

Huntingtin-lowering strategies for Huntington's disease

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ABSTRACT

Introduction: Huntington's disease (HD) is a currently incurable, autosomal dominant neurodegenerative disease caused by an abnormally long polyglutamine tract in the huntingtin protein. As this mutation causes disease via gain-of-function, lowering huntingtin levels represents a rational therapeutic strategy.

Areas covered: We searched MEDLINE, CENTRAL and other trial databases, and relevant company and HD funding websites for press releases until April 2020 to review different strategies for huntingtin lowering, including autophagy and PROTACs, which have been studied in preclinical models. We focussed our analyses on oligonucleotide (ASOs) and miRNA approaches, which have entered or are about to enter clinical trials.

Expert opinion: ASO and mRNA approaches aiming to lower mutant huntingtin protein production and strategies aiming to increase mutant huntingtin clearance are attractive, as they target the cause of disease. However, these strategies present some unresolved questions, including the optimal mode of delivery and associated safety issues. It is unclear if the human CNS coverage with intrathecal or intraparenchymal delivery will be sufficient for efficacy. The extent that one needs to lower mutant huntingtin levels for it to be therapeutic in relation to disease course is uncertain. Finally, the extent to which CNS lowering of wild-type huntingtin is safe is unclear.

Keywords: Allele specific oligonucleotide; Autophagy; huntingtin; Huntington's disease; miRNA; neurodegeneration; polyglutamine; PROTAC

1. Introduction

1.1. *Introduction to Huntington's disease*

Huntington disease (HD) is an autosomal dominant neurodegenerative disease that manifests with abnormal movements, including chorea, cognitive impairment and psychiatric disturbances. While HD can strike at any age, its median age-of-onset is around 40 years of age. It has a prevalence of 3-10/100,000 in populations of European descent but varies a lot in different ethnic groups and countries [1]. Pathologically, the disease causes neuronal loss in many brain regions, particularly the caudate, putamen and cortex. Currently, while there are treatments that have some impact on the motor signs of the disease (like tetrabenazine for chorea), there are no proven disease-modifying therapies.

HD is caused by a CAG trinucleotide repeat expansion at the N-terminus of the huntingtin gene (HTT), which encodes an expanded polyglutamine tract in the huntingtin protein. The polyglutamine tract length is polymorphic in different wild-type alleles and expansions of 38 or more successive glutamines can cause disease. There is an inverse correlation between the polyglutamine tract length and age-at-onset of disease.

HD is characterised by the formation of neuronal inclusions/aggregates comprising mutant huntingtin and other proteins associated with it. In rare juvenile-onset HD, the aggregates are found in the nucleus, while in most adult-onset cases the aggregates are seen in the cytoplasm. While there has been discussion as to whether the large aggregates are toxic or relatively protective compared to the “soluble” protein [2,3], the toxicity does seem to be associated with the propensity of the mutant protein to aggregate and it is possible that the process of aggregation is a key component of toxicity.

Extensive data argue that the HD mutation causes disease predominantly via gain-of-function mechanisms at the protein level [4]. These include mouse studies showing that hemizygous loss-of-function of huntingtin is well tolerated, while mutant transgenes expressed on a wild-type background cause HD-like pathologies. Importantly, switching off mutant huntingtin expression results in a reversal of signs of disease in a conditional mouse model [5], as is also seen with a Cre-Lox-mediated excision of mutant exon 1 of huntingtin with the polyglutamine expansion in a BAC mouse model [6]. It may be beneficial to reduce mutant huntingtin levels not only in neurons but also in astrocytes and oligodendrocytes [7,8].

Accordingly, strategies for lowering mutant huntingtin have been explored for therapeutic purposes. These will be considered in this review.

2.. Increasing mutant huntingtin degradation

2.1. Introduction to huntingtin degradation pathways

One approach that can lower mutant huntingtin is to enhance its degradation. Mutant huntingtin can be degraded by macroautophagy (henceforth autophagy) or the ubiquitin-proteasome pathway. Macroautophagy (generally referred to as autophagy) is a bulk degradation system that engulfs proteins or organelles into double-membrane autophagosomes associated with microtubule-associated protein 1A/1B light chain 3 (LC3), for subsequent lysosomal degradation [9,10]. The clearance of mutant huntingtin has a much greater dependence on autophagy than the wild-type protein. The ubiquitin-proteasome system (UPS) can also assist mutant huntingtin degradation, but this pathway cannot clear oligomeric or higher-order aggregated forms of the protein, as the narrow entrance to the proteasome requires substrates to be monomeric and unfolded in order to pass through. However, there clearing monomeric forms is likely to have benefit.

2.2. Autophagy

Inhibition of the mammalian target of rapamycin complex 1 (mTORC1), which negatively regulates the autophagic pathway, using rapamycin, an inhibitor of mTOR, and CC1-779, an analog of rapamycin, enhanced the clearance of mutant huntingtin, resulting in decreased toxicity in cell, fly and mouse models of HD [11,12]. Similarly, induction of autophagy through mTOR-independent pathways facilitates the clearance of mutant huntingtin in mammalian cell, fly and zebrafish models [13,14], indicating that autophagy induction may be a rational therapeutic strategy for HD through lowering mutant huntingtin [15-19].

The clinical approach exploiting autophagy has been encouraged by recent repurposing studies using felodipine, an L-type calcium channel blocker that crosses the blood-brain barrier (BBB) that induces autophagy through an mTOR-independent pathway [13]. In this study, felodipine induced autophagy and lowered mutant huntingtin and other autophagy substrates when the drug was administered via subcutaneous minipumps in mice in order that

their plasma drug concentrations mimicked those seen in humans taking the drug at standard doses for its conventional antihypertensive use. This compound also protected against mutant htt toxicity in mice [20]. Thus, it may be possible to induce autophagy in mammalian brains with compounds that are already in clinical use that are neuroprotective in HD models.

2.3. Autophagosome-tethering compound (ATTEC)

Since mutant htt is degraded by autophagy, molecular glue-like molecules have also been developed that interact with both mutant htt and autophagosome-associated LC3 and by so doing facilitate engulfment of mutant HTT by autophagosomes [21] (Figure 1). These so-called autophagosome-tethering compounds (ATTEC) enhance mutant htt degradation via autophagy. ATTECs can cross the blood-brain barrier and lower mutant HTT without affecting WT htt in cultured neurons and *in vivo* brain tissue, thereby ameliorating mutant htt toxicity in *Drosophila* and mouse models.

2.4. Proteasome/PROTACs

The UPS can also be exploited to target specific substrates for degradation via proteolysis-targeting chimeras (PROTACs) [22]. This approach utilises E3 ligase ligands that are fused through a flexible chemical linker to another ligand that binds a target protein, in order to induce artificial ubiquitination, leading to degradation of the protein by the proteasome (Figure 2). In this way, PROTACs provide the potential for rapid and targeted degradation of proteins to which one generates a specific ligand. Hybrid molecules that bind a ligand for a ubiquitin E3 ligase (cellular inhibitor of apoptosis protein 1; cIAP1) to ligands for mutant HTT, reduced levels of mutant HTT through induction of selective degradation by the proteasome in cell lines [23].

3. Reducing mutant huntingtin synthesis

3.1. Introduction to strategies that may reduce huntingtin synthesis

As a monogenic disorder, HD pathology results from translation of mutant huntingtin, which has allowed development of targeted therapies along this pathway. Although gene editing would seem the logical place to start by using zinc finger nucleases or CRISPR-Cas9 [24], the tools for doing this across the human brain with the required fidelity are not currently

available, although these are being developed [25]. Some groups have investigated whether one can target huntingtin gene expression epigenetically through histone deacetylase inhibitors. This is being assessed in a phase 1 clinical trial in South Korea using CKD-504 (NCT03713892) and was investigated using phenylbutyrate in a Phase 1 trial (NCT00212316) [26].

The feasibility of reducing mutant huntingtin synthesis as a therapeutic strategy for HD has been supported by a series of different RNAi experiments in HD animal models [27]. Cleveland and colleagues showed that antisense oligonucleotides lowered both wild-type and mutant htt in 3 different mouse models of HD and thereby ameliorated signs of disease. They also reported effective huntingtin lowering in a non-human primate, and thereby set the scene for antisense therapeutics for HD [28].

As a result, a number of non-selective and selective DNA and RNA gene-silencing or gene-editing approaches for lowering mHTT are being explored, some of which have now entered clinical trials. Non-selective approaches, including some antisense oligonucleotides (ASO), will lower both mutant and wild-type huntingtin (wtHTT) proteins. The advantages and disadvantages of this strategy are unknown, as the impact of lowering wtHTT in people has not been extensively explored [29] outside of early clinical trials with ASO therapies (see below) and rare individuals with isolated genetic abnormalities within the HD gene [30]. In animal models, studies initially suggested that knocking down wtHTT has no deleterious consequences in the mammalian brain [31], but more recently it has reported that huntingtin is needed for both striatal cell survival and connectivity [32] and for regeneration at other sites in the adult CNS [33]. As such, allele-selective lowering of mHTT (e.g. using viral-encoded small interfering RNAs (siRNAs) and microRNAs) may be a safer option, as this will preserve wtHTT levels.

3.2. Antisense oligonucleotides (ASO)

ASOs are short, single-stranded synthetic oligomers that are made up of nucleotides which bind to specific sections of RNA. The purpose of ASOs is to either enable degradation of the target RNA, or terminate its translation. The nucleotides may be chemically modified to facilitate specific enzymatic functions following binding with the target RNA. In preclinical experiments with HD models, ASOs have demonstrated dose-dependent lowering of mHTT

by arresting translation of the mutant protein, resulting in long-lasting phenotypic and survival benefits [28,34-36]. This has now led to clinical trials which are discussed in more detail below [37].

3.2.1. *HTT_{rx}* / *RG6042*

RG6042 (initially known as *HTT_{rx}*) is an ASO developed by IONIS Pharmaceuticals to degrade the target RNA sequence that encodes huntingtin. RNA degradation is facilitated by triggering of RNase H1 once the ASO has bound to the target RNA, thereby reducing translation of both mHTT and wtHTT. RG6042 is the first such putative disease-modifying therapy using this strategy that has been trialled in HD. In order for the drug to work at the site where it is needed, it had to be delivered intrathecally, which is associated with logistical as well as compliance issues, especially as injections are currently given every few months.

The first-in-human HD clinical trial with RG6042 had a randomised, double-blinded, placebo-controlled multiple ascending dose design and was published in 2019 [38]. This international multicentre phase I/IIa study evaluated RG6042 in early-stage HD patients (n=46). The participants were randomised in a 3:1 ratio to receive either the ASO or placebo every 4 weeks over a 4-month period via intrathecal injection, followed by a 4-month follow-up period with no dosing. Cerebrospinal fluid (CSF) samples were collected at each dosing visit prior to administering the RG6042, and a further CSF sample was collected during the follow-up period. Repeated intrathecal administration of RG6042 was found to be well-tolerated over this short period of time and resulted in a dose-dependent reduction in CSF mHTT concentrations. Clinical efficacy could not be assessed in such a small short-term trial, but treatment did lead to a paradoxical increase in CSF neurofilament light chain levels, as well as ventricular volume - both of these measures are thought to be associated with greater neuronal loss.

At the conclusion of this trial, an open-label extension study was initiated where all participants who completed the initial study were invited to participate and were dosed with RG6042 either every 4 or 8 weeks (NCT03342053). The results of this study have yet to be formally published although the 15-month data have been presented at meetings and continue to show ~70% reduction in CSF mHtt levels in the higher frequency dose group (<https://chdifoundation.org/2020-conference/#schobel>). A further open-label extension study

using this same patient cohort was commenced in October 2019 but dosing was reduced in frequency to either every 8 or 16 weeks, in view of some of the adverse events that were seen with the more frequent dosing regime (NCT03842969).

In parallel to these open label studies, an international, randomised, double-blinded, multicentre, pivotal phase III trial to evaluate efficacy and safety of RG6042 commenced in January 2019 (n=909) (GENERATION HD1, (NCT03761849). Recruitment concluded in April 2020 and the trial is expected to finish in 2022. The results of this trial will be instrumental in determining whether this ASO may offer true disease-modifying benefit to patients with early HD.

3.2.2. WVE120101 and WVE-120102

One of the theoretical disadvantages of RG6042 (above) is its inability to discriminate between the pathological allele and the normal allele. This is important, since complete loss of huntingtin in embryogenesis and early postnatal stages causes neurodegeneration in mice [39]. While neuronal loss of huntingtin in adult mice appears to be well tolerated, huntingtin has functions relevant to nervous system maintenance [39], and the medium- and long-term effects of reducing overall huntingtin levels in humans are unclear. To avoid this, allele-selective ASOs, such as WVE-120101 and WVE-120102 have been developed to selectively target the mHTT gene and not the wild-type allele, acting at the U variant of the single nucleotide polymorphism (SNP) rs362307 (SNP1) and the U variant of SNP rs362331 (SNP2) [40]. The company estimates that about 2/3rds of HD patients will be heterozygous for one of these SNPs and thus will be eligible for treatment with this agent. This therapy has now progressed to 2 clinical trials, PRECISION HD1 (NCT03225833) and PRECISION HD2 (NCT03225846), which have tested WVE-120101 and 120102, respectively, in a multicentre, randomised, double-blinded, placebo-controlled fashion in patients with early manifest HD. The interim results of these trials, after short periods of treatment, have been made public through press releases but both trials are still ongoing and no peer-reviewed publication has appeared to date. In both trials, the agent appears to be well tolerated (with no serious adverse events reported) with a dose-dependent reduction in CSF mutant huntingtin levels of around 12% [41] with no difference seen in the level of CSF neurofilament light chain. The relatively modest reduction in CSF mtHTT levels has now led the company to test higher doses of its therapeutic agents in

both of these ongoing trials with “topline results” expected in the second half of 2020, although this may be delayed because of the current COVID-19 virus pandemic.

3.3. MicroRNAs against huntingtin

3.3.1. AMT-130

Another approach being taken by a company called uniQure is to use an AAV5 vector to deliver a specific microRNA into selective brain areas (striatum) which works to inhibit the production of mtHTT [37], especially the toxic exon1 HTT fragment. This approach has been trialled preclinically in human iPSC models, as well as in transgenic mice [42] and more recently minipigs [43]. In these latter studies, it was shown that convection enhanced delivery of the agent to the porcine striatum, leading to significant effects on mutant huntingtin expression 6 and 12 months post-injection. The reductions in mtHTT were highly significant at around 80-85% in the caudate and putamen with smaller reductions in the amygdala, thalamus and cortex, along with a 30% reduction in CSF mtHTT levels. The first in-human clinical trial using this approach has now commenced in the US with the first 2 patients enrolled and treated in June 2020 (NCT04120493). This trial is designed to compare 2 doses of AMT-130 in 26 patients over 18 months, 16 of whom will receive the active agent and 10 sham surgery.

3.3.2. VY-HTT01

A similar therapeutic approach to AMT-130 is being developed by Voyager Therapeutics in collaboration with Sanofi-Genzyme and the CHDI foundation. As for AMT-130, this again uses an AAV vector to deliver a miRNA against HTT and has been shown to work in mouse models of HD [44], as well as non-human primates [45], with reductions of HTT protein levels of up to 50% in various brain regions. As a result, the company are now filing to take this agent to clinical trials [46], which will be done on the back of an observational cohort study in peri-manifest patients, who are thought most likely to benefit from the early phase trials with this agent.

3.4. Other agents being developed:

Other companies working on therapies designed to lower huntingtin include Vybion, who are working on an intrabody (INT41) that will be delivered using an AAV virus; PTC Therapeutics and Novartis who are aiming to develop an oral therapy designed to lower

huntingtin mRNA; Takeda and Sangamo who are trying to develop zinc finger nucleases to target the mHtt gene; and Biomarín who are aiming to develop an anti-sense oligonucleotide (ASO) that targets CAG repeats in all genes not just huntingtin. (More details can be found on these agents at <https://hdsa.org/hd-research/therapies-in-pipeline/#>).

4. Conclusion

As Huntington's disease is caused primarily by a gain-of-function mutation, this disease may be ameliorated by strategies that either reduce the synthesis or enhance the degradation of mutant huntingtin. Early clinical studies with antisense oligonucleotides suggest that huntingtin lowering can be achieved relatively safely over a 15 month period and follow-up studies are in progress. In parallel, other antisense oligonucleotide and miRNA studies are about to enter the clinic. Preclinical studies are revealing a number of strategies that can be used to enhance huntingtin clearance using small molecules/non-nucleic acid strategies. Thus, this is a rapidly moving field and promising progress is occurring on both the clinical and preclinical fronts.

5. Comment

The approaches looking to lower mutant huntingtin protein production are clearly attractive as a therapeutic strategy in HD as they target the root cause of the disorder. However, there are a number of unresolved questions with this approach at the moment and these include:

- (i) **Mode of delivery** of ASOs and iRNAs, as these require either repeated intrathecal injections or a single intrastriatal injection. Both of these procedures are invasive and are not without complications, in particular with repeated lumbar punctures, as problems can arise due to fibrosis around the site of needle insertion and drug administration. In addition, the ASO backbone can induce a chemical meningitis in some patients (personal observation). Oral drug repurposing approaches avoid such issues assuming the drug can get into the CNS at therapeutic doses.
- (ii) **Coverage of the CNS** is limited with current approaches. With intrathecal injections, it is unlikely that the ASO can penetrate deep within the adult brain parenchyma and for stereotactic injections of AAV-delivered agents, the volume of distribution will be even more limited to the target structure. However, at least in preclinical studies with AMT 130, there is some axonal transport away from the site of injection with AAV delivery, which would increase the brain areas exposed to the therapeutic. However, it is unknown whether such

axonal transport occurs in HD, given problems that have been reported in this disease state [47]. Again orally-administered blood-brain-barrier-penetrating agents avoid such issues given the less invasive nature of their administration and the fact that in theory all cells in the CNS will be exposed to them via the circulation.

(iii) ***Degree and extent that one needs to lower mutant huntingtin levels*** for it to be efficacious in relation to disease course. In mouse models of HD, transient reductions of htt lead to sustained improvements [28]. However, what this means in the clinical setting is unknown and has major implications for the frequency and dosing of patients, which is likely to be required over decades. With this also comes financial implications – e.g. the ASO therapy for spinal muscular atrophy costs \$750,000 for the first year and \$375,000 per year thereafter.

(iv) ***Stage of disease to target therapeutically.*** This partly relates to point iii above, in that the extent to which one needs to lower huntingtin to achieve clinical benefit likely depends on the stage when lowering is induced in relation to the disease course – one may be able to delay onset of disease if one lowers huntingtin modestly but long before the expected age-of-onset. On the other hand, one may need greater degrees of huntingtin lowering to achieve clinical benefits, if one initiates this strategy well into the clinical course. Indeed, there may be a point in the disease after which huntingtin lowering has no effect on the clinical trajectory [48], similar to what has been described with mouse models of another polyglutamine disease, spinocerebellar ataxia type 1 [49]. Ideally one would seek a strategy that could be employed at an early stage to delay the onset of disease. This is particularly feasible in HD, when most cases have family histories and most individuals at risk can be identified. While challenges remain in order to gain regulatory approval in such scenarios, there has been considerable progress in identifying biomarkers of early premanifest disease in HD [50]. Thus, this disease is better placed than most for developing disease-delaying agents. That being said, there are likely to be limits on when one can start such therapies including in paediatric populations. Such individuals are typically excluded from trials because of issues around consent and in part because these patients possess very high CAG repeats with a more aggressive clinical course. Thus, it is unknown whether these huntingtin-lowering strategies will be effective in these cohorts as in older patients with less aggressive disease. Interestingly, elderly populations are also excluded, although paradoxically these patients appear to advance less rapidly than those with younger onset disease [51].

(v) *Extent to which one needs to retain near-normal levels of wild type Htt* for CNS function to not be affected is unknown and thus it is unclear whether allele specific approaches are really needed. This is a particularly important issue when comparing the AAV approaches, which results in irreversible loss of HTT, versus the ASO strategy, where the depletion is only ever transient, versus oral drug clearance therapies where the drug can easily be discontinued.

(vi) *Extent to which one needs to treat peripheral Htt expression.*

The huntingtin gene is expressed throughout the body with a number of clinical features that may relate to its expression outside the CNS, such as weight loss, metabolic disturbances and muscle atrophy [52,53]. CNS-targeted therapies are unlikely to affect expression of mtHTT peripherally, which again would argue for the advantages of oral systemically-delivered therapies that also have significant CNS penetration. However, the added benefits of lowering mutant huntingtin in the whole body versus the brain alone are not known.

Overall agents that directly seek to lower huntingtin in the adult HD brain hold great promise and could be combined with agents that seek to enhance the clearance of mtHTT, such as up-regulators of autophagy. Indeed, such a combined approach has some clear advantages as it would enable:

- (i) the ASO therapies to be given less frequently, which would have much implications for improving compliance and costs of such an approach;
- (ii) and for AAV miRNA therapies they could be given in lower doses, which would enable larger margins of safety around concerns over the irreversible loss or reduction in CNS huntingtin levels.

However, the multisystem nature of Huntington's disease, and the currently unresolved questions about the efficacy and safety of various huntingtin-lowering strategies, suggests that it will be wise to continue exploring other therapeutic strategies for this disease. Indeed, polypharmacy may be powerful both for ameliorating signs and symptoms in affected individuals, as well as in preventing/delaying onset and progression.

Highlights:

Huntington's disease is an autosomal dominant disease caused by a gain-of-function mutation

Extensive data in preclinical models argues that lowering mutant huntingtin levels may ameliorate disease onset and progression

Huntingtin levels can be lowered by either enhancing degradation via autophagy of the ubiquitin-proteasome routes, strategies supported in preclinical models or reducing its formation via allele-specific oligonucleotides or mRNAs, strategies that are being tested in the clinic.

Current data suggest that these approaches are not associated with overt liabilities in the short-term and could in theory be combined.

New technologies and approaches being developed will lead to refinement of approaches leading to huntingtin lowering and have the potential to bring strategies aiming to enhance huntingtin degradation into the clinic.

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CONFLICTS OF INTEREST:

DCR is on the SABs of Aladdin Healthcare Technologies and Nido Biosciences.

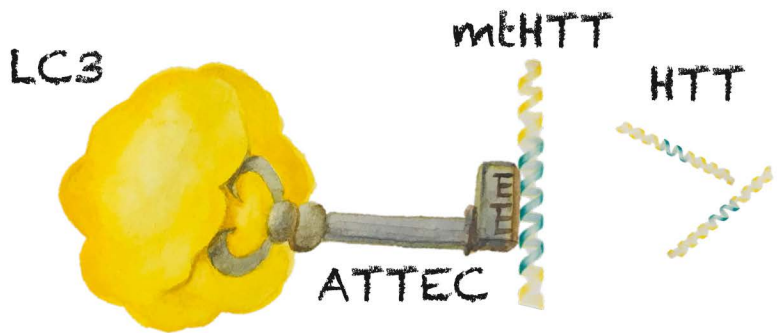
RAB advises the following companies on some of their therapeutic developments – Living Cell Technologies; Fujifilm Cellular Dynamics Inc; Novo Nordisk; BlueRock Therapeutics; Aspen Neuroscience and UCB

Figure Legends:

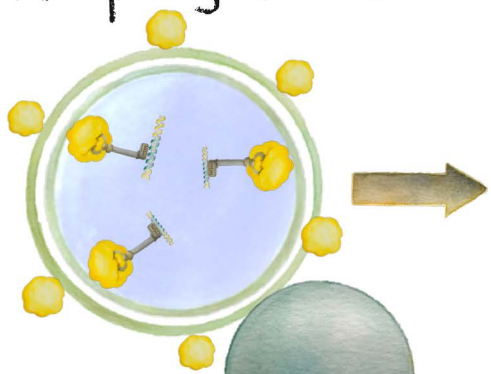
Figure 1. Schematic showing Autophagosome-tethering compound (ATTEC). These molecules act by tethering targets like mutant huntingtin to LC3, a component in the autophagosome membrane. This enables preferential capture of the substrates by autophagosomes.

Figure 2. Schematic illustrating the principle behind PROTACs. These comprise E3 ligase ligands that are fused through a flexible chemical linker to another ligand that binds a target protein, in order to induce artificial ubiquitination, leading to degradation of the protein by the proteasome.

Drug name	Phase	Indication	Pharmacology description	Route of administration	Pivotal trials
RG6042	Phase III ongoing	Allele non-selective	Degradation of HTT RNA bound with ASO	Intrathecal administration	NCT03761849 NCT03842969
WVE120101	Phase I/II ongoing	Allele-selective	Degradation of mtHTT RNA recognized with ASO via specific SNPs for mtHTT	Intrathecal administration	NCT03225833
WVE120102	Phase I/II ongoing				NCT03225846
AMT-130	Phase I/II ongoing	Allele non-selective Injection into the brain	Degradation of HTT RNA targeted with a microRNA	Intrastriatal injection	NCT04120493
VY-HTT01	Preclinical study	Allele non-selective Injection into the brain	Inhibition of HTT production through RNA interference	Injection into thalamus and putamen	Ref [44, 45]



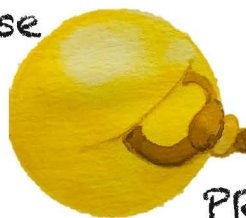
Autophagosome



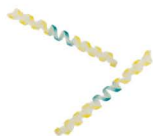
Ubiquitin
Ligase

mEHTT

HTT



PROTAC



polyubiquitin
chain



mEHTT

degradation

