Adeno-Associated Viral Vectors as Versatile Tools for Therapeutic Perspectives

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Abstract: It is without doubt that the gene therapy field is currently in the spotlight for the development of new therapeutics targeting unmet medical needs. Thus, considering the gene therapy scenario, neurological diseases in general and neurodegenerative disorders in particular are emerging as the most appealing choices for new therapeutic arrivals intended to slow down, stop, or even revert the natural progressive course that characterizes most of these devastating neurodegenerative processes. Since an extensive coverage of all available literature is not feasible in practical terms, here emphasis was made in providing some advice to beginners in the field with a narrow focus on elucidating the best delivery route available for fulfilling any given AAV-based therapeutic approach. Furthermore, it is worth noting that the number of ongoing clinical trials is increasing at a breathtaking speed. Accordingly, a landscape view of preclinical and clinical initiatives is also provided here in an attempt to best illustrate what is ongoing in this quickly expanding field.

Keywords: AAV; gene therapy; disease-modifying therapeutics; neuroprotection; precision medicine

1. Introduction

Adeno-associated viral vectors (AAVs) are members of Dependoparvovirus in the Parvoviridae family. AAVs require co-infection with adenovirus (Ad), baculovirus, or herpes simplex virus to complete the replication cycle, with Ad being the natural helper virus in clinical isolates. An AAV without helper can integrate into the genome, but cannot be propagated by itself, which makes it a safer choice for gene therapy [1]. AAVs were first identified by electron microscopy [2], and they are formed by an icosahedral capsid carrying a single-stranded linear DNA genome that contains two open-reading frames encoding for Rep (40, 52, 68, and 78) involved in replication and integration, the capsid (Cap), three structural proteins (VP1, VP2, and VP3), and a small viral cofactor for assembly-activating protein [3]. Rep-independent recombinant AAVs are used for gene therapy purposes, in order to avoid the preference of integration of Rep proteins into the AAVS1 site inside of Ch19 [1,4].

The origin of AAV-based technologies started when the plasmid clone of wild AAV showed infective behavior when transfected into human cells after Ad helper co-infection [5].
This discovery demonstrated the feasibility of a transient and persistent expression for a marker gene lasting for 6 months or more with AAVs [6,7].

When designing any given AAV-based experiment in the central nervous system (CNS), there are two important prerequisites to be taken into consideration at first glance: (i) choosing the best suited AAV, with a proper balance between the AAV serotype and its expected neurotropism, and (ii) selection of the promoter driving the desired transgene expression (e.g., either ubiquitous or cell-specific). The most commonly used promoters for CNS applications are CAG, CBA, JeT, GusB, and EF1, among others [8]. Different promoters may have different potencies when driving transgene expression. Indeed, for late-stage preclinical developments, ensuring a proper balance between efficacy and safety often represents a critical issue. The use of small-sized promoters is a convenient strategy in order to leave enough cargo space when accommodating large-sized genes [9,10]. Once the choice of best AAV serotype and promoter is made, the most critical decision to be reached before pushing forward any given successful therapeutic approach is to elucidate the most adequate route for AAV delivery, as described below.

2. AAV Delivery Routes

When coming to design any AAV-based therapeutics, the choice of the delivery route represents the most critical decision for achieving the best balance of safety, efficacy, and target engagement. In other words, evidence supporting that any given therapeutic product enters the brain and reaches the right target in a concentration high enough to be efficient needs to be provided. In addition to delivery routes targeting neurosensory organs such as the eye or the cochlea, the most frequently used approaches for CNS applications can be broadly categorized into (i) intraparenchymal, (ii) intra-CSF (intrathecal or lumbar administration, intracisternal, and intracerebroventricular), (iii) intravenous, (iv) intramuscular, and (v) intranasal [11]. Final choice for delivery also needs to be tailored taking into consideration the CNS disorder to be dealing with. In recent years, the gene therapy field has witnessed an exponential increase in initiatives rising up to unprecedented levels, particularly when dealing with CNS applications, as summarized in Table 1.

**Table 1.** Summary of selected ongoing initiatives which have been available in recent years for different CNS disorders approached by with AAV-based therapeutics, such as Alzheimer disease (AD), Huntington disease (HD), amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy (SMA), as well as either vision- or hearing-related diseases. Abbreviations: Aβ (β-amyloid), APOE (apolipoprotein E), shIRS1 (short hairpin RNA against insulin receptor substrate 1), NTR (neurotrophin receptor), CCL2 (chemokine L2), ECE (endothelin-converting enzyme), NGF (nerve growth factor), scFv (semisynthetic anti-Aβ antibody), PHF1 (monoclonal antibody against TAU), IL-10 (interleukin-10), BDNF (brain-derived neurotrophic factor), GDNF (glial cell-derived neurotrophic factor), HTT (huntingtin protein), SIRT3 (mitochondrial protein deacetylase), XBP1 (X-box binding protein 1), ZNF10 (zinc finger protein 10), SREBP2 (sterol regulatory element-binding protein 2), AAT (α-1 antitrypsin), SOD1 (superoxide dismutase 1), HGF (hepatocyte growth factor), hIGF1 (insulin-like growth factor 1), DOK7 (tyrosine kinase 7), GLT1 (glutamate transporter 1), NMJ (neuromuscular junction), TGF-β1 (transforming growth factor beta 1), CAD180 (calreticulin anti-angiogenic domain), SYNE4 (spectrin repeat containing nuclear envelope family member 4), XIAP (X-linked inhibitor of apoptosis).

| Disease         | Delivery Routes     | Target                        | Species | AAV Serotype | References |
|-----------------|---------------------|-------------------------------|---------|--------------|------------|
| Alzheimer       | Intraparenchymal    | Aβ                            | Mice    | AAV1         | [12]       |
| Alzheimer       | Intraparenchymal    | APOE2                         | Mice    | AAV9 and AArh10 | [13]       |
| Alzheimer       | Intraparenchymal    | shIRS1 (IRS1: neuroprotective role) | Rats    | AAV2/DJ8 | [14]       |
| Alzheimer       | Intraparenchymal    | CCL2 (diffuse amyloid plaques) | Mice    | AAV1/2      | [15]       |
| Disease   | Delivery Routes                     | Target                                      | Species | AAV Serotype | References |
|-----------|-------------------------------------|---------------------------------------------|---------|--------------|------------|
| Alzheimer | Intraparenchymal                    | ECE (protease involved in Aβ degradation)   | Mice    | AAV5         | [16]       |
| Alzheimer | Intraparenchymal                    | NGF (improving cholinergic activity)        | Rats    | AAV2 and AAV5 | [17]       |
| Alzheimer | Intraparenchymal                    | NGF                                          | Mice    | CERE-110 (AAV2) | [18]       |
| Alzheimer | Intraparenchymal                    | PHF1 (anti-phospho-TAU antibody)            | Mice    | AAVrh10      | [19]       |
| Alzheimer | Intraparenchymal                    | CascFv59 (anti-Aβ antibody)                 | Mice    | AAV2         | [20]       |
| Alzheimer | Intraparenchymal                    | IL-10 (inhibition of proinflammatory cytokines) | Mice    | AAV1         | [21]       |
| Alzheimer | Intramuscular and intravenous       | GFP                                          | Mice    | AAV9, exo-AAV9 (IM) and AAV8 (IV) | [22]       |
| Huntington| Intramuscular                       | scFv (anti-Aβ antibody)                     | Mice    | AAV1         | [23]       |
| Huntington| Intramuscular                       | P75NTR (protective against Aβ)              | Mice    | AAV8         | [24]       |
| Huntington| Intracerebroventricular             | GFP                                          | Mice    | AAV1, AAV5, AAV8, AAV9, AAV2-BR1 and AAV2-PHP.eB | [25]       |
| Huntington| Intraparenchymal                    | 82Q (mutant Htt)                            | Rats    | AAV2         | [26]       |
| Huntington| Intraparenchymal                    | BDNF and GDNF                               | Rats    | AAV2         | [27]       |
| Huntington| Intraparenchymal                    | CRISPR/Cas9 (Htt)                           | Mice    | AAV1         | [28]       |
| Huntington| Intraparenchymal                    | SIRT3 (protective against oxidative and mitochondrial stress) | Mice    | AAV-DJ       | [29]       |
| Huntington| Intraparenchymal                    | XBP1 (involved in the splicing events of Htt) | Mice    | AAV2         | [30]       |
| Huntington| Intraparenchymal                    | mRNA or siRNA (Htt)                         | Mice    | AAV9         | [31]       |
| Huntington| Intraparenchymal                    | iRNA (Htt)                                  | Mice    | AAV8         | [32]       |
| Huntington| Intraparenchymal                    | Exon1-Q138 and wildtype Htt                 | Mice    | AAV9         | [33]       |
| Huntington| Intraparenchymal                    | Human KRAB domain from KOX1 (ZNF10); ZNF10 represses mutant Htt expression | Mice    | AAV9         | [34]       |
| Huntington| Intraparenchymal                    | GFP                                          | Rats    | AAV1, AAV2 and AAV5 | [35]       |
| Huntington| Intraparenchymal                    | GDNF (neurturin)                            | Mice    | AAV8         | [36]       |
| Huntington| Intraparenchymal                    | miHDS1 (Htt)                                | Mice    | AAV1         | [37]       |
| Huntington| Intraparenchymal                    | SREBP2 (to reverse synaptic defects in Huntington disease) | Mice    | AAV5         | [38]       |
| Huntington| Intravenous                         | siRNA (Htt)                                 | Sheep   | AAV serotype not disclosed | [39]       |
| Huntington| Intramuscular and intravenous       | iRNA (Htt)                                  | Mice    | AAV1         | [40]       |
| Huntington| Intrathecal                         | miRNA based on endogenous mir135 backbone (Htt) | Sheep   | AAV9         | [42]       |
| Disease                          | Delivery Routes                  | Target              | Species  | AAV Serotype                  | References |
|---------------------------------|----------------------------------|---------------------|----------|-------------------------------|------------|
| **Amyotrophic lateral sclerosis** | Intraparenchymal and intramuscular | GFP                 | Mice     | AAV1, AAV2, AAV5, AAV6, AAV7, AAV8 | [43]       |
|                                 | Intravenous and intracisternal   | SOD1                | Mice     | AAVrh10                       | [44]       |
|                                 | Intravenous                      | IGF1                | Mice     | AAV9                          | [45]       |
|                                 | Intraocular                      | GDNF                | Rat      | AAV9                          | [46]       |
|                                 | Intracerebroventricular          | GFP                 | Mice     | AAV9                          | [47]       |
|                                 | Intramuscular                    | HGF in SOD1 model   | Mice     | AAV6                          | [48]       |
|                                 | Intramuscular                    | hIGF1 in SOD1 model | Mice     | AAV9                          | [49]       |
|                                 | Intramuscular                    | GDNF                | Mice     | AAV2                          | [50]       |
|                                 | Intramuscular                    | GDNF                | Mice     | AAV2                          | [51]       |
|                                 | Intradural                       | GFP                 | Mice     | AAV1, AAV5, AAV8 and AAV9     | [52]       |
|                                 | Intramuscular                    | SOD1                | Mice     | AAV6                          | [53]       |
|                                 | Intramuscular                    | IGF1 and GDNF       | Mice     | AAV2                          | [54]       |
|                                 | Intramuscular                    | IGF1                | Mice     | AAV9                          | [55]       |
|                                 | Intrathecal                      | GLT1 overexpression in SOD1 animal model | Mice | AAV8 | [56] |
|                                 | Intrathecal                      | SOD1                | Mice     | AAV9                          | [57]       |
|                                 | Intracerebroventricular and intraperitoneal | C9orf72 hexanucleotide repeat expansions (generates neuropathology) | Mice | AAV9 | [58] |
| **Spinal muscular atrophy**     | Intracerebroventricular          | GFP                 | Mice     | AAV9                          | [59]       |
|                                 | Intracerebroventricular          | SMN1 (gene replacement strategy) | Mice | AAV9 | [60] |
|                                 | Intracerebroventricular (mice) and intracisternal (pigs and NHP) | hSMN1 | Mice, Pigs, and NHPs | AAV9 | [61] |
|                                 | Intracerebroventricular and intravenous | SMN1 | Mice | AAV9 | [62] |
|                                 | Intramuscular                    | DOK7 (tuning down disease severity) | Mice | AAV9 | [63] |
|                                 | Intravenous                      | SMN transgene       | Piglets and NHPs | AAVhu68 | [64] |
|                                 | Intramuscular                    | GFP                 | Mice     | AAV9                          | [65]       |
|                                 | Intrathecal                      | SMN2 (to rescue the SMA model) | Mice | AAV9 | [66] |
|                                 | Intracisternal                   | miRNA               | Mice     | AAVrh10                       | [67]       |
| Disease                        | Delivery Routes                      | Target          | Species | AAV Serotype                        | References |
|-------------------------------|--------------------------------------|----------------|---------|-------------------------------------|------------|
| Subconjunctival Vision disorders | GFP                                  | Mice           |         | AAV2, AAV6 and AAV8                | [68]       |
| Intravenous Vision disorders | CRISPR/Cas9 (retinitis pigmentosa)   | Mice           |         | AAV2, AAV6 and AAV8                | [69]       |
|                               | TGF-β1 (retinitis pigmentosa)        | Mice           | NHPs    | AAV7m8 and AAV8BP2                 | [71]       |
| Subretinal Vision disorders   | GFP                                  | Mice           | NHPs    | AAV2, AAV9, AAV-HP.B, AAV-PhBe     | [72]       |
|                               | GFP                                  | Mice           |         | AAV8                               | [73]       |
| Retinal Vision disorders      | CRISPR/Cas9 (retinal editing)        | Mice           |         | AAV2 and AAV7                      | [74]       |
| Intravitreal Vision disorders | CAD180 (endogenous inhibitor of angiogenesis) retinal neovascularization (RNV) | Mice           |         | AAV2                               | [75]       |
|                               | GFP                                  | NHPs           |         | AAV2                               | [76]       |
|                               | GFP                                  | Mice and NHPs  |         | AAV2                               | [77]       |
|                               | GFP                                  | Mice           |         | AAV2, AAV5, AAV8 and AAV9          | [78]       |
| Cochlear Hearing disorders    | CRISPR/Cas9 (gene editing)           | Mice           |         | AAV2                               | [79]       |
|                               | SYNE4 (to rescue in a deafness model)| Mice           |         | AAV9-HP.B                          | [80]       |
|                               | GFP                                  | Mice           |         | AAV2, AAV6, AAV8, AAV/Anc80L65     | [81]       |
|                               | GFP                                  | Mice           |         | AAV2, AAV9 and Anc80L65            | [82]       |
|                               | GFP                                  | Mice           |         | AAV1, AAV2, AAV6.2, AAV8, AAV9, AAVr.h.39, AAVr.h.43 and Anc80L65 | [83]       |
| Canalastomy (inner ear cells) | CRISPR/Cas9 (GFP, Biodistribution)   | Mice           |         | AAV8                               | [84]       |
|                               | XIAP against Cisplatin (chemotherapeutic agent) | Mice        |         | AAV2                               | [85]       |
| Round window membrane Utricle (inner and outer cells) | GFP                                  | Mice           |         | AAV9-HP.B, Anc80L65 and AAV2.7m8   | [89]       |
|                               | Harmonin-a1 and harmonin-b1 (To rescue Usher syndrome type 1c) | Mice     |         | AAV1 and AAV/Anc80L65              | [87]       |
|                               | GFP                                  | Mice           |         | AAV1 and exo-AAV1                  | [88]       |
The most commonly used routes for AAV delivery in the brain are intraparenchymal and intra-CSF (lumbar, intracisternal, or intracerebroventricular). Although less commonly used, a subpial delivery route has also been reported elsewhere [91,92]. Other ways to cope with CNS disorders bypassing the blood–brain barrier (BBB) are intranasal delivery [93], systemic eye delivery, and ear delivery [68–70,73,81,83,84]. In the case of disorders engaging motor neurons of the spinal cord, intramuscular delivery can also be viewed as a feasible approach [53,54,63,65].

3. Intraparenchymal Deliveries

Intraparenchymal AAV delivery requires stereotaxic surgery, a procedure where a needle or cannula is inserted directly into the desired target area, as defined with three coordinates (e.g., rostrocaudal, mediolateral, and dorsoventral coordinates). By delivering the viral vector this focused way, a high transduction efficiency is expected; therefore, the intraparenchymal delivery is the choice most frequently used in the treatment of brain disorders such as Alzheimer disease (AD), Huntington disease (HD) (see Tables 1 and 2), or Parkinson disease (PD) [11]. When translating preclinical research toward clinical uses, the use of pressurized convection-enhanced delivery (CED) is the procedure most often used [94–96]. Compared to any other available delivery route, the intraparenchymal approach holds several advantages, such as (i) high transduction efficacy within the target region, (ii) reduced amounts of AAV needed (both in terms of total delivered volume and titration), (iii) BBB bypassing, (iv) little concern—if any—when dealing with neutralizing antibodies, and (v) off-target effects (e.g., transduction of peripheral organs) very unlikely.

Regarding intraparenchymal deliveries, the recent availability of AAV capsid variants engineered to enhance retrograde spread of the encoded transgene also represents an appealing choice. Among others, AAV2-retro [97], AAV-TT [98], and AAV-MNM008 [99] are well suited for multiple transduction of neurons innervating the injected site.

4. Intra-CSF Deliveries

Intra-CSF AAV deliveries collectively represent another feasible way for viral vector administration. This administration is less invasive than intraparenchymal delivery. Furthermore, compared to intravenous administration, a reduced immune response together with fewer off-target effects in peripheral organs is expected. It can be achieved through lumbar puncture, cisterna magna injection, or administration into the lateral ventricles [100] (Table 1). However, a potential toxic effect at the level of the dorsal root ganglia needs to be taken into consideration [101]. Although this delivery route has its own inherent advantages, vector dilution and the limited penetration/transduction in deep brain structures collectively represent important limiting factors that need to be properly balanced before pushing forward any therapeutic development [11]. In this regard, it is worth noting that the CSF volume is replaced five times per day in humans, and the pattern of CSF circulation indeed needs to be properly understood when tailoring therapeutic uses. In our experience, intra-CSF deliveries of AAV resulted in highly variable patterns of neuronal transduction throughout the cerebral cortex, only affording a desired consistent pattern when dealing with efficient transduction of neurons in the spinal cord.

5. Intravenous Delivery Routes

Intravenous AAV deliveries have been widely used in the past (see Table 1). Although some AAV serotypes—AAV9 in particular—have been reported to be efficient when transducing the CNS upon systemic delivery, some concerns still remain regarding BBB passage. Highest efficacy rates were obtained in newborn animals, whereas there is a limited BBB penetration in adult animals. In an attempt to circumvent this limitation, years ago Viviana Gradinaru and Benjamin Deverman developed the AAV9 variant known as AAV9-PHP.B and AAV9-PHP.eB (making reference to “enhanced B”, introduced later on), a capsid variant specifically designed for enhancing BBB bypass [102]. Although initial results afforded an impressive performance for AAV9-PHP.B in C57BL6 mice, some limitations in terms
of BBB penetrance were reported later on when using different strains of mice, as well as in NHPs [103,104]. Regardless of BBB passage, main limitations inherent to systemic deliveries can be broadly summarized as (i) need for high volume of AAV to be injected, with high titration levels, (ii) undesired off-target effects, in particular potential liver toxicity, and (iii) limited CNS transduction, at least when relying on most of the currently available AAV capsid variants.

6. AAV Delivery in Sensory Organs

Direct AAV delivery into the eye currently represents a good example of preclinical experiments translated to several ongoing clinical trials. There are several different delivery options, such as (i) subretinal, (ii) intravitreal, (iii) intracameral, (iv) subchoroidal, or (v) topical (Figure 1). Both the subretinal and the intravitreal choices are those most commonly used [70,75], somewhat predictable considering the isolation and compartmentalization of the eye and the specificity of an injection in these areas. When considering targeting the inner ear, AAV delivery can be achieved through cochlear injection, transcanal administration, oval window, or the row window membrane (RWM) (Table 1 and Figure 1). Unlike AAV eye delivery, the ear delivery of AAVs has still not yet entered into clinical practice, although a number of promising preclinical studies are currently ongoing (Table 1).

Figure 1. Illustration of most commonly used AAV delivery routes. For CNS diseases (e.g., Parkinson, Alzheimer, Huntington), the intraparenchymal administration of viral particles is by far the strategy most commonly used, followed by intra-CSF administration (intraventricular, intracisternal, and intrathecal). Several ongoing gene therapy studies are focused on targeting blindness and deafness disorders, and, in these scenarios, eye delivery (e.g., subretinal, intravitreal, intracameral, etc.) and ear delivery (e.g., cochleostomy or RWM) have proven preclinical success.
7. AAV-Mediated Therapeutic Uses: The Path to the Clinical Scenario

The use of AAVs for the treatment of CNS disorders exemplifies translation of preclinical evidence toward clinical trials, beginning with pioneer experiences [105,106], up to a quickly growing list of clinical trials. Indeed, a broad majority of the ongoing AAV clinical trials are targeting several neurological diseases. Among the different AAV serotypes available, AAV2 and AAV9 rank as the most commonly used within the context of PD [11]. AAV2 undergoes anterograde axonal transport in rat and non-human primate brain [107,108], while AAV9 shows both anterograde and retrograde transport [109]. The use of AAV2 is often the main option in the case of AD, eye delivery-related diseases, and other neurological disease as Batten disease. On the other hand, AAV9 is the most popular choice for neuromuscular dystrophies or atrophies such as ALS or SMA.

When considering PD under a simplistic view as a basal ganglia-related disorder primarily affecting the nigrostriatal pathway, the most rationale scenario implies an intraparenchymal delivery route administering a given therapeutic AAV either into the substantia nigra pars compacta (SNc) or into the striatum [11,110,111]. Considering AD as a whole-brain disorder, intraparenchymal, intracisternal, or intrathecal administrations are the options most commonly used. Lastly, diseases such as SMA are usually approached through either intravenous or intramuscular injections (Table 1).

Ongoing gene therapy clinical trials for PD can be broadly categorized on the basis of the chosen target: (i) dopamine-related, (ii) neurotrophic factors, (iii) neuromodulators, and (iv) specific genetic mutations. Dopamine-related approaches take advantage of AAVs coding for l-aromatic acid decarboxylase (AADC), the enzyme converting levodopa into dopamine [112–114]. Neurotrophic factors such as GDNF or NRTN have also been introduced into the clinical path [115–119], with GDNF AAV-based therapies currently witnessing a revival. Regarding, neuromodulation, some clinical trials have been carried out using the enzyme glutamic acid decarboxylase (GAD) [120–124], with the purpose of switching the functional activity of the STN from excitation to inhibition. Lastly, targeting particular genetic mutations in disease-related genes has recently opened a completely new scenario. This is the case of glucocerebrosidase (GCase), a lysosomal enzyme encoded by the GBA1 gene [125]. When going this way, promising results were obtained in several different preclinical studies carried out in mice and in NHP [126–128].

Similarly to PD, gene therapy ongoing clinical trials in the AD field can also be categorized on the basis of the selected target: (i) neurotrophic factors from the GDNF family, brain-derived neurotrophic factor (BDNF), and beta-nerve growth factor (NGF), (ii) neuromodulators such as GAD, and (iii) specific mutations, particularly in apolipoprotein E (APOE).

Within the field of motor-related neurological disorders, SMA is a good example of ongoing clinical trials with AAVs. When considering SMA, the survival of motor neuron (SMN) is the preferred choice (Table 2). Treatments intended to overexpress cytotoxic T cell GalNAc transferase (GALGT2) in skeletal muscles for the purpose of inhibiting the development of muscular dystrophy have been explored in mice [129]. Moreover, the use of human alpha-sarcoglycan (hαSG) has shown efficacy for treatment of muscular dystrophies. Despite several preclinical attempts made for testing AAV-related therapies for the treatment of ALS, ongoing clinical trials challenging this devastating disorder are still lacking. A single dose of a DNA-based gene therapy (AVXS-101 or Zolgensma®) has been approved for the clinical treatment of SMA type 1. Although the beneficial effect of this treatment is clear, increases in AST and ALT liver enzymes have been reported. Resulting from this therapy, life expectancy increased for children enrolled in the trial. The clinical results suggested persistence of the transgene activity in the treated patients [130–132]; however, thrombotic microangiopathy (TMA) has been reported as an undesired side effect sometimes observed. The expected beneficial effect for gene therapy-based treatments targeting genetic disorders needs to be properly balanced with issues such as liver toxicity, vascular injury, and neurotoxicity.
Table 2. AAV-based clinical trials for neurological disorders with AAV for PD, AD, HD, SMA and blindness related diseases. (http://www.genetherapynet.com/clinical-trials.html; last access: 10 February 2022). Abbreviations: hTERT (active telomerase), CM (cisterna magna), STN (subthalamic nucleus), NBM (nucleus basalis of Meynert), TH (thalamus), AADC (aromatic l-amino acid decarboxylase), GDNF (glial cell-derived neurotrophic factor), GAD (glutamic acid decarboxylase), NRTN (neurturin), GBA (lysosomal enzyme glucocerebrosidase), APOE (apolipoprotein E), NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), HTT (huntingtin), RPGR (retinitis pigmentosa GTPase regulator), MCO-I (multi-characteristic opsip 1), ND4 (NADH-ubiquinone oxidoreductase chain 4, IP (intraparenchymal), ICV (intracerebroventricular), IV (intravenous), IT (intrathecal), IC (intracisternal), IM (intramuscular), IVT (intravitreal), SR (subretinal), REP1 (Rab escort protein 1), RPE (retinal pigment epithelium), MERTK (proto-oncogene tyrosine kinase MER), PDE6B (phosphodiesterase 6B), RS1 (retinoschisin 1), Ab (antibody), VEGF (vascular endothelial growth factor), CNGA3 (cyclic nucleotide-gated cation channel alpha-3), CNGB3 (cyclic nucleotide-gated cation channel beta-3).

| Disease       | Clinical Trial | Duration   | Phase | Target | AAV Serotype | Delivery Routes | Status                          | Company References |
|---------------|----------------|------------|-------|--------|--------------|----------------|---------------------------------|-------------------|
| Parkinson     | NCT01973543    | 2013–2020  | I     | AADC   | AAV2         | IP in the Putamen | Completed                      | [112] University of California |
|               | NCT02418598    | 2015–2018  | I/II  | AADC   | AAV2         | IP in the Putamen | Terminated (another clinical study for regulatory approval is planned) | [113] Jichi Medical University |
|               | NCT03065192    | 2017–2021  | I     | AADC01 | AAV2         | IP in the Putamen | Active, not recruiting          | Neurocrine Biosciences |
|               | NCT03562494    | 2018–2022  | II    | AADC02 | AAV2         | IP               | Active, not recruiting          | [114] Voyager Therapeutics (Neurocrine Biosciences) |
|               | NCT03733496    | 2018–2026  | IV    | AADC01 | AAV2         | IP in the Putamen | Enrolling, by invitation        | [112,133,134] Voyager Therapeutics (Neurocrine Biosciences) |
|               | NCT04167540    | 2020–2022  | I     | GDNF   | AAV2         | IP in the Putamen | Recruiting                      | Ask Bio (formerly Brain Neurotherapy Bio, Inc.) |
|               | NCT01621581    | 2013–2022  | I     | GDNF   | AAV2         | IP in the Putamen | Completed                      | [114–117] National Institute of Neurological Disorders and Stroke |
| Disease          | Clinical Trial | Duration | Phase | Target | AAV Serotype | Delivery Routes | Status                                      | Company                  | References                  |
|------------------|----------------|----------|-------|--------|--------------|----------------|--------------------------------------------|--------------------------|-----------------------------|
| Parkinson        | NCT00643890    | 2008–2010| II    | GAD    | AAV2         | IP in the STN | Terminated (due to financial reasons)      | Neurologix, Inc.         | [120–123]                  |
|                  | NCT00195143    | 2003–2005| I     | GAD    | AAV2         | IP in the STN | Completed                                  | Neurologix, Inc.         | [121–124]                  |
|                  | NCT01301573    | 2011–2012| IV    | GAD    | AAV2         | IP in the STN | Terminated (due to financial reasons)      | Neurologix, Inc.         |                             |
|                  | NCT00252850    | 2005–2007| I     | NRTN   | CERE-120 (AAV2) | IP in the Putamen | Completed                                  | Ceregene                 | [118]                       |
|                  | NCT00985517    | 2009–2017| I/II  | NRTN   | CERE-120 (AAV2) | IP in the Putamen | Completed                                  | Sangamo Therapeutics      | [119]                       |
|                  | NCT00400634    | 2006–2008| II    | NRTN   | CERE-120 (AAV2) | IP in the Putamen | Completed                                  | Ceregene                 | [118]                       |
|                  | NCT04127578    | 2020–2027| I/II  | GBA1   | AAV9         | IC in the CM  | Recruiting                                  | Prevail Therapeutics      |                             |
| Alzheimer        | NCT03634007    | 2019–2023| I     | APOE2  | AAVrh.10h    | IC in the CM  | Recruiting                                  | Lexeo Therapeutics        |                             |
|                  | NCT04133454    | 2019–2021| I     | hTERT  | N.A.         | IV and IT     | The status was recruiting; currently unknown| Libella Gene Therapeutics |                             |
|                  | NCT00087789    | 2004–2010| I     | NGF    | CERE-110 (AAV2) | IP in the NBM | Completed                                  | Ceregene                 |                             |
|                  | NCT00876863    | 2008–2015| II    | NGF    | CERE-110 (AAV2) | IP in the NBM | Completed                                  | Sangamo Therapeutics      | [135]                       |
|                  | NCT05040217    | 2021–2025| I     | BDNF   | AAV2         | IP            | Recruiting                                  |                         | [136,137]                  |
| Huntington's disease | NCT04885114  | 2021–2024| I     | miHtt   | AAV1         | IP in the Putamen and TH | Withdrawn (novel AAV that may enable IV delivery) | Voyager Therapeutics     |                             |
|                  | NCT04120493    | 2019–2026| I/II  | miHtt   | AAV5         | IP in the striatum | Recruiting                                  | UniQure Biopharma B.V.   | [138]                       |
### Table 2. Cont.

| Disease                      | Clinical Trial | Duration       | Phase | Target | AAV Serotype | Delivery Routes | Status              | Company References                  |
|------------------------------|----------------|----------------|-------|--------|--------------|-----------------|---------------------|-------------------------------------|
| Spinal muscular atrophy      | NCT03306277    | 2017–2019      | III   | SMN    | AAV9         | IV              | Completed           | Novartis Gene Therapies [139]       |
|                              | NCT04042025    | 2020–2035      | IV    | SMN    | AAV9         | IV              | Enrolling by invitation | Novartis Gene Therapies             |
|                              | NCT03837184    | 2019–2021      | III   | SMN    | AAV9         | IV              | Completed           | Novartis Gene Therapies [139]       |
|                              | NCT02122952    | 2014–2017      | I     | AVXS-101| AAV9        | IV              | Completed           | [140,141]                           |
|                              | NCT03461289    | 2018–2020      | III   | SMN    | AAV9         | IV              | Completed           | Novartis Gene Therapies             |
|                              | NCT03381729    | 2017–2024      | I     | SMN    | AAV9         | IT              | Completed           | Novartis Gene Therapies             |
| Vision-related diseases      | NCT02781480    | 2016–2018      | I/II  | RPE65  | AAV2/5       | SR              | Completed           | MeiraGTx UK II [142]                |
| Leber’s congenital amaurosis | NCT01496040    | 2011–2014      | I/II  | RPE65  | AAV2/4       | SR              | Completed           | Nantes University Hospital          |
|                              | NCT00516477    | 2007–2018      | I     | RPE65  | AAV2         | SR              | Completed           | Spark Therapeutics                  |
|                              | NCT00999609    | 2012–2029      | III   | RPE65  | AAV2         | SR              | Active, not recruiting | [142,143] Spark Therapeutics       |
|                              | NCT00821340    | 2016–2017      | I     | RPE65  | AAV2         | SR              | Completed           | [144,145] Hadassah Medical Organization |
|                              | NCT00481546    | 2007–2026      | I     | RPE65  | AAV2         | SR              | Active, not recruiting | [146,147] University of Pennsylvania |
|                              | NCT02946879    | 2016–2023      | I/II  | RPE65  | AAV2/5       | SR              | Recruiting          | MeiraGTx UK II [148]                |
|                              | NCT00749957    | 2009–2017      | I/II  | RPE65  | AAV2         | SR              | Completed           | [144,148] Applied Genetic Technologies Corp |
|                              | NCT02161380    | 2014–2023      | I     | ND4    | AAV2         | IVT             | Active, not recruiting | [149] University of Miami          |
|                              | NCT02652767    | 2016–2019      | III   | ND4    | AAV2/2       | IVT             | Completed           | [150] GenSight Biologics            |
|                              | NCT02652780    | 2016–2018      | III   | ND4    | AAV2/2       | IVT             | Completed           | [150] GenSight Biologics            |
|                              | NCT03153293    | 2017–2025      | II/III| ND4    | AAV2         | IVT             | Active, not recruiting | [151,152]                        |
| Disease                                      | Clinical Trial     | Duration      | Phase | Target                  | AAV Serotype | Delivery Routes | Status            | Company                          | References                  |
|----------------------------------------------|--------------------|---------------|-------|-------------------------|--------------|-----------------|--------------------|----------------------------------|-----------------------------|
| Vision-related diseases                      | NCT01482195        | 2011–2019     | I     | MERTK                  | AAV2         | SR              | Completed          | King Khalid Eye Specialist Hospital | [153]                       |
| Retinitis pigmentosa                         | NCT03116113        | 2017–2020     | III   | BIIB112 (RPGR)         | AAV8         | SR              | Enrolling by invitation | NightstaRx, Biogen Company       | [154]                       |
|                                             | NCT03252847        | 2017–2020     | I/II  | RPGR                   | AAV2/5       | SR              | Completed          | MeiraGTx UK II                  |                             |
|                                             | NCT03326336        | 2018–2025     | I/II  | GS030-DP               | AAV2.7m8     | IVT             | Recruiting         | GenSight Biologics               |                             |
|                                             | NCT04919473        | 2019–2020     | I/II  | vMCO-I                 | AAV2         | IVT             | Recruiting         | Nanoscope Therapeutics           |                             |
|                                             | NCT03328130        | 2017–2026     | I/II  | PDE6B                  | AAV2/5       | SR              | Recruiting         | [155,156] Horama                 |                             |
|                                             | NCT04945772        | 2021–2023     | II    | vMCO-010               | AAV2         | IVT             | Recruiting         | Nanoscope Therapeutics           |                             |
|                                             | NCT04850118        | 2021–2029     | II/III| RPGR                   | AAV2         | SR              | Not yet recruiting | Applied Genetic Technologies    |                             |
|                                             | NCT03316560        | 2018–2026     | I/II  | RPGR                   | AAV2         | SR              | Recruiting         | Applied Genetic Technologies    |                             |
|                                             | NCT04312672        | 2019–2023     | I/II  | RPGR                   | AAV2         | SR              | Recruiting         | MeiraGTx UK II                  |                             |
| Retinitis pigmentosa/choroideremia           | NCT03584165        | 2018–2027     | III   | BIIB111 (REP1) and BIIB112 (RPGR) | AAV2 and AAV8 | SR              | Enrolling by invitation | NightstaRx, Biogen Company       |                             |
| Choroideremia                                | NCT02161380        | 2011–2017     | I/II  | REP1                   | AAV2         | SR              | Active, not recruiting | University of Oxford            | [157–160]                   |
|                                             | NCT02553135        | 2015–2018     | III   | REP1                   | AAV2         | SR              | Enrolling by invitation | University of Miami              | [161]                       |
|                                             | NCT03507686        | 2018–2022     | III   | BIIB111 (REP1)         | AAV2         | SR              | Enrolling by invitation | NightstaRx, Biogen Company       | [161]                       |
|                                             | NCT02077361        | 2015–2025     | III   | REP1                   | AAV2         | SR              | Enrolling by invitation | University of Alberta            | [147,162]                   |
|                                             | NCT02671539        | 2016–2018     | III   | REP1                   | AAV2         | SR              | Enrolling by invitation | STZ eyetrial                    | [163]                       |
|                                             | NCT03496012        | 2017–2020     | III   | BIIB111 (REP1)         | AAV2         | SR              | Enrolling by invitation | NightstaRx, Biogen Company       | [161]                       |
|                                             | NCT02341807        | 2015–2022     | I/II  | REP1                   | AAV2         | SR              | Active, not recruiting | Spark Therapeutics               |                             |
|                                             | NCT02407678        | 2016–2021     | III   | REP1                   | AAV2         | SR              | Enrolling by invitation | University of Oxford            |                             |
Table 2. Cont.

| Disease                          | Clinical Trial     | Duration     | Phase | Target       | AAV Serotype | Delivery Routes | Status           | Company                        | References                  |
|----------------------------------|--------------------|--------------|-------|--------------|--------------|----------------|------------------|-------------------------------|------------------------------|
| Achromatopsia                    | NCT03758404        | 2019–2021    | I/II  | CNGA3        | AAV2/8       | SR             | Completed        | MeiraGTx UK II               | [164] Applied Genetic Technologies Corp |
| Achromatopsia                    | NCT02935517        | 2017–2025    | I/II  | CNGA3        | AAV2         | SR             | Recruiting       | Applied Genetic Technologies Corp |
| Achromatopsia                    | NCT02599922        | 2016–2025    | I/II  | hCNGB3       | AAV2         | SR             | Recruiting       | Applied Genetic Technologies Corp |
| Achromatopsia                    | NCT03001310        | 2017–2019    | I/II  | CNGB3        | AAV2/8       | SR             | Completed        | MeiraGTx UK II               | [165] Applied Genetic Technologies Corp |
| Achromatopsia                    | NCT03278873        | 2017–2024    | I/II  | CNGB3 & CNGA3| AAV2/8       | SR             | Active, not recruiting | MeiraGTx UK II               |                              |
| Retinal degeneration             | NCT00643747        | 2007–2014    | I/II  | RPE65        | AAV2/2       | SR             | Completed        | [145] University College, London |
| Retinal dystrophy                | NCT04516369        | 2020–2026    | III   | RPE65        | AAV2         | SR             | Active, not recruiting | Novartis Pharmaceuticals |
| Retinoschisis                    | NCT02416622        | 2015–2023    | I/II  | RS1          | AAV2         | IVT            | Active, not recruiting | Applied Genetic Technologies Corp |
| Age-related macular degeneration | NCT03748784        | 2018–2022    | I     | aflibercept  | AAV.7m8      | IVT            | Active, not recruiting | Adverum Biotechnologies       |
| Age-related macular degeneration | NCT04645212        | 2020–2025    | IV    | aflibercept  | AAV.7m8      | IVT            | Enrolling by invitation | Adverum Biotechnologies       |
| Age-related macular degeneration | NCT03066258        | 2017–2021    | I/II  | RGX-314 (Ab against VEGF) | AAV8 | SR | Active, not recruiting | Regenxbio |
| Diabetic macular edema/diabetic  | NCT04832724        | 2021–2022    | II    | RGX-314      | AAV8         | SR             | Recruiting       | Regenxbio                    |
| Retinopathy                      | NCT04418427        | 2020–2022    | II    | aflibercept  | AAV.7m8      | IVT            | Active, not recruiting | Adverum Biotechnologies       |
The clinical trials against HD are usually focused on the specific mutation of the huntingtin protein (Htt). Htt is the main cause of the disease, and it is involved in axonal transport, related to vesicles and microtubules. Currently, there are two ongoing clinical trials on early stages (Table 2).

Vision loss and retinal degeneration processes are appealing choices for AAV therapeutics, considering that peripheral sensory organs such as the eye are easily accessible and, therefore, fully approachable through a direct AAV delivery. Luxturna® was the first gene therapy treatment receiving FDA approval (NCT00999609). Intravitreal and subretinal injection are useful choices when targeting disorders such as Leber’s congenital amaurosis, retinositis pigmentosa, choroideremia, achromatopsia, retinal neurodegeneration, retinal dystrophy, retinoschisis, and age-related macular degeneration (Table 2).

8. Conclusions

The field of gene therapy has witnessed the arrival of new viral serotypes and capsids which have contributed to bringing AAV-based therapies closer than ever to the clinical scenario. More arrivals to the field have been constantly incorporated at a breathtaking speed. Considering gene therapy overall, main expectancies for therapeutic success are currently represented by CNS applications. Although the best is yet to come, for the very first time, the potential success of disease-modifying treatments is achievable. When implementing AAV-based therapeutics for neurological considerations, there are at least three important items to be properly balanced: (i) biosafety, (ii) selection of the most appropriate target gene, and (iii) disease-tailored delivery route. Furthermore, rare disorders are creating a completely new scenario for gene therapy application; indeed, it is worth nothing that roughly half of the lysosomal storage disorders have a neurological impact, most often related to neurodegenerative pathologies. Lastly, incoming advanced novel therapeutics such as gene therapies are demanding a clear regulatory scenario, to properly preserve patient and pharmaceutical expectations, reaching an adequate balance across all engaged stakeholders. Accordingly, recent advice issued by the FDA is a good step forward in this direction, clarifying underlying rules and regulations within the adequate framework.

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