Current Opinion in Neurology

Therapeutic strategies for Huntington’s disease

--Manuscript Draft--

Manuscript Number:	WCO330402
Full Title:	Therapeutic strategies for Huntington’s disease
Article Type:	Review Article
Corresponding Author:	Sarah J Tabrizi, MRCP PhD UCL Institute of Neurology London, UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	UCL Institute of Neurology
Corresponding Author's Secondary Institution:	
First Author:	Sarah J Tabrizi, MRCP PhD
First Author Secondary Information:	
Order of Authors:	Sarah J Tabrizi, MRCP PhD
	Carlos Estevez-Fraga, MD
	Michael D Flower, MD, PhD
Order of Authors Secondary Information:	

Title page:

Title: Therapeutic strategies for Huntington's disease

Authors: Carlos Estevez-Fraga[1,2], Michael D Flower[1,2,3] and Sarah J Tabrizi[1,2,3*]

1. University College London (UCL) Huntington's Disease Centre

2. Department of Neurodegenerative Disease, Queen Square Institute of Neurology, UCL

3. UK Dementia Research Institute at UCL, London

Corresponding author: Sarah J Tabrizi

Address: Huntington's Disease Centre, 2nd floor Russell Square House. 10-12 Russell Square. WC1B 5EH. London. United Kingdom.

Telephone: 020 3456 7890

E-mail: s.tabrizi@ucl.ac.uk

Structured Abstract

Purpose of review

Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by a trinucleotide expansion in the *HTT* gene, and current therapies focus on symptomatic treatment. This review explores therapeutic approaches that directly target the pathogenic mutation, disrupt *HTT* mRNA or its translation.

Recent findings

Zinc-finger transcription repressors and CRISPR-Cas9 therapies target *HTT* DNA, thereby preventing all downstream pathogenic mechanisms. These therapies, together with RNA interference (RNAi) require intraparenchymal delivery to the brain in viral vectors, with only a single delivery potentially required, though they may carry the risk of irreversible side effects.

Along with RNAi, antisense oligonucleotides (ASOs) target mRNA, but are delivered periodically and intrathecally. ASOs have safely decreased mHTT levels in the central nervous system of patients, and a phase 3 clinical trial is currently under way.

Finally, orally available small molecules, acting on splicing or posttranslational modification, have recently been shown to decrease mHTT in animal models.

Summary

Huntingtin lowering approaches act upstream of pathogenic mechanisms and therefore have a high *a priori* likelihood of modifying disease course. ASOs are already in late stage clinical development, whilst other strategies are progressing rapidly towards human studies.

Keywords

Huntington’s disease, gene therapy, antisense oligonucleotides, RNA interference, small molecules.

Therapeutic strategies for Huntington's disease

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by CAG trinucleotide repeat expansion in the *HTT* gene, which is fully penetrant when the number of CAG repeats exceeds 39. The mutation leads to the production of the mutant Huntingtin protein (mHTT). Clinically, HD is characterised by adult-onset, progressive cognitive, motor and neuropsychiatric symptoms, ultimately leading to death around two decades later [1*].

Biology of wild-type Huntingtin

HTT is ubiquitously expressed in the adult, taking part in numerous cellular processes such as vesicle trafficking, cell division, ciliogenesis, transcription regulation, production of brain-derived neurotrophic factor and autophagy. It is present in both the cytoplasm and the nucleus and interacts with a large number of proteins and genes [2]. During embryogenesis, it is essential for neurogenesis and neuronal migration.

It has long been known that *Htt* knockout in mice is embryonically lethal [3], and its inactivation in the early postnatal period causes progressive neurodegeneration, suggesting HTT is essential for neurodevelopment [4]. Loss of *Htt* in young mice causes pancreatitis, but no neurodegeneration, and has no detectable effect over 4 months of age [5].

Several studies have evaluated the effects of partial lowering of wild-type HTT (wtHTT) in the larger brains of adult non-human primates (NHP). Protein reduction of 45% in the striatum of the adult rhesus monkey has been shown to be clinically safe [6] with another study achieving similar levels of wtHTT lowering also in the basal ganglia not revealing side effects or neuropathological changes after 6 months [7]. Moreover, wtHTT reduction in cortical areas and spinal cord of adult NHP was not associated with adverse histological findings [8,9].

These differential effects of *Htt* deletion during different stages and throughout animal models suggest it is safe to knockdown HTT in adult patients, though may have implications for how young we are able to intervene.

Pathogenesis in HD

The chronic expression of mutant HTT (mHTT) causes protein aggregation in the nucleus and the cytosol, leading to inclusions that are the pathological hallmark of the disease. Neuronal death can also result from oligomeric stages of aggregation and there is evidence suggesting the formation of inclusions may even be protective [10].

Many factors contribute to disease pathogenesis in HD. The expanded CAG repeat can alter splicing, generating a small exon 1 HTT protein that is highly toxic. Furthermore, cell to cell spread of the protein, inflammation, mitochondrial dysfunction, RNA toxicity, alteration of DNA repair mechanisms and somatic instability also contribute to molecular pathogenesis [11].

HUNTINGTIN LOWERING THERAPIES

HD is most likely caused by a toxic gain-of-function mechanism [2], so decreasing CNS mHTT expression is expected to mitigate pathology and improve symptoms [12]. As neurodegeneration in HD follows a topographically specific pattern [13], regional mHTT lowering may have distinct effects on pathology and phenotype. This was investigated by Wang et al. (2014) who showed that reduction of mHTT in the cortex improved behaviour, while reduction in striatal neurons slowed brain atrophy rates. However, knockdown in both had synergistic effects, suggesting more widespread lowering is beneficial [14].

HTT lowering therapies can be classified as non allele-specific, reducing levels of both mHTT and wtHTT, or allele-specific, selectively lowering the mutant allele.

In this review, we focus on therapeutic reduction of *HTT* expression through a range of different approaches, including those targeting RNA, such as RNA interference (RNAi), ASOs or small molecules, and those acting on DNA, such as zinc finger proteins (ZFP) and the “clustered regularly interspaced short palindromic repeats associated caspase 9” (CRISPR-Cas9) system (Table 1) (Fig. 1).

Therapies targeting DNA

These treatments either edit the *HTT* gene or alter its transcription. Targeting the pathogenic mutation itself has the potential to prevent all downstream mechanisms [15,16]. These compounds tend to be formed of two constituents; a DNA binding element that targets the *HTT* locus, and an effector that edits the genome or modulates expression.

Zinc-finger proteins

Zinc finger domains are one of the most frequent DNA-binding motifs found in eukaryotic transcription factors [17]. In zinc finger domains a zinc ion acts as structural stabiliser [18], allowing the molecule to bind a three to five base pair DNA array while the effector element can be modified to repress transcription [17]. This approach has is a risk of production of non-human proteins prompting an immunogenic response. Furthermore, ZFP DNA binding may not completely accurate, resulting in potential off-target binding [19]. ZFPs need to be integrated into an adeno-associated virus (AAV) or lentiviral vector, and delivered intraparenchymally in order to provide stable expression. These vectors have gained increased popularity due to their long term effects, low immunogenicity and inability to replicate [20].

ZFPs, delivered by intrastriatal injection of AAV-ZFP in an HD mouse model, achieved 98% mHTT protein and 78% mRNA reduction, without lowering wtHTT [21]. Another study by Zeitler et al. (2019) evaluated an AAV-ZFP targeting the *HTT* CAG repeat in cell and mouse models. Following intrastriatal delivery, the ZFP transfected 50-70% of the striatum and achieved dose-dependent mHTT knockdown, which in turn significantly reduced mHTT aggregates, particularly if administered early in disease course. There was mild improvement in behavioural phenotypes, though there was no significant change in brain volumes, weight loss or survival. Importantly, allele specificity was only achieved when there were large differences between the number of repeats on each allele [22**].

CRISPR-Cas9

CRISPR-Cas9 technology is based on the bacterial analogue of an immune system, which protects against viral infections. It is composed of two elements; a guide RNA that is complementary to the target DNA, and a Cas9 nuclease that cleaves DNA at that location, introducing a strand break which is then repaired by endogenous mechanisms[23]. There are several potential approaches in HD, including blocking *HTT* transcription, excising CAG repeats, or targeting associated SNPs. It has been used to successfully lower mHTT in patient-derived cells [23,24], and following intrastriatal delivery in HD mouse models, the *mHTT* allele has been excised, thereby reducing expression, improving neuropathology, motor function and prolonging survival [25–27*, 28].

However, CRISPR-Cas9 has several significant disadvantages. Repair mechanisms are not entirely precise, so mutations can be introduced. There is also risk of off-target effects at similar sequences elsewhere in the genome and finally, its bacterial origin entails a risk of immune response in the human brain [23,24]. All these drawbacks will have to be assessed before administering CRISPR-Cas9 to HD patients in the context of clinical trials.

Therapies targeting RNA

There are three main approaches being investigated, each one acting at different stages of mRNA maturation; ASOs trigger the degradation of pre-mRNA in the nucleus, RNA interference (RNAi) binds to mature mRNA in the cytosol, and small molecules can alter pre-mRNA splicing to encode a protein that is not viable.

Antisense oligonucleotides

ASOs are synthetic oligomers that bind to pre-mRNA through Watson-Crick base pairing [29]. Their effects are reversible, which is advantageous in case of side effects, but they require repetitive administration. They freely enter cells, rather than requiring a viral vector, but cannot cross the blood brain barrier (BBB), so have thus far been delivered by intrathecal injection [12].

Non allele-specific ASOs

Kordasiewicz et al. (2012) showed that intraventricular infusion of a non-allele specific *HTT* ASO in HD mouse models persistently lowered HTT by 66%, restored motor function and increased survival, particularly if administered early. Furthermore, good distribution was shown in the brain of NHP [8].

$\mathrm{HTT}_{\mathrm{Rx}}$ (also known as ISIS443139 and RG6042) is an ASO that targets a nucleotide sequence common to both the mutant and the wild-type allele. Tabrizi et al. recently published the results of the first in-human phase 1-2a study evaluating the safety and tolerability of $\mathrm{HTT}_{\mathrm{Rx}}$. It included 46 patients with stage 1 HD, randomised to receive four infusions of active drug at escalating doses, or placebo. There were no significant safety concerns. Importantly, CSF mHTT levels showed a dose-dependent decrease by up to 40%. Though this study was not powered to detect changes in clinical outcomes (Fig. 2), a post-hoc analysis showed a correlation between CSF mHTT lowering and a composite functional, cognitive and motor score [30**, 31*, 32]

CSF levels of neurofilament light chain (NfL), a marker of neuroaxonal damage, showed an increase at the final visit of the phase 1-2a study which resolved at the beginning of the open-label extension (OLE). In the OLE, this increase improved by 9 months, despite continued dosing [31*]. This finding is as yet unexplained, and it remains to be seen whether levels will ultimately fall below baseline, or levels expected accounting for progression. However, resolution despite continued treatment suggests it is not an adverse effect of total HTT lowering [33]. Ventricular volumes increased in those treated with higher doses, without parallel decreases in whole brain volume, which may reflect the resolution of disease-associated inflammation, or increased CSF outflow due to the removal of mHTT from neurons [30]. In 2019, a large phase 3 study evaluating HTT_{Rx} commenced, recruiting 801 patients at over 90 sites worldwide, to be dosed for 24 months; this study is sufficiently powered to assess the drug's efficacy in symptomatic patients [34].

Allele-specific ASOs

A molecule that selectively targets the mutant allele would not carry the theoretical risks of lowering wtHTT. Allele-specificity can be achieved by either targeting the CAG repeat itself, or genetic variants inherited along with it [12].

Targeting the CAG repeat carries a substantial risk of binding other CAG containing genes, with undesirable side effects [12]. However, this approach was successfully tested in two different mouse models showing a significant decrease in mHTT mRNA, together with improved performance, decreased atrophy and reduced mHTT aggregates [35].

Although targeting linked variants is potentially safer, several ASOs would need to be independently developed in order to be able to treat the majority of HD patients [36]. Skotte et al. (2014) showed that two ASOs could potentially be used in all HD patients, though with allele specificity in only 50% [37].

Two phase 1b/2a human studies PRECISION-HD1 and PRECISION-HD2, have already commenced, targeting two SNPs enriched on the mutant allele. Each study has enrolled 48 patients with early HD for intrathecal injection of escalating doses of active drug, or placebo, with completion planned in 2020 [38*–40].

RNA interference

RNA interference (RNAi) is a natural process by which RNA molecules target mRNA for degradation by the RNA-induced silencing complex (RISC) reducing protein expression. They act further downstream in mRNA processing than ASOs, degrading mature mRNA in the cytosol. Similar to ZFPs, they require lentiviral or AAVs vectors and intrastriatal infusion in order to provide stable expression of the drug [41*], but are expected to provide long term benefit after a single delivery.

Miniarikova et al. [41] effectively suppressed HTT using microRNA (miRNA) in a rat model. The same group also injected AAV-miHTT into the thalamus and striatum of minipigs, achieving widespread distribution. After three months mHTT levels were halved in the striatum, and decreased by 21.2% in the cortex, though there was a paradoxical increase in CSF mHTT after infusion and an inflammatory response, with the production of cytokines [42**].

UniQure recently administered their AAV-miHTT (AMT-130) by striatal injection to NHPs. They saw no neurological side effects, and identified the dose that would be required to achieve knockdown in the human brain [43]. The FDA recently granted it a Fast-Track designation, permitting the initiation of the first-in-human phase 1/2 trial of AAV gene therapy in HD targeting the caudate and putamen in early manifest HD [44,45*].

Spark therapeutics employed a non-allele selective approach, with intraputaminal infusion of an AAV-miHTT that reduced mHTT by 45% in the putamen, without significant side effects [46*]. Voyager Therapeutics, used the a similar method in the rhesus macaque, achieving good distribution and 50% improvement in motor function [47*,48].

In summary, RNAi is an attractive approach for mHTT lowering, and the first human studies are planned. The main challenge is delivery, as they require the intraparenchymal injection of viral vectors into relatively small parts of the brain, which may be immunogenic and are potentially irreversible [20].

Small molecules

A small molecule drug is a compound with a low molecular weight that regulates a biological process. They are of significant interest in neurology because they can be administered orally and cross the blood-brain barrier (BBB). The main drawback is their lack of target specificity [49*].

Recently, PTC therapeutics [50], identified a small molecule that lowers both cortical and striatal mHTT in HD mice; it acts through the inclusion of a poison exon that leads to the degradation of *HTT* mRNA [51*].

Another study by Li et al. (2019) has identified compounds that interact with both the mHTT protein and the autophagosome protein microtubule-associated protein 1A/1B light chain 3. These substances targeted mHTT for allele-specific autophagy, resulting in mHTT lowering and improved phenotypes in animal models [52**].

Challenges for HTT lowering therapies

Delivery and distribution

Delivery is a major challenge facing all HTT lowering therapies, and may significantly influence each methods' therapeutic potential (Fig. 3). Current ASOs have to be administered intrathecally, and would require repeated dosing throughout life. Following CSF dosing, ASO concentration is expected to be higher in cortex than striatum, though encouragingly postmortem studies in spinal muscular atrophy (SMA) show they can distribute more widely [53]. Tabrizi et al. achieved 40-60% CSF HTT lowering, which exceeds the lowering that improved phenotype in animal models, and reflects around 20-50% reduction in striatum [30]. LPs were generally well tolerated [31], though new delivery methods, including intraventricular and subcutaneous catheters could potentially improve safety, tolerability and efficacy [54*–56]. Following CSF delivery, parenchymal distribution is limited, so approaches such as convection-enhanced delivery [56], transient BBB disruption and Focused Ultrasound (FUS) coupled with microbubbles, are gaining interest [57]. Such requirements could be overcome by small molecules that freely cross the BBB following oral intake.

ZFP, CRISPR-cas9 and miRNA require a viral vector to achieve long term transduction. These have relatively limited distribution to isolated brain regions, could potentially disrupt the host genome, and may provoke immunogenicity or neutralising antibodies [28,42,58], but have the advantage of potentially only needing a single injection.

Off-target binding

The DNA binding specificity of each approach varies, but is critical to their success. Off-target activity could disrupt other critical genes and transcripts, with potentially deleterious consequences [59]. Trials of the small molecule Risdiplam, for example, were stopped due to retinal abnormalities in animal models linked to off-target binding [60].

ASOs have good target affinity, and their reversibility is beneficial, in case of side effects. RNAi, ZFP and CRISPR-Cas9, still remain in preclinical stages of development and bear a significant risk of off target editing and knockdown, though methods are continually developing in order to avoid these risks [14,61].

Lowering of wtHTT

Preclinical animal studies suggest that HTT lowering in adults is safe and well tolerated, though it will be important to monitor for side effects in human trials from long term total HTT lowering [62]. It is important to note that HTT lowering strategies, such as ASOs, reduce, but do not completely deplete HTT. Therefore, the physiological roles of wtHTT may be preserved [19].

Clinical endpoints and when to start treatment

There is little point in developing therapies for HD if we lack the sensitivity to measure whether they modify disease course. As such, the availability of reliable biomarkers is critical. HD slowly progresses over two decades; a timeframe which is far too long for clinical trials [1]. However, imaging studies have shown regional brain atrophy precedes and then parallels symptomatic onset and disease progression [63], and CSF biomarkers, rise with disease progression [64].

The fact that imaging and CSF biomarkers are abnormal many years before clinical onset suggests intervention in the premanifest period may be necessary to prevent neurodegeneration. To this end, the HD Young Adult Study is currently investigating the earliest detectable changes in premanifest subjects far from onset [65].

Conclusion

The history of therapeutic trials in HD is beset with failures, largely because of the difficulty in influencing a complex web of downstream pathogenic mechanisms. Novel therapeutic approaches target the RNA or the pathogenic mutation itself, and have the potential to alleviate all of these. Cell and animal studies have produced encouraging results, and human trials of HTT lowering approaches, including ASOs and RNAi, are underway. Each method faces its own challenges, though delivery to the most affected brain regions is common to all.

Key points:

- Following promising results in HD animal models, several therapies targeting the causative mutation or its mRNA transcript are in clinical development.
- CRISPR-Cas9, ZFP and RNAi require one-off intraparenchymal delivery, with potentially long-term effects.
- Potential side effects from total HTT lowering include the knockdown of the wild type allele.
- ASOs have produced a dose-dependent HTT reduction in HD patients’ CNS, and a large phase 3 clinical trial is currently under way to determine whether this modifies disease course.

Acknowledgements: None

Financial Support and sponsorships: This work was in part supported by the UK Dementia Research Institute, and research grant funding from the Wellcome Trust (ref 200181/Z/15/Z) (CEF, MF, SJT)

Conflicts of interest: CEF is a subinvestigator in the active $\mathrm{HTT}_{\mathrm{Rx}}$ trials. MF was a subinvestigator in the Phase 1-2a study with $\mathrm{HTT}_{\mathrm{Rx}}$. SJT has been on scientific advisory boards with Hoffmann-La Roche Ltd, Ionis Pharmaceuticals, Shire, Teva Pharmaceuticals, GSK, Takeda Pharmaceuticals, and Heptares Therapeutics and is the global principal investigator on the $\mathrm{HTT}_{\mathrm{Rx}}$ trials, for which she receives no personal salary or fees. All honoraria for these advisory boards were paid through University College London (UCL) Consultants Ltd—a wholly owned subsidiary of UCL. The authors' host clinical institution, UCL Hospitals NHS Foundation Trust, receives funds as compensation for conducting clinical trials for Hoffman-La Roche, Ionis Pharmaceuticals, Pfizer, and Teva Pharmaceuticals.

Figures titles and legends

Figure 1 **mHTT lowering mechanisms**

Yellow sections of DNA, RNA, and protein represent the pathogenic expanded CAG tract. Therapeutic compounds are represented in pink. ZFP, zinc- finger protein; ASO, antisense oligonucleotide; RISC, RNA-induced silencing complex. Adapted with permission from Tabrizi, Gosh and Leavitt [19].

Figure 2 **CSF mHTT reduction in the ASO HTTRx phase 1-2a trial**

Percentage change in the concentration of mutant HTT in CSF, by dose group, from baseline (dotted line) to final time point, 28 days after the previous dose. Circles indicate individual patients, and horizontal lines indicate group means; 95% confidence intervals are also shown for the active-agent dose groups. Reproduced with permission from Tabrizi et al [30].

Figure 3 **Delivery methods for the different mHTT therapies**

ZFP, CRISPR-Cas9 and RNAi are integrated into AAV vectors and administered intraparenchymally. ASOs have been administered intrathecally by LP, and could potentially be delivered by portacath or Ommaya reservoir via an intrathecal or intraventricular catheter. ZFP, zinc- finger protein; ASO, antisense oligonucleotides.

Table 1 **Huntingtin lowering therapies targeting DNA and RNA**

Adapted from Wild and Tabrizi [12] with permission.

References:

*[1] McColgan P, Tabrizi SJ. Huntington's disease: a clinical review. Eur J Neurol 2018;25:24–34.

Excellent clinical review about HD.

[2] Saudou F, Humbert S. The Biology of Huntingtin. Neuron 2016;89:910–26.

[3] Nasir J, Floresco S, O'Kusky J. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell 1995;81:811–23.

[4] Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nat Genet 2000;26:300–6.

[5] Wang G, Liu X, Gaertig MA, *et al*. Ablation of huntingtin in adult neurons is nondeleterious but its depletion in young mice causes acute pancreatitis. Proc Natl Acad Sci U S A 2016;113:3359–64.

[6] Stiles DK, Zhang Z, Ge P, *et al*. Widespread suppression of huntingtin with convection-enhanced delivery of siRNA. Exp Neurol 2012;233:463–71.

[7] Grondin R, Kaytor MD, Ai Y, *et al*. Six-month partial suppression of Huntingtin is well tolerated in the adult rhesus striatum. Brain 2012;135:1197–209.

[8] Kordasiewicz HB, Stanek LM, Wancewicz E V., *et al*. Sustained Therapeutic Reversal of Huntington's Disease by Transient Repression of Huntingtin Synthesis. Neuron 2012;74:1031–44.

[9] Kaemmerer WF, Grondin R. The effects of huntingtin-lowering: what do we know so far? Degener Neurol Neuromuscul Dis 2019;Volume 9:3–17.

[10] Nucifora LG, Burke KA, Feng X, *et al*. Identification of novel potentially toxic oligomers formed in vitro from mammalian-derived expanded huntingtin exon-1 protein. J Biol Chem 2012;287:16017–28.

[11] Bates GP, Dorsey R, Gusella JF, *et al*. Huntington disease. Nat Rev Dis Prim 2015;1:15005.

[12] Wild EJ, Tabrizi SJ. Therapies targeting DNA and RNA in Huntington's disease. Lancet Neurol 2017;16:837–47.

[13] McColgan P, Seunarine KK, Razi A, *et al*. Selective vulnerability of Rich Club brain regions is an organizational principle of structural connectivity loss in Huntington's disease. Brain 2015;138:3327–44.

[14] Wang N, Gray M, Lu XH, *et al*. Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of Huntington's disease. Nat Med 2014;20:536–41.

[15] Martí E. RNA toxicity induced by expanded CAG repeats in Huntington's disease. Brain Pathol 2016;26:779–86.

[16] Neueder A, Landles C, Ghosh R, *et al*. The pathogenic exon 1 HTT protein is produced by incomplete splicing in Huntington's disease patients. Sci Rep 2017;7:1–10.

[17] Cassandri M, Smirnov A, Novelli F, *et al*. Zinc-finger proteins in health and disease. Cell Death Discov 2017;3.

[18] Laity JH, Lee BM, Wright PE. Zinc finger proteins: New insights into structural and functional diversity. Curr Opin Struct Biol 2001;11:39–46.

[19] Tabrizi SJ, Ghosh R, Leavitt BR. Huntingtin Lowering Strategies for Disease Modification in Huntington's Disease. Neuron 2019;101:801–19.

[20] Miniarikova J, Evers MM, Konstantinova P. Translation of MicroRNA-Based Huntingtin-Lowering Therapies from Preclinical Studies to the Clinic. Mol Ther 2018;26:947–62.

[21] Garriga-Canut M, Agustín-Pavón C, *et al.* Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice. Proc Natl Acad Sci U S A 2012;109.

**[22] Zeitler B, Froelich S, Marlen K, *et al*. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease. Nat Med 2019;25.

In this paper allele-selective transcription repression of mHTT using ZFP targeting the CAG expansion is studied. Treated mice showed improved pathology and increased motor function without significant neuroinflammation

[23] Malankhanova TB, Malakhova AA, Medvedev SP, *et al*. Modern Genome Editing Technologies in Huntington's Disease Research. J Huntingtons Dis 2017;6:19–31.

[24] Heman-Ackah SM, Bassett AR, Wood MJA. Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons. Sci Rep 2016;6:1–12. .

[25] Monteys AM, Ebanks SA, Keiser MS, Davidson BL. CRISPR/Cas9 Editing of the Mutant Huntingtin Allele In Vitro and In Vivo. Mol Ther 2017;25:12–23.

[26] Stanek LM, Yang W, Angus S, *et al*. Antisense oligonucleotide-mediated correction of transcriptional dysregulation is correlated with behavioral benefits in the YAC128 mouse model of huntington's disease. J Huntingtons Dis

*[27] Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer D V., Gaj T. CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model. Mol Ther - Nucleic Acids 2019;17:829–39.

This paper shows that a AAV-CRISPR-cas9 can disrupt the expression of mHTT and decrease the neuronal inclusions

*[28] Dabrowska M, Juzwa W, Krzyzosiak WJ, Olejniczak M. Precise Excision of the CAG Tract from the Huntingtin Gene by Cas9 Nickases. Front Neurosci 2018;12:1–8.

This paper shows excision of the CAG tract from the HTT gene using CRISPR-cas9 in HD patient-derived fibroblasts.

[29] Bennett CF, Swayze EE. RNA Targeting Therapeutics: Molecular Mechanisms of Antisense Oligonucleotides as a Therapeutic Platform. Annu Rev Pharmacol Toxicol 2010;50:259–93.

**[30] Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, *et al*. Targeting huntingtin expression in patients with Huntington's disease. N Engl J Med 2019;380:2307–16.

This article shows for the first time in-human, dose dependent decreases of CSF mHTT in early HD patients using a non-allele specific ASO without significant side effects.

This article shows for the first time in-human, dose dependent decreases of CSF mHTT in early HD patients using a non-allele specific ASO without significant side effects.

*[31] S. Tabrizi, B. Leavitt, P. Sanwald Ducray, *et al*. A safety, tolerability and biomarker update from an ongoing open-label extension study of RG6042 in adults with early manifest Huntington's disease. https://www.mdsabstracts.org/abstract/a-safety-tolerability-and-biomarker-update-from-an-ongoing-open-label-extension-study-of-rg6042-in-adults-with-early-manifest-huntingtons-disease/ (accessed November 13, 2019).

Results of the 9-month timepoint of the Open Label Study with HTTRx . It shows a good safety profile together with an early peak in the CSF NfL concentration that improves with follow up despite continued dosing.

[32] Trundell D, Palermo G, Schobel S, *et al*. Validity, reliability, ability to detect change and meaningful within-patient change of the CUHDRS. J Neurol Neurosurg Psychiatry 2018;89.

[33] Ducray PS, Frances N, Smart K, *et al*. Translational Pharmacokinetic/Pharmacodynamic (PK/PD) Modeling Strategy to Support RG6042 Dose Selection in Huntington's Disease (HD) (S16.005). Neurology 2019;92:S16.005.

[34] Hoffman La Roche. A Study to Evaluate the Efficacy and Safety of Intrathecally Administered RO7234292 (RG6042) in Patients With Manifest Huntington's Disease 2019. https://clinicaltrials.gov/ct2/show/study/NCT03761849?term=RO7234292&rank=3&show_locs=Y (accessed April 19, 2019).

[35] Datson NA, González-Barriga A, Kourkouta E, *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.

[36] Rodrigues FB, Wild EJ. Huntington ' s Disease Clinical Trials Corner : February 2018 2018;7:89–98.

[37] Skotte NH, Southwell AL, Østergaard ME, *et al*. Allele-specific suppression of mutant huntingtin using antisense oligonucleotides: Providing a therapeutic option for all Huntington disease patients. PLoS One 2014;9.

*[38] Panzara M. PRECISION-HD: Phase 1b/2a clinical trials of investigational

stereopure antisense oligonucleotides WVE-120101 and WVE-120102 for the treatment of Huntington’s disease. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2018 2018. https://chdifoundation.org/2018-conference/ (accessed January 20, 2020).

Outline of the first in-human allele selective trial with intrathecal ASOs

[39] Safety and Tolerability of WVE-120101 in Patients With Huntington’s Disease. https://clinicaltrials.gov/ct2/show/NCT03225833 (accessed November 13, 2019).

[40] Safety and Tolerability of WVE-120102 in Patients With Huntington’s Disease. https://clinicaltrials.gov/ct2/show/NCT03225846 (accessed November 13, 2019).

*[41] Miniarikova J, Zimmer V, Martier R, *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.

Extensive review about therapies with miRNAs lowering mHTT. It covers different delivery vectors, strengths limitations and necessary preclinical measures to be assessed before reaching human phases.

**[42] Evers MM, Miniarikova J, Juhas S, *et al.* AAV5-miHTT Gene Therapy Demonstrates Broad Distribution and Strong Human Mutant Huntingtin Lowering in a Huntington’s Disease Minipig Model. Mol Ther 2018;26:2163–77.

First publication of miRNA lowering mHTT in a large animal models of HD. THe authors used combined intrastriatal and intrathalamic delivery of a miRNA bound to an AAV resulting in widespread diffusion of the drug. They achieved decreased mHTT even in cortical regions. There was however an increase in CSF mHTT one week after injection.

[43] M. Evers, M. de Haan, A. Valles-Sanchez, E. Sawyer, S. Gill, R. Roos, S. van Deventer, P. Konstantinova JH. Translating Preclinical Data to a Human Equivalent Dose for AMT-130 AAV Gene Therapy for Early Manifest Huntington's Disease https://www.globenewswire.com/news-release/2018/10/16/1621781/0/en/Voyager-Therapeutics-Announces-Preclinical-Data-for-Huntington-s-Disease-and-Amyotrophic-Lateral-Sclerosis-Programs-at-the-Congress-of-the-European-Society-of-Gene-and-Cell-Therapy.html (accessed November 6, 2019).

[44] http://uniqure.com/gene-therapy/huntingtons-disease.php (accessed November 13, 2019).

*[45] J. Higgins, B. Blits, L. Spronck, A. Valles-Sanchez, *et al*. MRI, Clinical, and Neuropathological Findings after Bilateral Intra-striatal Administration of rAAV5-miHTT in Non-human Primates [abstract]. Mov Disord. 2019; 34 (suppl 2). https://www.mdsabstracts.org/abstract/mri-clinical-and-neuropathological-findings-after-bilateral-intra-striatal-administration-of-raav5-mihtt-in-non-human-primates/. Accessed January 27, 2020

Results of the AAV-miRNA from UniQure, AMT-130. After single infusion there was a 75% reduction of mHTT in the striatum and up to 50% in the cortex without associated toxicology and with improved pathologic and clinical phenotype. Following these and other simultaneous results FDA allowed UniQure to develop a phase 1-2 clinical trial.

*[46] McBride JL. AAV-miRNA mediated HTT lowering as a potential treatment for Huntington's disease: Dosing and biodistribution studies in mice and rhesus macaques. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 26–Mar 28, 2018 2018. https://chdifoundation.org/2018-conference/ (accessed January 20, 2020).

Study showing dose-dependent decreases in HTT-mRNA expression following administration of an AAV-miRNA using Clearpoint, an intra MRI neurosurgical delivery platform into the putamen of rhesus macaques.

[47] Sah D. VY-HTT01, an AAV miRNA gene therapy targeting huntingtin for the treatment of Huntington's disease. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2019 2019. https://chdifoundation.org/2019-conference/ (accessed January 20, 2020).

[48] Stanek LM, Sardi SP, Mastis B, *et al*. Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's Disease. Hum Gene Ther 2014;25:461–74.

Report of an mHTT lowering AAV-miRNA developed by Voyager therapeutics. The authors showed good distribution in NHP with improved pathology and good tolerance.

*[49] Banks WA, Greig NH. Small molecules as central nervous system therapeutics: old challenges, new directions, and a philosophic divide. Future Med Chem 2019;11:489–93.

Overview of the mechanism of action of small molecules in neurodegenerative diseases.

[50] https://chdifoundation.org/ptc-therapeutics-and-chdi-foundation-announce-a-collaboration-on-a-small-molecule-therapeutic-for-huntingtons-disease/ (accessed November 13, 2019).

*[51] Bhattacharyya A. Identification and development of orally administered, CNS-penetrant small molecules that lower huntingtin protein levels by inducing a novel splicing event that alters the stability of huntingtin mRNA. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2019 2019. https://chdifoundation.org/2019-conference/ (accessed January 20, 2020).

First report of a small molecule orally available decreasing mHTT levels in mouse brains through the inclusion of a toxic exon in mHTT mRNA.

**[52] Li Z, Wang C, Wang Z, *et al*. Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. Nature 2019;575:203–9.

Paper showing the feasibility of decreasing mHTT with small molecules through autophagic degradation.

[53] Finkel RS, Mercuri E, Darras BT, *et al*. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med 2017;377:1723–32.

*[54] Lakhotia A, Bhalla S, Doll E, Gump W. Use of Ommaya Reservoir with a Thoracic Spinal Catheter for Intrathecal Delivery of Nusinersen in a Patient with Spinal Muscular Atrophy Type 2 (P4.464). Neurology 2018;90.

Case report of a Ommaya reservoir connected to a intrathecal catheter as effective and safe option to deliver an ASO in SMA.

[55] Strauss KA, Carson VJ, Brigatti KW, *et al*. Preliminary Safety and Tolerability of a

Novel Subcutaneous Intrathecal Catheter System for Repeated Outpatient Dosing of Nusinersen to Children and Adults with Spinal Muscular Atrophy. J Pediatr Orthop 2018;38:e610–7.

[56] Zhou Z, Singh R, Souweidane MM. Convection-Enhanced Delivery for Diffuse Intrinsic Pontine Glioma Treatment. Curr Neuropharmacol. 2017;15(1):116-128.

[57] Karakatsani ME, Blesa J, Konofagou EE. Blood–brain barrier opening with focused ultrasound in experimental models of Parkinson's disease. Mov Disord 2019;34:1252–61.

[58] Hocquemiller M, Giersch L, Audrain M, *et al*. Adeno-Associated Virus-Based Gene Therapy for CNS Diseases. Hum Gene Ther 2016;27:478–96.

[59] Fedorov Y, Anderson EM, Birmingham A, *et al*. Off-target effects by siRNA can induce toxic phenotype. RNA 2006;12:1188–96.

[60] Hoffman La Roche. Roche community update on our molecule, RG7916 https://www.sma-europe.eu/wp-content/uploads/2017/08/Update_RG7916_Eye_document_08.08.17.pdf (accessed January 10, 2020).

[61] Tycko J, Wainberg M, Marinov GK, *et al* Mitigation of off-target toxicity in CRISPR-Cas9 screens for essential non-coding elements. Nat Commun 2019;10:4063.

[62] McBride JL, Pitzer MR, Boudreau RL, *et al*. Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. Mol Ther 2011;19:2152–62.

[63] Tabrizi SJ, Scahill RI, Durr A, *et al*. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: The 12-month

longitudinal analysis. Lancet Neurol 2011;10:31–42.

[64] Byrne LM, Rodrigues FB, Johnson EB, *et al.* Evaluation of mutant huntingtin and neurofilament proteins as potential markers in Huntington's disease. Sci Transl Med. 2018 Sep 12;10(458).

[65] Zeun P, Lowe J, Osborne-Crowley K, *et al*. F59 Huntington's disease young adult study (HD-YAS). J Neurol Neurosurg Psychiatry 2018; 89:A60 LP-A61.

Selected references:

* [1] McColgan P, Tabrizi SJ. Huntington’s disease: a clinical review. Eur J Neurol 2018;25:24–34.

Excellent clinical review about HD.

** [22] Zeitler B, Froelich S, Marlen K, Shivak DA, Yu Q, Li D, et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington’s disease. Nat Med 2019;25. https://doi.org/10.1038/s41591-019-0478-3.

In this paper allele-selective transcription repression of mHTT using ZFP targeting the CAG expansion is studied. Treated mice showed improved pathology and increased motor function without significant neuroinflammation.

* [27] Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer D V., Gaj T. CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington’s Disease Mouse Model. Mol Ther - Nucleic Acids 2019;17:829–39.

This paper shows that a AAV-CRISPR-cas9 can disrupt the expression of mHTT and decrease the neuronal inclusions

* [28] Dabrowska M, Juzwa W, Krzyzosiak WJ, Olejniczak M. Precise Excision of the CAG Tract from the Huntingtin Gene by Cas9 Nickases. Front Neurosci 2018;12:1–8.

This paper shows excision of the CAG tract from the HTT gene using CRISPR-cas9 in HD patient-derived fibroblasts.

**[30] Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, Wild EJ, Saft C, Barker RA, et al. Targeting huntingtin expression in patients with Huntington’s disease. N Engl J Med 2019;380:2307–16.

This article shows for the first time in-human, dose dependent decreases of CSF mHTT in early HD patients using a non-allele specific ASO without significant side effects.

* [31] S. Tabrizi, B. Leavitt, P. Sanwald Ducray, E. Wild, V. Schlegel, G. Hooper, A. Nicotra, J. Chevure, A. Smith, R. Lane, F. Bennett, L. Boak, R. Doody SS. A safety, tolerability and biomarker update from an ongoing open-label extension study of RG6042 in adults with early manifest Huntington's disease [abstract] n.d. https://www.mdsabstracts.org/abstract/a-safety-tolerability-and-biomarker-update-from-an-ongoing-open-label-extension-study-of-rg6042-in-adults-with-early-manifest-huntingtons-disease/ (accessed November 13, 2019).

Results of the 9-month timepoint of the Open Label Study with HTT_{Rx}. It shows a good safety profile together with an early peak in the CSF NfL concentration that improves with follow up despite continued dosing.

*[38] Panzara M. PRECISION-HD: Phase 1b/2a clinical trials of investigational stereopure antisense oligonucleotides WVE-120101 and WVE-120102 for the treatment of Huntington's disease. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2018 2018. https://chdifoundation.org/2018-conference/ (accessed January 20, 2020).

Outline of the first in-human allele selective trial with intrathecal ASOs

* [41] 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.

Extensive review about therapies with miRNAs lowering mHTT. It covers different delivery vectors, strengths limitations and necessary preclinical measures to be assessed before reaching human phases.

**[42] Evers MM, Miniarikova J, Juhas S, Vallès A, Bohuslavova B, Juhasova J, et al. AAV5-miHTT Gene Therapy Demonstrates Broad Distribution and Strong Human Mutant Huntingtin Lowering in a Huntington's Disease Minipig Model. Mol Ther 2018;26:2163–77.

First publication of miRNA lowering mHTT in a large animal models of HD. THe authors used combined intrastriatal and intrathalamic delivery of a miRNA bound to an AAV resulting in widespread diffusion of the drug. They achieved decreased mHTT even in cortical regions. There was however an increase in CSF mHTT one week after injection.

*[45] J. Higgins, B. Blits, L. Spronck, A. Valles-Sanchez, *et al*. MRI, Clinical, and Neuropathological Findings after Bilateral Intra-striatal Administration of rAAV5-miHTT in Non-human Primates [abstract]. Mov Disord. 2019; 34 (suppl 2). https://www.mdsabstracts.org/abstract/mri-clinical-and-neuropathological-findings-after-bilateral-intra-striatal-administration-of-raav5-mihtt-in-non-human-primates/. Accessed January 27, 2020

Results of the AAV-miRNA from UniQure, AMT-130. After single infusion there was a 75% reduction of mHTT in the striatum and up to 50% in the cortex without associated toxicology and with improved pathologic and clinical phenotype. Following these and other simultaneous results FDA allowed UniQure to develop a phase 1-2 clinical trial.

[46] McBride JL. AAV-miRNA mediated HTT lowering as a potential treatment for Huntington’s disease: Dosing and biodistribution studies in mice and rhesus macaques. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 26–Mar 28, 2018 2018. https://chdifoundation.org/2018-conference/(accessed January 20, 2020).

Study showing dose-dependent decreases in HTT-mRNA expression following administration of an AAV-miRNA using Clearpoint, an intra MRI neurosurgical delivery platform into the putamen of rhesus macaques.

*[47] Sah D. VY-HTT01, an AAV miRNA gene therapy targeting huntingtin for the treatment of Huntington’s disease. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2019 2019. https://chdifoundation.org/2019-conference/ (accessed January 20, 2020).

Report of an mHTT lowering AAV-miRNA developed by Voyager therapeutics. The authors showed good distribution in NHP with improved pathology and good tolerance.

*[49] Banks WA, Greig NH. Small molecules as central nervous system therapeutics: old challenges, new directions, and a philosophic divide. Future Med Chem 2019;11:489–93. https://doi.org/10.4155/fmc-2018-0436.

Overview of the mechanism of action of small molecules in neurodegenerative diseases.

*[51] Bhattacharyya A. Identification and development of orally administered, CNS-penetrant small molecules that lower huntingtin protein levels by inducing a novel splicing event that alters the stability of huntingtin mRNA. CHDI Found Annu Ther Conf Palm Springs, CA, USA; Feb 25–28, 2019 2019.

https://chdifoundation.org/2019-conference/ (accessed January 20, 2020).

First report of a small molecule orally available decreasing mHTT levels in mouse brains through the inclusion of a toxic exon in mHTT mRNA.

**[52] Li Z, Wang C, Wang Z, Zhu C, Li J, Sha T, et al. Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. Nature 2019;575:203–9.

Paper showing the feasibility of decreasing mHTT with small molecules through autophagic degradation.

*[54] Lakhotia A, Bhalla S, Doll E, Gump W. Use of Ommaya Reservoir with a Thoracic Spinal Catheter for Intrathecal Delivery of Nusinersen in a Patient with Spinal Muscular Atrophy Type 2 (P4.464). Neurology 2018;90.

Case report of a Ommaya reservoir connected to a intrathecal catheter as effective and safe option to deliver an ASO in SMA.

Figure 1

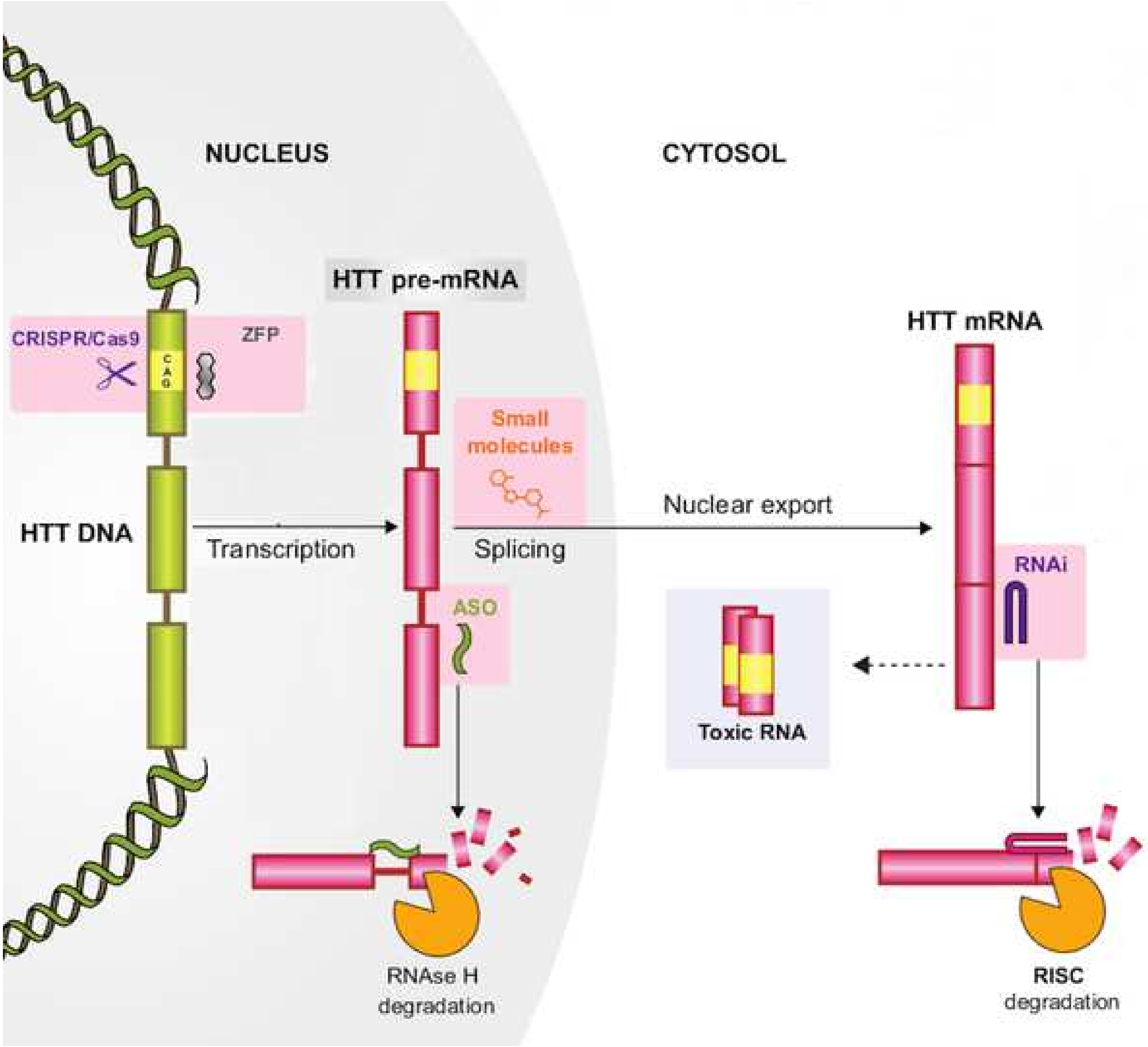
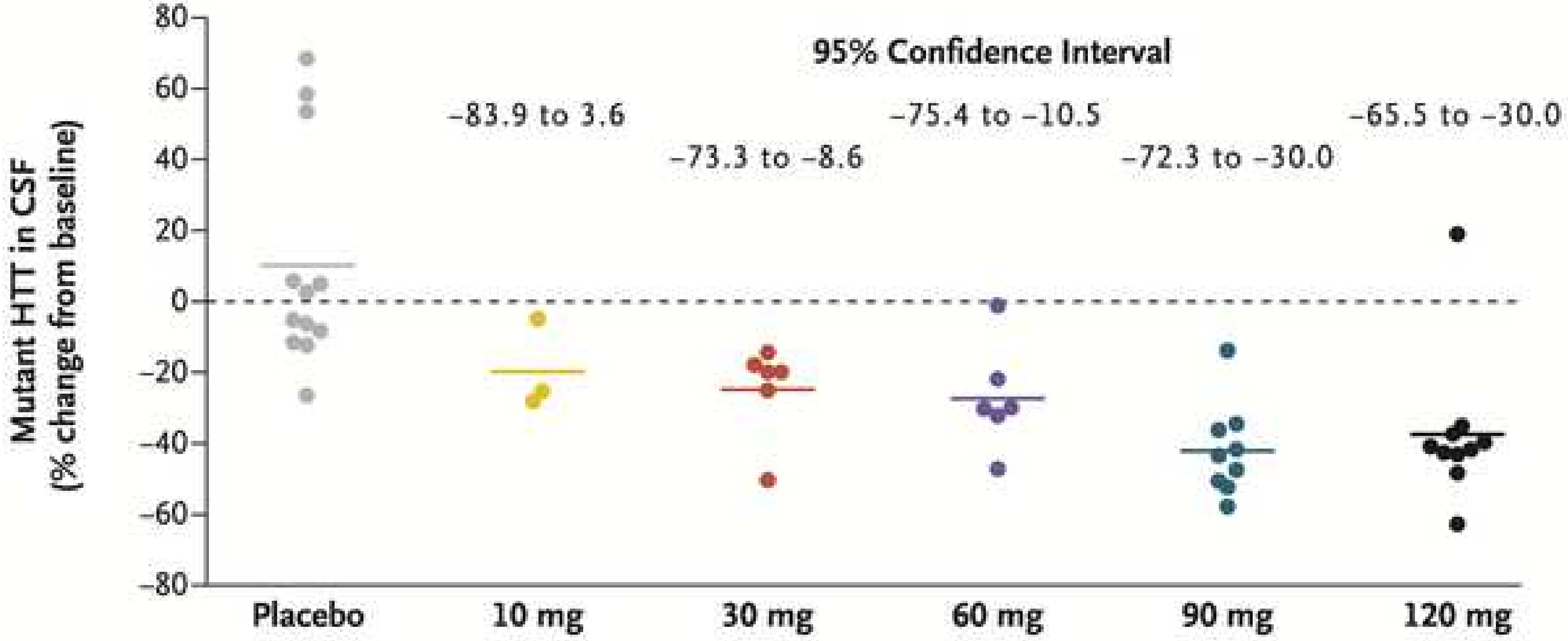


Figure 1 - mHTT lowering mechanisms copia.jpg

Figure 2



Percentage Change in CSF Concentration of Mutant HTT, According to Dose Group

Figure 3

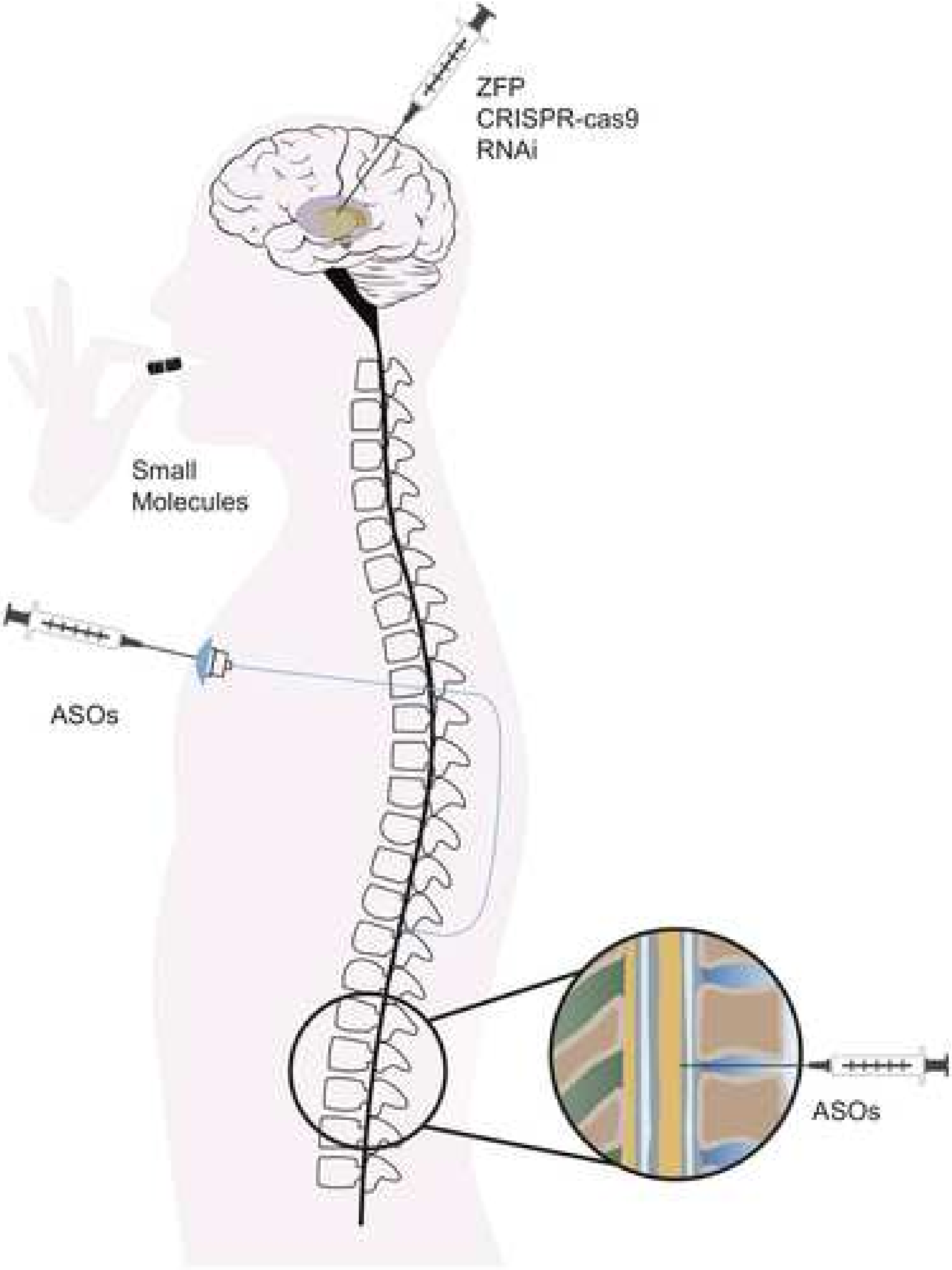


Table 1 Huntingtin lowering therapies targeting DNA and RNA

Target	Drug class	Delivery	Sponsor	Mechanism	Allele-selectivity	Stage	Advantages	Disadvantages	References
DNA	ZFP	Intracranial (AAV)	Imperial College London	Repression of transcription	Yes, CAG repeat	Preclinical	One drug for all HD patients Long-term effects after single infusion Decreasing all toxic species	Invasive Risk of persistent side effects Risk of off-target binding Risk of production of non-human proteins	Garriga-Canut 2012, Agustin-Pavon 2016
DNA	ZFP	Intracranial (AAV)	Sangamo Therapeutics/ Takeda	Repression of transcription	Yes, CAG repeat	Preclinical	Same as above	Same as above	Zeitler 2014, Zeitler 2019
DNA	CRISPR-cas9	Intracranial (AAV)	Harvard University/ University of Pennsylvania	Genome editing	Yes, SNP	Preclinical	Accurate binding to targeted DNA region Long-term effects after single infusion Still in early stages of development Decreasing all toxic species	Immunogenicity derived from bacterial proteins Risk of persistent side effects Risk of production of non-human proteins	Shin 2016, Monteys 2017, Davidson 2019
DNA	CRISPR-cas9	Intracranial (AAV)	Emory University/ University of California	Genome editing	Not allele selective, eliminates CAG expansion	Preclinical	Same as above	Same as above	Yang 2017, Ekman 2019
RNA	ASO	Intrathecal	Ionis pharmaceuticals/Hoffmann-La Roche	pre-mRNA degradation	Not allele selective	Phase 3	Reversible, titratable One drug for all HD patients	Requires repeated administration Risks of reducing wild-type protein	Kordasiewicz 2012, Tabrizi 2019

RNA	ASO	Intrathecal	Wave Life Sciences	pre-mRNA degradation	Yes, SNP	Phase 1b/2a	Reversible, titratable	Requires repeated administration Does not target all HD population with one drug	Panzara 2018, Hersch 2017,
RNA	ASO	Intrathecal	Biomarin	pre-mRNA degradation	Yes, CAG repeat	Preclinical	Reversible, titratable One drug for all HD population	Requires repeated administration May target other CAG containing genes	Datson 2017
RNA	RNAi	Intracranial (AAV)	UniQure	mRNA degradation	Not allele selective	Phase 1/2	One drug for all HD patients. Long-term effects after single infusion Limited volume distribution	Invasive Risk of persistent side effects	Miniarikova 2017, Evers 2018, Evers 2019, Valles - Sanchez 2019, Higgins 2019, Konstantinova 2019
RNA	RNAi	Intracranial (AAV)	Genzyme/Voyager therapeutics	mRNA degradation	Not allele selective	Preclinical	Same as above	Same as above	Sah 2019, Stanek 2015
RNA	RNAi	Intracranial (AAV)	Spark therapeutics	mRNA degradation	Not allele selective	Preclinical	Same as above	Same as above	McBride 2018
RNA	Small molecules	Oral	PTC therapeutics	Splicing modification	Unknown	Preclinical	Orally available	Unknown	Bhattacharya 2019, Doherty 2017

AAV=Adeno-associated virus. ASO= Antisense oligonucleotides. HD=Huntington's disease. RNAi=RNA interference. ZFP=Zinc-finger proteins