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A Detailed Look into the Gene-based Therapeutic Approaches in Huntington's Disease

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List of Abbreviations

AAV(#)	Adeno-associated virus (serotype #)
AON	Antisense oligonucleotide
ASO	Antisense oligonucleotide
Cas9	CRISPR-associated protein 9
CED	Convection-enhanced delivery
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
FANCD2	Fanconi anemia group D2 protein
FANCI	Fanconi anemia, complementation group I
FAN1	FANCD2 and FANCI associated nuclease 1
GWAS	Genome wide associated study
HTT	Huntingtin
HD	Huntington's Disease
mHTT	Mutant huntingtin
miRNA	microRNA
MLH1/3	mutL homolog 1/3
mRNA	Messenger RNA
MSH3	mutS homolog 3
NfL	Neurofilament light
pre-mRNA	Precursor mRNA
RAN	Repeat-associated non-AUG
RNA	Ribonucleic acid
RNAi	RNA interference
RNase H	Ribonuclease H
shRNA	Short hairpin RNA
siRNA	Small interfering RNA
SMA	Spinal muscular atrophy
SNP	Single-nucleotide polymorphisms
Spt4/Spt5	Suppressor of Ty 4/5
TALEN	Transcription activator-like effector nucleases
wtHTT	Wild-type HTT

ZFP	Zinc finger protein

Abstract

Huntington's disease (HD) is the most common form of genetic dementia and is caused by a mutation in the first exon of the huntingtin (*HTT*) gene. This mutation leads to the production of an abnormal protein that causes cognitive decline and neurodegeneration. In recent years, several genetic-based therapies have been developed to treat Huntington's disease. These therapies can act either on the RNA level and include the use of antisense oligonucleotides (ASOs) or RNA interference (RNAi) to reduce the production of the toxic species by targeting the RNA machinery or on the DNA level including gene editing techniques such as CRISPR/Cas9, zinc-finger proteins (ZFPs), or transcription activator-like effector nucleases (TALENs) to target the underlying DNA mutation. Small molecule approaches targeting the genetic machinery and affecting the expression of genes are also under development. This review will discuss the current state of these gene-based therapies for HD, including their efficacy and limitations. It will provide an overview of conducted preclinical and clinical trials of these approaches, highlighting ongoing trials, such as the recent discontinuation of branaplam in HD, and discussing future directions for research in this field.

1. Introduction

The mutation in *HTT* is an expansion of a sequence of repetitive DNA (a CAG region); consequently, the number of the CAG repeats is a crucial determinant in the penetrance of HD. Specifically, <26 CAG repeats are considered unexpanded and normal, 27-35 repeats are intermediate with no penetrance, 36-39 repeats are associated with semi penetrance, and >40 results in full penetrance. (Killoran et al., 2013). The number of CAG repeats is also known to determine the onset/severity of HD (Lee et al., 2019). Furthermore, HD is prone to anticipation, where the repeats increase (especially in the male-line) in each generation (Ridley et al., 1988); hence, leads to earlier onset and greater penetrance. The mutation results in multiple RNA species and huntingtin protein (HTT) fragments that could be toxic and enable the pathology leading to significant neurodegeneration, including progressive loss of neurons in the striatum and other brain areas. As the disease progresses, individuals with HD may experience difficulty with movement control, memory, and decision making, leading to significant impairment in their daily activities.

1.1.1. The toxic species of Huntington's Disease

The pathogenesis of HD is complex, although most literature suggests a toxic gain-of-function of the mutant huntingtin (mHTT) protein. The mHTT protein has significant evidence of its association with the progression of HD; for example, a study by Rodrigues et al. (2020) shows how the concentration of mHTT correlates with disease stages. This is furthered by many studies linking the amelioration of motor dysfunction, synaptic dysfunction, various electrophysiology deficits, and intraneuronal protein aggregation to the lowering of mHTT, such as Zeitler et al. (2019). Studies suggesting a loss-of-function by mHTT contributing to HD should also be considered (Saudou & Humbert, 2016); it is likely there are both gain and loss of function dynamics in play. Over the years, the exon 1 HTT protein fragment has been increasingly suggested as a primary pathogenic species for HD (Wild & Tabrizi, 2017; Tabrizi et al., 2022) known to be produced due to incomplete splicing of the *HTT* mRNA and accumulated (primarily in the striatum) (Neueder et al., 2017). Evidence for this has been shown by Sathasivam et al. (2013) linking the presence of the exon 1 HTT protein to increased severity and earlier onset of HD using mouse models, and furthered with post-mortem HD patient samples (Neueder et al., 2017). It is likely that both mHTT protein and exon 1 HTT protein fragment play a role in HD. Additionally, although less studied, RAN proteins can be produced via RAN translation, which were shown to be toxic (Bañez-Coronel et al., 2015). Furthermore, toxic RNA species have been shown to contribute to HD development in mice models (Rué et al., 2016). Hence, an ideal therapeutic treatment should take all the toxic species into account.

1.1.2. Current HD Gene Therapies

HD gene therapies (shown in Figure 1) such as antisense oligonucleotides (ASOs) or RNA interference (RNAi) target the RNA machinery, whereas CRISPR/Cas9, zinc-finger proteins (ZFPs), or transcription activator-like effector nucleases (TALENs) target the underlying DNA mutation, and small molecule approaches target the genetic machinery and affect the expression of genes. By targeting the RNA machinery, it should be possible to prevent the formation of toxic proteins involved in the development of HD; hence, potentially preventing symptomatic onset if treated early enough, or helping to reduce disease-related symptoms. Gapmer ASOs are typically inserted via a cerebrospinal fluid injection to bind to pre-mRNA and target it for removal by RNase H. They disrupt the formation of full-length *mHTT*, which helps to prevent toxic protein fragments forming. RNA-based therapeutic molecules, such as small interfering RNA (siRNA), short hairpin RNA (shRNA), or microRNA (miRNA) are either modified to enhance distribution or are inserted by an enhanced delivery method (such as an adeno-associated virus; AAV). They bind to mature mRNA to target it for removal by Argonaute 2, an enzyme within the RNA-induced silencing complex (RISC). Both ASOs and RNAi molecules can reduce the formation of toxic mRNA species, consequent mHTT/HTT protein, and RAN translation proteins after target engagement; however, this depends on their target sequence. DNA approaches have the potential to prevent the entire pathogenesis of HD. CRISPR/Cas9 can edit genes by creating double-strand breaks in DNA and utilising DNA repair pathways. ZFPs work to selectively target DNA by using a DNA-binding element created with zinc finger peptides (hence, the name) which is bound to an active element (e.g., a protein for repressing transcription or a nuclease). TALENs selectively bind to DNA nucleotides (using their DNA-binding domains of repeated peptides) and cause double-strand breakage via their artificial nucleases, enabling them to delete or correct specific DNA segments. Small molecule approaches are often involved in modulating or modifying the splicing of pre-mRNA to mRNA, in this case for reducing huntingtin expression.

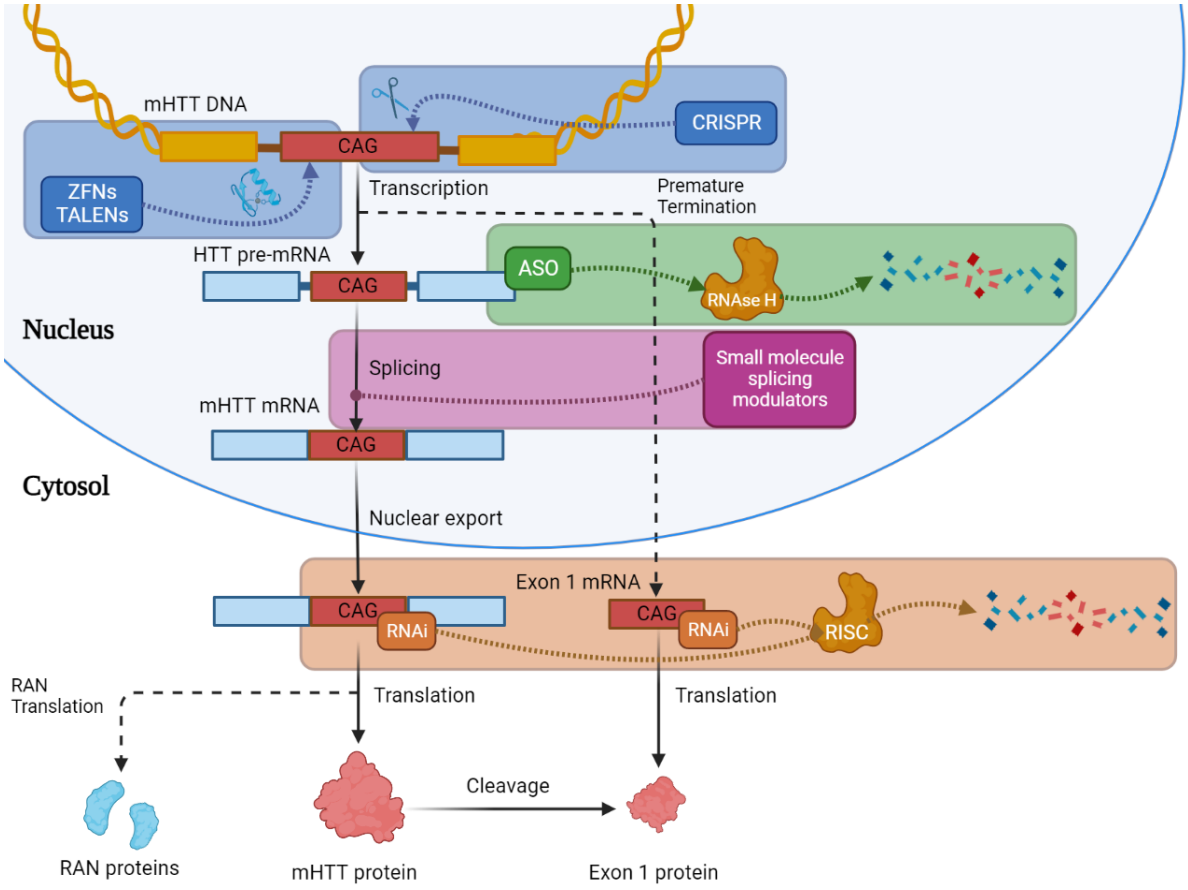

Figure 1. *Production of known HD toxic species and therapy targets.*
Red DNA/RNA marks the expanded CAG tract. DNA therapies are in blue boxes. RNA therapies are green (for ASOs) and orange (for RNAi). Small molecule splicing modulators are in purple. Figure created in BioRender for the purpose of this review.

2. Targeting the RNA Machinery

Both ASOs and RNAi have shown success in many animal models (Keiser et al., 2016). Effectively widely showing that the reduction of *HTT* mRNA expression reduces overall HTT/mHTT; hence, alleviating symptoms and postponing onset (Keiser et al., 2016). However, the limitations of these models are highlighted by the lack of success shown in the follow-up of human trials; this is a difficulty of neurodegenerative disease research mostly due to the vast difference in complexity of the human CNS to non-human animal models.

2.1. ASOs

ASOs can be either allele-selective or non-allele selective; allele selectivity currently works by targeting either single-nucleotide polymorphisms (SNPs) or expanded CAG repeats. This selectivity allows it to avoid lowering of both *mHTT* and *HTT* which is increasingly understood as problematic due to *HTT* likely having important functions (Saudou & Humbert, 2016). Another advantage of ASOs is that their distribution has been shown to be widespread relative to RNAi approaches (Wild & Tabrizi, 2017), likely due to how single-stranded DNA diffuses well in the body. However, ASOs can have off-target effects resulting in liver dysfunction, or can trigger immune response, resulting in platelet deficiencies (Chi et al., 2017); hence, highlighting the importance of phase 1 clinical trials for safety. Additionally, unless the ASO targets exon 1, the formation of exon 1 truncated mRNA could be unaffected by the ASOs (due to premature termination of the *HTT* DNA transcription) and therefore the exon 1 HTT protein fragment would still be produced.

2.1.1. Tominersen

Tominersen is the first ASO administered in human trials for HD. It targets exon 36 of *HTT* mRNA (for binding and removal via RNase H); however, it is not allele specific and as a result it equally affects the levels of both mutant and wild-type HTT (wtHTT). The results for early phase 1/2 trials (NCT02519036) were positive, with no serious adverse side effects and a 40% reduction of mHTT (Figure 2) measured in the CSF (even outperforming its reduction in animal studies) (Tabrizi et al., 2019). However, increases in neurofilament light (NfL) concentrations were recorded, potentially a sign of off-target effects like nerve damage (due to the non-selectivity of tominersen). Furthermore, the following phase 3 study (NCT03761849) was halted as the administered group was performing worse on clinical scales and had more frequent serious adverse side effects (Boak & McColgan, 2022). The participants also had statistically significant concentrations of NfL proteins, which decayed with time, leading to the study concluding that younger participants might reap the benefits from lower/less frequent doses of tominersen due to their lower disease burden (Boak & McColgan, 2022). The programme is currently set to continue in a new phase 2 trial in younger patients and to explore the effects of varying dosage levels (Boak & McColgan, 2022). This could potentially result in

tominersen safely reducing mHTT levels in younger patients at lower doses and a consequent delay in symptomatic onset of HD.

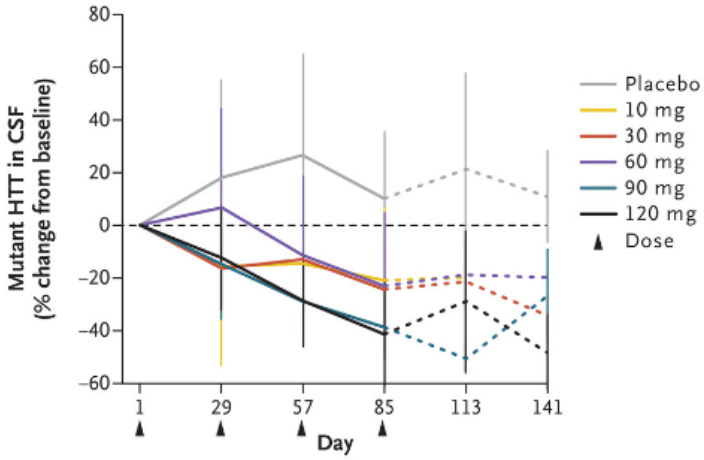

Figure 2. *HTT lowering using tominersen. The relative concentration of mHTT in CSF over 141 days shown in patients administered with different doses of the ASO dosed at days 1, 29, 57 and 85. Adapted from Tabrizi et al., (2019).*

2.1.2. WVE-120101, WVE-120102, and WVE-003

WVE-120101 and WVE-120102 were the second ASOs to enter human trials, even without any preclinical animal studies, and were the first to be potentially allele-specific to SNPs unique to *mHTT* (avoiding adverse effects by selectively reducing the mutant transcript). However, their phase 1/2 trials (NCT03225833 and NCT03225846, respectively) were suspended as neither drug reported any significant lowering of mHTT (across all doses) (Kingwell, 2021). WVE-003 is another allele-selective (for SNP) ASO in phase 1/2 trials (NCT05032196) (and the only ASO other than tominersen currently awaiting further human trials), with recent positive preliminary results evidenced by preserved wtHTT whilst showing a mean 22% reduction in mHTT. However, increases in raised NfL concentrations in some participants as well as mild-moderate adverse effects were observed (Wave Life Sciences USA Inc., 2022). This is the first evidence of allele-selective mHTT reduction with ASOs working in humans, with more data from the trial coming in early 2023.

2.1.3. VO659

VO659 (referred to as (CUG)7 AON in preclinical studies) is another ASO shown to exhibit allele-selectivity, targeting the CAG repeats (instead of SNPs like WVE ASOs) (Datson et al., 2017). In preclinical studies, it has shown to preferentially reduce mHTT levels (by 24-47% depending on brain region) in both mouse models (Figure 3) and HD patient fibroblasts (Datson et al., 2017). It is currently awaiting phase 1/2 trials for use in both HD and spinocerebellar atrophy type 1 (VICO Therapeutics, 2022).

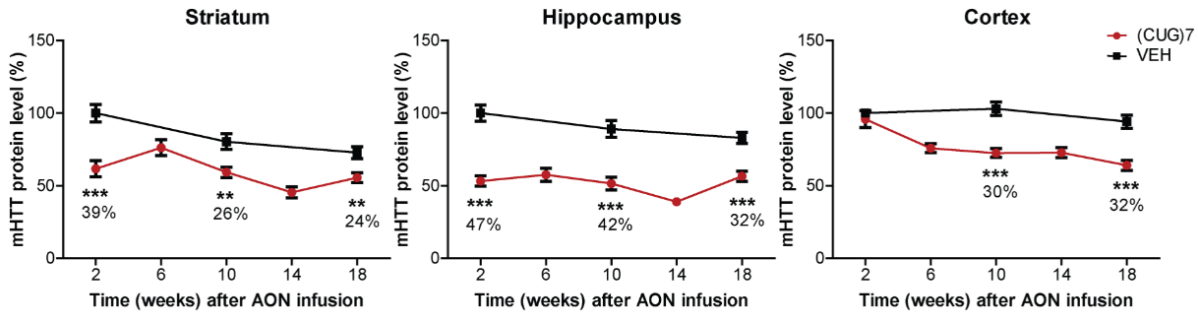

Figure 3. *mHTT levels using TR-FRET analysis. The relative mHTT protein levels as mean averages across brain regions over time from 2-18 weeks of infusion are shown. Significance **p<0.01, ***p<0.001 assessed using 2-tailed t-test. Adapted from Datson et al. (2017).*

2.1.4. TTX-3360

TTX-3360 is an ASO currently in development (Antonijevic, 2021) which does not target *mHTT*, instead it targets mutS homolog 3 gene (*MSH3)* which encodes the protein competitor for FAN1 binding with MLH1; hence, a potential therapy for all CAG-related diseases. In preclinical trials, TTX-3360 showed safe and tolerable results in mouse models with the reduction in MSH3 expectedly inhibiting *mHtt* repeat expansion and is currently awaiting human trials (Antonijevic, 2021).

2.1.5. Novel ASOs in preclinical stages

Several novel approaches in formulating ASOs are being developed for the treatment of HD. Firstly, a study by Thadke et al. (2018) has developed a CAG-selective ASO using ligands made with Janus bases (nucleic acid recognition elements which can target both DNA and RNA at once). The study proposed several advantages for their ligands, such as being small and easy to synthesise, with more favourable pharmacokinetics, the base recognition is more sequence-specific, selective, and their binding interactions are better defined compared to small molecules (Thadke et al., 2018). Although this study has implications for the design of these nucleic acid ligands, no example of their use in preclinical studies is available. Secondly, a more recent study utilised a set of guanine-rich aptamers for selective *mHTT* recognition (Riccardi et al., 2022) called MS3, which is safely uptaken in cell models, and showed significant motor neuron improvement in HD fly models. In a follow-up study, they found shorter truncated analogues of MS3 with improved bioactivity, especially MS3-17, in both cell lines and HD fly models (Riccardi et al., 2022). Hence, the studies showed that MS3-17 could be a candidate for selective targeting of *mHTT* in HD gene therapies. Thirdly, gapmer ASOs made up of tricyclo-DNA (tcDNA) exhibit unique uptake in the CNS (being able to cross the blood-brain barrier) and were shown to successfully lower HTT concentrations in mouse models with the human *HTT* gene (Imbert et al., 2019). However, this study still used intracerebroventricular injections (an invasive and common technique for ASO injection). The use of intravenous or subcutaneous injections would be beneficial in both potentially enabling targeting toxicity in central and peripheral tissue and providing a less invasive treatment for patients (which would typically require regular lumbar punctures) (Imbert et al., 2019). Although this study shows a proof of concept for its

potential in reducing HTT concentrations, future studies are needed to test systemic administration methods.

2.2. RNAi

In comparison to ASOs, RNAi distribution is poor, due to the limited cellular uptake and diffusion of double-stranded RNA in the CNS (Wild & Tabrizi, 2017); hence, why modifications or AAVs/enhanced delivery methods are necessary. Although more invasive, specific site administration has the advantage of reaching the primary sites of HD (i.e., intrastriatal), where ASOs via intrathecal might not. Furthermore, an important advantage of AAV-mediated RNAi is that they should provide lifelong treatment from a single dose. The potential for other RNAi delivery methods to provide more lasting effects might be something to look forward to.

2.2.1. Modified siRNA approaches

ALN-HTT is an siRNA for targeting the exon 1 of the *Htt* mRNA, which has been infused into the striatum of mouse models (using an implant) and ameliorated motor dysfunction (DiFiglia et al., 2007). However, gene silencing only lasted approximately 3 days, and consequently, the motor benefit lasted up to 1 week (DiFiglia et al., 2007). Stiles et al. (2012) used convection-enhanced delivery (CED) with siRNA targeting *HTT* in non-human primates and with significant effects, showing wide distribution of the siRNA (after 7 days of CED) and well-tolerated gene silencing. Another study used hydrophobically modified siRNAs to enhance their distribution in mouse models, by a single intrastriatal injection which significantly silenced the gene with minimal toxicity (Alterman et al., 2015). Lastly, a more recent study showed sustained *Htt* silencing (over 4-6 months) via divalent siRNAs in deep regions of the brain of mouse models (Alterman et al., 2019). This study used a single intracerebroventricular injection and non-human primate models which exhibited significant distribution, no toxicity detected, few off-target effects, and robust *HTT* silencing. Crucially, the main advantage is the silencing lasting 4-6 months, a significant improvement on 3 days (DiFiglia et al., 2007), as well as not requiring 7 days of infusion like Stiles et al. (2012). In conclusion, modified multivalent siRNAs could be a potential candidate for RNAi-based treatment in neurological disorders.

2.2.2. AAV-mediated RNAi therapies

Early studies of RNAi via AAV therapy in HD mouse models showed it as a promising therapy, highlighting tolerable HTT protein lowering, increased lifespan, and alleviated motor symptoms, even in later stage HD (Drouet et al., 2009). Furthermore, to test the safety of HTT reduction (both wtHTT and mHTT) in RNAi via AAV therapy, a study used non-human primate models with a 45% sustained reduction in HTT protein and found no observed toxic effects or adverse symptoms (Grondin et al.,

2012). These studies provided a foundation for the potential utilisation of AAV-mediated RNAi in the treatment of HD.

2.2.2.1. AMT-130

AMT-130 is a RNAi therapy engineered using AAV5 vector (serotype 5) that expresses a miRNA for targeting *HTT* mRNA (both mHTT and wtHTT) (Evers et al., 2018). AMT-130 showed a widely distributed reduction of 41.5% in HTT protein concentration (Figure 4) in mini-pig models, and these effects were sustained for up to 1 year (Evers et al., 2018). More recently, it was shown to be well-tolerated in rats and non-human primate models, with no adverse clinical effects across species, and widespread distribution (Figure 5), especially in HD-related brain areas (Spronck et al., 2021). Hence, it is now the first RNAi therapy in clinical trials, currently in phase 1b/2 (NCT04120493) with an open-label extension (NCT05243017). A disadvantage of AMT-130 is the invasiveness of the procedure; specifically, it is administered via intrastriatal injection, which involves burr holes through the skull and a direct injection into the brain.

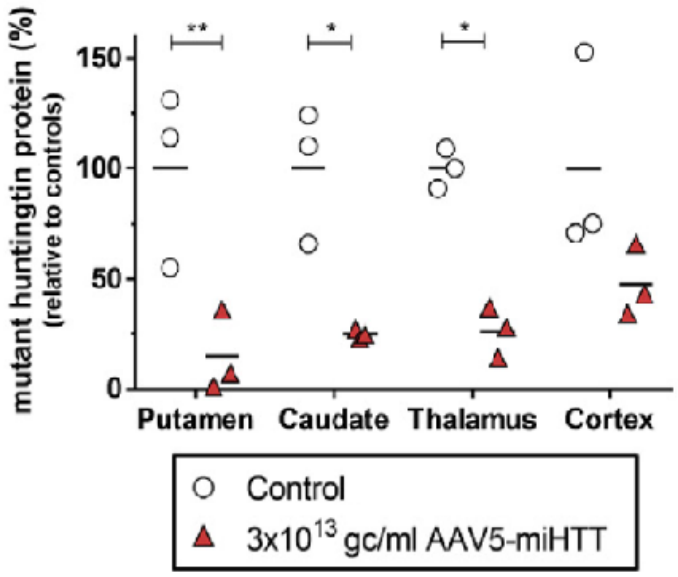

***Figure 4**. HTT lowering using AMT-130. The relative levels of mutant HTT across brain regions in mini pigs after AMT-130 administration are shown. (Adapted from Evers et al., 2018).*

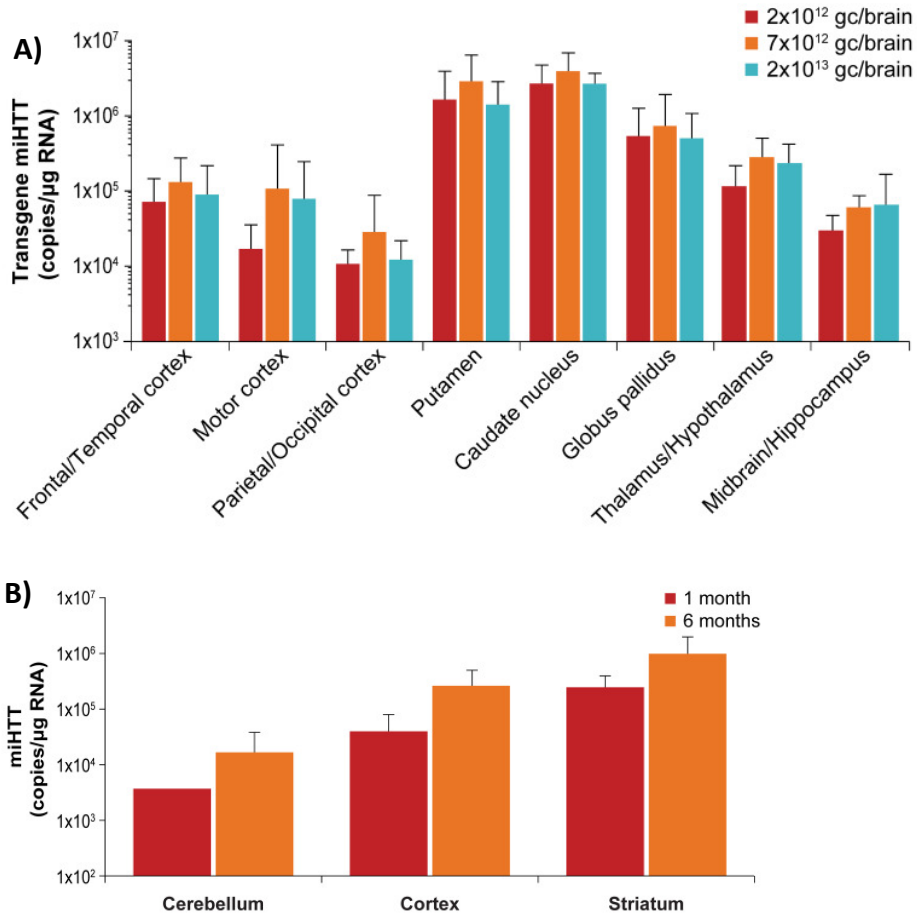

Figure 5. *miRNA levels across brain areas in both non-human primates and rats. (A) The average distribution of the miRNA targeting HTT (miHTT) copies per brain region and dose group in non-human primates is shown. (B) The levels of miHTT in different rat brain regions per dose group and per timepoint as average are shown. Adapted from Spronck et al. (2021).*

2.2.2.2. VY-HTT01

VY-HTT01 is an RNAi via AAV vector (serotype 1) that expresses miRNA for targeting *HTT* mRNA (both mHTT and wtHTT). During preclinical trials, it showed significant (~40%) mHTT reduction (Figure 6) and motor/behavioural improvements in a mouse model with human *mHTT* (Stanek et al., 2014). However, it was withdrawn during phase 1/2 trials (NCT04885114) with future trials planned to use a new AAV-packaged *HTT*-targeting molecule for potentially intravenous administration. Hence, it has the potential to be a substantial improvement on intrastriatal injections like AMT-130.

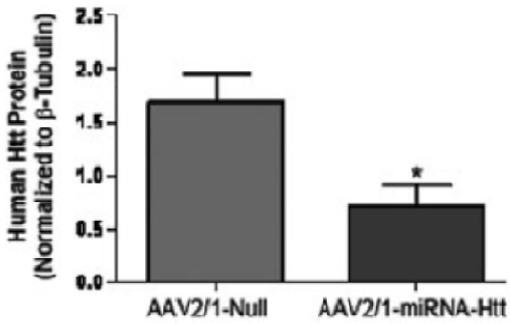

Figure 6. *HTT lowering using VY-HTT01 in a mouse model with the human mHTT gene. Quantification of HTT protein after administration of VY-HTT01 using western blotting techniques. (Adapted from Stanek et al., 2014).*

2.2.2.3. Primary artificial microRNA

A recent study by Wang et al. (2022) used primary artificial microRNA (pri-amiRNA) in order to target *HTT* mRNA (both mHTT and wtHTT). The optimised pri-amiRNA via AAV therapy exhibited potent mRNA lowering (~40% low dose and ~52% high dose; Figure 7) and general tolerability in both mice and non-human primate models; hence, providing future grounds for both effective pri-amiRNA processing and safe AAV gene therapy.

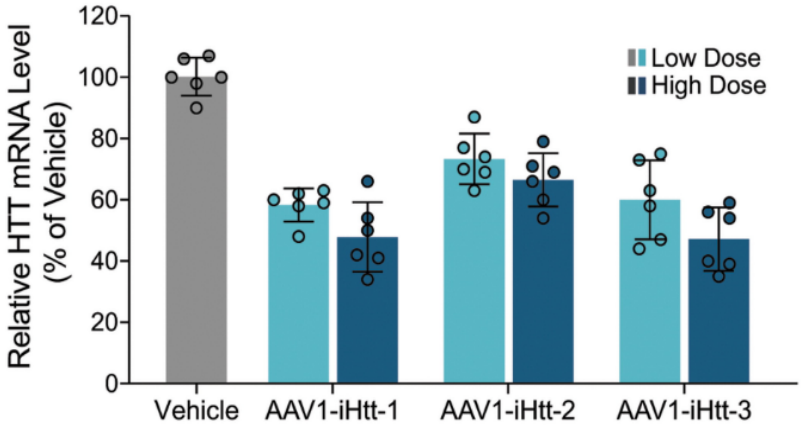

Figure 7. *HTT lowering using pri-amiRNA. Relative levels of HTT mRNA in non-human primates are presented as % of vehicle after administration of the molecules at different doses. (Adapted from Wang et al., 2022).*

3. Targeting Mutant DNA

By correcting the underlying genetic mutation, it would be possible to prevent the entire pathogenesis of HD.

3.1. CRISPR/Cas9 systems

CRISPR/Cas9 systems are a potential option for a DNA targeting treatment. CRIPSR gene editing has shown successful selective inactivation of the *mHtt* gene in mouse models (Monteys et al., 2017). It has also shown this capability in both HD patient-derived fibroblasts (Shin et al., 2016), and human stem cells (Heman-Ackah et al., 2016). However, there are yet to be any clinical trials as more testing is required before CRISPR trials are performed on HD patients. A recent study for the treatment of hereditary transthyretin amyloidosis (a fatal monogenic disease) used CRISPR-Cas9 therapy via lipid nanoparticles and had positive trial results (87% reduction of toxic protein in higher doses) and only mild adverse effects (Gillmore et al., 2021). Hence, although not concluded, it provides promising future insight for the use of CRISPR in other neurological diseases. Furthermore, the relatively low cost, design simplicity, efficiency, and specificity of CRISPR/Cas9 (Malankhanova et al., 2017) makes it the most investigated of the three DNA-based approaches in HD treatment. Other CRISPR systems that can be even more advantageous for certain applications (such as CRISPR-Cpf1 for a simpler design; Malankhanova et al., 2017) should also be considered.

3.2. Zinc Finger Proteins

There are currently only two ZFP treatments for HD in development and both are in preclinical stages. Firstly, TAK-686 (referred to as ZFP-TFs in preclinical studies) is a ZFP with promising evidence in cells lines, having lowered mHTT expression selectively without significant effect on the wild-type transcript and protein (Zeitler et al., 2019). Furthermore, TAK-686 via AAV administration was shown to reduce mHTT concentrations and improve cognitive symptoms in mouse models (Zeitler et al., 2019). Secondly, ZF-KOX1 is another ZFP delivered via AAV which was shown (using a mouse analogue version) to slow down onset of HD symptoms and reduce mHTT protein by 77% in mice via bilateral intraventricular injection (Agustín-Pavón et al., 2016). The results of this study also suggested that sustained effects are possible. There are however several disadvantages to the use of ZFPs including the high cost and complexity of constructing the DNA-binding domain, and a relatively high chance of off-target effects due to possibility of incorrect domain interaction and single-nucleotide substitutions (Malankhanova et al., 2017).

3.3. TALENs

TALEN strategies have been shown to remove CAG repeats safely and successfully in yeast cells (Mosbach et al., 2018). Importantly, another study has shown an allele-specific reduction of mHTT using TALEN in human fibroblasts by targeting SNPs in the mutant allele and causing the CAG to collapse (Fink et al., 2016). Although TALEN is more efficient and specific than ZFPs (Malankhanova et

al., 2017) as a technique and these studies provide potential for more TALEN-based HD treatments, it is more limited in target sequence options (Malankhanova et al., 2017).

4. Small Molecule Approaches

Small molecule approaches are unique as they are orally delivered and they distribute well into the CNS; hence, the most convenient and least intrusive form of gene-based therapy in HD.

4.1.1. Branaplam

Branaplam is a splicing modulator which was recently identified to decrease the expression of HTT protein by causing the mis-splicing of *HTT* mRNA (between exon 49-50) (Keller et al., 2022). Branaplam has been designated as an "Orphan Drug" by the FDA for HD treatment and was active in its phase 2 trial (NCT05111249) which was expected to complete in 2025. However, very recently the development of branaplam for HD ended. This was announced shortly after the abrupt suspension of the phase 2 trial due to safety issues with symptoms of peripheral neuropathy. Further investigation showed increases in NfL concentrations, and increased size of lateral ventricle with more information planned to be released later (Novartis Pharmaceuticals, 2022). It is possible that these symptoms, which were not observed in cases of SMA treated with branaplam (Novartis Pharmaceuticals, 2022), might provide insight into mechanisms of action upon branaplam administration. Hence, although branaplam is discontinued for use in HD, the data it has provided (and will provide later) will continue to inform for future therapies in the field.

4.1.2. PTC518

Drug discovery is now taking place to identify more potential small molecules to target mechanisms in decreasing *HTT* mRNA and HTT protein (Bhattacharyya et al., 2021). PTC518 is a splicing modulator identified through this process which has been shown to cross the BBB in both humans and non-human primates, and had positive results in phase 1 trials, with 30-50% reduction HTT protein, as well as being well-tolerated and with predictable pharmacology (PTC Therapeutics, 2021). It is therefore a promising drug which is in an ongoing phase 2 trial (NCT05358717) to test safety and efficacy, with a report of no adverse side effects (6 months in); although it is still awaiting FDA approval for US enrolment (but is continuing globally) (PTC Therapeutics, 2022). Since these results seem promising, PTC518 is a hopeful candidate for being the first-approved small molecule approach in HD.

4.1.3. Inhibitor Molecules

Studies have been investigating the possibility of using small molecules for the inhibition of various processes which might contribute to HD. Spt5 inhibitors have been shown to reproduce effects similar to Spt5 knockdown on the *Htt* gene by reducing their transcription (Bahat et al., 2019) and therefore showing potential as a novel approach to targeting HD. Future small molecules approaches could work as a potential therapy by encouraging FAN1-MLH1 binding (Goold et al., 2021); for

instance, by inhibiting MSH3 (a competitor for FAN1 binding) (Tabrizi et al., 2022). However, there have been no announcements for future studies for these inhibitors and no clinical trials have been planned.

5. Conclusion and future perspectives

In conclusion, gene-based therapies have shown promise as a potential treatment for Huntington's disease (HD), a genetic form of dementia caused by a mutation in the *HTT* gene. These therapies, summarised in Table 1, include the use of antisense oligonucleotides (ASOs) and RNA interference (RNAi) to reduce the production of toxic proteins, gene editing techniques to target the underlying DNA mutation, and small molecule approaches to affect the expression of genes involved in HD. While some of these therapies have shown promising results in animal models and early-stage clinical trials, they have also faced limitations and setbacks. ASOs have demonstrated efficacy in human trials but have also faced safety concerns (such as tominersen) and lack of clinical efficacy (such as WVE-120101 and WVE-120102). However, WVE-003, VO659, TTX-3360 are promising ASOs awaiting human trials, with several other ASOs in preclinical testing. RNAi therapies, including small interfering RNAs (siRNAs) and microRNAs (miRNAs) have shown success in animal models and early-stage clinical trials, but face challenges in delivery and distribution in the brain. Current approaches to overcome the delivery issues use various adeno-associated viruses (AAVs) or modifications. With AMT-130 phase 1/2 trials having only recently begun and awaiting news on the development of intravenous AAV capsids, RNAi therapy research is still in early stages and newer approaches like multivalent siRNAs could seem promising with more testing. Gene editing techniques, such as CRISPR/Cas9, ZFPs, and TALENs, have shown promise in animal models, but their use in human trials is still in the early stages and their efficacy and safety need to be further evaluated. Despite the recent discontinuation of branaplam for the treatment of HD, preclinical studies have demonstrated the potential of small molecule approaches, and their potential for use in human HD remains to be determined; although PTC518 and its phase 2 trial appears promising at this point.

Table 1. Current therapeutics still in development and their strengths and limitations
✔=Studies suggest yes, ✖=Studies suggest no, ICV=intracerebroventricular.

Target site	Type	Name	Current Stage	Reduces mHTT	Target exon 1	Selective lowering	Non-invasive	Single dose
mHTT RNA	ASO	WVE-003	Ongoing phase 1/2	✔	✖	✔ SNP-	✖ ICV	✖
		Tominersen	Planning phase 2 trial	✔	✖	✖	✖ ICV	✖
		VO659	Planning phase 1/2	✔	✖	✔	✖ ICV	✖
		TTX-3360	Planning phase 1/2	✔	✔	✔	✖ ICV	✖
		MS3-17	Preclinical	✔	✖	✔	✖ ICV	✖
		Tricyclo-DNA base	Preclinical	✔	✖	✖	Potentially intravenous	✖
	RNAi	AMT-130	Ongoing phase 1/2	✔	✔	✖	✖ Intrastriata	✔
		VY-HTT01	Planning new trial	✔	✔	✖	Potentially intravenous	✔
		Divalent siRNAs	Preclinical	✔	✔	✖	✖ ICV	✔
	Small molecule	PTC518	Ongoing phase 2 trial	✔	✔	✖	✔ Oral	✖
		MSH3/Spt5 inhibitors	Preclinical	✔	✔	✔	✔ Oral	✖
DNA	ZFP	TAK-686	Preclinical	✔	✔	✔	✖ Intrastriata	✔
		ZF-KOX1	Preclinical	✔	✔	✔	✖ Intrastriata	✔
	CRISPR/ Cas9	Still in development	Preclinical	✔	✔	✔	✖ Intracranial	✔
	TALEN	Still in development	Preclinical	✔	✔	✔	Not known	✔

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