Targeting for success: mechanistic insights into microRNA-based gene therapy for Huntington disease

Sogorb Gonzalez, M.

Chapter 6

General discussion and future perspectives

Main findings of this thesis

In order to have clinical benefit, therapies need to address the precise disease-related pathogenic mechanism at the right place and the right time. Focusing on the first two requirements, the work in this thesis aimed to contribute to our understanding of the mechanism of action of adeno-associated virus (AAV)-delivered micro(mi)RNA-based gene therapy (AAV-miRNA) for Huntington's disease.

We proposed and demonstrated four relevant mechanistic aspects related to AAV-miRNA: (1) the lowering of the highly toxic mis-spliced exon 1 HTT fragment (HTTex1) by engineered miRNAs targeting exon 1 sequence, (2) the sustained and widespread lowering of mHTT in large animal model using this approach, (3) the secretion of engineered miRNAs within extracellular vesicles as sources of translational biomarkers to monitor efficacy *in vivo* and (4) the inter-cellular dissemination of engineered miRNAs as a mechanism of therapeutic spread to cover all cells exposed to mHTT toxicity. These data provide a scientific basis for an ongoing clinical trial of the first AAV-miRNA-based gene therapy in HD patients. Above all, this work emphasizes the importance of meticulously investigating and validating the critical molecular pathological drivers of the disease in conjunction with the development of technologies that enable targeting of the majority of the degenerating neurons that cause the disease phenotype.

Below, we summarize these findings and discuss the implications of these novel concepts for the HD field and the potential use of AAV-miRNA gene therapy for other neurodegenerative diseases.

Reducing the toxic exon 1 HTT fragment

The classical view that germ-line CAG repeat expansions directly cause HD through the expression of mutant HTT (mHTT) protein expression has been challenged in recent years. It has now become apparent that somatic instability can cause a dramatic increase of the CAG repeats, and it is thought that this somatic repeat expansion, rather than the germ-line CAG repeat number, may be the driver of the accelerated neurodegeneration observed in many HD patients during mid-life (Swami *et al.*, 2009; Ciosi *et al.*, 2019). There also is ample data supporting the notion that rather than the full-length mutant huntingtin protein, an aberrantly spliced glutamine rich exon-1 fragment drives HD pathology (Sathasivam *et al.*, 2013; Franich *et al.*, 2019). Both observations are likely related, because CAG repeat expansion is known to be correlated to increased generation of aberrantly spliced exon-1 protein fragments (Neueder *et al.*, 2017). These observations have important consequences for the design of HTT lowering therapies.

The finding that HTTex1 is generated by aberrant splicing raised the alarm that nucleic-acid-based approaches currently being developed might not reduce the most toxic fragment, because most compounds target sequences distant from the CAG-containing exon 1 (Sathasivam *et al.*, 2013). The work presented in **Chapter 2** demonstrated that our AAV-delivered engineered miRNA targeting HTT exon 1 sequence (AAV-miHTT) effectively suppressed translation of both the mutant full-length HTT protein and the highly pathogenic HTTex1 fragment. In contrast to most other nucleic-acid-based approaches for HD, AAV5-miHTT targets exon 1 sequence, thereby lowering HTTex1 transcript, as demonstrated in this thesis (**Chapter 2**). To our knowledge, this is the first study that demonstrates the efficacy of a HTT lowering-based treatment to successfully reduce the pathogenic HTTex1 in a HD splicing context, which may be a critical determinant of clinical efficacy. This work also highlights the importance of the choice of the mRNA target sequence as well as considering potential pathogenic splicing events when designing silencing therapeutics for HD and other diseases. Indeed, engineered miRNAs targeting sequences close to the repeat expansion have also been effective in other CAG-repeat expansion disorders, such as spinocerebellar ataxin 3 (SCA3) (Martier *et al.*, 2019).

Apart from exon 1-targeting technologies, other approaches may reduce exposure to toxic polyglutamine species. Antisense-oligonucleotides (ASO) and short hairpin RNA (shRNA) targeting the CAG repeat expansion directly have showed selective lowering of full-length mHTT protein, reduction of RNA foci and are expected to reduce expanded HTTex1 fragments (Evers *et al.*, 2011; Datson *et al.*, 2017; Urbanek *et al.*, 2017; Kotowska-Zimmer *et al.*, 2020) (**Chapter 1**, Table 1). However, these therapies are likely to downregulate the expression of other important CAG-containing genes, leading to off-target adverse effects. Protein-targeting approaches, including intrabodies that target the proline-rich region in

exon 1, have showed reduced HTTex1 aggregation and reduced gene dysregulation, but the efficacy of these approaches in the context of alternative exon-1 splicing is not yet known (Southwell *et al.*, 2009; Amaro and Henderson, 2016).

Exposure to toxic HTTex1 levels may also be prevented by suppressing somatic instability. In contrast to FL-HTT protein, the levels of the pathogenic HTTex1 are CAG length-dependent and correlate with somatic instability in HD transgenic mice (Gu *et al.*, 2022). Hence, CAG expansion in striatal neurons due to somatic instability leads to a greatly increased production of HTTex1, causing cell death and neurodegeneration. Based on this two-step model of HD pathogenesis, blocking somatic instability, when applied in early stages of the disease and in striatal cells, could slow down the disease progression. Since DNA mismatch repair (MMR) genes have been associated with disease severity and somatic instability, initial approaches to silence MMR genes, including MSH3 and MLH3, are investigated (Pinto *et al.*, 2013; Flower *et al.*, 2019; Goold *et al.*, 2019; Roy *et al.*, 2021). Interestingly, lowering of HTT protein can result in suppression of somatic repeat expansion in several genes. Preliminary data have shown that, in the liver of HD mice, lowering of full-length HTT by antisense oligonucleotides, results in reduction of somatic expansion CAG tracts in *HTT* as well as in ataxin 2 (*ATXN2*) genes (Coffey *et al.*, 2020). This suggests that HTT protein itself plays a role in regulating somatic instability occurring at several repeat expanded loci.

Widespread coverage and therapeutic effect in all affected brain regions

One important question for all HTT lowering therapies is whether the main brain areas affected in the disease, striatum and cortex, are effectively covered. Biodistribution data obtained in small animals such as mice and rats, poorly translate into larger brains and even non-human primates have much smaller brains than humans (Eaton and Wishart, 2017). In transgenic HD minipigs (Baxa *et al.*, 2013), which have a relatively large brain, we demonstrated that intrastriatal convention-enhanced delivery is an effective and well-tolerated approach to achieve widespread biodistribution and long-lasting mHTT lowering in the disease-relevant areas in a large brain (**Chapter 3**) (Valles *et al.*, 2021). This study was essential for dose finding for the ongoing phase 1b/2 clinical study in HD patients (clinicaltrial.gov, NCT04120493).

The favorable distribution of the vector DNA throughout the brain is likely related to the AAV serotype 5 (AAV5) used in these studies. Compared to other frequently used AAV serotypes, AAV5 has the ability to be transported in a retrograde and anterograde direction

along the neuronal tracts, resulting in a therapeutically relevant coverage of the brain in rodents and in non-human primates (NHP) (Colle *et al.*, 2010; Gerits *et al.*, 2015; Samaranch *et al.*, 2017). Since the striatum is connected to thalamus and cortical regions, high levels of AAV5-delivered therapeutic miRNA were also observed in these connected areas in minipigs after intrastriatal administration (**Chapter 3**), which correlated with mHTT protein lowering effect (**Chapter 5**) (Valles *et al.*, 2021).

In order to achieve global gene correction in CNS and avoid multiple-injection strategies, the injection site and volume are important factors that contribute to AAV spread and maximal efficacy (Cearley and Wolfe, 2007). Although less invasive delivery routes would be preferable, such as intrathecal or intravenous infusions, these currently available options lead to poor coverage of the striatum. For instance, following intrathecal administration in NHP, HTT-targeting ASOs showed an opposite pattern of HTT lowering, with predominant effects in cortex and spinal cord (Kordasiewicz *et al.*, 2012) and much less lowering in deep brain regions (such as the caudate and putamen) which are relevant for HD. Not targeting the right areas might be one of the reasons why trials with ASOs have recently lacked efficacy in early-manifest HD patients (Kingwell, 2021).

Since gene therapy treatments result in steady levels of therapeutic transgene for more than 7 years after one-time dosing (Yuan *et al.*, 2020; Marcó *et al.*, 2021), accessible measurements of durability and efficacy in the brain are needed. In **Chapter 3** we assessed pharmacokinetic and brain damage markers (such as neurofilament light chain, NFL) in plasma and cerebrospinal fluid (CSF) after striatal infusion, demonstrating the persistence as well as the safety of AAV-miHTT approach. These measurements in biofluids importantly contributed to the rationale for the first clinical trial (clinicaltrial.gov, NCT04120493). Unfortunately, HTTex1 targeting could not be assessed in the minipig model since it was generated using HTT cDNA without splicing signals and the animals, that were still relatively young, lacked a disease-related phenotype.

Nonetheless, the results in HD transgenic minipigs, together with separate preclinical studies in iPSC-derived cultures (Keskin *et al.*, 2019), murine models (Miniarikova *et al.*, 2017; Spronck *et al.*, 2019; Caron *et al.*, 2020) and large animal GLP toxicology studies (Spronck *et al.*, 2021) provided strong support for the further development of the AAV5-miHTT program into the clinic and identified candidate biomarkers to assess transgene expression and persistence.

Extracellular vesicles and the concept of cross-corrective silencing

Even the best AAV vectors currently available transduce <30% of target neurons (Blits *et al.*, 2010; Hammond *et al.*, 2017), which may be too low to translate into a clinically meaningful effect in neurodegenerative diseases such as HD. Hence, we have explored technologies to ensure a wider exposure to the miRNA-related mHTT silencing.

Engineered miRNAs are small interfering RNA (siRNA) sequences embedded in a pre-miRNA scaffold, which is then processed in the same manner as their endogenous counterparts (Lam *et al.*, 2015). Engineered miRNAs delivered by AAV are expressed in the nucleus and processed in the cytoplasm of target cells, where they target and degrade complementary mRNA sequences via the activation of RNA-induced silencing complect (RISC) (O'Brien *et al.*, 2018). Endogenous miRNAs have also been found to be enriched in extracellular vesicles (EV) secreted by almost all cell types (Hu *et al.*, 2012). Circulating endogenous miRNAs contained in EVs, are protected from degradation and can be taken up by other cells where they exert their biological function contributing to intercellular communication (Valadi *et al.*, 2007; Li *et al.*, 2019). The work in this thesis demonstrated that AAV-delivered therapeutic engineered miRNAs, expressed and functional within neuronal cells, are secreted within EVs (**Chapter 4**) (Sogorb-Gonzalez *et al.*, 2021) and induce gene lowering in recipient cells upon internalization (**Chapter 5**). These findings have important implications for AAV-miRNA-based gene therapies targeting brain diseases, such as AAV-miHTT for the treatment HD.

Secretion of engineered miRNAs may seem self-evident, but sorting of miRNA into EVs is a highly regulated process leading to a selected subset of cellular miRNAs being loaded into EVs (Zhang *et al.*, 2015). Among those, miR-451 has been reported as one of the most highly enriched in EVs (Guduric-Fuchs *et al.*, 2012). The short-stem miR-451 is the only miRNA which follows a non-canonical dicer-independent processing mediated by argonaute 2 (Ago2) protein (Cheloufi *et al.*, 2010; Herrera-Carrillo and Berkhout, 2017). Phosphorylation of Ago2 is known to affect miRNA sorting, suggesting that the efficient EV export of miR-451 might be mediated by Ago2 (McKenzie *et al.*, 2016). Our engineered therapeutic miRNAs targeting HTT (miHTT) and ataxin 3 (miATXN3) were designed by embedding the complementary target sequence in a pre-miR-451 backbone (Miniarikova *et al.*, 2016; Martier *et al.*, 2019). Extracellular dose-dependent levels of mature miHTT and miATXN3 were detected in EV-enriched fractions secreted by neuronal cells (**Chapter 4)** (Sogorb-Gonzalez *et al.*, 2021). The scaffold-dependent secretion of engineered miRNAs is supported by Reshke et al. who showed that pre-miR-451, when compared to preferentially intracellular pre-miR-16, was indeed efficiently loaded in EVs (Reshke *et al.*, 2020). This, together with dicer-independent processing, which results in the absence of a passenger

strand, makes the selection of pre-miR-451 an attractive candidate for the design of miRNA-based therapeutics.

The secretion of EV-protected therapeutic miRNAs into biofluids offers a source of pharmacokinetic biomarkers in CNS diseases. This is especially important for AAV-based gene therapies, where a one-time administration results in long-term expression of active therapeutic molecules in the inaccessible brain. In **chapter 4**, we detected dose-dependent levels of miHTT in the CSF of NHP up to years after AAV-miHTT striatal treatment, supporting the use of engineered miRNA profiles in CSF as useful pharmacokinetic measurements of AAV-based miRNA therapies administered to the brain parenchyma (Sogorb-Gonzalez *et al.*, 2021). These findings are critical for the assessment of transgene expression and persistence in the first clinical trial of AAV-miHTT for HD.

Extracellular vesicles were discovered as intercellular communicators due to the functional transfer of their cargo to neighboring cells (Valadi *et al.*, 2007). The work in this thesis demonstrates that EV-mediated transfer of engineered miRNAs can be used as a novel mechanism of dissemination of gene therapeutics in the brain (**Chapter 5**). We demonstrated that, upon AAV transduction and secretion of the engineered miRNA by neuronal cells, EV-enriched therapeutic miRNAs (miHTT and miATXN3) are internalized by neighboring cells where they remain functional and cause gene silencing of target genes (**Chapter 5**). This innovative "cross-corrective silencing" concept implies that AAV-transduced cells might become local "factories" of therapeutic engineered miRNAs, which can distribute and treat all disease-affected neighboring cells. A similar mechanism of dissemination is widely used for the treatment of lysosomal storage disease, where cross-correction is mediated by the secretion of AAV-expressed enzymes (Kosuga *et al.*, 2000).

For HD and other neurodegenerative diseases, considering the extend of neuropathology in multiple brain regions, the intercellular dissemination of engineered miRNAs might importantly contribute to a meaningful therapeutic effect. Moreover, the progression of neurodegenerative diseases from initially affected brain areas to other regions is known to be partially mediated by the transfer of pathological proteins within EVs (Russo *et al.*, 2012). In HD, the secretion and intercellular transfer of mHTT fragments and aggregates is thought to contribute to neuropathology progression (Pecho-Vrieseling *et al.*, 2014; Jeon *et al.*, 2016). Assuming that the dissemination of therapeutic miRNAs within EV's would follow the same route as the pathological molecules, this might also contribute to a greater cover of affected brain areas.

Concluding remarks and future perspectives

Gene therapies have evolved as a potential treatment for neurodegenerative disease, especially those with a genetically identified mutation such as HD. Compared to other treatments, AAV-based gene therapies offer a lifelong treatment after a one-time administration. The approach investigated in this thesis, AAV-miHTT, was designed to target relevant pathogenic molecules (FL-HTT and Httex1) in the deep structures of the brain (striatum) that are most affected in HD patients. Furthermore, we demonstrated that intrastriatal delivery in a large brain is well-tolerated and results in widespread efficacy, mediated by both axonal transfer (AAV) and inter-cellular transfer via extracellular vesicles. AAV-miHTT is currently being tested in a Phase1b/2 clinical trial in USA and recently in Europe as well (clinicaltrial.gov, NCT04120493). No adverse effects have been observed at one-year follow-up upon AAV-miHTT intrastriatal infusion. Although the efficacy of AAV-miHTT to improve the symptoms and behavioral signs of HD still has to be shown, the findings presented in this thesis demonstrate important mechanistic insights of the treatment, increasing the odds of hopefully reaching therapeutic efficacy for HD patients.

During the last decades, the knowledge in the field of HD has greatly advanced due to the enormous effort from academic, private and clinical teams, leading to the development and testing of potential therapeutics in HD patients. Unfortunately, the lack of efficacy in recent trials have raised concern about the potential of HTT-lowering therapies as a treatment for HD. As reviewed in this thesis, in order to achieve therapeutic success, it is critical to understand the disease pathological aspects as well as the mechanism of action of the therapeutic approach. Therefore, developing therapies should aim at targeting the right species in the right location and moment in time. Novel pathological mechanisms have been described as potential drivers of the disease in early stages, including the tissue-specific somatic instability of the CAG repeat and the consequent formation of toxic fragment and other CAG-dependent processes. In view of these findings, disease-modifying therapies are now shifting to suppressing somatic instability and reducing pathogenic HTT fragments, rather than silencing full-length mHTT. These approaches are expected to better prevent neuronal loss and brain damage hopefully resulting in greater therapeutic effects. As previously indicated, a critical aspect is the timing of treatment. The disease course is characterized by a long pre-symptomatic phase that, in mid adulthood, dramatically progresses into a severe state with progressive neuronal loss Therefore, early treatment, before irreversible somatic expansion, is likely to achieve better protection. The success of novel therapeutics will hopefully extend to pre-manifest mutation carriers. Future studies should investigate the optimal therapeutic window to ensure clinical benefit.

These concepts regarding the right target, location and timing have important consequences for clinical trials. Development of markers that correlate with levels of pathological drivers and disease progression in affected areas and in early stages are needed to assess efficacy of potential therapeutics. For instance, somatic instability or genetic modifiers such as DNA damage repair genes might become early markers to effectively predict HD progression. The main challenge is that these processes are mainly confined to deep brain structure, especially during early phases of the disease. Therefore, development of markers in accessible biofluids will contribute to advance the treatment of pre-symptomatic carriers. Moreover, recent advances in brain imaging of mHTT aggregates and fragments by positron emission tomography (PET) tracers also offer promising prospects as biomarkers (Prime *et al.*, 2020).

Finally, yet importantly, advances in the treatment HD could be highly valuable to apply to other neurodegenerative diseases. The mechanistic insights of AAV-miRNA-based gene therapy for HD demonstrated in this work will hopefully open the door to the successful and lifelong treatment of other brain diseases.

References

Amaro IA, Henderson LA. An Intrabody Drug (rAAV6-INT41) Reduces the Binding of N-Terminal Huntingtin Fragment(s) to DNA to Basal Levels in PC12 Cells and Delays Cognitive Loss in the R6/2 Animal Model. J Neurodegener Dis 2016; 2016

Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J, et al. A transgenic minipig model of Huntington's Disease. J Huntingtons Dis 2013; 2: 47–68.

Blits B, Derks S, Twisk J, Ehlert E, Prins J, Verhaagen J. Adeno-associated viral vector (AAV)-mediated gene transfer in the red nucleus of the adult rat brain: comparative analysis of the transduction properties of seven AAV serotypes and lentiviral vectors. J Neurosci Methods 2010; 185: 257–63.

Caron NS, Southwell AL, Brouwers CC, Cengio LD, Xie Y, Black HF, et al. Potent and sustained huntingtin lowering via AAV5 encoding miRNA preserves striatal volume and cognitive function in a humanized mouse model of Huntington disease. Nucleic Acids Res 2020; 48: 36–54.

Cearley CN, Wolfe JH. A Single Injection of an Adeno-Associated Virus Vector into Nuclei with Divergent Connections Results in Widespread Vector Distribution in the Brain and Global Correction of a Neurogenetic Disease. J Neurosci 2007; 27: 9928–40.

Cheloufi S, Dos Santos CO, Chong MMW, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature 2010; 465: 584–9.

Ciosi M, Maxwell A, Cumming SA, Hensman Moss DJ, Alshammari AM, Flower MD, et al. A genetic association study of glutamine-encoding DNA sequence structures, somatic CAG expansion, and DNA repair gene variants, with Huntington disease clinical outcomes. EBioMedicine 2019; 48: 568–80.

Coffey S, Andrew M, Ging H, Hamilton J, Flower M, Kovalenko M, et al. Huntingtin lowering reduces somatic instability at CAG-expanded loci. bioRxiv 2020; preprint

Colle MA, Piguet F, Bertrand L, Raoul S, Bieche I, Dubreil L, et al. Efficient intracerebral delivery of AAV5 vector encoding human ARSA in non-human primate. Hum Mol Genet 2010; 19: 147–58.

Datson NA, González-Barriga A, Kourkouta E, Weij R, Van De Giessen J, Mulders S, et al. The expanded CAG repeat in the huntingtin gene as target for therapeutic RNA modulation throughout the HD mouse brain. PLoS One 2017; 12

Eaton SL, Wishart TM. Bridging the gap: large animal models in neurodegenerative research. Mamm Genome 2017; 28: 324–37.

Evers MM, Pepers BA, van Deutekom JCT, Mulders SAM, den Dunnen JT, Aartsma-Rus A, et al. Targeting several CAG expansion diseases by a single antisense oligonucleotide. PLoS One 2011; 6

Flower M, Lomeikaite V, Ciosi M, Cumming S, Morales F, Lo K, et al. MSH3 modifies somatic instability and disease severity in Huntington's and myotonic dystrophy type 1. Brain 2019; 142: 1876.

Franich NR, Hickey MA, Zhu C, Osborne GF, Ali N, Chu T, et al. Phenotype onset in Huntington's disease knock-in mice is correlated with the incomplete splicing of the mutant huntingtin gene. J Neurosci Res 2019; 97: 1590–605.

Gerits A, Vancraeyenest P, Vreysen S, Laramée M-E, Michiels A, Gijsbers R, et al. Serotype-dependent transduction efficiencies of recombinant adeno-associated viral vectors in monkey neocortex. Neurophotonics 2015; 2: 031209.

Goold R, Flower M, Moss DH, Medway C, Wood-Kaczmar A, Andre R, et al. FAN1 modifies Huntington's disease progression by stabilizing the expanded HTT CAG repeat. Hum Mol Genet 2019; 28: 650–61.

Gu X, Richman J, Langfelder P, Wang N, Zhang S, Bañez-Coronel M, et al. Uninterrupted CAG repeat drives striatum-selective transcriptionopathy and nuclear pathogenesis in human Huntingtin BAC mice. Neuron 2022; 110: 1–20.

Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. BMC Genomics 2012; 13: 357.

Hammond SL, Leek AN, Richman EH, Tjalkens RB. Cellular selectivity of AAV serotypes for gene delivery in neurons and astrocytes by neonatal intracerebroventricular injection. PLoS One 2017; 12

Herrera-Carrillo E, Berkhout B. Survey and summary: Dicer-independent processing of small RNA duplexes: Mechanistic insights and applications. Nucleic Acids Res 2017; 45: 10369–79.

Hu G, Drescher KM, Chen XM. Exosomal miRNAs: Biological properties and therapeutic potential. Front Genet 2012; 3: 56.

Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, Bae J, et al. Human - to - mouse prion - like propagation of mutant huntingtin protein. Acta Neuropathol 2016; 132: 577–92.

Keskin S, Brouwers CC, Sogorb-Gonzalez M, Martier R, Depla JA, Vallès A, et al. AAV5-miHTT Lowers Huntingtin mRNA and Protein without Off-Target Effects in Patient-Derived Neuronal Cultures and Astrocytes. Mol Ther - Methods Clin Dev 2019; 15: 275–84.

Kingwell K. Double setback for ASO trials in Huntington disease. Nat Rev Drug Discov 2021; 20: 412–3.

Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis. Neuron 2012; 74: 1031–44.

Kosuga M, Takahashi S, Sasaki K, Li XK, Fujino M, Hamada H, et al. Adenovirus-mediated gene therapy for mucopolysaccharidosis VII: involvement of cross-correction in wide-spread distribution of the gene products and long-term effects of CTLA-4Ig coexpression. Mol Ther 2000; 1: 406–13.

Kotowska-Zimmer A, Ostrovska Y, Olejniczak M. Universal RNAi Triggers for the Specific Inhibition of Mutant Huntingtin, Atrophin-1, Ataxin-3, and Ataxin-7 Expression. Mol Ther - Nucleic Acids 2020; 19: 562–71.

Lam JKW, Chow MYT, Zhang Y, Leung SWS. siRNA Versus miRNA as Therapeutics for Gene Silencing. Mol Ther - Nucleic Acids 2015; 4: e252.

Li J, Jiang X, Wang K. Exosomal mirna: An alternative mediator of cell-to-cell communication. ExRNA 2019; 1: 1–6.

Marcó S, Haurigot V, Jaén ML, Ribera A, Sánchez V, Molas M, 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: 370–89.

Martier R, Sogorb-Gonzalez M, Stricker-Shaver J, Hübener-Schmid J, Keskin S, Klima J, et al. Development of an AAV-Based MicroRNA Gene Therapy to Treat Machado-Joseph Disease. Mol Ther - Methods Clin Dev 2019; 15: 343–58.

McKenzie AJ, Hoshino D, Hong NH, Cha DJ, Franklin JL, Coffey RJ, et al. KRAS-MEK Signaling Controls Ago2 Sorting into Exosomes. Cell Rep 2016; 15: 978–87.

Miniarikova J, Zanella I, Huseinovic A, van der Zon T, Hanemaaijer E, Martier R, et al. Design, Characterization, and Lead Selection of Therapeutic miRNAs Targeting Huntingtin for Development of Gene Therapy for Huntington's Disease. Mol Ther - Nucleic Acids 2016; 5: e297.

Miniarikova J, Zimmer V, Martier R, Brouwers CC, Pythoud C, Richetin K, et al. AAV5-miHTT gene therapy demonstrates suppression of mutant huntingtin aggregation and neuronal dysfunction in a rat model of Huntington's disease. Gene Ther 2017; 24: 630–9.

Neueder A, Landles C, Ghosh R, Howland D, Myers RH, Faull RLM, et al. The pathogenic exon 1 HTT protein is produced by incomplete splicing in Huntington's disease patients. Sci Rep 2017; 7

O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne) 2018; 9: 402.

Pecho-Vrieseling E, Rieker C, Fuchs S, Bleckmann D, Esposito MS, Botta P, et al. Transneuronal propagation of mutant huntingtin contributes to non–cell autonomous pathology in neurons. Nat Neurosci 2014; 17: 1064–72.

Pinto RM, Dragileva E, Kirby A, Lloret A, Lopez E, St. Claire J, et al. Mismatch Repair Genes Mlh1 and Mlh3 Modify CAG Instability in Huntington's Disease Mice: Genome-Wide and Candidate Approaches. PLoS Genet 2013; 9

Prime ME, Liu L, Lee MR, Khetarpal V, Brown CJ, Johnson PD, et al. Imaging Mutant Huntingtin Aggregates: Development of a Potential PET Ligand. J Med Chem 2020; 63: 8608–33.

Reshke R, Taylor JA, Savard A, Guo H, Rhym LH, Kowalski PS, et al. Reduction of the therapeutic dose of silencing RNA by packaging it in extracellular vesicles via a pre-microRNA backbone. Nat Biomed Eng 2020; 4: 52–68.

Roy JCL, Vitalo A, Andrew MA, Mota-Silva E, Kovalenko M, Burch Z, et al. Somatic CAG expansion in Huntington's disease is dependent on the MLH3 endonuclease domain, which can be excluded via splice redirection. Nucleic Acids Res 2021; 49: 3907–18.

Russo I, Bubacco L, Greggio E. Exosomes-associated neurodegeneration and progression of Parkinson's disease. Am J Neurodegener Dis 2012; 1: 217.

Samaranch L, Blits B, San Sebastian W, Hadaczek P, Bringas J, Sudhakar V, et al. MR-guided parenchymal delivery of adeno-associated viral vector serotype 5 in non-human primate brain. Gene Ther 2017; 24: 253–61.

Sathasivam K, Neueder A, Gipson TA, Landles C, Benjamin AC, Housman DE, et al. Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. PNAS 2013; 110: 2366–70.

Sogorb-Gonzalez M, Vendrell-Tornero C, Snapper J, Stam A, Keskin S, Miniarikova J, et al. Secreted therapeutics: monitoring durability of microRNA-based gene therapies in the central nervous system. Brain Commun 2021; 3

Southwell AL, Ko J, Patterson PH. Intrabody Gene Therapy Ameliorates Motor, Cognitive, and Neuropathological Symptoms in Multiple Mouse Models of Huntington's Disease. J Neurosci 2009; 29: 13589–602.

Spronck EA, Brouwers CC, Vallès A, de Haan M, Petry H, van Deventer SJ, et al. AAV5-miHTT Gene Therapy Demonstrates Sustained Huntingtin Lowering and Functional Improvement in Huntington Disease Mouse Models. Mol Ther - Methods Clin Dev 2019; 13: 334–43.

Spronck EA, Vallès A, Lampen MH, Montenegro-Miranda PS, Keskin S, Heijink L, et al. Intrastriatal administration of AAV5-MIHTT in non-human primates and rats is well tolerated and results in MIHTT transgene expression in key areas of huntington disease pathology. Brain Sci 2021; 11: 1–18.

Swami M, Hendricks AE, Gillis T, Massood T, Mysore J, Myers RH, et al. Somatic expansion of the Huntington's disease CAG repeat in the brain is associated with an earlier age of disease onset. Hum Mol Genet 2009; 18: 3039–47.

Urbanek MO, Fiszer A, Krzyzosiak WJ. Reduction of Huntington's Disease RNA Foci by CAG Repeat-Targeting Reagents. Front Cell Neurosci 2017; 11: 1–13.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 645–59.

Valles A, Evers MM, Stam A, Gonzalez MS, Brouwers C, Tornero CV, et al. Widespread and sustained target engagement in Huntington s disease minipigs upon intrastriatal microRNA-based gene therapy. Sci Transl Med 2021; 13: 1–51.

Yuan J, Zhang Y, Liu H, Wang D, Du Y, Tian Z, et al. Seven-Year Follow-up of Gene Therapy for Leber's Hereditary Optic Neuropathy. Ophthalmology 2020; 127: 1125–7.

Zhang J, Li S, Mi S, Li L, Li M, Guo C, et al. Exosome and Exosomal MicroRNA: Trafficking , Sorting , and Function. Genomics, Proteomics Bioinforma 2015; 13: 17–24.