentials for AAT treatment have been shown in a variety of other preclinical studies, including rAAV1-AAT, rAAV2/9-AAT, and rAAV6 [46,120,175,305].

AAVs have also been tested to develop therapeutic approaches for other lung diseases, such as asthma and surfactant B deficiency [306,307]. A preclinical study by Zavorotinskaya et al., investigated the efficacy of rAAV carrying the interleukin 4 gene (*IL-4*) as a vector for gene therapy of allergic asthma, upon intratracheal delivery into the murine model [306]. Showing a significant inhibition of airway eosinophilia and mucus production along with a reduction in airway hyper-responsiveness and asthma-associated cytokines levels, obtained data suggested rAAV efficacy for gene therapy of the studied disease. Kang et al., have recently tested an engineered rAAV6/2 vector carrying human or murine surfactant protein B gene (*SFTPB*) in a murine model [307]. Mutations of this gene cause surfactant protein B deficiency (SPB) in humans, a rare genetic condition with a very poor prognosis [284,308]. In their study, Kang et al., demonstrated efficient transduction of the airway and alveolar epithelium by engineered rAAV6/2-*SFTPB* following intratracheal administration into a murine model of SPB. As a result, the administered vector was well-tolerated with no adverse effects. Moreover, rapid, long-term restoration of the deficient SPB protein was reported, along with an improvement of lung function, leading, subsequently, to an extended survival [307].

5.3. AAV Viral Vectors for Gene Therapy of Muscle Diseases

Having a large, body-distributed mass, and myofibers with a long half-life time makes muscles an attractive target for gene therapy, also considering the minimal invasiveness of the intramuscular delivery route [133,309]. Muscular dystrophies (MD) constitute a large group of muscle diseases that have been studied for a long time and proved to be a good target for AAV-mediated gene therapy [310]. For example, AAV1 carrying the follistatin gene, an antagonist of muscle growth negative regulator, has been shown to promote sustained improvement in muscle size and strength in NHPs following intramuscular administration [311]. There has also been many reports of both histopathological and functional sustained muscle restoration as a result of using AAV-mediated therapeutic approaches in animal models of Duchenne muscular dystrophy (DMD) [312,313], and

Limb-Girdle muscular dystrophies (LGMD) [314–318]. As for clinical research, there have been several registered clinical trials of AAV-mediated gene therapy for muscular dystrophies [319]. The first clinical study was a randomized, double-blind, placebo-controlled phase I clinical trial, conducted in 2012 by Bowles D et al., which used AAV2.5 carrying the mini-dystrophin gene for intramuscular administration in DMD patients [320]. As a result, the administered vector was found to be safe and well-tolerated, and transgene DNA was detected in all patients. Similarly, other clinical trials were started in 2017 and 2018 to test AAV-micro-dystrophin efficacy for treatment of DMD [319] (details in Table 2).

5.4. AAV Viral Vectors for Gene Therapy of Cardiovascular and Blood Diseases

Cardiovascular diseases make another attractive target for gene therapy, being a leading cause of death globally with their high incidence and mortality rates [128,321]. As mentioned before, AAV serotypes 6, 8, and 9 have been shown to efficiently transduce cardiac cells in different animal models, and accordingly, they have been used for therapeutic purposes in different cardiovascular diseases [126,128,167,168,322].

In the search for an effective gene therapy platform for heart failure (HF), White J et al., published a study in 2011, in which they suggested a novel technique for AAV-mediated myocardial gene therapy using molecular cardiac surgery [128]. Using AAV6 in an ovine model, the suggested approach resulted in a global transgene expression, that was cardiac-tropic and substantially more robust and targeted, compared to that of intramuscular or intracoronary injection. Furthermore, a randomized phase I/II AAV1-based clinical trial for heart failure treatment was conducted in 2013, where the researchers used a sarcoplasmic reticulum calcium ATPase gene (SERCA2a), the product of which was suggested to play a key role in HF pathology [323]. As a result, adverse effects, including death, were found to be highest in the placebo group, and lowest in the high-dose group, with evidence of long-term transgene expression. However, in the low-dose and mid-dose groups, adverse effects were also found to be high but delayed.

Hemophilia, an inherited disease that is caused either by deficiency of blood clotting factor VIII (type A) or IX (type B), is another example of diseases that have been targeted by AAV-mediated gene therapy [168,322]. rAAV6 and 8 expressing the canine gene of FVIII have been shown to restore physiologic levels of the deficient factor in a canine model for three years following intravenous administration, without any toxicity or immune reactions [322]. The same vectors could result in a similar effect in the murine model; however, neutralizing antibodies against cFVIII were detected in the mice sera [322]. As for hemophilia B, intravenous administration of rAAV8 and 9 carrying the IX factor gene in a murine model resulted in a significant increase in transgene expression and therefore in IX factor levels, with a decreased proinflammatory risk [168].

5.5. AAV Viral Vectors for Gene Therapy of Liver Diseases

AAV-based gene therapy plays a significant role in liver diseases, as it is, in some cases, an alternative to the only effective therapy, which is liver transplantation [324]. In addition to the previously mentioned diseases affecting the liver, that have been targeted by AAV-base gene therapy, such as CF, AAT deficiency, and hemophilia, there are other liver diseases that have been targeted and continue to be, using AAV8, mainly.

Wilson's disease (WD) is a rare autosomal recessive disease caused by mutations in the copper transporter gene, *ATP7B*, resulting in copper accumulation mainly in the liver, along with some other organs [325,326]. A recent in vivo study, conducted by Murillo et al., proved the efficacy of an AAV-based gene therapy for WD in a murine model of the disease [327]. The vector used in the study was AAV8 carrying an optimized short version of the *ATP7B* gene (AAV8-mini *ATP7B*) adjusted to the right size, so it could be produced and delivered more efficiently. As a result, intravenous administration of the recombinant vector to 6- and 12-week-old WD mice could restore copper homeostasis, with 20% hepatocyte transduction being sufficient for correction.

Crigler-Najjar syndrome (CNs) type 1 is another autosomal recessive liver disease caused by deficiency of uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), which leads to severe inherited unconjugated hyperbilirubinemia [328]. There is currently an ongoing clinical trial investigating AAV8-UGT1A1 as a vector for gene therapy in children with CNs [329]; however, as the preclinical data of the trial suggest a negative effect of liver cells' proliferation on the efficacy of gene therapy in children, Shi et al., suggested to optimize the approach by starting therapy at a specific age and combining it with an immune suppression regimen [330]. In their in vivo study, Shi et al., found that a stable correction was achieved when AAV8-UGT1A1 was administered to CNs rats at the 28th postnatal day, coupled with a rapamycin-based immunosuppression regimen delivered intraperitoneally.

5.6. AAV Viral Vectors for Gene Therapy of Endocrine Disorders

Being a major health issue for humans all around the world, with relatively high prevalence estimates, endocrine disorders have always been in the focus of research for therapeutic approaches and techniques [331,332]. Accordingly, rapid developments of diagnostic techniques, along with the better understanding of endocrine disorders' pathophysiology and molecular bases, helped in developing different therapies, such as hormonal replacement therapies, for example [332,333]. However, as these approaches have mostly focused on improving the patient's quality of life, reducing or reversing symptoms, rather than targeting the underlying defect, the resulting effect has not always been sufficient, which highlighted the need for gene therapy approaches, including AAV-based ones [333].

Type 1 diabetes mellitus (T1DM) is one of the endocrine autoimmune disorders that has been extensively studied as a target of gene therapy [334]. T1DM is characterized by self-destruction of insulin-secreting islet β cells, resulting from a wide variety of causative factors [335]. In non-obese diabetic (NOD) mice, administration of recombinant adeno-associated virus serotype 8 carrying DNA of mouse insulin promoter (dsAAV8-mIP) has been shown to prevent hyperglycemia in a dose-dependent manner [336]. High levels of mouse interleukin 10 (mIL-10) achieved following rAAV2-IL-10 intramuscular administration also proved to have a positive effect in NOD mice by decreasing autoimmunity, and thereby hyperglycemia [337]. Similarly, negative regulation of the immune response by programmed death ligand 1 (PDL1) was achieved in NOD mice following intraperitoneal delivery of AAV8-PDL1, which protected β cells [338]. Currently, there is an ongoing clinical trial investigating AAV8 containing a transgene for the fragment antigen-binding region of anti-vascular endothelial growth factor (anti-VEGF fab), as a vector for gene therapy of diabetic retinopathy, delivered in the suprachoroidal space [339].

Autoimmune polyglandular syndrome type-1 (APS1) is another disorder characterized by multiple endocrine abnormalities, resulting from a monogenic defect in the autoimmune regulator (AIRE) gene [340]. Recently, Almaghrabi tested AAV9-AIRE as a potential gene therapy for APS1 [341]. As a result, AAV9-AIRE, following intra-thymic administration in a murine model, showed high transduction efficiency, along with restoration of AIRE expression in the thymus. Subsequently, a significant reduction of serum auto-antibodies was detected in treated mice, with a relatively normal tissue morphology showing no lymphocytic infiltrations.

5.7. AAV Viral Vectors for Gene Therapy of Cancer

Despite the variety of therapeutic approaches developed so far for cancer, including chemotherapy, radiotherapy, surgery, and different medications, cancer is still a huge health issue and a leading cause of death worldwide [342,343]. Several AAV-mediated cancer gene therapy approaches have been reported so far, including suicide gene, RNA-interference, and anti-angiogenesis gene therapies [342].

In AAV-mediated suicide gene therapy, an AAV-based system with herpes simplex virus type-1 thymidine kinase and ganciclovir (AAVtk/GCV) has been used. This system was found to exhibit a significant in vitro and in vivo tumor-suppressor efficacy in human

head and neck cancer xenografts in a murine model [344–346], and murine models of bile duct cancer [347] and bladder carcinoma [348].

As for AAV-mediated RNA-interference cancer gene therapy, an AAV vector is used to provide a stable expression of short hairpin-structured RNA (shRNA) for gene knockdown applications [349]. This approach could induce a strong androgen receptor (AR) gene silencing in a murine model of prostate cancer following intravenous administration [350]. It has also been tested in a murine model of hepatocellular carcinoma and was found to induce tumor-specific apoptosis, resulting in a significant inhibition of cancer cell proliferation without toxicity [351].

AAV-mediated anti-angiogenesis cancer gene therapy targets factors that can regulate a cancer cell angiogenesis process. VEGF, being an important factor for angiogenesis, has been targeted in a murine model of breast carcinoma using AAV2 carrying transgene of VEGF Trap protein (a pseudo high-affinity receptor) [352]. The approach resulted in significant suppression of tumor growth and prevention of spontaneous pulmonary metastases. Another factor that has been targeted by this approach is pigment epithelium-derived factor (PEDF), a highly potent angiogenesis inhibitor. In a murine colorectal peritoneal carcinomatosis model, AAV2 carrying the human gene of PEDF was found to induce significant tumor suppression, inhibit metastases, and prolong survival time [353]. Similarly, kallistatin is another anti-angiogenesis factor that has been targeted using an AAV-mediated murine model of hepatocellular carcinoma, and it provided a similar efficacy as AAV-kallistatin-induced potent cancer cell apoptosis [354].

6. Challenges and Limitations of AAVs for Gene Therapy AAV-Mediated Applications

Despite the many advantages AAVs provide as viral vectors for gene therapy of various diseases, and the many improvements and molecular modifications they have undergone since their first discovery, AAVs still have some limitations that can result in impeding impacts on their applications in some cases. One example of such major limitations is the limited cloning capacity of AAVs, that is ~4.5 Kb [355,356]. Moreover, AAVs need to converse into double-stranded DNA, and may cause a delay in transgene expression following delivery, compared to double-stranded vectors [357]. Although it can be dose-dependent in some cases, preexisting immunity against some serotypes can also be a limiting factor [358,359].

Preexisting immunity, or an immunity response occurring after the first introduction of a viral vector, can be a major obstacle in the development of gene therapy approaches based on AAV or other viral vectors, and their application in clinical practice. Neutralizing antibodies are often found in the human population with a high prevalence that varies depending on the virus serotype and the geographic location of the population. Moreover, in a number of diseases, multiple administration of an AAV-based drug may be required, which can result in developing an immune response against the viral vector. Neutralizing antibodies can have a significant impact on the efficiency of gene transfer in gene therapy. Consequently, many patients with genetic diseases will not be able to benefit from such gene therapy medications.

Furthermore, although AAVs have not been linked to human pathologies or diseases, wild-type AAVs are widely distributed in the human population, and despite having a low immunogenicity profile, an immune response was observed in humans as a result of gene therapy using AAVs [358]. To date, the impact of such anti-AAV immune response on gene therapy is still not entirely understood, however, its mechanisms are being actively explored. For example, systemic delivery of AAV vectors, while having neutralizing antibodies, has been shown to result in accumulation of vector genomes in lymphoid organs. Whereas the efficiency of liver transduction while having binding antibodies (indicative of a previous infection, but not neutralizing the virus), on the contrary, increases [360]. The role of neutralizing antibodies in activating the complement system has also been studied [361]. Individuals seropositive for AAV have high titers of IgG1, IgG2, and IgG3. It is known that IgG1, IgG2, and IgM highly correlate with the level of neutralizing antibodies, and

IgG1 levels increase after gene therapy with AAV [362]. Additionally, IgG3 correlates with the level of T-cell reactivity against AAV vectors [363]. From a clinical point of view, neutralizing antibody titers are of a great significance, as having neutralizing antibodies against a specific AAV serotype highly affects gene therapy effectiveness upon using this serotype. Although methods for detecting neutralizing antibodies are poorly standardized, existing research data indicate that the prevalence of neutralizing antibodies against AAV2 is about 40% [364]. It has been shown that neutralizing antibodies can trigger elimination of the vector by the immune system. At the same time, viral vectors can accumulate in the spleen, not reaching the liver [365]. Therefore, researching mechanisms to establish and develop effective methods for preexisting immunity assessment can help overcome many problems arising in gene therapy applications. Tackling such an urgent problem can expand the possibilities of therapy for many incurable human hereditary diseases [366].

Another challenge is the need to deeply understand the exact mechanisms and pathways of AAV cellular uptake, trafficking, and transduction, which are still unknown for most AAV serotypes [367]. Such understanding can help in addressing and explaining the changes of transduction potency and efficacy that can be found between different species in different *in vivo* studies, or between treatment efficacy *in vivo* and in clinical trials [368]. In other words, differences between humans and various animal models in terms of body size, genomic and anatomic specificity, immunogenicity, and disease process should be taken into account, as they can affect the effective translation of preclinical investigations [369].

Table 1. Characteristics of different AAV serotypes.

AAV Serotype	Primary Receptor	Other/Coreceptors	Post Translational Modifications	Recommended Purification Method	Tropism
AAV1	Sialic acid	AAV receptor (AAVR)	-	iodixanol gradient centrifugation, anion-exchange chromatography, and mucin column affinity chromatography	Skeletal muscles [35,43–46], heart [48,49], glial and ependymal cells in the murine brain [47], endothelial and vascular smooth muscles [50], retina [51]
AAV2	HSPG	FGFR1, α V β 5 and α 5 β 1 integrins, HGFR, LR, and CD9	ubiquitination, phosphorylation, SUMOylation, and multiple-site-glycosylation	heparin column affinity chromatography	renal tissue [62–64], hepatocytes [65,66], retina [67–69], non-mitotic cells of central nervous system (CNS) [70,71], and skeletal muscles [45,71]
AAV3	HSPG	FGFR1, LR, and HGFR	acetylation, phosphorylation, and glycosylation	iodixanol gradient centrifugation	human liver cancer cells as well as human and NHP hepatocytes [76,77], murine cochlear inner hair cells [82].
AAV4	Sialic acid	-	ubiquitination	ion-exchange chromatography, mucin column affinity chromatography	ependymal cells of mammalian CNS [90], RPE cells of the retina (canine, rodent, and NHP origins) [91], murine kidney, lung, and heart cells [92,99].
AAV5	Sialic acid	PDGFR (α and β)	ubiquitination, phosphorylation, SUMOylation, and glycosylation	ion-exchange chromatography, mucin column affinity chromatography	murine: retinal cells [38,51], mainly photoreceptors [106], airway epithelia [98,110], liver cells [92,112], vascular endothelial cells and smooth muscles [50], and neurons (murine and NHP) [107,370]
AAV6	Sialic acid and HSPG	EGFR	acetylation	heparin or mucin column affinity chromatography	airway epithelia of murine and canine models [119,120], murine liver cells [92,121], skeletal muscles of murine and canine models [92,122–124], cardiomyocytes in murine [92,125], porcine [126], canine [127], and in sheep [128] models.

Table 1. Cont.

AAV Serotype	Primary Receptor	Other/Coreceptors	Post Translational Modifications	Recommended Purification Method	Tropism
AAV7	-	-	glycosylation, phosphorylation, SUMOylation, and acetylation	-	murine skeletal muscle cells [133], murine and human hepatocytes [92], murine and NHPs CNS [134,135], murine photoreceptor cells [136], murine vascular endothelial cells (limited tropism) [130], murine epicardium cells [49].
AAV8	LR	-	phosphorylation, glycosylation, and acetylation	Dual-ion-exchange chromatography, iodixanol gradient centrifugation	murine, canine, and 115 hepatocytes [92,112,141–147], murine skeletal and cardiac muscles [148], murine pancreatic cells [149,150], murine renal cells [151], and different cells in the murine retina [152–154]
AAV9	terminal N-linked galactose	putative integrin, LR	glycosylation, ubiquitination, phosphorylation, SUMOylation, and acetylation	Sucrose gradient centrifugation, and ion-exchange chromatography	murine, NHP, and feline neuronal and non-neuronal cells, including astrocytes [27,159–162], murine and NHP retinal photoreceptors cells [163], murine, NHP, and porcine cardiac muscle tissue [164–169], murine hepatocytes, skeletal muscles, and pancreatic cells [81,169], photoreceptor cells [170], renal tubular epithelium cells [171–173], Leydig cells in the testicular interstitial tissue [174], and alveolar and nasal epithelia [175,176].
AAV10	-	-	glycosylation, ubiquitination, phosphorylation, SUMOylation, and acetylation	iodixanol gradient centrifugation	NHP intestinal cells, hepatocytes, lymph nodes, and less frequently, renal cells and adrenal glands [180], murine small intestine and colon cells [181], retinal cells (including RPE, cells in the ganglion cell layer, several cell types in the inner nuclear layer, photoreceptors, and a highly efficient transduction of horizontal cells) [152], murine liver cells, lung cells [182], renal, and pancreatic cells [183].

Table 1. Cont.

AAV Serotype	Primary Receptor	Other/Coreceptors	Post Translational Modifications	Recommended Purification Method	Tropism
AAV11	-	-	-	iodixanol gradient centrifugation	NHP intestinal cells, hepatocytes, lymph nodes, and less frequently, renal cells and adrenal glands [180], murine projection neurons and astrocytes [184], and mild tropism to NHP CNS (cerebrum and spinal cord, mainly) [180].
AAV12	mannose and mannosamine have been suggested as components of a potential receptor complex		-	AVB Sepharose affinity chromatography	murine salivary glands and muscles [185], murine nasal epithelia (mainly after intranasal administration) [187].
AAV13	-	HSPG	-	iodixanol gradient centrifugation, heparin column affinity chromatography	-

Table 2. Preclinical and clinical studies using AAVs as viral vectors for gene therapy of different diseases.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
CNS	PD	AAV2-GAD	phase I clinical trial for advanced PD patients	safe and well-tolerated approach, providing significant improvement in motor function scores up to 12 months after unilateral subthalamic delivery	[229]
			double-blind, controlled, randomized clinical trial for advanced PD patients	safe and well-tolerated approach, along with improved motor function scores following bilateral subthalamic delivery	[230]
		AAV2-neurturin	open-label clinical trial for PD patients	suggested feasibility, safety, and good tolerance of the approach	[231]
		rAAV2-AADC	clinical trial for moderately advanced PD patients	good tolerance, improvement on motor rating scales, however, accompanied with an increased risk of intracranial hemorrhages and headache	[232]
	AADC deficiency	rAAV2-AADC	open-label, phase I/II trial in children	good tolerance and a stable expression of the transgene that lasted for the following 4 years, although higher vector doses were suggested for further studies	[233]
				good tolerance in general, with evidence for potential improvement of motor function	[234]
	SMA	AA9-SMN	open-label, phase I clinical trial	significant improvement of the motor function in all 15 patients following single-dose intravenous administration, reflected by their ability to perform different activities, such as unassisted sitting and walking, oral feeding, and speaking, with no reported motor function regression at two-year follow-up. A long-term safety assessment, however, was recommended.	[236]

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
LSD	MPS VII	(rAAV-GUS β)	in vivo study, murine model	stable expression of the deficient enzyme upon single administration, that was adequate for phenotype correction in the liver	[238,239]
		(rAAV-GUS β)	in vivo study, neonatal murine model	therapeutic levels of the deficient enzyme in multiple organs, including ones of the CNS, with the gene expression not being affected by rapid growth and differentiation of tissues	[240]
		rAAV9-GUS β and rAAVrh10-GUS β	in vivo study, canine model	significantly high expression levels of GUS β in the CNS tissues, with the enzyme in brain tissue homogenates showing over 100% normal activity	[221]
	MPS IIIA	AAV9-Sgsh (canine sulfamidase gene)	in vivo study, canine model	sustained and widely distributed transgene expression with no toxicity for a duration of 7 years after therapy	[241]
	MLD	AAV1-ARSA	in vivo study, murine model	significant elevation of ARSA levels and activity, resulting in reduction of accumulated sulfatides	[244]
		AAV5-ARSA	in vivo study, murine model	rapid, abundant, and sustained restoration of ARSA levels in the brain and brainstem up to 15 months after administration, reduction of accumulated sulfatides, and preservation of neurologic function.	[246]
		AAV5-ARSA	in vivo study, NHP model	good tolerance, distribution of the transgene in the brain with elevated activity of the deficient enzyme	[370]
		AAV9-ARSA	in vivo study, neonatal murine model	global expression of the transgene in the brain and spinal cord, along with muscles and heart, inhibition of sulfatide accumulation, and improvement of neurologic/motor function	[245]
		AAVrh10-ARSA AAVrh10-ARSA AAVrh10-ARSA	in vivo study, murine model	correction of sulfatide accumulation following single administration, with a transduction efficacy higher than that of AAV5 as it transduced both neurons and oligodendrocytes	[243]
			in vivo study, NHP model	good tolerance, neuroinflammation 3 months following the fifth dose but none after the first, detection of transgene expression after the first dose along with increased ARSA activity, and detection of the enzyme in other organs but not in gonads	[371]
			clinical trial for children with asymptomatic or early-stage MLD	significant elevation in ARSA levels in the cerebrospinal fluid (CSF) following intracerebral vector delivery, but no clinical improvement has been noticed compared to the control group.	[253]
	GM2-gangliosidose	rAAV2-HEXA + rAAV2-HEXB	in vivo study, murine model	delay of disease clinical onset, maintained motor function, good tolerance, stable and abundant levels of the deficient enzyme, resulting in a significant reduction of gangliosides' storage in the CNS	[250]
		AAVrh8-HEX	in vivo study, feline model	prevention or reduction of tremors that is characteristic of improvement in the neurologic function, and a significant elevation of the deficient enzyme levels (HEX), resulting in a significant reduction of gangliosides' storage in different tissues of the CNS.	[252]
		AAVrh8-HEXA + AAVrh8-HEXB	in vivo study, ovine model	delay of disease clinical onset and progression, improved neurologic function and clinical biomarkers. However, lifespan was not significantly higher.	[251]
		AAVrh8-HEXA + AAVrh8-HEXB	first clinical trial for children with TSD	good tolerance and broad distribution of the transgene in the CNS	[248]

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
	CD	rAAV2- <i>ASPA</i>	in vivo study, murine model	increased <i>ASPA</i> activity and, thereby, decreased NAA accumulation and white matter degeneration. However, areas remote from injection site, such as the cerebellum, were not affected	[256]
	GLD	rAAV1- <i>GALC</i>	in vivo study, murine model	sustained expression of the deficient enzyme, improved myelination status, and prolonged lifespan. However, both treated and untreated mice died with similar symptoms, suggesting that the used approach should be initiated prior to symptoms' onset.	[262]
		rAAV2/5- <i>GALC</i>	in vivo study, murine model	wide dispersion of <i>GALC</i> transgene across the CNS reaching areas remote from the injection site, reduced loss of oligodendrocytes and Purkinje cells, along with a significant improvement of neuromotor function and a prolonged lifespan of treated mice.	[263]
		AAV9- <i>GALC</i> AAVrh10- <i>GALC</i> AAVOlig001- <i>GALC</i>	in vivo study, murine model	All three serotypes provided wide distribution of the transgene across the CNS and liver, resulting in a significant improvement of myelination, and a prolonged lifespan, with AAV9 being the most effective when combined with bone marrow transplantation.	[265]
		AAVrh10-c <i>GALC</i>	in vivo study, canine model	delayed symptoms onset, prolonged lifespan, correction of biochemical defects, and a positive effect on neuropathology in treated animals	[266].
		AAV9-c <i>GALC</i>	in vivo study, canine model	increased activity of the deficient enzyme and, therefore, normal levels of its substrate, improved myelination, and decreased inflammation both in the CNS and PNS, which, along with prevention of clinical neurological dysfunction, resulted in a significantly prolonged lifespan of treated dogs, compared to the control group. However, sufficient dosing was found to be critical, as high doses significantly extended the lifespan even for post-symptomatic subjects, and a 5-fold lower dose of the vector resulted in an attenuated form of disease	[267].
	Retinal degeneration and ocular neurovascular diseases	AAV2, 8, and 9	in vivo study, C57BL/6 mice	transduction of retinal cells, as well as efficient transduction of ganglion cell layer by AAV8 and AAV9	[170]
	Inherited photoreceptor diseases	hybrid serotypes AAV2/7 and AAV2/8	in vivo study, C57BL/6 mice	high transduction rates of the murine photoreceptors	[136]
	Retinal blindness caused by LCA	AAV2/6	in vivo study, wild-type 129Sv/Ev (Taconic) mice	efficient transduction of murine cone photoreceptors following subretinal injection	[268]
		AAV2/4- RPE65	clinical trial for patients with LCA	systemic and local good tolerance with no adverse effects, along with improvement of visual function presented by different parameters in different patients, including improvement of visual acuity and color vision, as well as reduction of visual fatigue or photophobia, over a follow-up period between 1 and 3 years	[269]
	Retinal blindness caused by choroideremia	AAV2- REP1	phase I/II clinical trial	improvement of retinal sensitivity in all six patients, following subretinal vector delivery, with two of them having significant increases in visual acuity, supporting further consideration of the tested therapeutic approach.	[272]

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
	RPE65-mediated inherited retinal dystrophy	AAV2-hRPE65v2	open-label, randomized, controlled phase III trial	good tolerance with no adverse effects, restoration of RPE65 enzymatic activity reflected by significant and sustained improvement in light perception and navigational abilities	[274]
	Leber Hereditary Optic Neuropathy (LHON)	rAAV2-ND4 (gene encoding nicotinamide adenine dinucleotide dehydrogenase subunit IV)	open-label, phase I/II randomized clinical trial	good tolerance, although a mild, intraocular inflammation was detected after vector administration, but it was responsive to treatment and suggested to be overcome in further studies by systemic vector delivery instead of local.	[275]
			open-label, phase I clinical trial	minor adverse events, improvement in visual activity in some but not all patients, no detection of vector DNA in patients' blood samples	[276]
	RPGR-related X-linked retinitis pigmentosa	AAV8-RPGR	phase I/II clinical trial	no adverse effects other than steroid-responsive subretinal inflammation following administration of higher doses, sustained improvements in visual function in 6/18 patients.	[277]
	X-Linked Retinoschisis	AAV8-RS1 (Retinoschisin gene)	phase I/IIa single-center, open-label, clinical trial	good tolerance in general, although steroid-responsive, dose-related inflammation was observed, and a dose-related increase of systemic antibodies against AAV8, but none against RS1	[278,279]
	CNGA3-linked achromatopsia	AAV8-CNGA3	nonrandomized controlled clinical trial	no substantial safety issues, successful targeting of cone photoreceptors reflected by reported improvement of color vision ability, along with improvements in visual acuity and contrast sensitivity (although a cause–effect relationship was not established)	[280,281]
	Hearing disorders	AAV9	in vivo study, porcine model	persistent expression of the transgene within the mammalian inner ear following intracochlear delivery	[282]
			in vivo study-rabbits	efficient and stable gene transfer of CFTR into airway epithelium, indicating, as a result, the vector potential to be used for gene therapy	[286]
	CF	AAV2-CFTR	phase I clinical trial for CF patients	safety, successful transduction of targeted cells, and a detected function restoration of sinuses.	[288]
			phase II, double-blind, randomized, placebo-controlled clinical trial for CF patients	safety and good tolerance, but no effective clinical treatment of disease was achieved	[289]
			phase I clinical trial for mild CF patients	safety, but no effective clinical treatment of disease was achieved	[290]
Respiratory organs (airway epithelia)		rAAV-hAAT	in vivo study, intravenous delivery into murine model	Serum levels of AAT that are potentially therapeutic	[297,298]
	α 1AT deficiency	rAAV2-hAAT and rAAV5-hAAT	in vivo study, intrapleural vs. intramuscular delivery into murine model	AAT lung and serum levels higher than the ones achieved by intravenous delivery, with rAAV5 showing 10-fold higher effectiveness than rAAV2	[299,300]
		rhAAV10-hAAT	in vivo study, intrapleural delivery into murine and NHP models	safety, and persistent expression of the transgene in the chest cavity cells of both models	[303,304]

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
		rAAV6/2-hAAT	in vivo study, intratracheal delivery into C57/Bl6 mice, and in vitro study, cultures of human airway epithelial cells	lung cells' transduction, even more efficient than rAAV5 both in vivo and in vitro	[301]
		rAAV8-hAAT	in vivo study, intratracheal delivery into C57/Bl6 mice	lung cells' transduction superior to that of rAAV5, as it resulted in 6-fold and 2.5-fold higher AAT levels in serum and broncho-alveolar fluid, respectively.	[302]
		rAAV2/8-hAAT	in vivo study, intravenous delivery into C57/Bl6 mice	high transduction rate of hepatocytes	[298,372]
		rAAV1-AAT	in vivo study, intramuscular delivery into C57/Bl6 mice and rabbits	dose-dependent inflammatory infiltrates at injection sites not affecting expression of transgene, along with dose-dependent detection of vector DNA in most animals, both at injection sites and in distal organs	[46,305]
		rAAV2/9-AAT	in vivo study, intratracheal delivery into C57/Bl6 mice	efficient, relatively stable transduction of alveolar and nasal epithelia, that was not affected by high levels of neutralizing antibodies upon following re-administration	[175]
		rAAV6-hAAT	in vivo study, nasal delivery into C57/Bl6 mice, and intratracheal delivery into canine model	therapeutic hAAT concentrations in both studied animal models, with levels being higher in lungs than serum, accompanied with an immune response against the vector capsid in some animals despite being immunosuppressed	[120]
	Allergic asthma	rAAV-IL-4	in vivo study, intratracheal delivery into Balb/cByJ mice	significant inhibition of airway eosinophilia and mucus production along with a reduction in airway hyper-responsiveness and asthma-associated cytokine levels	[306]
	SPB	rAAV6/2-SFTPB	in vivo study, intratracheal delivery into murine model	efficient transduction of airway and alveolar epithelium, good tolerance of administered vector with no adverse effects, rapid and long-term restoration of the deficient SPB protein, along with an improvement of lung function, leading, subsequently, to an extended survival	[307]
Muscles	Degenerative muscle disorders	AAV1-FS344 (follistatin gene)	in vivo study, NHP model	safety and good tolerance of administered vector, promotion of sustained improvement in muscle size and strength	[311]
	DMD	rAAV6-micro-dystrophin	in vivo study, dystrophin/utrophin double-knockout murine model	no serious adverse events, improvement of muscle function along with prolongation of lifespan, explained by sustained restoration of deficient protein (dystrophin) in the respiratory, cardiac, and limb muscles	[312]
		rAAV1-mini-dystrophin	in vivo study, dystrophin/utrophin double knockout murine model	prolongation of lifespan, highly efficient expression of transgene, improvement in muscle histopathology and function, reflected by improved growth and motility, along with prevention of spine and extremities' deformation	[313]
		AAV2.5-mini-dystrophin	Randomized, double-blind, placebo-controlled phase I clinical trial	safety and good tolerance of the vector, and detection of transgene DNA in all patients following intramuscular administration	[320]
		AAV9-micro-dystrophin	phase I/II open-label, randomized, controlled clinical trial	safety and successful expression of transgene	[319]
		AAV-rh74-micro-dystrophin	phase I/II, open-label, non-randomized clinical trial	safety and successful expression of transgene, along with improvement of motor function	

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
Cardiovascular system	LGMD	rAAV8-h δ -SG (human δ -sarcoglycan gene)	in vivo study, murine model	prolongation of lifespan, efficient and sustained transduction of cardiac and skeletal muscles along with improvement in their histopathology and function	[314]
		rAAV1-h α -SG	in vivo study, murine model	efficient and sustained expression of the transgene, histological and functional improvement of musculature reflected by correction of contractile force deficits and stretch sensibility, along with increase of animal activity	[316]
		rAAV-h γ -SG	in vivo study, murine model	significantly efficient transduction of muscle fibers, and general histopathological improvement, that were achieved only upon early intervention	[317]
	HF	AAV6-EGFP	in vivo study, ovine model	global expression of the transgene, that was cardiac-tropic and substantially more robust and targeted, compared to that of intramuscular or intracoronary injection.	[128]
		AAV1-SERCA2a	randomized phase I/II clinical trial	highest adverse effects, including death, in the placebo group, and lowest in the high-dose group, with evidence of long-term transgene expression. However, in the low-dose and mid-dose groups, adverse effects were found to be high but delayed.	[323]
	Hemophilia A	rAAV6-cFVIII and rAAV8-cFVIII	in vivo study, murine and canine models	restoration of physiologic levels of the deficient factor in the canine model for three years following intravenous administration, without any toxicity or immune reactions. A similar effect in the murine model was found, however, with detection of neutralizing antibodies against cFVIII in the mice sera	[322]
	Hemophilia B	rAAV8-FIX and rAAV9-FIX	in vivo study, murine model	significant increase in transgene expression and therefore in IX factor levels, with decreased proinflammatory risk following intravenous administration	[168]
	WD	AAV8-mini ATP7B	in vivo study, murine model	restoration of copper homeostasis, with 20% hepatocyte transduction being sufficient for correction	[327]
	Liver	AAV8- UGT1A1	ongoing clinical trial	-	[329]
			in vivo study, murine model	stable correction was achieved when the vector was administered at the 28th postnatal day, coupled with a rapamycin-based immunosuppression regimen delivered intraperitoneally	[330]
Endocrine system	T1DM	dsAAV8-mIP	in vivo study, murine model	prevention of hyperglycemia in a dose-dependent manner	[336]
		rAAV2-IL-10	in vivo study, murine model	a positive effect, decreasing autoimmunity, and thereby hyperglycemia	[337]
		AAV8-PDL1	in vivo study, murine model	pancreatic β cells' protection	[338]
		AAV8-anti-VEGF fab	ongoing clinical trial for diabetic retinopathy patients	-	[339]
	APS1	AAV9-AIRE	in vivo study, murine model	high transduction efficiency, along with restoration of AIRE expression in the thymus, following intra-thymic administration, and a subsequent significant reduction of serum auto-antibodies, with relatively normal tissue morphology showing no lymphocytic infiltrations.	[341]

Table 2. Cont.

Gene Therapy Target	Disease **	AAV Viral Vector	Study Type	Outcome	Ref.
Cancer	laryngeal cancer cell line (HEp-2)	AAVtk/GCV system	in vitro and in vivo study, murine model	significant tumor-suppressor efficacy in human head and neck cancer xenografts, and markedly prolonged survival	[344–346]
	bile duct cancer	AAVtk/GCV system	in vivo study, murine model	increased anti-tumor effect upon simultaneous administration with 5-fluorouracil, and better survival	[347]
	bladder carcinoma	AAVtk/GCV system	in vivo study, murine model	control of tumor cell growth, a strong anti-tumor efficacy	[348]
	prostate cancer	AAV2-ARHP8	in vivo study, murine model	strong androgen receptor (AR) gene silencing following intravenous administration	[350]
	hepatocellular carcinoma	AAV8-miR-26a	in vivo study, murine model	Induction of tumor-specific apoptosis, resulting in a significant inhibition of cancer cell proliferation without toxicity	[351]
	breast carcinoma	AAV2-VEGF Trap	in vivo study, murine model	significant suppression of tumor growth and prevention of spontaneous pulmonary metastases	[352]
	colorectal peritoneal carcinomatosis	AAV2-hPEDF	in vivo study, murine model	significant tumor suppression, inhibition of metastases, and prolonged survival time	[353]
	hepatocellular carcinoma	AAV-kallistatin	in vivo study, murine model	induced potent cancer cell apoptosis, and prolonged survival time	[354]

** Disease abbreviations: PD: Parkinson's disease, AADC deficiency: amino acid decarboxylase deficiency, SMA: spinal muscular atrophy, MPS: mucopolysaccharidosis, MLD: metachromatic leukodystrophy, CD: Canavan's disease, GLD: globoid cell leukodystrophy, LCA: Leber congenital amaurosis, CF: cystic fibrosis, α 1AT deficiency: Alpha-1 antitrypsin deficiency, SPB deficiency: surfactant protein B deficiency, DMD: Duchenne muscular dystrophy, LGMD: Limb-Girdle muscular dystrophies, HF: heart failure, WD: Wilson's disease, CNs: Crigler-Najjar syndrome, T1DM: Type 1 diabetes mellitus, APS1: autoimmune polyglandular syndrome type-1.

7. Conclusions

AAVs were discovered over five decades ago and have since represented a potent tool for gene therapy, that still needs to be better understood and developed for broader and larger therapeutic applications. Further optimizations should cover vector design, tropism modifications, and delivery routes. In this article, we have covered different serotypes of adeno-associated viruses and their applications in gene therapy for different diseases in preclinical and clinical studies, with a brief overview of AAVs' limitations and challenges.

Author Contributions: Conceptualization, S.S.I., A.A.S. and V.V.S.; writing—original draft preparation, S.S.I. and A.A.S.; writing—review and editing, V.V.S.; supervision, A.A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This article has been supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the Center for Precision Genome Editing and Genetic Technologies for Biomedicine, IGB RAS, for scientific advice on development of gene therapy drugs.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Ozelo, M.C.; Mahlangu, J.; Pasi, K.J.; Giermasz, A.; Leavitt, A.D.; Laffan, M.; Symington, E.; Quon, D.V.; Wang, J.D.; Peerlinck, K.; et al. Valoctocogene Roxaparvec Gene Therapy for Hemophilia A. *N. Engl. J. Med.* **2022**, *386*, 1013–1025. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zolgensma—One-time gene therapy for spinal muscular atrophy. *Med. Lett. Drugs Ther.* **2019**, *61*, 113–114.
- Prado, D.A.; Acosta-Acero, M.; Maldonado, R.S. Gene therapy beyond luxturna: A new horizon of the treatment for inherited retinal disease. *Curr. Opin. Ophthalmol.* **2020**, *31*, 147–154. [\[CrossRef\]](#) [\[PubMed\]](#)
- Keeler, A.M.; Flotte, T.R. Recombinant Adeno-Associated Virus Gene Therapy in Light of Luxturna (and Zolgensma and Glybera): Where Are We, and How Did We Get Here? *Annu. Rev. Virol.* **2019**, *6*, 601–621. [\[CrossRef\]](#)
- Atchison, R.W.; Casto, B.C.; Hammon, W.M. Adenovirus-associated defective virus particles. *Science* **1965**, *149*, 754–756. [\[CrossRef\]](#)
- Mezzina, M.; Merten, O.W. Adeno-associated viruses. *Methods Mol. Biol.* **2011**, *737*, 211–234. [\[CrossRef\]](#)
- Hoggan, M.D.; Blacklow, N.R.; Rowe, W.P. Studies of small DNA viruses found in various adenovirus preparations: Physical, biological, and immunological characteristics. *Proc. Natl. Acad. Sci. USA* **1966**, *55*, 1467–1474. [\[CrossRef\]](#)
- Berns, K.I.; Giraud, C. Biology of adeno-associated virus. *Curr. Top. Microbiol. Immunol.* **1996**, *218*, 1–23. [\[CrossRef\]](#)
- Yalkinoglu, A.O.; Heilbronn, R.; Bürkle, A.; Schlehofer, J.R.; zur Hausen, H. DNA amplification of adeno-associated virus as a response to cellular genotoxic stress. *Cancer Res.* **1988**, *48*, 3123–3129.
- Gonçalves, M.A.F.V. Adeno-associated virus: From defective virus to effective vector. *Virol. J.* **2005**, *2*, 43. [\[CrossRef\]](#)
- Erles, K.; Sebökova, P.; Schlehofer, J.R. Update on the prevalence of serum antibodies (IgG and IgM) to adeno-associated virus (AAV). *J. Med. Virol.* **1999**, *59*, 406–411. [\[CrossRef\]](#)
- Büning, H.; Perabo, L.; Coutelle, O.; Quad-Humme, S.; Hallek, M. Recent developments in adeno-associated virus vector technology. *J. Gene Med.* **2008**, *10*, 717–733. [\[CrossRef\]](#)
- Tijssen, P. *Handbook of Parvoviruses Vol. I and Vol. II Continued*; CRC Press, Inc.: Boca Raton, FL, USA, 1990.
- Grimes, T.; King, D.; Fletcher, O. Serologic and pathogenicity studies of avian adenovirus isolated from chickens with inclusion body hepatitis. *Avian Dis.* **1978**, *22*, 177–180. [\[CrossRef\]](#)
- Alvarado, I.; Villegas, P.; El-Attrache, J.; Jensen, E.; Rosales, G.; Perozo, F.; Purvis, L.J. Genetic characterization, pathogenicity, and protection studies with an avian adenovirus isolate associated with inclusion body hepatitis. *Avian Dis.* **2007**, *51*, 27–32. [\[CrossRef\]](#)
- Bossis, I.; Chiorini, J.A. Cloning of an avian adeno-associated virus (AAAV) and generation of recombinant AAAV particles. *J. Virol.* **2003**, *77*, 6799–6810. [\[CrossRef\]](#)
- Arbetman, A.E.; Lochrie, M.; Zhou, S.; Wellman, J.; Scallan, C.; Doroudchi, M.M.; Randlev, B.; Patarroyo-White, S.; Liu, T.; Smith, P. Novel caprine adeno-associated virus (AAV) capsid (AAV-Go. 1) is closely related to the primate AAV-5 and has unique tropism and neutralization properties. *J. Virol.* **2005**, *79*, 15238–15245. [\[CrossRef\]](#)
- Olson, E.J.; Haskell, S.R.; Frank, R.K.; Lehmkuhl, H.D.; Hobbs, L.A.; Warg, J.V.; Landgraf, J.G.; Wünschmann, A. Isolation of an adenovirus and an adeno-associated virus from goat kids with enteritis. *J. Vet.-Diagn. Investig.* **2004**, *16*, 461–464. [\[CrossRef\]](#)
- Schmidt, M.; Katano, H.; Bossis, I.; Chiorini, J.A. Cloning and characterization of a bovine adeno-associated virus. *J. Virol.* **2004**, *78*, 6509–6516. [\[CrossRef\]](#)

20. Coria, M.; Lehmkuhl, H. Isolation and identification of a bovine adenovirus type 3 with an adenovirus-associated virus. *J. Vet.-Diagn. Investig.* **1978**, *39*, 1904–1906.
21. Dutta, S.K. Isolation and characterization of an adenovirus and isolation of its adenovirus-associated virus in cell culture from foals with respiratory tract disease. *Am. J. Vet. Res.* **1975**, *36*, 247–250.
22. Qiu, J.; Pintel, D.J. Alternative polyadenylation of adeno-associated virus type 5 RNA within an internal intron is governed by the distance between the promoter and the intron and is inhibited by U1 small nuclear RNP binding to the intervening donor. *J. Biol. Chem.* **2004**, *279*, 14889–14898. [[CrossRef](#)] [[PubMed](#)]
23. Merten, O.W.; Gény-Fiamma, C.; Douar, A.M. Current issues in adeno-associated viral vector production. *Gene Ther.* **2005**, *12* (Suppl. S1), S51–S61. [[CrossRef](#)] [[PubMed](#)]
24. Potter, M.; Lins, B.; Mietzsch, M.; Heilbronn, R.; Van Vliet, K.; Chipman, P.; Agbandje-McKenna, M.; Cleaver, B.D.; Clément, N.; Byrne, B.J.; et al. A simplified purification protocol for recombinant adeno-associated virus vectors. *Mol. Ther. Methods Clin. Dev.* **2014**, *1*, 14034. [[CrossRef](#)] [[PubMed](#)]
25. Carter, P.J.; Samulski, R.J. Adeno-associated viral vectors as gene delivery vehicles. *Int. J. Mol. Med.* **2000**, *6*, 17–27. [[CrossRef](#)] [[PubMed](#)]
26. Cataldi, M.P.; McCarty, D.M. Hairpin-end conformation of adeno-associated virus genome determines interactions with DNA-repair pathways. *Gene Ther.* **2013**, *20*, 686–693. [[CrossRef](#)]
27. Michelfelder, S.; Trepel, M. Adeno-associated viral vectors and their redirection to cell-type specific receptors. *Adv. Genet.* **2009**, *67*, 29–60. [[CrossRef](#)]
28. Dubielzig, R.; King, J.A.; Weger, S.; Kern, A.; Kleinschmidt, J.A. Adeno-associated virus type 2 protein interactions: Formation of pre-encapsidation complexes. *J. Virol.* **1999**, *73*, 8989–8998. [[CrossRef](#)]
29. Kyöstiö, S.R.; Owens, R.A.; Weitzman, M.D.; Antoni, B.A.; Chejanovsky, N.; Carter, B.J. Analysis of adeno-associated virus (AAV) wild-type and mutant Rep proteins for their abilities to negatively regulate AAV p5 and p19 mRNA levels. *J. Virol.* **1994**, *68*, 2947–2957. [[CrossRef](#)]
30. Galibert, L.; Hyvönen, A.; Eriksson, R.A.E.; Mattola, S.; Aho, V.; Salminen, S.; Albers, J.D.; Peltola, S.K.; Weman, S.; Nieminen, T.; et al. Functional roles of the membrane-associated AAV protein MAAP. *Sci. Rep.* **2021**, *11*, 21698. [[CrossRef](#)]
31. Kronenberg, S.; Kleinschmidt, J.A.; Böttcher, B. Electron cryo-microscopy and image reconstruction of adeno-associated virus type 2 empty capsids. *EMBO Rep.* **2001**, *2*, 997–1002. [[CrossRef](#)]
32. Rabinowitz, J.E.; Samulski, R.J. Building a better vector: The manipulation of AAV virions. *Virology* **2000**, *278*, 301–308. [[CrossRef](#)]
33. Wu, Z.; Asokan, A.; Samulski, R.J. Adeno-associated virus serotypes: Vector toolkit for human gene therapy. *Mol. Ther. J. Am. Soc. Gene Ther.* **2006**, *14*, 316–327. [[CrossRef](#)]
34. Mori, S.; Wang, L.; Takeuchi, T.; Kanda, T. Two novel adeno-associated viruses from cynomolgus monkey: Pseudotyping characterization of capsid protein. *Virology* **2004**, *330*, 375–383. [[CrossRef](#)]
35. Gao, G.P.; Alvira, M.R.; Wang, L.; Calcedo, R.; Johnston, J.; Wilson, J.M. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11854–11859. [[CrossRef](#)]
36. Wu, Z.; Miller, E.; Agbandje-McKenna, M.; Samulski, R.J. Alpha2,3 and alpha2,6 N-linked sialic acids facilitate efficient binding and transduction by adeno-associated virus types 1 and 6. *J. Virol.* **2006**, *80*, 9093–9103. [[CrossRef](#)]
37. Li, C.; Samulski, R.J. Engineering adeno-associated virus vectors for gene therapy. *Nat. Rev. Genet.* **2020**, *21*, 255–272. [[CrossRef](#)]
38. Rabinowitz, J.E.; Rolling, F.; Li, C.; Conrath, H.; Xiao, W.; Xiao, X.; Samulski, R.J. Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity. *J. Virol.* **2002**, *76*, 791–801. [[CrossRef](#)]
39. Zolotukhin, S.; Potter, M.; Zolotukhin, I.; Sakai, Y.; Loiler, S.; Fraites, T.J., Jr.; Chiodo, V.A.; Phillipsberg, T.; Muzyczka, N.; Hauswirth, W.W.; et al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* **2002**, *28*, 158–167. [[CrossRef](#)]
40. Robert, M.A.; Chahal, P.S.; Audy, A.; Kamen, A.; Gilbert, R.; Gaillet, B. Manufacturing of recombinant adeno-associated viruses using mammalian expression platforms. *Biotechnol. J.* **2017**, *12*, 1600193. [[CrossRef](#)]
41. Mary, B.; Maurya, S.; Arumugam, S.; Kumar, V.; Jayandharan, G.R. Post-translational modifications in capsid proteins of recombinant adeno-associated virus (AAV) 1-rh10 serotypes. *FEBS J.* **2019**, *286*, 4964–4981. [[CrossRef](#)]
42. Huang, L.-Y.; Patel, A.; Ng, R.; Miller, E.B.; Halder, S.; McKenna, R.; Asokan, A.; Agbandje-McKenna, M.; Banks, L. Characterization of the Adeno-Associated Virus 1 and 6 Sialic Acid Binding Site. *J. Virol.* **2016**, *90*, 5219–5230. [[CrossRef](#)] [[PubMed](#)]
43. Chao, H.; Liu, Y.; Rabinowitz, J.; Li, C.; Samulski, R.J.; Walsh, C.E. Several log increase in therapeutic transgene delivery by distinct adeno-associated viral serotype vectors. *Mol. Ther. J. Am. Soc. Gene Ther.* **2000**, *2*, 619–623. [[CrossRef](#)] [[PubMed](#)]
44. Hauck, B.; Xiao, W. Characterization of tissue tropism determinants of adeno-associated virus type 1. *J. Virol.* **2003**, *77*, 2768–2774. [[CrossRef](#)] [[PubMed](#)]
45. Lisowski, L.; Tay, S.S.; Alexander, I.E. Adeno-associated virus serotypes for gene therapeutics. *Curr. Opin. Pharmacol.* **2015**, *24*, 59–67. [[CrossRef](#)]
46. Lu, Y.; Choi, Y.K.; Campbell-Thompson, M.; Li, C.; Tang, Q.; Crawford, J.M.; Flotte, T.R.; Song, S. Therapeutic level of functional human alpha 1 antitrypsin (hAAT) secreted from murine muscle transduced by adeno-associated virus (rAAV1) vector. *J. Gene Med.* **2006**, *8*, 730–735. [[CrossRef](#)]

47. Wang, C.; Wang, C.M.; Clark, K.R.; Sferra, T.J. Recombinant AAV serotype 1 transduction efficiency and tropism in the murine brain. *Gene Ther.* **2003**, *10*, 1528–1534. [\[CrossRef\]](#)
48. Su, H.; Yeghiazarians, Y.; Lee, A.; Huang, Y.; Arakawa-Hoyt, J.; Ye, J.; Orcino, G.; Grossman, W.; Kan, Y.W. AAV serotype 1 mediates more efficient gene transfer to pig myocardium than AAV serotype 2 and plasmid. *J. Gene Med.* **2008**, *10*, 33–41. [\[CrossRef\]](#)
49. Palomeque, J.; Chemaly, E.R.; Colosi, P.; Wellman, J.A.; Zhou, S.; Del Monte, F.; Hajjar, R.J. Efficiency of eight different AAV serotypes in transducing rat myocardium in vivo. *Gene Ther.* **2007**, *14*, 989–997. [\[CrossRef\]](#)
50. Chen, S.; Kapturczak, M.; Loiler, S.A.; Zolotukhin, S.; Glushakova, O.Y.; Madsen, K.M.; Samulski, R.J.; Hauswirth, W.W.; Campbell-Thompson, M.; Berns, K.I.; et al. Efficient transduction of vascular endothelial cells with recombinant adeno-associated virus serotype 1 and 5 vectors. *Hum. Gene Ther.* **2005**, *16*, 235–247. [\[CrossRef\]](#)
51. Auricchio, A.; Kobinger, G.; Anand, V.; Hildinger, M.; O'Connor, E.; Maguire, A.M.; Wilson, J.M.; Bennett, J. Exchange of surface proteins impacts on viral vector cellular specificity and transduction characteristics: The retina as a model. *Hum. Mol. Genet.* **2001**, *10*, 3075–3081. [\[CrossRef\]](#)
52. Srivastava, A. In vivo tissue-tropism of adeno-associated viral vectors. *Curr. Opin. Virol.* **2016**, *21*, 75–80. [\[CrossRef\]](#)
53. Summerford, C.; Samulski, R.J. Membrane-Associated Heparan Sulfate Proteoglycan Is a Receptor for Adeno-Associated Virus Type 2 Virions. *J. Virol.* **1998**, *72*, 1438–1445. [\[CrossRef\]](#)
54. Opie, S.R.; Warrington, K.H., Jr.; Agbandje-McKenna, M.; Zolotukhin, S.; Muzyczka, N. Identification of amino acid residues in the capsid proteins of adeno-associated virus type 2 that contribute to heparan sulfate proteoglycan binding. *J. Virol.* **2003**, *77*, 6995–7006. [\[CrossRef\]](#)
55. Auricchio, A.; Sena-Esteves, M.; Gao, G. Purification of Recombinant Adeno-Associated Virus 2 (rAAV2) by Heparin Column Affinity Chromatography. *Cold Spring Harb. Protoc.* **2020**, *2020*, 095620. [\[CrossRef\]](#)
56. Qing, K.; Mah, C.; Hansen, J.; Zhou, S.; Dwarki, V.; Srivastava, A.J. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. *Nat. Med.* **1999**, *5*, 71–77. [\[CrossRef\]](#)
57. Summerford, C.; Bartlett, J.S.; Samulski, R.J.J. $\alpha V\beta 5$ integrin: A co-receptor for adeno-associated virus type 2 infection. *Nat. Med.* **1999**, *5*, 78–82. [\[CrossRef\]](#)
58. Asokan, A.; Hamra, J.B.; Govindasamy, L.; Agbandje-McKenna, M.; Samulski, R.J.J. Adeno-associated virus type 2 contains an integrin $\alpha 5\beta 1$ binding domain essential for viral cell entry. *J. Virol.* **2006**, *80*, 8961–8969. [\[CrossRef\]](#)
59. Kashiwakura, Y.; Tamayose, K.; Iwabuchi, K.; Hirai, Y.; Shimada, T.; Matsumoto, K.; Nakamura, T.; Watanabe, M.; Oshimi, K.; Daida, H.J. Hepatocyte growth factor receptor is a coreceptor for adeno-associated virus type 2 infection. *J. Virol.* **2005**, *79*, 609–614. [\[CrossRef\]](#)
60. Akache, B.; Grimm, D.; Pandey, K.; Yant, S.R.; Xu, H.; Kay, M.A.J. The 37/67-kilodalton laminin receptor is a receptor for adeno-associated virus serotypes 8, 2, 3, and 9. *J. Virol.* **2006**, *80*, 9831–9836. [\[CrossRef\]](#)
61. Kurzeder, C.; Koppold, B.; Sauer, G.; Pabst, S.; Kreienberg, R.; Deissler, H.J. CD9 promotes adeno-associated virus type 2 infection of mammary carcinoma cells with low cell surface expression of heparan sulphate proteoglycans. *Int. J. Mol. Med.* **2007**, *19*, 325–333. [\[CrossRef\]](#)
62. Takeda, S.-i.; Takahashi, M.; Mizukami, H.; Kobayashi, E.; Takeuchi, K.; Hakamata, Y.; Kaneko, T.; Yamamoto, H.; Ito, C.; Ozawa, K.J. Successful gene transfer using adeno-associated virus vectors into the kidney: Comparison among adeno-associated virus serotype 1–5 vectors in vitro and in vivo. *Nephron Exp. Nephrol.* **2004**, *96*, e119–e126. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Qi, Y.F.; Li, Q.H.; Shenoy, V.; Zingler, M.; Jun, J.Y.; Verma, A.; Katovich, M.J.; Raizada, M.K. Comparison of the transduction efficiency of tyrosine-mutant adeno-associated virus serotype vectors in kidney. *Clin. Exp. Pharmacol. Physiol.* **2013**, *40*, 53–55. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Haddad, G.; Kölling, M.; Wegmann, U.A.; Dettling, A.; Seeger, H.; Schmitt, R.; Soerensen-Zender, I.; Haller, H.; Kistler, A.D.; Dueck, A.J. Renal AAV2-mediated overexpression of long non-coding RNA H19 attenuates ischemic acute kidney injury through sponging of microRNA-30a-5p. *J. Am. Soc. Nephrol.* **2021**, *32*, 323–341. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Logan, G.J.; Dane, A.P.; Hallwirth, C.V.; Smyth, C.M.; Wilkie, E.E.; Amaya, A.K.; Zhu, E.; Khandekar, N.; Ginn, S.L.; Liao, S.H. Identification of liver-specific enhancer–promoter activity in the 3′ untranslated region of the wild-type AAV2 genome. *Nat. Genet.* **2017**, *49*, 1267–1273. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Niemeyer, G.P.; Herzog, R.W.; Mount, J.; Arruda, V.R.; Tillson, D.M.; Hathcock, J.; van Ginkel, F.W.; High, K.A.; Lothrop, C.D., Jr. The Journal of the American Society of Hematology. Long-term correction of inhibitor-prone hemophilia B dogs treated with liver-directed AAV2-mediated factor IX gene therapy. *Blood J. Am. Soc. Hematol.* **2009**, *113*, 797–806.
67. Petrs-Silva, H.; Dinculescu, A.; Li, Q.; Deng, W.-T.; Pang, J.-j.; Min, S.-H.; Chiodo, V.; Neeley, A.W.; Govindasamy, L.; Bennett, A.; et al. Novel Properties of Tyrosine-mutant AAV2 Vectors in the Mouse Retina. *Mol. Ther.* **2011**, *19*, 293–301. [\[CrossRef\]](#)
68. Yin, L.; Greenberg, K.; Hunter, J.J.; Dalkara, D.; Kolstad, K.D.; Masella, B.D.; Wolfe, R.; Visel, M.; Stone, D.; Libby, R.T.; et al. Intravitreal injection of AAV2 transduces macaque inner retina. *Investig. Ophthalmol. Vis. Sci.* **2011**, *52*, 2775–2783. [\[CrossRef\]](#)
69. Wassmer, S.J.; Carvalho, L.S.; György, B.; Vandenbergh, L.H.; Maguire, C.A. Exosome-associated AAV2 vector mediates robust gene delivery into the murine retina upon intravitreal injection. *J. Sci. Rep.* **2017**, *7*, 45329. [\[CrossRef\]](#)
70. Griffey, M.; Bible, E.; Vogler, C.; Levy, B.; Gupta, P.; Cooper, J.; Sands, M.S. Adeno-associated virus 2-mediated gene therapy decreases autofluorescent storage material and increases brain mass in a murine model of infantile neuronal ceroid lipofuscinosis. *Neurobiol. Dis.* **2004**, *16*, 360–369. [\[CrossRef\]](#)

71. Griffey, M.A.; Wozniak, D.; Wong, M.; Bible, E.; Johnson, K.; Rothman, S.M.; Wentz, A.E.; Cooper, J.D.; Sands, M.S. CNS-directed AAV2-mediated gene therapy ameliorates functional deficits in a murine model of infantile neuronal ceroid lipofuscinosis. *Mol. Ther.* **2006**, *13*, 538–547. [\[CrossRef\]](#)
72. Pankajakshan, D.; Makinde, T.O.; Gaurav, R.; Del Core, M.; Hatzoudis, G.; Pipinos, I.; Agrawal, D.K. Successful Transfection of Genes Using AAV-2/9 Vector in Swine Coronary and Peripheral Arteries. *J. Surg. Res.* **2012**, *175*, 169–175. [\[CrossRef\]](#)
73. Rabinowitz, J.E.; Bowles, D.E.; Faust, S.M.; Ledford, J.G.; Cunningham, S.E.; Samulski, R.J. Cross-Dressing the Virion: The Transcapsidation of Adeno-Associated Virus Serotypes Functionally Defines Subgroups. *J. Virol.* **2004**, *78*, 4421–4432. [\[CrossRef\]](#)
74. Blackburn, S.D.; Steadman, R.A.; Johnson, F.B. Attachment of adeno-associated virus type 3H to fibroblast growth factor receptor 1. *Arch. Virol.* **2006**, *151*, 617–623. [\[CrossRef\]](#)
75. Ling, C.; Wang, Y.; Zhang, Y.; Ejigani, A.; Yin, Z.; Lu, Y.; Wang, L.; Wang, M.; Li, J.; Hu, Z.; et al. Selective in vivo targeting of human liver tumors by optimized AAV3 vectors in a murine xenograft model. *Hum. Gene Ther.* **2014**, *25*, 1023–1034. [\[CrossRef\]](#)
76. Ling, C.; Yin, Z.; Li, J.; Zhang, D.; Aslanidi, G.; Srivastava, A. Strategies to generate high-titer, high-potency recombinant AAV3 serotype vectors. *Mol. Ther. Methods Clin. Dev.* **2016**, *3*, 16029. [\[CrossRef\]](#)
77. Handa, A.; Muramatsu, S.I.; Qiu, J.; Mizukami, H.; Brown, K.E. Adeno-associated virus (AAV)-3-based vectors transduce haematopoietic cells not susceptible to transduction with AAV-2-based vectors. *J. Gen. Virol.* **2000**, *81*, 2077–2084. [\[CrossRef\]](#)
78. Cheng, B.; Ling, C.; Dai, Y.; Lu, Y.; Glushakova, L.G.; Gee, S.W.Y.; McGoogan, K.E.; Aslanidi, G.V.; Park, M.; Stacpoole, P.W.; et al. Development of optimized AAV3 serotype vectors: Mechanism of high-efficiency transduction of human liver cancer cells. *Gene Ther.* **2012**, *19*, 375–384. [\[CrossRef\]](#)
79. Muramatsu, S.; Mizukami, H.; Young, N.S.; Brown, K.E. Nucleotide sequencing and generation of an infectious clone of adeno-associated virus 3. *Virology* **1996**, *221*, 208–217. [\[CrossRef\]](#)
80. Li, S.; Ling, C.; Zhong, L.; Li, M.; Su, Q.; He, R.; Tang, Q.; Greiner, D.L.; Shultz, L.D.; Brehm, M.A.; et al. Efficient and Targeted Transduction of Nonhuman Primate Liver With Systemically Delivered Optimized AAV3B Vectors. *Mol. Ther.* **2015**, *23*, 1867–1876. [\[CrossRef\]](#)
81. Vercauteren, K.; Hoffman, B.E.; Zolotukhin, I.; Keeler, G.D.; Xiao, J.W.; Basner-Tschakarjan, E.; High, K.A.; Ertl, H.C.J.; Rice, C.M.; Srivastava, A.; et al. Superior In vivo Transduction of Human Hepatocytes Using Engineered AAV3 Capsid. *Mol. Ther.* **2016**, *24*, 1042–1049. [\[CrossRef\]](#)
82. Liu, Y.; Okada, T.; Sheykhosslami, K.; Shimazaki, K.; Nomoto, T.; Muramatsu, S.; Kanazawa, T.; Takeuchi, K.; Ajalli, R.; Mizukami, H.; et al. Specific and efficient transduction of Cochlear inner hair cells with recombinant adeno-associated virus type 3 vector. *Mol. Ther. J. Am. Soc. Gene Ther.* **2005**, *12*, 725–733. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Padron, E.; Bowman, V.; Kaludov, N.; Govindasamy, L.; Levy, H.; Nick, P.; McKenna, R.; Muzyczka, N.; Chiorini, J.A.; Baker, T.S.; et al. Structure of adeno-associated virus type 4. *J. Virol.* **2005**, *79*, 5047–5058. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Parks, W.P.; Boucher, D.W.; Melnick, J.L.; Taber, L.H.; Yow, M.D. Seroepidemiological and ecological studies of the adenovirus-associated satellite viruses. *Infect. Immun.* **1970**, *2*, 716–722. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Blacklow, N.R.; Hoggan, M.D.; Rowe, W.P. Serologic evidence for human infection with adenovirus-associated viruses. *J. Natl. Cancer Inst.* **1968**, *40*, 319–327. [\[PubMed\]](#)
86. Dreizin, R.S.; Zhuravel, T.F.; Tarasova, A.B.; Sobolev, S.G.; Kozlov, V.G. Experimental infection of green monkeys with adeno-associated virus. *Vopr. Virusol.* **1981**, *1*, 82–89.
87. Chiorini, J.A.; Yang, L.; Liu, Y.; Safer, B.; Kotin, R.M. Cloning of adeno-associated virus type 4 (AAV4) and generation of recombinant AAV4 particles. *J. Virol.* **1997**, *71*, 6823–6833. [\[CrossRef\]](#)
88. Kaludov, N.; Brown, K.E.; Walters, R.W.; Zabner, J.; Chiorini, J.A. Adeno-associated virus serotype 4 (AAV4) and AAV5 both require sialic acid binding for hemagglutination and efficient transduction but differ in sialic acid linkage specificity. *J. Virol.* **2001**, *75*, 6884–6893. [\[CrossRef\]](#)
89. Kaludov, N.; Handelman, B.; Chiorini, J.A. Scalable purification of adeno-associated virus type 2, 4, or 5 using ion-exchange chromatography. *Hum. Gene Ther.* **2002**, *13*, 1235–1243. [\[CrossRef\]](#)
90. Davidson, B.L.; Stein, C.S.; Heth, J.A.; Martins, I.; Kotin, R.M.; Derksen, T.A.; Zabner, J.; Ghodsi, A.; Chiorini, J.A. Recombinant adeno-associated virus type 2, 4, and 5 vectors: Transduction of variant cell types and regions in the mammalian central nervous system. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3428–3432. [\[CrossRef\]](#)
91. Weber, M.; Rabinowitz, J.; Provost, N.; Conrath, H.; Folliot, S.; Briot, D.; Chérel, Y.; Chenuaud, P.; Samulski, J.; Moullier, P.; et al. Recombinant adeno-associated virus serotype 4 mediates unique and exclusive long-term transduction of retinal pigmented epithelium in rat, dog, and nonhuman primate after subretinal delivery. *Mol. Ther. J. Am. Soc. Gene Ther.* **2003**, *7*, 774–781. [\[CrossRef\]](#)
92. Zincarelli, C.; Soltys, S.; Rengo, G.; Rabinowitz, J.E. Analysis of AAV serotypes 1–9 mediated gene expression and tropism in mice after systemic injection. *Mol. Ther.* **2008**, *16*, 1073–1080. [\[CrossRef\]](#)
93. Bantel-Schaal, U.; zur Hausen, H. Characterization of the DNA of a defective human parvovirus isolated from a genital site. *Virology* **1984**, *134*, 52–63. [\[CrossRef\]](#)
94. Stutika, C.; Hüser, D.; Weger, S.; Rutz, N.; Heßler, M.; Heilbronn, R. Definition of herpes simplex virus helper functions for the replication of adeno-associated virus type 5. *J. Gen. Virol.* **2015**, *96*, 840–850. [\[CrossRef\]](#)
95. Bantel-Schaal, U.; Delius, H.; Schmidt, R.; Hausen, H.Z. Human Adeno-Associated Virus Type 5 Is Only Distantly Related to Other Known Primate Helper-Dependent Parvoviruses. *J. Virol.* **1999**, *73*, 939–947. [\[CrossRef\]](#)

96. Chiorini, J.A.; Kim, F.; Yang, L.; Kotin, R.M. Cloning and characterization of adeno-associated virus type 5. *J. Virol.* **1999**, *73*, 1309–1319. [[CrossRef](#)]
97. Bantel-Schaal, U.; Hub, B.; Kartenbeck, J. Endocytosis of adeno-associated virus type 5 leads to accumulation of virus particles in the Golgi compartment. *J. Virol.* **2002**, *76*, 2340–2349. [[CrossRef](#)]
98. Zabner, J.; Seiler, M.; Walters, R.; Kotin, R.M.; Fulgeras, W.; Davidson, B.L.; Chiorini, J.A. Adeno-associated virus type 5 (AAV5) but not AAV2 binds to the apical surfaces of airway epithelia and facilitates gene transfer. *J. Virol.* **2000**, *74*, 3852–3858. [[CrossRef](#)]
99. Grimm, D.; Kay, M.A. From virus evolution to vector revolution: Use of naturally occurring serotypes of adeno-associated virus (AAV) as novel vectors for human gene therapy. *Curr. Gene Ther.* **2003**, *3*, 281–304. [[CrossRef](#)]
100. Walters, R.W.; Yi, S.M.; Keshavjee, S.; Brown, K.E.; Welsh, M.J.; Chiorini, J.A.; Zabner, J. Binding of adeno-associated virus type 5 to 2,3-linked sialic acid is required for gene transfer. *J. Biol. Chem.* **2001**, *276*, 20610–20616. [[CrossRef](#)]
101. Henckaerts, E.; Linden, R.M. Adeno-associated virus: A key to the human genome? *Future Virol.* **2010**, *5*, 555–574. [[CrossRef](#)]
102. Di Pasquale, G.; Davidson, B.L.; Stein, C.S.; Martins, I.; Scudiero, D.; Monks, A.; Chiorini, J.A. Identification of PDGFR as a receptor for AAV-5 transduction. *Nat. Med.* **2003**, *9*, 1306–1312. [[CrossRef](#)] [[PubMed](#)]
103. Nonnenmacher, M.; Weber, T. Intracellular transport of recombinant adeno-associated virus vectors. *Gene Ther.* **2012**, *19*, 649–658. [[CrossRef](#)] [[PubMed](#)]
104. Auricchio, A.; O'Connor, E.; Hildinger, M.; Wilson, J.M. A Single-Step Affinity Column for Purification of Serotype-5 Based Adeno-associated Viral Vectors. *Mol. Ther.* **2001**, *4*, 372–374. [[CrossRef](#)] [[PubMed](#)]
105. Walters, R.W.; Pilewski, J.M.; Chiorini, J.A.; Zabner, J. Secreted and Transmembrane Mucins Inhibit Gene Transfer with AAV4 More Efficiently than AAV5*. *J. Biol. Chem.* **2002**, *277*, 23709–23713. [[CrossRef](#)]
106. Yang, G.S.; Schmidt, M.; Yan, Z.; Lindbloom, J.D.; Harding, T.C.; Donahue, B.A.; Engelhardt, J.F.; Kotin, R.; Davidson, B.L. Virus-mediated transduction of murine retina with adeno-associated virus: Effects of viral capsid and genome size. *J. Virol.* **2002**, *76*, 7651–7660. [[CrossRef](#)]
107. Alisky, J.M.; Hughes, S.M.; Sauter, S.L.; Jolly, D.; Dubensky, T.W., Jr.; Staber, P.D.; Chiorini, J.A.; Davidson, B.L. Transduction of murine cerebellar neurons with recombinant FIV and AAV5 vectors. *Neuroreport* **2000**, *11*, 2669–2673. [[CrossRef](#)]
108. Burger, C.; Gorbatyuk, O.S.; Velardo, M.J.; Peden, C.S.; Williams, P.; Zolotukhin, S.; Reier, P.J.; Mandel, R.J.; Muzyczka, N. Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. *Mol. Ther. J. Am. Soc. Gene Ther.* **2004**, *10*, 302–317. [[CrossRef](#)]
109. Ding, W.; Yan, Z.; Zak, R.; Saavedra, M.; Rodman, D.M.; Engelhardt, J.F. Second-strand genome conversion of adeno-associated virus type 2 (AAV-2) and AAV-5 is not rate limiting following apical infection of polarized human airway epithelia. *J. Virol.* **2003**, *77*, 7361–7366. [[CrossRef](#)]
110. Auricchio, A.; O'Connor, E.; Weiner, D.; Gao, G.P.; Hildinger, M.; Wang, L.; Calcedo, R.; Wilson, J.M. Noninvasive gene transfer to the lung for systemic delivery of therapeutic proteins. *J. Clin. Investig.* **2002**, *110*, 499–504. [[CrossRef](#)]
111. Seiler, M.P.; Miller, A.D.; Zabner, J.; Halbert, C.L. Adeno-associated virus types 5 and 6 use distinct receptors for cell entry. *Hum. Gene Ther.* **2006**, *17*, 10–19. [[CrossRef](#)]
112. Pañeda, A.; Vanrell, L.; Mauleon, I.; Crettaz, J.S.; Berraondo, P.; Timmermans, E.J.; Beattie, S.G.; Twisk, J.; van Deventer, S.; Prieto, J.; et al. Effect of adeno-associated virus serotype and genomic structure on liver transduction and biodistribution in mice of both genders. *Hum. Gene Ther.* **2009**, *20*, 908–917. [[CrossRef](#)]
113. Xiao, W.; Chirmule, N.; Berta, S.C.; McCullough, B.; Gao, G.; Wilson, J.M. Gene Therapy Vectors Based on Adeno-Associated Virus Type 1. *J. Virol.* **1999**, *73*, 3994–4003. [[CrossRef](#)]
114. Rutledge, E.A.; Halbert, C.L.; Russell, D.W. Infectious Clones and Vectors Derived from Adeno-Associated Virus (AAV) Serotypes Other Than AAV Type 2. *J. Virol.* **1998**, *72*, 309–319. [[CrossRef](#)]
115. Korneyenkov, M.A.; Zamyatnin, A.A., Jr. Next Step in Gene Delivery: Modern Approaches and Further Perspectives of AAV Tropism Modification. *Pharmaceutics* **2021**, *13*, 750. [[CrossRef](#)]
116. Ng, R.; Govindasamy, L.; Gurda, B.L.; McKenna, R.; Kozyreva, O.G.; Samulski, R.J.; Parent, K.N.; Baker, T.S.; Agbandje-McKenna, M. Structural characterization of the dual glycan binding adeno-associated virus serotype 6. *J. Virol.* **2010**, *84*, 12945–12957. [[CrossRef](#)]
117. Wu, Z.; Asokan, A.; Grieger, J.C.; Govindasamy, L.; Agbandje-McKenna, M.; Samulski, R.J. Single amino acid changes can influence titer, heparin binding, and tissue tropism in different adeno-associated virus serotypes. *J. Virol.* **2006**, *80*, 11393–11397. [[CrossRef](#)]
118. Weller, M.L.; Amornphimoltham, P.; Schmidt, M.; Wilson, P.A.; Gutkind, J.S.; Chiorini, J.A. Epidermal growth factor receptor is a co-receptor for adeno-associated virus serotype 6. *Nat. Med.* **2010**, *16*, 662–664. [[CrossRef](#)]
119. Halbert, C.L.; Allen, J.M.; Miller, A.D. Adeno-associated virus type 6 (AAV6) vectors mediate efficient transduction of airway epithelial cells in mouse lungs compared to that of AAV2 vectors. *J. Virol.* **2001**, *75*, 6615–6624. [[CrossRef](#)]
120. Halbert, C.L.; Madtes, D.K.; Vaughan, A.E.; Wang, Z.; Storb, R.; Tapscott, S.J.; Miller, A.D. Expression of human alpha1-antitrypsin in mice and dogs following AAV6 vector-mediated gene transfer to the lungs. *Mol. Ther. J. Am. Soc. Gene Ther.* **2010**, *18*, 1165–1172. [[CrossRef](#)]

121. Grimm, D.; Zhou, S.; Nakai, H.; Thomas, C.E.; Storm, T.A.; Fuess, S.; Matsushita, T.; Allen, J.; Surosky, R.; Lochrie, M.; et al. Preclinical in vivo evaluation of pseudotyped adeno-associated virus vectors for liver gene therapy. *Blood* **2003**, *102*, 2412–2419. [\[CrossRef\]](#)
122. Blankinship, M.J.; Gregorevic, P.; Allen, J.M.; Harper, S.Q.; Harper, H.; Halbert, C.L.; Miller, A.D.; Chamberlain, J.S. Efficient transduction of skeletal muscle using vectors based on adeno-associated virus serotype 6. *Mol. Ther. J. Am. Soc. Gene Ther.* **2004**, *10*, 671–678. [\[CrossRef\]](#)
123. Wang, Z.; Kuhr, C.S.; Allen, J.M.; Blankinship, M.; Gregorevic, P.; Chamberlain, J.S.; Tapscott, S.J.; Storb, R. Sustained AAV-mediated dystrophin expression in a canine model of Duchenne muscular dystrophy with a brief course of immunosuppression. *Mol. Ther. J. Am. Soc. Gene Ther.* **2007**, *15*, 1160–1166. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Qiao, C.; Zhang, W.; Yuan, Z.; Shin, J.H.; Li, J.; Jayandharan, G.R.; Zhong, L.; Srivastava, A.; Xiao, X.; Duan, D. Adeno-associated virus serotype 6 capsid tyrosine-to-phenylalanine mutations improve gene transfer to skeletal muscle. *Hum. Gene Ther.* **2010**, *21*, 1343–1348. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Zincarelli, C.; Soltys, S.; Rengo, G.; Koch, W.J.; Rabinowitz, J.E. 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.* **2010**, *3*, 81–89. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Raake, P.W.; Hinkel, R.; Müller, S.; Delker, S.; Kreuzpointner, R.; Kupatt, C.; Katus, H.A.; Kleinschmidt, J.A.; Boekstegers, P.; Müller, O.J. Cardio-specific long-term gene expression in a porcine model after selective pressure-regulated retroinfusion of adeno-associated viral (AAV) vectors. *Gene Ther.* **2008**, *15*, 12–17. [\[CrossRef\]](#)
127. Bish, L.T.; Sleeper, M.M.; Reynolds, C.; Gazzara, J.; Withnall, E.; Singletary, G.E.; Buchlis, G.; Hui, D.; High, K.A.; Gao, G.; et al. Cardiac gene transfer of short hairpin RNA directed against phospholamban effectively knocks down gene expression but causes cellular toxicity in canines. *Hum. Gene Ther.* **2011**, *22*, 969–977. [\[CrossRef\]](#)
128. White, J.D.; Thesier, D.M.; Swain, J.B.; Katz, M.G.; Tomasulo, C.; Henderson, A.; Wang, L.; Yarnall, C.; Fargnoli, A.; Sumaroka, M.; et al. Myocardial gene delivery using molecular cardiac surgery with recombinant adeno-associated virus vectors in vivo. *Gene Ther.* **2011**, *18*, 546–552. [\[CrossRef\]](#)
129. Gao, G.; Vandenberghe, L.H.; Alvira, M.R.; Lu, Y.; Calcedo, R.; Zhou, X.; Wilson, J.M. Clades of Adeno-associated viruses are widely disseminated in human tissues. *J. Virol.* **2004**, *78*, 6381–6388. [\[CrossRef\]](#)
130. Denby, L.; Nicklin, S.A.; Baker, A.H. Adeno-associated virus (AAV)-7 and -8 poorly transduce vascular endothelial cells and are sensitive to proteasomal degradation. *Gene Ther.* **2005**, *12*, 1534–1538. [\[CrossRef\]](#)
131. Mietzsch, M.; Broecker, F.; Reinhardt, A.; Seeberger, P.H.; Heilbronn, R. Differential Adeno-Associated Virus Serotype-Specific Interaction Patterns with Synthetic Heparins and Other Glycans. *J. Virol.* **2014**, *88*, 2991–3003. [\[CrossRef\]](#)
132. Calcedo, R.; Vandenberghe, L.H.; Gao, G.; Lin, J.; Wilson, J.M. Worldwide Epidemiology of Neutralizing Antibodies to Adeno-Associated Viruses. *J. Infect. Dis.* **2009**, *199*, 381–390. [\[CrossRef\]](#)
133. Louboutin, J.-P.; Wang, L.; Wilson, J.M. Gene transfer into skeletal muscle using novel AAV serotypes. *J. Gene Med. A Cross-Discip. J. Res. Sci. Gene Transf. Clin. Appl.* **2005**, *7*, 442–451. [\[CrossRef\]](#)
134. Samaranch, L.; Salegio, E.A.; San Sebastian, W.; Kells, A.P.; Bringas, J.R.; Forsayeth, J.; Bankiewicz, K.S. Strong cortical and spinal cord transduction after AAV7 and AAV9 delivery into the cerebrospinal fluid of nonhuman primates. *Hum. Gene Ther.* **2013**, *24*, 526–532. [\[CrossRef\]](#)
135. Taymans, J.M.; Vandenberghe, L.H.; Haute, C.V.; Thiry, I.; Deroose, C.M.; Mortelmans, L.; Wilson, J.M.; Debyser, Z.; Baekelandt, V. Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain. *Hum. Gene Ther.* **2007**, *18*, 195–206. [\[CrossRef\]](#)
136. Allocca, M.; Mussolino, C.; Garcia-Hoyos, M.; Sanges, D.; Iodice, C.; Petrillo, M.; Vandenberghe, L.H.; Wilson, J.M.; Marigo, V.; Surace, E.M.; et al. Novel adeno-associated virus serotypes efficiently transduce murine photoreceptors. *J. Virol.* **2007**, *81*, 11372–11380. [\[CrossRef\]](#)
137. Okada, T.; Nonaka-Sarukawa, M.; Uchibori, R.; Kinoshita, K.; Hayashita-Kinoh, H.; Nitahara-Kasahara, Y.; Takeda, S.i.; Ozawa, K. Scalable purification of adeno-associated virus serotype 1 (AAV1) and AAV8 vectors, using dual ion-exchange adsorptive membranes. *Hum. Gene Ther.* **2009**, *20*, 1013–1021. [\[CrossRef\]](#)
138. Davidoff, A.M.; Ng, C.Y.; Sleep, S.; Gray, J.; Azam, S.; Zhao, Y.; McIntosh, J.H.; Karimipoor, M.; Nathwani, A.C. Purification of recombinant adeno-associated virus type 8 vectors by ion exchange chromatography generates clinical grade vector stock. *J. Virol. Methods* **2004**, *121*, 209–215. [\[CrossRef\]](#)
139. Lane, M.D.; Nam, H.-J.; Padron, E.; Gurda-Whitaker, B.; Kohlbrenner, E.; Aslanidi, G.; Byrne, B.; McKenna, R.; Muzyczka, N.; Zolotukhin, S.; et al. Production, purification, crystallization and preliminary X-ray analysis of adeno-associated virus serotype 8. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2005**, *61*, 558–561. [\[CrossRef\]](#)
140. Miyake, K.; Miyake, N.; Yamazaki, Y.; Shimada, T.; Hirai, Y.J. Serotype-independent method of recombinant adeno-associated virus (AAV) vector production and purification. *J. Nippon. Med. Sch.* **2012**, *79*, 394–402. [\[CrossRef\]](#)
141. Nam, H.-J.; Lane, M.D.; Padron, E.; Gurda, B.; McKenna, R.; Kohlbrenner, E.; Aslanidi, G.; Byrne, B.; Muzyczka, N.; Zolotukhin, S.; et al. Structure of Adeno-Associated Virus Serotype 8, a Gene Therapy Vector. *J. Virol.* **2007**, *81*, 12260–12271. [\[CrossRef\]](#)
142. Cabanes-Creus, M.; Navarro, R.G.; Zhu, E.; Baltazar, G.; Liao, S.H.Y.; Drouyer, M.; Amaya, A.K.; Scott, S.; Nguyen, L.H.; Westhaus, A.; et al. Novel human liver-tropic AAV variants define transferable domains that markedly enhance the human tropism of AAV7 and AAV8. *Mol. Ther. Methods Clin. Dev.* **2022**, *24*, 88–101. [\[CrossRef\]](#) [\[PubMed\]](#)

143. Nakai, H.; Fuess, S.; Storm, T.A.; Muramatsu, S.; Nara, Y.; Kay, M.A. Unrestricted hepatocyte transduction with adeno-associated virus serotype 8 vectors in mice. *J. Virol.* **2005**, *79*, 214–224. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Thomas, C.E.; Storm, T.A.; Huang, Z.; Kay, M.A. Rapid uncoating of vector genomes is the key to efficient liver transduction with pseudotyped adeno-associated virus vectors. *J. Virol.* **2004**, *78*, 3110–3122. [\[CrossRef\]](#) [\[PubMed\]](#)
145. Nam, H.-J.; Gurda, B.L.; McKenna, R.; Potter, M.; Byrne, B.; Salganik, M.; Muzyczka, N.; Agbandje-McKenna, M. Structural Studies of Adeno-Associated Virus Serotype 8 Capsid Transitions Associated with Endosomal Trafficking. *J. Virol.* **2011**, *85*, 11791–11799. [\[CrossRef\]](#)
146. Monahan, P.E.; Lothrop, C.D.; Sun, J.; Hirsch, M.L.; Kafri, T.; Kantor, B.; Sarkar, R.; Tillson, D.M.; Elia, J.R.; Samulski, R.J. Proteasome inhibitors enhance gene delivery by AAV virus vectors expressing large genomes in hemophilia mouse and dog models: A strategy for broad clinical application. *Mol. Ther. J. Am. Soc. Gene Ther.* **2010**, *18*, 1907–1916. [\[CrossRef\]](#)
147. Jiang, H.; Couto, L.B.; Patarroyo-White, S.; Liu, T.; Nagy, D.; Vargas, J.A.; Zhou, S.; Scallan, C.D.; Sommer, J.; Vijay, S.; et al. Effects of transient immunosuppression on adenoassociated, virus-mediated, liver-directed gene transfer in rhesus macaques and implications for human gene therapy. *Blood* **2006**, *108*, 3321–3328. [\[CrossRef\]](#)
148. Wang, Z.; Zhu, T.; Qiao, C.; Zhou, L.; Wang, B.; Zhang, J.; Chen, C.; Li, J.; Xiao, X. Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart. *Nat. Biotechnol.* **2005**, *23*, 321–328. [\[CrossRef\]](#)
149. Wang, A.Y.; Peng, P.D.; Ehrhardt, A.; Storm, T.A.; Kay, M.A. Comparison of adenoviral and adeno-associated viral vectors for pancreatic gene delivery in vivo. *Hum. Gene Ther.* **2004**, *15*, 405–413. [\[CrossRef\]](#)
150. Loiler, S.A.; Tang, Q.; Clarke, T.; Campbell-Thompson, M.L.; Chiodo, V.; Hauswirth, W.; Cruz, P.; Perret-Gentil, M.; Atkinson, M.A.; Ramiya, V.K.; et al. Localized gene expression following administration of adeno-associated viral vectors via pancreatic ducts. *Mol. Ther. J. Am. Soc. Gene Ther.* **2005**, *12*, 519–527. [\[CrossRef\]](#)
151. Rubin, J.D.; Nguyen, T.V.; Allen, K.L.; Ayasoufi, K.; Barry, M.A. Comparison of gene delivery to the kidney by adenovirus, adeno-associated virus, and lentiviral vectors after intravenous and direct kidney injections. *Hum. Gene Ther.* **2019**, *30*, 1559–1571. [\[CrossRef\]](#)
152. Giove, T.J.; Khankhel, Z.S.; Sena-Esteves, M.; Eldred, W.D. Cellular Tropism of Aav8, Aav9 and Aav10 in Mouse Retina. *Investig. Ophthalmol. Vis. Sci.* **2009**, *50*, 3013.
153. Ding, K.; Shen, J.; Hafiz, Z.; Hackett, S.F.; Silva, R.L.E.; Khan, M.; Lorenc, V.E.; Chen, D.; Chadha, R.; Zhang, M.; et al. AAV8-vectored suprachoroidal gene transfer produces widespread ocular transgene expression. *J. Clin. Investig.* **2019**, *129*, 4901–4911. [\[CrossRef\]](#)
154. Igarashi, T.; Miyake, K.; Asakawa, N.; Miyake, N.; Shimada, T.; Takahashi, H. Direct Comparison of Administration Routes for AAV8-mediated Ocular Gene Therapy. *Curr. Eye Res.* **2013**, *38*, 569–577. [\[CrossRef\]](#)
155. Gao, G.; Vandenberghe, L.H.; Wilson, J.M. New recombinant serotypes of AAV vectors. *Curr. Gene Ther.* **2005**, *5*, 285–297. [\[CrossRef\]](#)
156. Shen, S.; Bryant, K.D.; Brown, S.M.; Randell, S.H.; Asokan, A. Terminal N-Linked Galactose Is the Primary Receptor for Adeno-associated Virus 9*. *J. Biol. Chem.* **2011**, *286*, 13532–13540. [\[CrossRef\]](#)
157. Bell, C.L.; Gurda, B.L.; Van Vliet, K.; Agbandje-McKenna, M.; Wilson, J.M. Identification of the galactose binding domain of the adeno-associated virus serotype 9 capsid. *J. Virol.* **2012**, *86*, 7326–7333. [\[CrossRef\]](#)
158. Mitchell, M.; Nam, H.-J.; Carter, A.; McCall, A.; Rence, C.; Bennett, A.; Gurda, B.; McKenna, R.; Porter, M.; Sakai, Y.; et al. Production, purification and preliminary X-ray crystallographic studies of adeno-associated virus serotype 9. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2009**, *65*, 715–718. [\[CrossRef\]](#)
159. Foust, K.D.; Nurre, E.; Montgomery, C.L.; Hernandez, A.; Chan, C.M.; Kaspar, B.K. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat. Biotechnol.* **2009**, *27*, 59–65. [\[CrossRef\]](#)
160. Gray, S.J.; Matagne, V.; Bachaboina, L.; Yadav, S.; Ojeda, S.R.; Samulski, R.J. Preclinical Differences of Intravascular AAV9 Delivery to Neurons and Glia: A Comparative Study of Adult Mice and Nonhuman Primates. *Mol. Ther.* **2011**, *19*, 1058–1069. [\[CrossRef\]](#)
161. Bevan, A.K.; Duque, S.; Foust, K.D.; Morales, P.R.; Braun, L.; Schmelzer, L.; Chan, C.M.; McCrate, M.; Chicoine, L.G.; Coley, B.D.; et al. Systemic Gene Delivery in Large Species for Targeting Spinal Cord, Brain, and Peripheral Tissues for Pediatric Disorders. *Mol. Ther.* **2011**, *19*, 1971–1980. [\[CrossRef\]](#)
162. Duque, S.; Jousset, B.; Riviere, C.; Marais, T.; Dubreil, L.; Douar, A.M.; Fyfe, J.; Moullier, P.; Colle, M.A.; Barkats, M. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. *Mol. Ther. J. Am. Soc. Gene Ther.* **2009**, *17*, 1187–1196. [\[CrossRef\]](#)
163. Vandenberghe, L.H.; Bell, P.; Maguire, A.M.; Xiao, R.; Hopkins, T.B.; Grant, R.; Bennett, J.; Wilson, J.M.; Xiao, X. AAV9 targets cone photoreceptors in the nonhuman primate retina. *PLoS ONE* **2013**, *8*, e53463. [\[CrossRef\]](#)
164. Pacak, C.A.; Mah, C.S.; Thattaliyath, B.D.; Conlon, T.J.; Lewis, M.A.; Cloutier, D.E.; Zolotukhin, I.; Tarantal, A.F.; Byrne, B.J. Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction in vivo. *Circ. Res.* **2006**, *99*, e3–e9. [\[CrossRef\]](#) [\[PubMed\]](#)
165. Asokan, A.; Schaffer, D.V.; Samulski, R.J. The AAV vector toolkit: Poised at the clinical crossroads. *Mol. Ther.* **2012**, *20*, 699–708. [\[CrossRef\]](#) [\[PubMed\]](#)
166. Pleger, S.T.; Shan, C.; Ksienzyk, J.; Bekeredjian, R.; Boekstegers, P.; Hinkel, R.; Schinkel, S.; Leuchs, B.; Ludwig, J.; Qiu, G. Cardiac AAV9-S100A1 gene therapy rescues post-ischemic heart failure in a preclinical large animal model. *Sci. Transl. Med.* **2011**, *3*, 92ra64. [\[CrossRef\]](#)

167. Bish, L.T.; Morine, K.; Sleeper, M.M.; Sanmiguel, J.; Wu, D.; Gao, G.; Wilson, J.M.; Sweeney, H.L. 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.* **2008**, *19*, 1359–1368. [\[CrossRef\]](#)
168. Vandendriessche, T.; Thorrez, L.; Acosta-Sanchez, A.; Petrus, I.; Wang, L.; Ma, L.; De Waele, L.; Iwasaki, Y.; Gillijns, V.; Wilson, J.M.; et al. Efficacy and safety of adeno-associated viral vectors based on serotype 8 and 9 vs. lentiviral vectors for hemophilia B gene therapy. *J. Thromb. Haemost. JTH* **2007**, *5*, 16–24. [\[CrossRef\]](#)
169. Inagaki, K.; Fuess, S.; Storm, T.A.; Gibson, G.A.; McTiernan, C.F.; Kay, M.A.; Nakai, H. Robust systemic transduction with AAV9 vectors in mice: Efficient global cardiac gene transfer superior to that of AAV8. *Mol. Ther. J. Am. Soc. Gene Ther.* **2006**, *14*, 45–53. [\[CrossRef\]](#)
170. Petrs-Silva, H.; Dinculescu, A.; Li, Q.; Min, S.-H.; Chiodo, V.; Pang, J.-J.; Zhong, L.; Zolotukhin, S.; Srivastava, A.; Lewin, A.S.; et al. High-efficiency Transduction of the Mouse Retina by Tyrosine-mutant AAV Serotype Vectors. *Mol. Ther.* **2009**, *17*, 463–471. [\[CrossRef\]](#)
171. Shen, X.; Xu, Y.; Bai, Z.; Ma, D.; Niu, Q.; Meng, J.; Fan, S.; Zhang, L.; Hao, Z.; Zhang, X.J. Transparenchymal renal pelvis injection of recombinant adeno-associated virus serotype 9 vectors is a practical approach for gene delivery in the kidney. *Hum. Gene Ther. Methods* **2018**, *29*, 251–258. [\[CrossRef\]](#)
172. Konkalmatt, P.; Asico, L.; Feranil, J.; Jose, P.; Armando, I. Efficient in vivo gene transfer to murine renal cells using AAV9 vectors (912.7). *FASEB J.* **2014**, *28*, 917. [\[CrossRef\]](#)
173. Schievenbusch, S.; Strack, I.; Scheffler, M.; Nischt, R.; Coutelle, O.; Hösel, M.; Hallek, M.; Fries, J.W.U.; Dienes, H.-P.; Odenthal, M.; et al. Combined Paracrine and Endocrine AAV9 mediated Expression of Hepatocyte Growth Factor for the Treatment of Renal Fibrosis. *Mol. Ther.* **2010**, *18*, 1302–1309. [\[CrossRef\]](#)
174. Darbey, A.; Rebouret, D.; Curley, M.; Kilcoyne, K.; Jeffery, N.; Reed, N.; Milne, L.; Roesl, C.; Brown, P.; Smith, L.B. A comparison of in vivo viral targeting systems identifies adeno-associated virus serotype 9 (AAV9) as an effective vector for genetic manipulation of Leydig cells in adult mice. *Andrology* **2021**, *9*, 460–473. [\[CrossRef\]](#)
175. Limberis, M.P.; Wilson, J.M. Adeno-associated virus serotype 9 vectors transduce murine alveolar and nasal epithelia and can be readministered. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12993–12998. [\[CrossRef\]](#)
176. Bell, C.L.; Vandenberghe, L.H.; Bell, P.; Limberis, M.P.; Gao, G.-P.; Van Vliet, K.; Agbandje-McKenna, M.; Wilson, J.M. The AAV9 receptor and its modification to improve in vivo lung gene transfer in mice. *J. Clin. Invest.* **2011**, *121*, 2427–2435. [\[CrossRef\]](#)
177. Vance, M.A.; Mitchell, A.; Samulski, R.J. AAV Biology, Infectivity and Therapeutic Use from Bench to Clinic. In *Gene Therapy—Principles and Challenges*; IntechOpen: London, UK, 2015. [\[CrossRef\]](#)
178. Vandenberghe, L.H.; Miller, J.R.; Gao, G.-P.; Calcedo, R.; Wilson, J.M. 116. The Proposed AAV Serotypes 10 and 11 Serologically Cross-React with AAV8 and AAV4. *Mol. Ther.* **2006**, *13*, S47. [\[CrossRef\]](#)
179. Klein, R.L.; Dayton, R.D.; Tatom, J.B.; Henderson, K.M.; Henning, P.P. AAV8, 9, Rh10, Rh43 Vector Gene Transfer in the Rat Brain: Effects of Serotype, Promoter and Purification Method. *Mol. Ther.* **2008**, *16*, 89–96. [\[CrossRef\]](#)
180. Mori, S.; Takeuchi, T.; Enomoto, Y.; Kondo, K.; Sato, K.; Ono, F.; Sata, T.; Kanda, T. Tissue distribution of cynomolgus adeno-associated viruses AAV10, AAV11, and AAVcy.7 in naturally infected monkeys. *Arch. Virol.* **2008**, *153*, 375–380. [\[CrossRef\]](#)
181. Polyak, S.; Mach, A.; Porvasnik, S.; Dixon, L.; Conlon, T.; Erger, K.E.; Acosta, A.; Wright, A.J.; Campbell-Thompson, M.; Zolotukhin, I.; et al. Identification of adeno-associated viral vectors suitable for intestinal gene delivery and modulation of experimental colitis. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **2012**, *302*, G296–G308. [\[CrossRef\]](#)
182. Singh, M.K.; Uehara, H.; Zhang, X.; Vashist, D.; Carroll, L.; Archer, B.; Huang, Y.; Ambati, B. Systemic Distribution of AAV10.COMPAng1 in the Ins2Akita mouse. *Investig. Ophthalmol. Vis. Sci.* **2016**, *57*, 5056.
183. Tian, M.; Carroll, L.S.; Tang, L.; Uehara, H.; Westenfelder, C.; Ambati, B.K.; Huang, Y. Systemic AAV10. COMP-Ang1 rescues renal glomeruli and pancreatic islets in type 2 diabetic mice. *BMJ Open Diabetes Res. Care* **2020**, *8*, e000882. [\[CrossRef\]](#) [\[PubMed\]](#)
184. Han, Z.; Luo, N.; Kou, J.; Li, L.; Ma, W.; Peng, S.; Xu, Z.; Zhang, W.; Qiu, Y.; Wu, Y. AAV11 permits efficient retrograde targeting of projection neurons. *bioRxiv* **2022**. [\[CrossRef\]](#)
185. Schmidt, M.; Voutetakis, A.; Afione, S.; Zheng, C.; Mandikian, D.; Chiorini, J.A. Adeno-Associated Virus Type 12 (AAV12): A Novel AAV Serotype with Sialic Acid- and Heparan Sulfate Proteoglycan-Independent Transduction Activity. *J. Virol.* **2008**, *82*, 1399–1406. [\[CrossRef\]](#) [\[PubMed\]](#)
186. Schmidt, M.; Voutetakis, A.; Afione, S.; Zheng, C.; Chiorini, J.A. 745. AAV12, Isolated from Vervet Monkey, Has Unique Tropism and Biological as Well as Neutralization Properties. *Mol. Ther.* **2006**, *13*, S288. [\[CrossRef\]](#)
187. Quinn, K.; Quirion, M.R.; Lo, C.-Y.; Misplon, J.A.; Epstein, S.L.; Chiorini, J.A. Intranasal Administration of Adeno-associated Virus Type 12 (AAV12) Leads to Transduction of the Nasal Epithelia and Can Initiate Transgene-specific Immune Response. *Mol. Ther.* **2011**, *19*, 1990–1998. [\[CrossRef\]](#)
188. Mietzsch, M.; Jose, A.; Chipman, P.; Bhattacharya, N.; Daneshparvar, N.; McKenna, R.; Agbandje-McKenna, M. Completion of the AAV Structural Atlas: Serotype Capsid Structures Reveals Clade-Specific Features. *Viruses* **2021**, *13*, 101. [\[CrossRef\]](#)
189. Schmidt, M.; Govindasamy, L.; Afione, S.; Kaludov, N.; Agbandje-McKenna, M.; Chiorini, J.A. Molecular characterization of the heparin-dependent transduction domain on the capsid of a novel adeno-associated virus isolate, AAV(VR-942). *J. Virol.* **2008**, *82*, 8911–8916. [\[CrossRef\]](#)
190. Huang, L.-Y.; Halder, S.; Agbandje-McKenna, M. Parvovirus glycan interactions. *Curr. Opin. Virol.* **2014**, *7*, 108–118. [\[CrossRef\]](#)

191. Srivastava, A.J.C.; Insights, G.T. Advances and challenges in the use of recombinant AAV vectors for human gene therapy. *Cell Gene Ther. Insights* **2016**, *2*, 553–575. [\[CrossRef\]](#)
192. Muzyczka, N.; Warrington, K.H., Jr. Custom adeno-associated virus capsids: The next generation of recombinant vectors with novel tropism. *Hum. Gene Ther.* **2005**, *16*, 408–416. [\[CrossRef\]](#)
193. Zhang, L.; Rossi, A.; Lange, L.; Meumann, N.; Koitzsch, U.; Christie, K.; Nesbit, M.A.; Moore, C.B.T.; Hacker, U.T.; Morgan, M.; et al. Capsid Engineering Overcomes Barriers Toward Adeno-Associated Virus Vector-Mediated Transduction of Endothelial Cells. *Hum. Gene Ther.* **2019**, *30*, 1284–1296. [\[CrossRef\]](#)
194. Ward, P.; Walsh, C.E. Chimeric AAV Cap sequences alter gene transduction. *Virology* **2009**, *386*, 237–248. [\[CrossRef\]](#)
195. Yang, L.; Jiang, J.; Drouin, L.M.; Agbandje-McKenna, M.; Chen, C.; Qiao, C.; Pu, D.; Hu, X.; Wang, D.Z.; Li, J.; et al. A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3946–3951. [\[CrossRef\]](#)
196. White, A.F.; Mazur, M.; Sorscher, E.J.; Zinn, K.R.; Ponnazhagan, S. Genetic modification of adeno-associated viral vector type 2 capsid enhances gene transfer efficiency in polarized human airway epithelial cells. *Hum. Gene Ther.* **2008**, *19*, 1407–1414. [\[CrossRef\]](#)
197. Zhang, C.; Freistaedter, A.; Schmelas, C.; Gunkel, M.; Dao Thi, V.L.; Grimm, D. An RNA Interference/Adeno-Associated Virus Vector-Based Combinatorial Gene Therapy Approach Against Hepatitis E Virus. *Hepatol. Commun.* **2022**, *6*, 878–888. [\[CrossRef\]](#)
198. Van Vliet, K.M.; Blouin, V.; Brument, N.; Agbandje-McKenna, M.; Snyder, R.O. The role of the adeno-associated virus capsid in gene transfer. *Methods Mol. Biol.* **2008**, *437*, 51–91. [\[CrossRef\]](#)
199. Perabo, L.; Endell, J.; King, S.; Lux, K.; Goldnau, D.; Hallek, M.; Büning, H. Combinatorial engineering of a gene therapy vector: Directed evolution of adeno-associated virus. *J. Gene Med.* **2006**, *8*, 155–162. [\[CrossRef\]](#)
200. Maheshri, N.; Koerber, J.T.; Kaspar, B.K.; Schaffer, D.V. Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nat. Biotechnol.* **2006**, *24*, 198–204. [\[CrossRef\]](#)
201. Xiao, X.; deVlaminck, W.; Monahan, J. Adeno-associated virus (AAV) vectors for gene transfer. *Adv. Drug Deliv. Rev.* **1993**, *12*, 201–215. [\[CrossRef\]](#)
202. Han, Z.; Zhong, L.; Maina, N.; Hu, Z.; Li, X.; Chouthai, N.S.; Bischof, D.; Weigel-Van Aken, K.A.; Slayton, W.B.; Yoder, M.C. Stable integration of recombinant adeno-associated virus vector genomes after transduction of murine hematopoietic stem cells. *Hum. Gene Ther.* **2008**, *19*, 267–278. [\[CrossRef\]](#)
203. Tratschin, J.-D.; Miller, I.; Smith, M.; Carter, B.J.M.; Biology, C. Adeno-associated virus vector for high-frequency integration, expression, and rescue of genes in mammalian cells. *Mol. Cell. Biol.* **1985**, *5*, 3251–3260. [\[PubMed\]](#)
204. Samulski, R.; Zhu, X.; Xiao, X.; Brook, J.; Housman, D.; Epstein, N.A.; Hunter, L. Targeted integration of adeno-associated virus (AAV) into human chromosome 19. *EMBO J.* **1991**, *10*, 3941–3950. [\[CrossRef\]](#)
205. McCarty, D.M.; Young, S.M., Jr.; Samulski, R.J. Integration of adeno-associated virus (AAV) and recombinant AAV vectors. *Annu. Rev. Genet.* **2004**, *38*, 819–845. [\[CrossRef\]](#) [\[PubMed\]](#)
206. Walsh, C.E.; Liu, J.M.; Xiao, X.; Young, N.S.; Nienhuis, A.W.; Samulski, R.J. Regulated high level expression of a human gamma-globin gene introduced into erythroid cells by an adeno-associated virus vector. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7257–7261. [\[CrossRef\]](#) [\[PubMed\]](#)
207. Winocour, E.; Callahan, M.; Huberman, E. Perturbation of the cell cycle by adeno-associated virus. *Virology* **1988**, *167*, 393–399. [\[CrossRef\]](#)
208. Bantel-Schaal, U.; Stöhr, M.J. Influence of adeno-associated virus on adherence and growth properties of normal cells. *J. Virol.* **1992**, *66*, 773–779. [\[CrossRef\]](#)
209. Samulski, R.J. Adeno-associated virus: Integration at a specific chromosomal locus. *Curr. Opin. Genet. Dev.* **1993**, *3*, 74–80. [\[CrossRef\]](#)
210. Bijlani, S.; Pang, K.M.; Sivanandam, V.; Singh, A.; Chatterjee, S. The Role of Recombinant AAV in Precise Genome Editing. *Front. Genome Ed.* **2021**, *3*, 799722. [\[CrossRef\]](#)
211. Wang, D.; Tai, P.W.; Gao, G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat. Rev. Drug Discov.* **2019**, *18*, 358–378. [\[CrossRef\]](#)
212. Calcedo, R.; Wilson, J.M. Humoral immune response to AAV. *Front. Immunol.* **2013**, *4*, 341. [\[CrossRef\]](#)
213. Hirsch, M.L.; Samulski, R.J. AAV-mediated gene editing via double-strand break repair. In *Gene Correction*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 291–307.
214. Gray, S.J.; Nagabhushan Kalburgi, S.; McCown, T.J.; Jude Samulski, R. Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates. *Gene Ther.* **2013**, *20*, 450–459. [\[CrossRef\]](#)
215. Ge, L.-J.; Yang, F.-H.; Li, W.; Wang, T.; Lin, Y.; Feng, J.; Chen, N.-H.; Jiang, M.; Wang, J.-H.; Hu, X.-T. In vivo neuroregeneration to treat ischemic stroke through NeuroD1 AAV-based gene therapy in adult non-human primates. *Front. Cell Dev. Biol.* **2020**, *8*, 590008. [\[CrossRef\]](#)
216. Hu, C.; Lipshutz, G.S. AAV-based neonatal gene therapy for hemophilia A: Long-term correction and avoidance of immune responses in mice. *Gene Ther.* **2012**, *19*, 1166–1176. [\[CrossRef\]](#)
217. Suzuki, J.; Hashimoto, K.; Xiao, R.; Vandenberghe, L.H.; Liberman, M.C. Cochlear gene therapy with ancestral AAV in adult mice: Complete transduction of inner hair cells without cochlear dysfunction. *Sci. Rep.* **2017**, *7*, 45524. [\[CrossRef\]](#)

218. Akil, O.; Dyka, F.; Calvet, C.; Emptoz, A.; Lahlou, G.; Nouaille, S.; Boutet de Monvel, J.; Hardelin, J.-P.; Hauswirth, W.W.; Avan, P. Dual AAV-mediated gene therapy restores hearing in a DFNB9 mouse model. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 4496–4501. [\[CrossRef\]](#)
219. Acland, G.M.; Aguirre, G.D.; Ray, J.; Zhang, Q.; Aleman, T.S.; Cideciyan, A.V.; Pearce-Kelling, S.E.; Anand, V.; Zeng, Y.; Maguire, A.M. Gene therapy restores vision in a canine model of childhood blindness. *Nat. Genet.* **2001**, *28*, 92–95. [\[CrossRef\]](#)
220. Kodippili, K.; Hakim, C.H.; Pan, X.; Yang, H.T.; Yue, Y.; Zhang, Y.; Shin, J.-H.; Yang, N.N.; Duan, D. Dual AAV gene therapy for Duchenne muscular dystrophy with a 7-kb mini-dystrophin gene in the canine model. *Hum. Gene Ther.* **2018**, *29*, 299–311. [\[CrossRef\]](#)
221. Gurda, B.L.; De Guilhem De Lataillade, A.; Bell, P.; Zhu, Y.; Yu, H.; Wang, P.; Bagel, J.; Vite, C.H.; Sikora, T.; Hinderer, C.; et al. Evaluation of AAV-mediated Gene Therapy for Central Nervous System Disease in Canine Mucopolysaccharidosis VII. *Mol. Ther. J. Am. Soc. Gene Ther.* **2016**, *24*, 206–216. [\[CrossRef\]](#)
222. Gray-Edwards, H.L.; Maguire, A.S.; Salibi, N.; Ellis, L.E.; Voss, T.L.; Diffie, E.B.; Koehler, J.; Randle, A.N.; Taylor, A.R.; Brunson, B.L.; et al. 7T MRI predicts amelioration of neurodegeneration in the brain after AAV gene therapy. *Mol. Ther.-Methods Clin. Dev.* **2020**, *17*, 258–270. [\[CrossRef\]](#)
223. Choudhury, S.R.; Fitzpatrick, Z.; Harris, A.F.; Maitland, S.A.; Ferreira, J.S.; Zhang, Y.; Ma, S.; Sharma, R.B.; Gray-Edwards, H.L.; Johnson, J.A. In vivo selection yields AAV-B1 capsid for central nervous system and muscle gene therapy. *Mol. Ther.* **2016**, *24*, 1247–1257. [\[CrossRef\]](#)
224. McCown, T.J. Adeno-associated virus (AAV) vectors in the CNS. *Curr. Gene Ther.* **2005**, *5*, 333–338. [\[CrossRef\]](#) [\[PubMed\]](#)
225. Kaplitt, M.G.; Leone, P.; Samulski, R.J.; Xiao, X.; Pfaff, D.W.; O'Malley, K.L.; During, M.J. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nat. Genet.* **1994**, *8*, 148–154. [\[CrossRef\]](#) [\[PubMed\]](#)
226. McElroy, A.; Sena-Esteves, M.; Arjomandnejad, M.; Keeler, A.M.; Gray-Edwards, H.L. Redosing Adeno-Associated Virus Gene Therapy to the Central Nervous System. *Hum. Gene Ther.* **2022**, *33*, 889–892. [\[CrossRef\]](#) [\[PubMed\]](#)
227. Bartus, R.T.; Weinberg, M.S.; Samulski, R.J. Parkinson's Disease Gene Therapy: Success by Design Meets Failure by Efficacy. *Mol. Ther.* **2014**, *22*, 487–497. [\[CrossRef\]](#) [\[PubMed\]](#)
228. Marks, W.J.; Bartus, R.T.; Siffert, J.; Davis, C.S.; Lozano, A.; Boulis, N.; Vitek, J.; Stacy, M.; Turner, D.; Verhagen, L.; et al. Gene delivery of AAV2-neurturin for Parkinson's disease: A double-blind, randomised, controlled trial. *Lancet Neurol.* **2010**, *9*, 1164–1172. [\[CrossRef\]](#)
229. Kaplitt, M.G.; Feigin, A.; Tang, C.; Fitzsimons, H.L.; Mattis, P.; Lawlor, P.A.; Bland, R.J.; Young, D.; Strybing, K.; Eidelberg, D.; et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: An open label, phase I trial. *Lancet* **2007**, *369*, 2097–2105. [\[CrossRef\]](#)
230. LeWitt, P.A.; Rezai, A.R.; Leehey, M.A.; Ojemann, S.G.; Flaherty, A.W.; Eskandar, E.N.; Kostyk, S.K.; Thomas, K.; Sarkar, A.; Siddiqui, M.S.; et al. AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol.* **2011**, *10*, 309–319. [\[CrossRef\]](#)
231. Bartus, R.T.; Baumann, T.L.; Siffert, J.; Herzog, C.D.; Alterman, R.; Boulis, N.; Turner, D.A.; Stacy, M.; Lang, A.E.; Lozano, A.M.; et al. Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. *Neurology* **2013**, *80*, 1698–1701. [\[CrossRef\]](#)
232. Christine, C.W.; Starr, P.A.; Larson, P.S.; Eberling, J.L.; Jagust, W.J.; Hawkins, R.A.; VanBrocklin, H.F.; Wright, J.F.; Bankiewicz, K.S.; Aminoff, M.J. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology* **2009**, *73*, 1662–1669. [\[CrossRef\]](#)
233. Mittermeyer, G.; Christine, C.W.; Rosenbluth, K.H.; Baker, S.L.; Starr, P.; Larson, P.; Kaplan, P.L.; Forsayeth, J.; Aminoff, M.J.; Bankiewicz, K.S. Long-term evaluation of a phase 1 study of AADC gene therapy for Parkinson's disease. *Hum. Gene Ther.* **2012**, *23*, 377–381. [\[CrossRef\]](#)
234. Chien, Y.H.; Lee, N.C.; Tseng, S.H.; Tai, C.H.; Muramatsu, S.I.; Byrne, B.J.; Hwu, W.L. Efficacy and safety of AAV2 gene therapy in children with aromatic L-amino acid decarboxylase deficiency: An open-label, phase 1/2 trial. *Lancet Child Adolesc. Health* **2017**, *1*, 265–273. [\[CrossRef\]](#)
235. Kolb, S.J.; Kissel, J.T. Spinal muscular atrophy: A timely review. *Arch. Neurol.* **2011**, *68*, 979–984. [\[CrossRef\]](#)
236. Aguti, S.; Malerba, A.; Zhou, H. The progress of AAV-mediated gene therapy in neuromuscular disorders. *Expert Opin. Biol. Ther.* **2018**, *18*, 681–693. [\[CrossRef\]](#)
237. Spahiu, L.; Behluli, E.; Peterlin, B.; Nefic, H.; Hadziselimovic, R.; Liehr, T.; Temaj, G. Mucopolysaccharidosis III: Molecular basis and treatment. *Pediatr. Endocrinol. Diabetes Metab.* **2021**, *27*, 201–208. [\[CrossRef\]](#)
238. Watson, G.L.; Sayles, J.N.; Chen, C.; Elliger, S.S.; Elliger, C.A.; Raju, N.R.; Kurtzman, G.J.; Podsakoff, G.M. Treatment of lysosomal storage disease in MPS VII mice using a recombinant adeno-associated virus. *Gene Ther.* **1998**, *5*, 1642–1649. [\[CrossRef\]](#)
239. Skorupa, A.F.; Fisher, K.J.; Wilson, J.M.; Parente, M.K.; Wolfe, J.H. Sustained production of beta-glucuronidase from localized sites after AAV vector gene transfer results in widespread distribution of enzyme and reversal of lysosomal storage lesions in a large volume of brain in mucopolysaccharidosis VII mice. *Exp. Neurol.* **1999**, *160*, 17–27. [\[CrossRef\]](#)
240. Daly, T.M.; Vogler, C.; Levy, B.; Haskins, M.E.; Sands, M.S. Neonatal gene transfer leads to widespread correction of pathology in a murine model of lysosomal storage disease. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 2296–2300. [\[CrossRef\]](#)

241. Marcó, S.; Haurigot, V.; Jaén, M.L.; Ribera, A.; Sánchez, V.; Molas, M.; García, M.; León, X.; Roca, C.; Sánchez, X.; et al. Seven-year follow-up of durability and safety of AAV CNS gene therapy for a lysosomal storage disorder in a large animal. *Mol. Ther.-Methods Clin. Dev.* **2021**, *23*, 370–389. [\[CrossRef\]](#)
242. Shaimardanova, A.A.; Chulpanova, D.S.; Solovyeva, V.V.; Mullagulova, A.I.; Kitaeva, K.V.; Allegrucci, C.; Rizvanov, A.A. Metachromatic Leukodystrophy: Diagnosis, Modeling, and Treatment Approaches. *Front. Med.* **2020**, *7*, 576221. [\[CrossRef\]](#)
243. Piguet, F.; Sondhi, D.; Piraud, M.; Fouquet, F.; Hackett, N.R.; Ahouansou, O.; Vanier, M.-T.; Bieche, I.; Aubourg, P.; Crystal, R.G. Correction of brain oligodendrocytes by AAVrh. 10 intracerebral gene therapy in metachromatic leukodystrophy mice. *Hum. Gene Ther.* **2012**, *23*, 903–914. [\[CrossRef\]](#)
244. Kurai, T.; Hisayasu, S.; Kitagawa, R.; Migita, M.; Suzuki, H.; Hirai, Y.; Shimada, T. AAV1 mediated co-expression of formylglycine-generating enzyme and arylsulfatase efficiently corrects sulfatide storage in a mouse model of metachromatic leukodystrophy. *Mol. Ther.* **2007**, *15*, 38–43. [\[CrossRef\]](#) [\[PubMed\]](#)
245. Miyake, N.; Miyake, K.; Asakawa, N.; Yamamoto, M.; Shimada, T. Long-term correction of biochemical and neurological abnormalities in MLD mice model by neonatal systemic injection of an AAV serotype 9 vector. *Gene Ther.* **2014**, *21*, 427–433. [\[CrossRef\]](#) [\[PubMed\]](#)
246. Sevin, C.; Benraiss, A.; Van Dam, D.; Bonnain, D.; Nagels, G.; Verot, L.; Laurendeau, I.; Vidaud, M.; Gieselmann, V.; Vanier, M. Intracerebral adeno-associated virus-mediated gene transfer in rapidly progressive forms of metachromatic leukodystrophy. *Hum. Mol. Genet.* **2006**, *15*, 53–64. [\[CrossRef\]](#) [\[PubMed\]](#)
247. Shaimardanova, A.A.; Chulpanova, D.S.; Solovyeva, V.V.; Aimaletdinov, A.M.; Rizvanov, A.A. Functionality of a bicistronic construction containing HEXA and HEXB genes encoding β -hexosaminidase A for cell-mediated therapy of GM2 gangliosidosis. *Neural Regen. Res.* **2022**, *17*, 122–129. [\[CrossRef\]](#)
248. Taghian, T.; Marosfoi, M.G.; Puri, A.S.; Cataltepe, O.I.; King, R.M.; Diffie, E.B.; Maguire, A.S.; Martin, D.R.; Fernau, D.; Batista, A.R.; et al. A Safe and Reliable Technique for CNS Delivery of AAV Vectors in the Cisterna Magna. *Mol. Ther. J. Am. Soc. Gene Ther.* **2020**, *28*, 411–421. [\[CrossRef\]](#)
249. Solovyeva, V.V.; Shaimardanova, A.A.; Chulpanova, D.S.; Kitaeva, K.V.; Chakrabarti, L.; Rizvanov, A.A. New Approaches to Tay-Sachs Disease Therapy. *Front. Physiol.* **2018**, *9*, 1663. [\[CrossRef\]](#)
250. Cachón-González, M.B.; Wang, S.Z.; Lynch, A.; Ziegler, R.; Cheng, S.H.; Cox, T.M. Effective gene therapy in an authentic model of Tay-Sachs-related diseases. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10373–10378. [\[CrossRef\]](#)
251. Gray-Edwards, H.L.; Randle, A.N.; Maitland, S.A.; Benatti, H.R.; Hubbard, S.M.; Canning, P.F.; Vogel, M.B.; Brunson, B.L.; Hwang, M.; Ellis, L.E.; et al. Adeno-Associated Virus Gene Therapy in a Sheep Model of Tay-Sachs Disease. *Hum. Gene Ther.* **2018**, *29*, 312–326. [\[CrossRef\]](#)
252. Rockwell, H.E.; McCurdy, V.J.; Eaton, S.C.; Wilson, D.U.; Johnson, A.K.; Randle, A.N.; Bradbury, A.M.; Gray-Edwards, H.L.; Baker, H.J.; Hudson, J.A.; et al. AAV-mediated gene delivery in a feline model of Sandhoff disease corrects lysosomal storage in the central nervous system. *ASN Neuro* **2015**, *7*, 1759091415569908. [\[CrossRef\]](#)
253. Sevin, C.; Roujeau, T.; Cartier, N.; Baugnon, T.; Adamsbaum, C.; Piraud, M.; Martino, S.; Mouiller, P.; Couzinié, C.; Bellesme, C. Intracerebral Gene Therapy in Children with Metachromatic Leukodystrophy: Results of a Phase I/II Trial. *Mol. Genet. Metab.* **2018**, *123*, S129. [\[CrossRef\]](#)
254. Tenenbaum, L.; Chtarto, A.; Lehtonen, E.; Velu, T.; Brothi, J.; Levivier, M. Recombinant AAV-mediated gene delivery to the central nervous system. *J. Gene Med.* **2004**, *6*, S212–S222. [\[CrossRef\]](#)
255. Surendran, S.; Michals-Matalon, K.; Quast, M.J.; Tying, S.K.; Wei, J.; Ezell, E.L.; Matalon, R. Canavan disease: A monogenic trait with complex genomic interaction. *Mol. Genet. Metab.* **2003**, *80*, 74–80. [\[CrossRef\]](#)
256. Matalon, R.; Surendran, S.; Rady, P.L.; Quast, M.J.; Campbell, G.A.; Matalon, K.M.; Tying, S.K.; Wei, J.; Peden, C.S.; Ezell, E.L.; et al. Adeno-associated virus-mediated aspartoacylase gene transfer to the brain of knockout mouse for canavan disease. *Mol. Ther. J. Am. Soc. Gene Ther.* **2003**, *7*, 580–587. [\[CrossRef\]](#)
257. Janson, C.; McPhee, S.; Bilaniuk, L.; Haselgrove, J.; Testaiuti, M.; Freese, A.; Wang, D.J.; Shera, D.; Hurh, P.; Rupin, J.; et al. Clinical protocol. Gene therapy of Canavan disease: AAV-2 vector for neurosurgical delivery of aspartoacylase gene (ASPA) to the human brain. *Hum. Gene Ther.* **2002**, *13*, 1391–1412. [\[CrossRef\]](#)
258. rAAV-Olig001-ASPA Gene Therapy for Treatment of Children With Typical Canavan Disease. Available online: <https://ClinicalTrials.gov/show/NCT04833907> (accessed on 1 December 2022).
259. Canavan-Single Patient IND. Available online: <https://ClinicalTrials.gov/show/NCT05317780> (accessed on 1 December 2022).
260. A Study of AAV9 Gene Therapy in Participants With Canavan Disease. Available online: <https://ClinicalTrials.gov/show/NCT04998396> (accessed on 1 December 2022).
261. Nasir, G.; Chopra, R.; Elwood, F.; Ahmed, S.S. Krabbe Disease: Prospects of Finding a Cure Using AAV Gene Therapy. *Front. Med.* **2021**, *8*, 760236. [\[CrossRef\]](#)
262. Rafi, M.A.; Rao, H.Z.; Passini, M.A.; Curtis, M.; Vanier, M.T.; Zaka, M.; Luzi, P.; Wolfe, J.H.; Wenger, D.A. AAV-mediated expression of galactocerebrosidase in brain results in attenuated symptoms and extended life span in murine models of globoid cell leukodystrophy. *Mol. Ther.* **2005**, *11*, 734–744. [\[CrossRef\]](#)
263. Lin, D.-S.; Hsiao, C.-D.; Liao, I.; Lin, S.-P.; Chiang, M.-F.; Chuang, C.-K.; Wang, T.-J.; Wu, T.-Y.; Jian, Y.-R.; Huang, S.-F.; et al. CNS-targeted AAV5 gene transfer results in global dispersal of vector and prevention of morphological and function deterioration in CNS of globoid cell leukodystrophy mouse model. *Mol. Genet. Metab.* **2011**, *103*, 367–377. [\[CrossRef\]](#)

264. Lin, D.; Fantz, C.R.; Levy, B.; Rafi, M.A.; Vogler, C.; Wenger, D.A.; Sands, M.S. AAV2/5 vector expressing galactocerebrosidase ameliorates CNS disease in the murine model of globoid-cell leukodystrophy more efficiently than AAV2. *Mol. Ther.* **2005**, *12*, 422–430. [\[CrossRef\]](#)
265. Karumuthil-Meilethil, S.; Marshall, M.S.; Heindel, C.; Jakubauskas, B.; Bongarzone, E.R.; Gray, S.J. Intrathecal administration of AAV/GALC vectors in 10–11-day-old twitcher mice improves survival and is enhanced by bone marrow transplant. *J. Neurosci. Res.* **2016**, *94*, 1138–1151. [\[CrossRef\]](#)
266. Bradbury, A.M.; Rafi, M.A.; Bagel, J.H.; Brisson, B.K.; Marshall, M.S.; Pesayco Salvador, J.; Jiang, X.; Swain, G.P.; Prociuk, M.L.; O'Donnell, P.A. AAVrh10 gene therapy ameliorates central and peripheral nervous system disease in canine globoid cell leukodystrophy (Krabbe disease). *Hum. Gene Ther.* **2018**, *29*, 785–801. [\[CrossRef\]](#)
267. Bradbury, A.M.; Bagel, J.H.; Nguyen, D.; Lykken, E.A.; Salvador, J.P.; Jiang, X.; Swain, G.P.; Assenmacher, C.A.; Hendricks, I.J.; Miyadera, K. Krabbe disease successfully treated via monotherapy of intrathecal gene therapy. *J. Clin. Investig.* **2020**, *130*, 4906–4920. [\[CrossRef\]](#) [\[PubMed\]](#)
268. Hori, T.; Fukutome, M.; Maejima, C.; Matsushima, H.; Kobayashi, K.; Kitazawa, S.; Kitahara, R.; Kitano, K.; Kobayashi, K.; Moritoh, S.; et al. Gene delivery to cone photoreceptors by subretinal injection of rAAV2/6 in the mouse retina. *Biochem. Biophys. Res. Commun.* **2019**, *515*, 222–227. [\[CrossRef\]](#) [\[PubMed\]](#)
269. Le Meur, G.; Lebranchu, P.; Billaud, F.; Adjali, O.; Schmitt, S.; Béziau, S.; Péréon, Y.; Valabregue, R.; Ivan, C.; Darmon, C.; et al. Safety and Long-Term Efficacy of AAV4 Gene Therapy in Patients with RPE65 Leber Congenital Amaurosis. *Mol. Ther. J. Am. Soc. Gene Ther.* **2018**, *26*, 256–268. [\[CrossRef\]](#) [\[PubMed\]](#)
270. Le Meur, G.; Lebranchu, P.; Pereon, Y.; Billaud, F.; Ivan, C.; Chauveau, C.; Rolling, F.; Weber, M. Efficacy and safety of gene therapy with AAV4 in childhood blindness due to rpe65 mutations. *Acta Ophthalmol.* **2012**, *90*. [\[CrossRef\]](#)
271. Trapani, I.; Auricchio, A. Seeing the Light after 25 Years of Retinal Gene Therapy. *Trends Mol. Med.* **2018**, *24*, 669–681. [\[CrossRef\]](#)
272. MacLaren, R.E.; Groppe, M.; Barnard, A.R.; Cottrill, C.L.; Tolmachova, T.; Seymour, L.; Clark, K.R.; During, M.J.; Cremers, F.P.M.; Black, G.C.M.; et al. Retinal gene therapy in patients with choroideremia: Initial findings from a phase 1/2 clinical trial. *Lancet* **2014**, *383*, 1129–1137. [\[CrossRef\]](#)
273. Faynus, M.A.; Clegg, D.O. Chapter 7—Modeling inherited retinal dystrophies using induced pluripotent stem cells. In *Current Progress in iPSC Disease Modeling*; Birbrair, A., Ed.; Academic Press: Cambridge, MS, USA, 2022; pp. 157–184. [\[CrossRef\]](#)
274. Russell, S.; Bennett, J.; Wellman, J.A.; Chung, D.C.; Yu, Z.-F.; Tillman, A.; Wittes, J.; Pappas, J.; Elci, O.; McCague, S.; et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial. *Lancet* **2017**, *390*, 849–860. [\[CrossRef\]](#)
275. Bouquet, C.; Vignal Clermont, C.; Galy, A.; Fitoussi, S.; Blouin, L.; Munk, M.R.; Valero, S.; Meunier, S.; Katz, B.; Sahel, J.A.; et al. Immune Response and Intraocular Inflammation in Patients With Leber Hereditary Optic Neuropathy Treated With Intravitreal Injection of Recombinant Adeno-Associated Virus 2 Carrying the ND4 Gene: A Secondary Analysis of a Phase 1/2 Clinical Trial. *JAMA Ophthalmol.* **2019**, *137*, 399–406. [\[CrossRef\]](#)
276. Feuer, W.J.; Schiffman, J.C.; Davis, J.L.; Porciatti, V.; Gonzalez, P.; Koilkonda, R.D.; Yuan, H.; Lalwani, A.; Lam, B.L.; Guy, J. Gene Therapy for Leber Hereditary Optic Neuropathy: Initial Results. *Ophthalmology* **2016**, *123*, 558–570. [\[CrossRef\]](#)
277. Cehajic-Kapetanovic, J.; Xue, K.; Martinez-Fernandez de la Camara, C.; Nanda, A.; Davies, A.; Wood, L.J.; Salvetti, A.P.; Fischer, M.D.; Aylward, J.W.; Barnard, A.R.; et al. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR. *Nat. Med.* **2020**, *26*, 354–359. [\[CrossRef\]](#)
278. Cukras, C.; Wiley, H.E.; Jeffrey, B.G.; Sen, H.N.; Turrieff, A.; Zeng, Y.; Vijayasarathy, C.; Marangoni, D.; Ziccardi, L.; Kjellstrom, S.; et al. Retinal AAV8-RS1 Gene Therapy for X-Linked Retinoschisis: Initial Findings from a Phase I/IIa Trial by Intravitreal Delivery. *Mol. Ther.* **2018**, *26*, 2282–2294. [\[CrossRef\]](#)
279. Mishra, A.; Vijayasarathy, C.; Cukras, C.A.; Wiley, H.E.; Sen, H.N.; Zeng, Y.; Wei, L.L.; Sieving, P.A. Immune function in X-linked retinoschisis subjects in an AAV8-RS1 phase I/IIa gene therapy trial. *Mol. Ther.* **2021**, *29*, 2030–2040. [\[CrossRef\]](#)
280. Fischer, M.D.; Michalakakis, S.; Wilhelm, B.; Zobor, D.; Muehlfriedel, R.; Kohl, S.; Weisschuh, N.; Ochakovski, G.A.; Klein, R.; Schoen, C.; et al. Safety and Vision Outcomes of Subretinal Gene Therapy Targeting Cone Photoreceptors in Achromatopsia: A Nonrandomized Controlled Trial. *JAMA Ophthalmol.* **2020**, *138*, 643–651. [\[CrossRef\]](#)
281. Kahle, N.A.; Peters, T.; Zobor, D.; Kuehlewein, L.; Kohl, S.; Zhou, A.; Werner, A.; Seitz, I.P.; Sothilingam, V.; Michalakakis, S.; et al. Development of Methodology and Study Protocol: Safety and Efficacy of a Single Subretinal Injection of rAAV.hCNGA3 in Patients with CNGA3-Linked Achromatopsia Investigated in an Exploratory Dose-Escalation Trial. *Hum. Gene Ther. Clin. Dev.* **2018**, *29*, 121–131. [\[CrossRef\]](#)
282. Lalwani, A.K.; Walsh, B.J.; Reilly, P.G.; Muzyczka, N.; Mhatre, A.N. Development of in vivo gene therapy for hearing disorders: Introduction of adeno-associated virus into the cochlea of the guinea pig. *Gene Ther.* **1996**, *3*, 588–592.
283. Sondhi, D.; Stiles, K.M.; De, B.P.; Crystal, R.G. Genetic Modification of the Lung Directed Toward Treatment of Human Disease. *Hum. Gene Ther.* **2017**, *28*, 3–84. [\[CrossRef\]](#)
284. McLachlan, G.; Alton, E.; Boyd, A.C.; Clarke, N.K.; Davies, J.C.; Gill, D.R.; Griesenbach, U.; Hickmott, J.W.; Hyde, S.C.; Miah, K.M.; et al. Progress in Respiratory Gene Therapy. *Hum. Gene Ther.* **2022**, *33*, 893–912. [\[CrossRef\]](#)
285. Burney, T.J.; Davies, J.C. Gene therapy for the treatment of cystic fibrosis. *Appl. Clin. Genet.* **2012**, *5*, 29.

286. Flotte, T.R.; Afione, S.A.; Conrad, C.; McGrath, S.A.; Solow, R.; Oka, H.; Zeitlin, P.L.; Guggino, W.B.; Carter, B.J. Stable in vivo expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10613–10617. [\[CrossRef\]](#)
287. Guggino, W.B.; Cebotaru, L. Gene therapy for cystic fibrosis paved the way for the use of adeno-associated virus in gene therapy. *Hum. Gene Ther.* **2020**, *31*, 538–541. [\[CrossRef\]](#)
288. Wagner, J.A.; Reynolds, T.; Moran, M.L.; Moss, R.B.; Wine, J.J.; Flotte, T.R.; Gardner, P. Efficient and persistent gene transfer of AAV-CFTR in maxillary sinus. *Lancet* **1998**, *351*, 1702–1703. [\[CrossRef\]](#) [\[PubMed\]](#)
289. Wagner, J.A.; Nepomuceno, I.B.; Messner, A.H.; Moran, M.L.; Batson, E.P.; Dimiceli, S.; Brown, B.W.; Desch, J.K.; Norbash, A.M.; Conrad, C.K. A phase II, double-blind, randomized, placebo-controlled clinical trial of tgAAVCF using maxillary sinus delivery in patients with cystic fibrosis with antrastomies. *Hum. Gene Ther.* **2002**, *13*, 1349–1359. [\[CrossRef\]](#) [\[PubMed\]](#)
290. Aitken, M.; Moss, R.; Waltz, D.; Dovey, M.; Tonelli, M.; McNamara, S.; Gibson, R.; Ramsey, B.; Carter, B.; Reynolds, T. A phase I study of aerosolized administration of tgAAVCF to cystic fibrosis subjects with mild lung disease. *Hum. Gene Ther.* **2001**, *12*, 1907–1916. [\[CrossRef\]](#) [\[PubMed\]](#)
291. Flotte, T.R.; Zeitlin, P.L.; Reynolds, T.C.; Heald, A.E.; Pedersen, P.; Beck, S.; Conrad, C.K.; Brass-Ernst, L.; Humphries, M.; Sullivan, K. Phase I trial of intranasal and endobronchial administration of a recombinant adeno-associated virus serotype 2 (rAAV2)-CFTR vector in adult cystic fibrosis patients: A two-part clinical study. *Hum. Gene Ther.* **2003**, *14*, 1079–1088. [\[CrossRef\]](#)
292. Moss, R.B.; Rodman, D.; Spencer, L.T.; Aitken, M.L.; Zeitlin, P.L.; Waltz, D.; Milla, C.; Brody, A.S.; Clancy, J.P.; Ramsey, B. Repeated adeno-associated virus serotype 2 aerosol-mediated cystic fibrosis transmembrane regulator gene transfer to the lungs of patients with cystic fibrosis: A multicenter, double-blind, placebo-controlled trial. *Chest* **2004**, *125*, 509–521. [\[CrossRef\]](#)
293. Moss, R.B.; Milla, C.; Colombo, J.; Accurso, F.; Zeitlin, P.L.; Clancy, J.P.; Spencer, L.T.; Pilewski, J.; Waltz, D.A.; Dorkin, H.L. Repeated aerosolized AAV-CFTR for treatment of cystic fibrosis: A randomized placebo-controlled phase 2B trial. *Hum. Gene Ther.* **2007**, *18*, 726–732. [\[CrossRef\]](#)
294. Kurosaki, F.; Uchibori, R.; Mato, N.; Sehara, Y.; Saga, Y.; Urabe, M.; Mizukami, H.; Sugiyama, Y.; Kume, A. Optimization of adeno-associated virus vector-mediated gene transfer to the respiratory tract. *Gene Ther.* **2017**, *24*, 290–297. [\[CrossRef\]](#)
295. Cooney, A.L.; McCray, P.B.; Sinn, P.L. Cystic Fibrosis Gene Therapy: Looking Back, Looking Forward. *Genes* **2018**, *9*, 538. [\[CrossRef\]](#)
296. Stiles, K.M.; Sondhi, D.; Kaminsky, S.M.; De, B.P.; Rosenberg, J.B.; Crystal, R.G. Intrapleural Gene Therapy for Alpha-1 Antitrypsin Deficiency-Related Lung Disease. *Chronic Obstr. Pulm. Dis.* **2018**, *5*, 244–257. [\[CrossRef\]](#)
297. Song, S.; Embury, J.; Laipis, P.; Berns, K.; Crawford, J.; Flotte, T. Stable therapeutic serum levels of human alpha-1 antitrypsin (AAT) after portal vein injection of recombinant adeno-associated virus (rAAV) vectors. *Gene Ther.* **2001**, *8*, 1299–1306. [\[CrossRef\]](#)
298. Conlon, T.J.; Cossette, T.; Erger, K.; Choi, Y.-K.; Clarke, T.; Scott-Jorgensen, M.; Song, S.; Campbell-Thompson, M.; Crawford, J.; Flotte, T.R. Efficient Hepatic Delivery and Expression from a Recombinant Adeno-associated Virus 8 Pseudotyped α 1-Antitrypsin Vector. *Mol. Ther.* **2005**, *12*, 867–875. [\[CrossRef\]](#)
299. De, B.; Heguy, A.; Leopold, P.L.; Wasif, N.; Korst, R.J.; Hackett, N.R.; Crystal, R.G. Intrapleural administration of a serotype 5 adeno-associated virus coding for α 1-antitrypsin mediates persistent, high lung and serum levels of α 1-antitrypsin. *Mol. Ther.* **2004**, *10*, 1003–1010. [\[CrossRef\]](#)
300. Chulay, J.D.; Ye, G.J.; Thomas, D.L.; Knop, D.R.; Benson, J.M.; Hutt, J.A.; Wang, G.; Humphries, M.; Flotte, T.R. Preclinical evaluation of a recombinant adeno-associated virus vector expressing human alpha-1 antitrypsin made using a recombinant herpes simplex virus production method. *Hum. Gene Ther.* **2011**, *22*, 155–165. [\[CrossRef\]](#)
301. Limberis, M.P.; Vandenberghe, L.H.; Zhang, L.; Pickles, R.J.; Wilson, J.M. Transduction Efficiencies of Novel AAV Vectors in Mouse Airway Epithelium In Vivo and Human Ciliated Airway Epithelium In Vitro. *Mol. Ther.* **2009**, *17*, 294–301. [\[CrossRef\]](#)
302. Liqun Wang, R.; McLaughlin, T.; Cossette, T.; Tang, Q.; Foust, K.; Campbell-Thompson, M.; Martino, A.; Cruz, P.; Loiler, S.; Mueller, C.; et al. Recombinant AAV Serotype and Capsid Mutant Comparison for Pulmonary Gene Transfer of α 1-Antitrypsin Using Invasive and Noninvasive Delivery. *Mol. Ther.* **2009**, *17*, 81–87. [\[CrossRef\]](#)
303. Chiuchiolo, M.J.; Kaminsky, S.M.; Sondhi, D.; Hackett, N.R.; Rosenberg, J.B.; Frenk, E.Z.; Hwang, Y.; Van de Graaf, B.G.; Hutt, J.A.; Wang, G.; et al. Intrapleural administration of an AAVrh.10 vector coding for human α 1-antitrypsin for the treatment of α 1-antitrypsin deficiency. *Hum. Gene Ther. Clin. Dev.* **2013**, *24*, 161–173. [\[CrossRef\]](#)
304. De, B.P.; Heguy, A.; Hackett, N.R.; Ferris, B.; Leopold, P.L.; Lee, J.; Pierre, L.; Gao, G.; Wilson, J.M.; Crystal, R.G. High Levels of Persistent Expression of α 1-Antitrypsin Mediated by the Nonhuman Primate Serotype rh.10 Adeno-associated Virus Despite Preexisting Immunity to Common Human Adeno-associated Viruses. *Mol. Ther.* **2006**, *13*, 67–76. [\[CrossRef\]](#)
305. Flotte, T.R.; Conlon, T.J.; Poirier, A.; Campbell-Thompson, M.; Byrne, B.J. Preclinical characterization of a recombinant adeno-associated virus type 1-pseudotyped vector demonstrates dose-dependent injection site inflammation and dissemination of vector genomes to distant sites. *Hum. Gene Ther.* **2007**, *18*, 245–256. [\[CrossRef\]](#)
306. Zavorotinskaya, T.; Tomkinson, A.; Murphy, J.E. Treatment of experimental asthma by long-term gene therapy directed against IL-4 and IL-13. *Mol. Ther.* **2003**, *7*, 155–162. [\[CrossRef\]](#)
307. Kang, M.H.; van Lieshout, L.P.; Xu, L.; Domm, J.M.; Vadivel, A.; Renesme, L.; Mühlfeld, C.; Hurskainen, M.; Mižiková, I.; Pei, Y. A lung tropic AAV vector improves survival in a mouse model of surfactant B deficiency. *Nat. Commun.* **2020**, *11*, 3929. [\[CrossRef\]](#)
308. Cooney, A.L.; Wambach, J.A.; Sinn, P.L.; McCray, P.B., Jr. Gene therapy potential for genetic disorders of surfactant dysfunction. *Front. Genome Ed.* **2021**, *3*, 43. [\[CrossRef\]](#) [\[PubMed\]](#)

309. Guse, K.; Suzuki, M.; Sule, G.; Bertin, T.K.; Tyynismaa, H.; Ahola-Erkkilä, S.; Palmer, D.; Suomalainen, A.; Ng, P.; Cerullo, V.; et al. Capsid-modified adenoviral vectors for improved muscle-directed gene therapy. *Hum. Gene Ther.* **2012**, *23*, 1065–1070. [[CrossRef](#)] [[PubMed](#)]
310. Tang, Y.; Cummins, J.; Huard, J.; Wang, B. AAV-directed muscular dystrophy gene therapy. *Expert Opin. Biol. Ther.* **2010**, *10*, 395–408. [[CrossRef](#)] [[PubMed](#)]
311. Kota, J.; Handy, C.R.; Haidet, A.M.; Montgomery, C.L.; Eagle, A.; Rodino-Klapac, L.R.; Tucker, D.; Shilling, C.J.; Therlfall, W.R.; Walker, C.M.; et al. Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. *Sci. Transl. Med.* **2009**, *1*, 6ra15. [[CrossRef](#)]
312. Gregorevic, P.; Allen, J.M.; Minami, E.; Blankinship, M.J.; Haraguchi, M.; Meuse, L.; Finn, E.; Adams, M.E.; Froehner, S.C.; Murry, C.E.; et al. rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice. *Nat. Med.* **2006**, *12*, 787–789. [[CrossRef](#)]
313. Wang, B.; Li, J.; Fu, F.H.; Xiao, X. Systemic human minidystrophin gene transfer improves functions and life span of dystrophin and dystrophin/utrophin-deficient mice. *J. Orthop. Res. Off. Publ. Orthop. Res. Soc.* **2009**, *27*, 421–426. [[CrossRef](#)]
314. Zhu, T.; Zhou, L.; Mori, S.; Wang, Z.; McTiernan, C.F.; Qiao, C.; Chen, C.; Wang, D.W.; Li, J.; Xiao, X. Sustained Whole-Body Functional Rescue in Congestive Heart Failure and Muscular Dystrophy Hamsters by Systemic Gene Transfer. *Circulation* **2005**, *112*, 2650–2659. [[CrossRef](#)]
315. Pacak, C.A.; Walter, G.A.; Gaidosh, G.; Bryant, N.; Lewis, M.A.; Germain, S.; Mah, C.S.; Campbell, K.P.; Byrne, B.J. Long-term skeletal muscle protection after gene transfer in a mouse model of LGMD-2D. *Mol. Ther. J. Am. Soc. Gene Ther.* **2007**, *15*, 1775–1781. [[CrossRef](#)]
316. Fougereousse, F.; Bartoli, M.; Poupiot, J.; Arandel, L.; Durand, M.; Guerchet, N.; Gicquel, E.; Danos, O.; Richard, I. Phenotypic Correction of α -Sarcoglycan Deficiency by Intra-arterial Injection of a Muscle-specific Serotype 1 rAAV Vector. *Mol. Ther. J. Am. Soc. Gene Ther.* **2007**, *15*, 53–61. [[CrossRef](#)]
317. Cordier, L.; Hack, A.A.; Scott, M.O.; Barton-Davis, E.R.; Gao, G.; Wilson, J.M.; McNally, E.M.; Sweeney, H.L. Rescue of skeletal muscles of gamma-sarcoglycan-deficient mice with adeno-associated virus-mediated gene transfer. *Mol. Ther. J. Am. Soc. Gene Ther.* **2000**, *1*, 119–129. [[CrossRef](#)]
318. Bartoli, M.; Roudaut, C.; Martin, S.; Fougereousse, F.; Suel, L.; Poupiot, J.; Gicquel, E.; Noulet, F.; Danos, O.; Richard, I. Safety and efficacy of AAV-mediated calpain 3 gene transfer in a mouse model of limb-girdle muscular dystrophy type 2A. *Mol. Ther. J. Am. Soc. Gene Ther.* **2006**, *13*, 250–259. [[CrossRef](#)]
319. Duan, D. Systemic AAV Micro-dystrophin Gene Therapy for Duchenne Muscular Dystrophy. *Mol. Ther.* **2018**, *26*, 2337–2356. [[CrossRef](#)]
320. Bowles, D.E.; McPhee, S.W.J.; Li, C.; Gray, S.J.; Samulski, J.J.; Camp, A.S.; Li, J.; Wang, B.; Monahan, P.E.; Rabinowitz, J.E.; et al. Phase 1 Gene Therapy for Duchenne Muscular Dystrophy Using a Translational Optimized AAV Vector. *Mol. Ther.* **2012**, *20*, 443–455. [[CrossRef](#)]
321. World Health Organization. Cardiovascular Diseases (CVDs). Available online: <https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-cvds> (accessed on 1 December 2022).
322. Jiang, H.; Lillcrap, D.; Patarroyo-White, S.; Liu, T.; Qian, X.; Scallan, C.D.; Powell, S.; Keller, T.; McMurray, M.; Labelle, A.; et al. Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs. *Blood* **2006**, *108*, 107–115. [[CrossRef](#)]
323. Zsebo, K.; Yaroshinsky, A.; Rudy, J.J.; Wagner, K.; Greenberg, B.; Jessup, M.; Hajjar, R.J. Long-Term Effects of AAV1/SERCA2a Gene Transfer in Patients With Severe Heart Failure. *Circ. Res.* **2014**, *114*, 101–108. [[CrossRef](#)]
324. Nguyen, T.H.; Ferry, N. Liver gene therapy: Advances and hurdles. *Gene Ther.* **2004**, *11*, S76–S84. [[CrossRef](#)]
325. Członkowska, A.; Litwin, T.; Dusek, P.; Ferenci, P.; Lutsenko, S.; Medici, V.; Rybakowski, J.K.; Weiss, K.H.; Schilsky, M.L. Wilson disease. *Nat. Rev. Dis. Prim.* **2018**, *4*, 21. [[CrossRef](#)]
326. Murillo, O.; Collantes, M.; Gazquez, C.; Moreno, D.; Hernandez-Alcoceba, R.; Barberia, M.; Ecay, M.; Tamarit, B.; Douar, A.; Ferrer, V.; et al. High value of (64)Cu as a tool to evaluate the restoration of physiological copper excretion after gene therapy in Wilson's disease. *Mol. Ther. Methods Clin. Dev.* **2022**, *26*, 98–106. [[CrossRef](#)]
327. Murillo, O.; Moreno, D.; Gazquez, C.; Barberia, M.; Cenzano, I.; Navarro, I.; Uriarte, I.; Sebastian, V.; Arruebo, M.; Ferrer, V.; et al. Liver Expression of a MiniATP7B Gene Results in Long-Term Restoration of Copper Homeostasis in a Wilson Disease Model in Mice. *Hepatology* **2019**, *70*, 108–126. [[CrossRef](#)]
328. Crigler, J.F., Jr.; Najjar, V.A. Congenital familial nonhemolytic jaundice with kernicterus. *Pediatrics* **1952**, *10*, 169–180.
329. Gene Therapy for Severe Crigler Najjar Syndrome. Available online: <https://ClinicalTrials.gov/show/NCT03466463> (accessed on 1 December 2022).
330. Shi, X.; Aronson, S.J.; Ten Bloemendaal, L.; Duijst, S.; Bakker, R.S.; de Waart, D.R.; Bortolussi, G.; Collaud, F.; Oude Elferink, R.P.; Muro, A.F.; et al. Efficacy of AAV8-hUGT1A1 with Rapamycin in neonatal, suckling, and juvenile rats to model treatment in pediatric CNs patients. *Mol. Ther. Methods Clin. Dev.* **2021**, *20*, 287–297. [[CrossRef](#)] [[PubMed](#)]
331. Golden, S.H.; Robinson, K.A.; Saldanha, I.; Anton, B.; Ladenson, P.W. Clinical review: Prevalence and incidence of endocrine and metabolic disorders in the United States: A comprehensive review. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 1853–1878. [[CrossRef](#)] [[PubMed](#)]
332. Wilson, J.D. Prospects for research for disorders of the endocrine system. *JAMA* **2001**, *285*, 624–627. [[CrossRef](#)] [[PubMed](#)]

333. Barzon, L.; Bonaguro, R.; Palù, G.; Boscaro, M. New perspectives for gene therapy in endocrinology. *Eur. J. Endocrinol.* **2000**, *143*, 447–466. [CrossRef]
334. Chellappan, D.K.; Sivam, N.S.; Teoh, K.X.; Leong, W.P.; Fui, T.Z.; Chooi, K.; Khoo, N.; Yi, F.J.; Chellian, J.; Cheng, L.L.; et al. Gene therapy and type 1 diabetes mellitus. *Biomed. Pharmacother.* **2018**, *108*, 1188–1200. [CrossRef]
335. Atkinson, M.A. Chapter 32—Type 1 Diabetes Mellitus. In *Williams Textbook of Endocrinology*, 3rd ed.; Melmed, S., Polonsky, K.S., Larsen, P.R., Kronenberg, H.M., Eds.; Elsevier: Philadelphia, PA, USA, 2016; pp. 1451–1483. [CrossRef]
336. Flores, R.R.; Zhou, L.; Robbins, P.D. Expression of IL-2 in β cells by AAV8 gene transfer in pre-diabetic NOD mice prevents diabetes through activation of FoxP3-positive regulatory T cells. *Gene Ther.* **2014**, *21*, 715–722. [CrossRef]
337. Zhang, Y.C.; Pileggi, A.; Agarwal, A.; Molano, R.D.; Powers, M.; Brusko, T.; Wasserfall, C.; Goudy, K.; Zahr, E.; Poggioli, R.; et al. Adeno-Associated Virus-Mediated IL-10 Gene Therapy Inhibits Diabetes Recurrence in Syngeneic Islet Cell Transplantation of NOD Mice. *Diabetes* **2003**, *52*, 708–716. [CrossRef]
338. El Khatib, M.; Sakuma, T.; Tonne, J.M.; Mohamed, M.S.; Holditch, S.J.; Lu, B.; Kudva, Y.C.; Ikeda, Y. β -Cell-targeted blockage of PD1 and CTLA4 pathways prevents development of autoimmune diabetes and acute allogeneic islets rejection. *Gene Ther.* **2015**, *22*, 430–438. [CrossRef]
339. RGX-314 Gene Therapy Administered in the Suprachoroidal Space for Participants With Diabetic Retinopathy (DR) without Center Involved-Diabetic Macular Edema (CI-DME). Available online: <https://ClinicalTrials.gov/show/NCT04567550> (accessed on 1 December 2022).
340. Borden, K.L. Pondering the promyelocytic leukemia protein (PML) puzzle: Possible functions for PML nuclear bodies. *Mol. Cell. Biol.* **2002**, *22*, 5259–5269. [CrossRef]
341. Almaghrabi, S.; Azzouz, M.; Tazi Ahnini, R. AAV9-mediated AIRE gene delivery clears circulating antibodies and tissue T-cell infiltration in a mouse model of autoimmune polyglandular syndrome type-1. *Clin. Transl. Immunol.* **2020**, *9*, e1166. [CrossRef]
342. Luo, J.; Luo, Y.; Sun, J.; Zhou, Y.; Zhang, Y.; Yang, X. Adeno-associated virus-mediated cancer gene therapy: Current status. *Cancer Lett.* **2015**, *356*, 347–356. [CrossRef]
343. WHO. Cancer. Available online: <https://www.who.int/news-room/fact-sheets/detail/cancer> (accessed on 1 December 2022).
344. Kanazawa, T.; Mizukami, H.; Okada, T.; Hanazono, Y.; Kume, A.; Nishino, H.; Takeuchi, K.; Kitamura, K.; Ichimura, K.; Ozawa, K. Suicide gene therapy using AAV-HSVtk/ganciclovir in combination with irradiation results in regression of human head and neck cancer xenografts in nude mice. *Gene Ther.* **2003**, *10*, 51–58. [CrossRef]
345. Kim, J.Y.; Kim, J.H.; Khim, M.; Lee, H.S.; Jung, J.H.; Moon, D.H.; Jeong, S.; Lee, H. Persistent anti-tumor effects via recombinant adeno-associated virus encoding herpes thymidine kinase gene monitored by PET-imaging. *Oncol. Rep.* **2011**, *25*, 1263–1269. [CrossRef]
346. Mizuno, M.; Yoshida, J.; Colosi, P.; Kurtzman, G. Adeno-associated Virus Vector Containing the Herpes Simplex Virus Thymidine Kinase Gene Causes Complete Regression of Intracerebrally Implanted Human Gliomas in Mice, in Conjunction with Ganciclovir Administration. *Jpn. J. Cancer Res.* **1998**, *89*, 76–80. [CrossRef]
347. Kojima, Y.; Honda, K.; Hamada, H.; Kobayashi, N. Oncolytic gene therapy combined with double suicide genes for human bile duct cancer in nude mouse models. *J. Surg. Res.* **2009**, *157*, e63–e70. [CrossRef]
348. Pan, J.G.; Zhou, X.; Luo, R.; Han, R.F. The adeno-associated virus-mediated HSV-TK/GCV suicide system: A potential strategy for the treatment of bladder carcinoma. *Med. Oncol.* **2012**, *29*, 1938–1947. [CrossRef]
349. Borel, F.; Kay, M.A.; Mueller, C. Recombinant AAV as a platform for translating the therapeutic potential of RNA interference. *Mol. Ther. J. Am. Soc. Gene Ther.* **2014**, *22*, 692–701. [CrossRef]
350. Sun, A.; Tang, J.; Terranova, P.F.; Zhang, X.; Thrasher, J.B.; Li, B. Adeno-associated virus-delivered short hairpin-structured RNA for androgen receptor gene silencing induces tumor eradication of prostate cancer xenografts in nude mice: A preclinical study. *Int. J. Cancer* **2010**, *126*, 764–774. [CrossRef]
351. Kota, J.; Chivukula, R.R.; O'Donnell, K.A.; Wentzel, E.A.; Montgomery, C.L.; Hwang, H.W.; Chang, T.C.; Vivekanandan, P.; Torbenson, M.; Clark, K.R.; et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* **2009**, *137*, 1005–1017. [CrossRef]
352. Lu, L.; Luo, S.T.; Shi, H.S.; Li, M.; Zhang, H.L.; He, S.S.; Liu, Y.; Pan, Y.; Yang, L. AAV2-mediated gene transfer of VEGF-Trap with potent suppression of primary breast tumor growth and spontaneous pulmonary metastases by long-term expression. *Oncol. Rep.* **2012**, *28*, 1332–1338. [CrossRef]
353. Wu, Q.J.; Gong, C.Y.; Luo, S.T.; Zhang, D.M.; Zhang, S.; Shi, H.S.; Lu, L.; Yan, H.X.; He, S.S.; Li, D.D.; et al. AAV-mediated human PEDF inhibits tumor growth and metastasis in murine colorectal peritoneal carcinomatosis model. *BMC Cancer* **2012**, *12*, 129. [CrossRef] [PubMed]
354. Tse, L.Y.; Sun, X.; Jiang, H.; Dong, X.; Fung, P.W.; Farzaneh, F.; Xu, R. Adeno-associated virus-mediated expression of kallistatin suppresses local and remote hepatocellular carcinomas. *J. Gene Med.* **2008**, *10*, 508–517. [CrossRef] [PubMed]
355. Hunter, J.E.; Ramos, L.; Wolfe, J.H. Viral Vectors in the CNS. In *Reference Module in Neuroscience and Biobehavioral Psychology*; Elsevier: Amsterdam, The Netherlands, 2017. [CrossRef]
356. Hermonat, P.L.; Quirk, J.G.; Bishop, B.M.; Han, L. The packaging capacity of adeno-associated virus (AAV) and the potential for wild-type-plus AAV gene therapy vectors. *FEBS Lett.* **1997**, *407*, 78–84. [CrossRef] [PubMed]
357. Flotte, T.R.; Carter, B.J. Adeno-Associated Viral Vectors. In *Gene Therapy Technologies, Applications and Regulations*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 1999; pp. 109–125. [CrossRef]

358. Verdera, H.C.; Kuranda, K.; Mingozi, F. AAV Vector Immunogenicity in Humans: A Long Journey to Successful Gene Transfer. *Mol. Ther. J. Am. Soc. Gene Ther.* **2020**, *28*, 723–746. [[CrossRef](#)]
359. Ertl, H.C.J. Immunogenicity and toxicity of AAV gene therapy. *Front. Immunol.* **2022**, *13*, 975803. [[CrossRef](#)]
360. Fitzpatrick, Z.; Leborgne, C.; Barbon, E.; Masat, E.; Ronzitti, G.; van Wittenberghe, L.; Vignaud, A.; Collaud, F.; Charles, S.; Simon Sola, M.; et al. Influence of Pre-existing Anti-capsid Neutralizing and Binding Antibodies on AAV Vector Transduction. *Mol. Ther. Methods Clin. Dev.* **2018**, *9*, 119–129. [[CrossRef](#)]
361. Denard, J.; Marolleau, B.; Jenny, C.; Rao, T.N.; Fehling, H.J.; Voit, T.; Svinartchouk, F. 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.* **2013**, *87*, 10784–10791. [[CrossRef](#)]
362. Murphy, S.L.; Li, H.; Mingozi, F.; Sabatino, D.E.; Hui, D.J.; Edmonson, S.A.; High, K.A. Diverse IgG subclass responses to adeno-associated virus infection and vector administration. *J. Med. Virol.* **2009**, *81*, 65–74. [[CrossRef](#)]
363. Mingozi, F.; Meulenberg, J.J.; Hui, D.J.; Basner-Tschakarjan, E.; Hasbrouck, N.C.; Edmonson, S.A.; Hutnick, N.A.; Betts, M.R.; Kastelein, J.J.; Stroes, E.S.; et al. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. *Blood* **2009**, *114*, 2077–2086. [[CrossRef](#)]
364. Louis Jeune, V.; Joergensen, J.A.; Hajjar, R.J.; Weber, T. Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. *Hum. Gene Ther. Methods* **2013**, *24*, 59–67. [[CrossRef](#)]
365. Wang, L.; Calcedo, R.; Bell, P.; Lin, J.; Grant, R.L.; Siegel, D.L.; Wilson, J.M. Impact of pre-existing immunity on gene transfer to nonhuman primate liver with adeno-associated virus 8 vectors. *Hum. Gene Ther.* **2011**, *22*, 1389–1401. [[CrossRef](#)]
366. Herzog, R.W.; Biswas, M. Neutralizing the Neutralizers in AAV Gene Therapy. *Mol. Ther.* **2020**, *28*, 1741–1742. [[CrossRef](#)]
367. Zengel, J.; Carette, J.E. Structural and cellular biology of adeno-associated virus attachment and entry. *Adv. Virus Res.* **2020**, *106*, 39–84. [[CrossRef](#)]
368. Chowdhury, E.A.; Meno-Tetang, G.; Chang, H.Y.; Wu, S.; Huang, H.W.; Jamier, T.; Chandran, J.; Shah, D.K. Current progress and limitations of AAV mediated delivery of protein therapeutic genes and the importance of developing quantitative pharmacokinetic/pharmacodynamic (PK/PD) models. *Adv. Drug Deliv. Rev.* **2021**, *170*, 214–237. [[CrossRef](#)]
369. Kang, L.; Jin, S.; Wang, J.; Lv, Z.; Xin, C.; Tan, C.; Zhao, M.; Wang, L.; Liu, J. AAV vectors applied to the treatment of CNS disorders: Clinical status and challenges. *J. Control. Release* **2023**, *355*, 458–473. [[CrossRef](#)]
370. Colle, M.A.; Piguet, F.; Bertrand, L.; Raoul, S.; Bieche, I.; Dubreil, L.; Sloothak, D.; Bouquet, C.; Moullier, P.; Aubourg, P.; et al. Efficient intracerebral delivery of AAV5 vector encoding human ARSA in non-human primate. *Hum. Mol. Genet.* **2010**, *19*, 147–158. [[CrossRef](#)]
371. Zerah, M.; Piguet, F.; Colle, M.A.; Raoul, S.; Deschamps, J.Y.; Deniaud, J.; Gautier, B.; Toulgoat, F.; Bieche, I.; Laurendeau, I.; et al. Intracerebral Gene Therapy Using AAVrh.10-hARSA Recombinant Vector to Treat Patients with Early-Onset Forms of Metachromatic Leukodystrophy: Preclinical Feasibility and Safety Assessments in Nonhuman Primates. *Hum. Gene Ther. Clin. Dev.* **2015**, *26*, 113–124. [[CrossRef](#)]
372. Zieger, M.; Borel, F.; Greer, C.; Gernoux, G.; Blackwood, M.; Flotte, T.R.; Mueller, C. Liver-directed SERPINA1 gene therapy attenuates progression of spontaneous and tobacco smoke-induced emphysema in α 1-antitrypsin null mice. *Mol. Ther. Methods Clin. Dev.* **2022**, *25*, 425–438. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.