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PHARMACOLOGICAL TREATMENTS AND THERAPEUTIC APPROACHES FOR HUNTINGTON'S DISEASE

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ABSTRACT

The HTT gene's aberrantly enlarged CAG repeat expansion, which results in a dominantly toxic gain of function in the mutant huntingtin (mHTT) protein, is the etiology of Huntington's disease. While there aren't any disease-modifying treatments on the market right now, proximally targeting the pathophysiology of a disease holds a lot of promise. These include post-transcriptional huntingtin-lowering strategies like RNAi, antisense oligonucleotides, and small-molecule splicing modulators; DNA-targeting strategies like zincfinger proteins, transcription activator-like effector nucleases, and CRISPR/Cas9; and novel approaches to remove the mHTT protein like proteolysis-targeting chimeras. The establishment of objective biomarkers of disease and HTT reducing pharmacodynamic results, along with advancements in the delivery and distribution of these drugs, have elevated these possible therapeutics to the forefront of Huntington's disease research, with clinical trials underway.

INTRODUCTION

Hereditary chorea, or what is now known as Huntington's disease (HD), was described in detail by George Huntington in 1872. He explained the inherited basis of the condition, its correlation with psychiatric and cognitive symptoms, and how the illness manifests in adults between the ages of thirty and forty. He described how the illness progresses, saying, "Once it begins, it clings to the bitter end" 1. However, HD may be one of the most curable neurodegenerative diseases due to its monogenic character and complete penetration. This has been especially clear in the past ten years with the development of novel treatment

strategies that can directly target the HD gene and stop the harmful mutant huntingtin protein 2 from being produced.

An inherited autosomal-dominant neurodegenerative disease known as Huntington's disease (HD) is typified by three distinct features: motor, cognitive, and mental. HD usually shows the onset with symptoms that worsen irreversibly over a period of 10 to 15 years in midlife (Ross and Tabrizi, 2011). An aberrantly enlarged CAG repeat close to the huntingtin gene's (HTT) N terminus is the cause of all cases of HD. This repeat causes translation to produce mutant huntingtin protein (mHTT). After the genetic mutation was discovered in 1993, it has been 25 years, and extensive study has revealed numerous cellular pathogenic pathways that underlie the onset of disease. The mHTT protein, which is widely expressed and is assumed to induce disease via a dominant toxic gain-of-function mechanism, is the driving force behind nearly all of them.

The research and testing of treatments that target HTT DNA, RNA, and protein proximally in HD pathogenesis is currently a prominent area of study. The ultimate goal of these strategies is to lower mHTT levels and, as a result, lessen all of the numerous and diverse downstream harmful effects. Reduction of mHTT improves disease characteristics and reverses neuropathology in animal models of HD (Yamamoto et al., 2000; Wang et al., 2014), indicating the significance of mHTT lowering as a treatment goal.

Aetiology

An autosomal dominantly inherited expansion of the CAG trinucleotide repeats in the huntingtin (HTT) gene on chromosome 4 is the etiology of Huntington's disease. As a result, a mutant huntingtin (mHTT) protein with an atypically lengthy polyglutamine is produced. A person's likelihood of developing the condition increases with the number of CAG repeats they have; lower penetrance is shown between 36 and 39 repeats. It is possible for a father with a CAG repeat length in the intermediate range to have a child with an increased pathogenic repeat length, indicating anticipation when the gene is handed down the paternal line. This is because, in comparison to somatic tissues, male sperm exhibit higher repeat variability and bigger repeat sizes.

Epidemiology

In Western populations, the prevalence of Huntington's disease ranges from 10.6 to 13.7 cases per 100,000 people. With a prevalence of 1-2 cases per million, Japan, Taiwan, and Hong

Kong have substantially lower incidences of HD than do South Africa, where black populations have lower rates than white and mixed populations. Genetic variations in the HTT gene are responsible for the variation in illness prevalence throughout ethnic groups. High prevalence populations have average CAG repeat lengths that are longer. For instance, the average for people of European heritage is 18.4–18.5, but the average for people of Asian descent is 16.9–17.4.

Huntingtin-Lowering therapies

DNA-Targeting approaches

Currently under development, huntingtin-lowering medicinal treatments that target HTT DNA can function by directly modifying the HTT gene (Genome editing) or by influencing gene transcription. Conventional methods for DNA targeting involve the utilization of a particular type of DNA-binding component in conjunction with effector components like transcription factors, nucleases, or epigenetic modulators. The effectors change the expression or sequence of genes, and the DNA binding elements allow for accurate and efficient DNA targeting. Similar to genomic scissors, nuclease effectors cleave the targeted DNA to create a double-strand break, which then triggers the cell's natural DNA repair systems to mend the break utilizing homology-directed repair (HDR) or nonhomologous end joining (NHEJ). NHEJ is exceedingly error-prone and frequently causes the gene to become functionally disrupted as well as introduce deletion or insertion mutations. To fix the break, HDR makes use of a template from another DNA sequence. When using genome editing, a desired exogenous DNA sequence is delivered together with the nuclease and serves as a template for repair, inserting the new DNA sequence into the target region directly. Three primary kinds of nucleases can be designed for the goal of targeting DNA: transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and Cas9 or other bacterial nucleases directed by RNA. Each of them functions differently in terms of how it binds to DNA.

ZFNs are made up of a DNA binding element made up of a variety of zincfinger peptides attached to an active effector element, such as a transcriptional repressor protein or a particular nuclease. A sequence of three to five different nucleotides from the DNA strand can be bound by each zinc finger. Zinc finger arrays, which are highly specific to the DNA sequence of interest and span many ZFN peptides, are typically seen in ZFN-targeting constructs. Zinc-finger proteins (ZFPs) can lower gene expression levels by just attaching to

DNA and blocking the transcription of genes when they lack nuclease activity. In the context of HD, such According to Mittelman et al. (2009), ZFPs have the ability to attach more specifically to enlarged CAG repeats than to normal CAG repeat lengths. This allows for comparatively specific binding to the mutant HTT gene. According to results reported by one group (Garriga-Canut et al., 2012), ZFPs reduce the expression of mHTT in cell lines without appreciably changing the expression of the non-expanded HTT allele or other non-expanded CAG repeat-containing genes. Improved ZFPs with an active repressor element that were inserted using adeno-associated virus (AAV) vectors into the striatum of R6/2 mice similarly improved Table 1 and decreased mHTT levels.

Table 1. Huntingtin-Lowering Programs Targeting DNA and RNA

Sponsor	Stage	Delivery	Allele Selectivity	Advantages	Disadvantages	References
DNA-Targeting Approaches						
Zinc-Finger Transcription Factor						
Shire and Sangamo	preclinical	intracranial (AAV)	CAG repeat	single drug for all carriers of the HD mutation; single administration to provide long-term treatment; targeting transcription should ameliorate all pathogenic pathways	invasive; cannot be deactivated; small treatment volumes; risk of inflammation from non-host repressor proteins	Zeitler et al., 2014
Imperial College London	preclinical	intracranial (AAV)	CAG repeat	as above, plus use of host species proteins reduces inflammatory effects	use of human proteins in clinical candidate compound might limit utility of animal work	Garriga-Canut et al., 2012; Agustín-Pavón et al., 2016
CRISPR/Cas9						
Harvard University University of Pennsylvania	preclinical	direct to fibroblasts	SNP-targeted	permanent removal of genetic cause; highly specific and targeted	very early work in model systems only; irreversible, ethical concerns for germline alteration, delivery problems as with other virally delivered approaches, immunogenicity of bacterial proteins	Shin et al., 2016; Monteys et al., 2017
Emory University	preclinical	intracranial	nonselective HTT depletion by polyglutamine domain deletion	as above	as above	Yang et al., 2017
RNA-Targeting Approaches						
ASOs						
Ionis Pharmaceuticals	phase 1/2a completed	intrathecal	none	single drug for all HD mutation carriers	potential risk from reducing wild-type HTT; repeated administration	Bennett and Swayze, 2010; Kordasiewicz et al., 2012; Tabrizi et al., 2018
Wave Life Sciences	phase 1b/2a	intrathecal	SNP-targeted	selective silencing of mutant allele	several drugs required to treat majority of patients SNP-targeting limits choice of RNA binding sequences; repeated administration	Butler et al., 2015; Hersch et al., 2017
Biomarin	pre-clinical	intrathecal	CAG repeat	selective silencing of mutant allele with a single drug for all mutation carriers	will reduce expression of other important CAG-containing genes, risking off-target effects; repeated administration	Datson et al., 2017

These findings encourage the further research and development of ZFNs and allele-specific ZFP repressors as possible treatments for HD. Zincfinger transcriptional repressor strategies could target DNA without changing it, which could decrease mHTT levels, yet ZFNs could complement ZFPs' repressive impact by genuinely rupturing or repairing the faulty gene, in a manner akin to other direct genome editing techniques like CRISPR/Cas9, and may eventually rectify the disease-causing CAG expansion in HD.

Because they both use a nuclease effector domain connected to a DNA recognition domain, TALENs and ZFNs are highly similar. The TALEN DNA recognition domain makes use of a particular sequence of repeating amino acids.

It attaches to a particular nucleotide; distinct variants of these repeats of amino acids can be produced to identify particular DNA sequences. While ZFN-based methods may be less selective and theoretically more efficient than TALEN-based nucleases, TALENs require a certain nucleotide at the end of the DNA sequence, which may restrict the range of possible targets. The CAG repeat tract in yeast could be shortened with great specificity and efficiency using a TALEN construct that was engineered to target a CAG trinucleotide repeat (Richard et al., 2014). Fibroblasts generated from patients were employed in the lone published HD study on TALENs. This group employed two distinct strategies: CAG repeat-binding TALENs, which required the combined binding of two complementary TALEN monomers to re-constitute an active nuclease and cleave the target CAG repeat DNA, and SNP-based (allele-specific) TALEs lacking nuclease activity combined with a transcriptional repressor to reduce expression of the expanded HTT gene. In order to bind both monomers, these TALEN constructs needed a minimum of 15 CAG repetitions, which prevented gene editing in CAG tracts of typical length. In HD fibroblasts, a selective decline in mHTT expression and aggregation was shown using these two TALE-based methods. Despite the incredibly poor efficiency, this work offers proof of principle. It indicates TALEN-based techniques for allele-specific HTT gene editing are feasible and encourage additional research in this field. A bacterial immune system that detects and eliminates foreign DNA, most commonly viruses, is built on the CRISPR/Cas system (Savic and Schwank, 2016). "Clustered regularly interspaced short palindromic repeats" is what CRISPR stands for, and the Cas9. An RNA-guided nuclease called a protein cleaves double-strand breaks in particular DNA locations. Engineered variants of the Cas9 nuclease are used in therapeutic gene editing via the CRISPR/Cas9 system; however, this enzyme does not utilise a protein-based DNA recognition domain, in contrast to ZFNs or TALENs. Certain RNA constructions, or guide RNAs, direct the Cas9 protein to target particular DNA sequences. The DNA target sequence for gene-editing applications needs to be followed by a particular recognition site called a protospacer-adjacent motif (PAM) sequence. Most PAMs are made up of two to five highly conserved nucleotides. According to Jinek et al. (2012), the NGG or NAG nucleotides found in the PAM of the Streptococcus pyogenes Cas9 protein are the first ones employed in gene editing. Synthetic guide RNAs and other Cas9 nuclease forms can be combined to create

ribonucleoprotein (RNP) complexes that are highly precise in their targeting of certain DNA locations.

Research in this field is developing quickly, and there are already many distinct Cas9 variants (often derived from different organisms) with varying PAM sequence specificity, differential (nickase) or inactive nuclease activity, and the ability to complex with DNA effector molecules like histone deacetylases or DNA methyltransferases. HTT gene-directed editing using CRISPR/Cas9-type techniques has several potential mechanisms of action. These include non-allele-specific targeting of the HTT gene that would lower total HTT levels, targeted inactivation of the mutant allele that would result in a hemizygous null state, and direct excision of CAG repeats to correct the mutation and generate two wild-type HTT alleles. Furthermore, CRISPR/Cas9-targeted epigenetic editing might potentially be a successful treatment for HD by changing the transcription of the HTT gene without modifying the DNA permanently.

Preclinical development of CRISPR/Cas9 applications in HD is still in its early phases. A non-catalytic CRISPR/mutation was used to demonstrate the reduction of huntingtin in concept. Cas9 technique that prevented the transcription of the HTT gene in a human non-neuronal cell lineHD has reduced mHTT expression as a result of the precise removal of the CAG repeats from the HTT gene using paired guide RNA (gRNA) constructs that target the DNA flanking the CAG repeat using a modified "nickase" Cas9 method. Fibroblasts from patients (Dabrowska et al., 2018). In the Q140 transgenic mouse model of HD, a similar strategy utilizing Cas9 was similarly effective (Yang et al., 2017), leading to selective mutant HTT decrease, abatement of disease, and enhanced motor function.

Additionally, in patient-derived fibroblasts, this method has been used to selectively inactivate mutant HTT genes that target specific PAM sites to SNPs associated with the CAG-expanded allele. This has led to an almost complete reduction in both mutant HTT protein and RNA. Using a similar strategy, CRISPR/Cas9 selectively inhibited the production of mHTT in the brain of the BACHD mouse model and in human differentiated induced pluripotent stem (IPS) cells by targeting SNPs linked to the CAG expansion. There is a lot of promise for treating HD with CRISPR/Cas9-mediated targeted huntingtin reduction and HTT genome editing techniques. Preclinical results thus far indicate that this strategy is feasible for treating HD, but further investigation is required before these quickly developing technologies may be used in human clinical trials.

When treated early enough, bearers of HD gene mutations only need a single administration to give long-term treatment that should successfully block all harmful pathways. Unfortunately, these treatments will be invasive, irreversible, selective to specific brain regions, potentially targeting other CAG repeat-containing genes, and carry a long-term risk of inflammation from non-host repressor proteins due to the long-term expression of exogenous proteins (Agustı´n-Pavo´ n et al., 2016). Based on protein-guided DNA binding, ZFNs and TALENs demand specialized knowledge and labour-intensive design, assembly, selection, and validation processes. It is challenging and frequently costly to design these nucleases to be specific for a single genomic sequence since it necessitates joining the correct zinc finger combination in ZFNs or the correct amino acid repeat combination in TALENs. Additionally, as with RNA-targeted ZFNs and TALENs exhibit imprecise recognition of DNA targets during gene silencing, which can lead to notable off-target consequences. Additionally, ZFN and TALEN are typically regarded as being less effective than the CRISPR/Cas9 method in areas like ease of delivery and design simplicity.

Apart from the aforementioned BACHD model investigations, there are currently relatively few published data about CRISPR/Cas9 techniques in HD, and the majority of this work is still being done in basic cellular model systems. Off-target effects and the requirement for a particular PAM next to the target DNA sequence are potential drawbacks of CRISPR/Cas9 techniques. Similar to worries about ZFNs and TALENs, there are still unanswered questions about delivery in the brain, the use of irreversible viral transduction systems may put prolonged nuclease expression at risk, and the expression of bacterial proteins in the human brain may be immunogenic (Fu et al., 2013). One group has responded directly to worries about the possibility of long-term Cas9 nuclease expression. The KamiCas9 technique, which is a self-inactivating Cas9 system for temporary genome editing with the potential for increased security. While the lentivirus vector decreases long-term Cas9 expression, it also carries the danger of insertional mutagenesis. In an HD mouse model, our method was able to permanently disrupt HTT, indicating the viability and possible therapeutic benefit of CNS gene editing (Merienne et al., 2017).

Though a lot has been accomplished, there are still a lot of variables to be taken into account before genome editing is a practical treatment option for HD. A significant challenge for all HTT-targeted approaches has been getting the therapeutic drug into the central nervous system.

Targeting the CAG-expanded HTT DNA in HD with genome editing techniques could be extremely beneficial, and fixing the underlying genetic flaw should stop the disease's continuous pathogenic pathways. This would cover every other toxic species of mHTT as well as additional pathogenic pathways such RNA-mediated toxicity, non-RAN translation, and alternative splicing. Furthermore, the risk of HD in the future would vanish if genome editing was utilized to fix the genetic mutation in the germ cells, preventing the mutant allele from being passed down from generation to generation.

The field of genome editing for human neurologic illnesses is still relatively young and is developing extremely quickly. While numerous groups are pursuing this strategy as a potential cure for HD, more development and comprehensive pre-clinical investigations will be necessary before this is prepared for human clinical trials.

RNA-Targeting Approaches

Antisense oligonucleotides (ASOs), small-molecule modulators of RNA processing, and RNA interference (RNAi) are techniques that alter translation efficiency at the post-transcriptional stage. All of these techniques ultimately result in the cleavage, increased degradation, or translational suppression of mutant HTT mRNA, which lowers the quantity of mutant HTT protein that is generated.

In order to achieve the destruction of target mRNAs in the cytosol, RNAi technologies manipulate a natural and evolutionarily conserved process within cells.

RNA polymerase transcribes native non-coding microRNAs (miRNAs) inside the nucleus to create stem-loop structures called primary miRNAs (pri-miRNAs). The Drosha enzyme cleaves them to create a precursor miRNA (pre-miRNA) with a hairpin structure, which Exportin-5 exports to the cytoplasm. The Dicer enzyme further processes the pre-miRNA in the cytoplasm to produce a mature miRNA complex. The complex is incorporated into the RNA-induced silencing complex (RISC), where its antisense, also known as the "guide strand," directs the complex toward its corresponding target mRNA.

Ultimately, translational suppression results via Argonaut-2's (Ago-2) cleavage of the target mRNA (Ha and Kim, 2014). Similar to miRNAs, small interfering RNAs (siRNAs) are not short hairpins; instead, they originate from larger double-stranded RNA segments. While siRNAs usually show perfect base pair matching and cause complete translational inhibition

through cleavage of a single specific mRNA target, miRNAs often exhibit imperfect base pair complementarity, which results in translational suppression of multiple mRNA targets with similar sequences.

Artificial miRNAs and synthetic siRNAs have been created to block the translation of certain target proteins, including HTT. RNAi effectors work on fully spliced mRNA species in the cytosol and have a more downstream location of action than ASOs. siRNAs are unable to pass through the plasma membrane and penetrate CNS cells across the blood-brain barrier (BBB). To improve cellular penetration, liposome formulations, nanoparticles, and chemical modifications to the siRNA itself have all been used (de Fougerolles, 2008). Viral vectors are more frequently utilized to transduce cells permanently and produce stable expression of siRNAs that inhibit HTT translation.

RNA interference (RNAi) was first shown to lower HTT levels and improve survival in cellular models of HD (Chen et al., 2005). Harper et al. (2005) conducted positive initial in vivo investigations using HD transgenic mice. They found that motor function was improved by rotarod performance following bilateral striatal injections of anti-HTT short hairpin (shRNA) given by AAV vector. In the N171-82Q model, these therapies, on average, led to significant reductions in mutant HTT mRNA levels of 55% when compared with untreated mice. Similar results have been published using the R6/1 HD mouse model, demonstrating an increase in the prevalence of PR proenkephalin (ppENK) and striatal markers (DARPP-32) following therapy. In transgenic R6/2 mice, intraventricular infusion of a liposome-siRNA complex decreased mutant HTT mRNA, HTT inclusions, and brain atrophy while delaying the onset of motor symptoms and improving survival (Wang et al., 2005). Cholesterol-conjugated anti-HTT siRNA duplexes were injected into the striatum of a viral transgenic mouse model of HD, which prevented neuronal damage and delayed the onset of the behavioral abnormality.

These trials demonstrate how crucial timing of HTT reducing treatments may be in the management of HD. All of the RNA interference treatments previously mentioned were given to pre-symptomatic mice. Treatment of symptomatic transgenic HD mice (HD190QG) has also been demonstrated to lower HTT protein levels, decrease inclusion formation, and enhance histopathology (Machida et al., 2006); however, it is unclear what impact, if any, this will have on the actual reversal of the disease phenotype. As early as possible in the disease course, HTT lowering therapy should be implemented to either prevent or postpone

the emergence of symptoms, as pre-clinical animal studies have not yet demonstrated the possibility of a complete reversal of all symptoms at the time they manifest.

Predictive testing for HD is already available, enabling trials in pre-manifest populations; nevertheless, early symptomatic patients will still need to be used in initial investigations.

The amount of transgenic protein has been reduced in vivo investigations using transgenic mice; however, endogenous HTT levels, both wild-type and mutant, remained unaffected. "Allele selectivity" refers to the ability to target endogenous full-length mutant HTT while keeping wild-type HTT mRNA unchanged. While this is desirable, it has caused significant hurdles (see Allele Selectivity of Huntingtin-Lowering Approaches). A different approach would be to non-specifically reduce the levels of both mutant and wild-type HTT to a point where the wild-type HTT loss is safe and well-tolerated while simultaneously improving HD pathogenesis. Research using both full-length and fragmented mouse models of HD has demonstrated that reducing endogenous and mutant HTT by 40%–60% prolongs life and avoids motor symptoms without adding to toxicity. This also applied to rodents that were exhibiting symptoms at the time.

It has also been demonstrated that partial RNAi reduction of wild-type HTT is safe and well-tolerated in non-human primates. Adult rhesus monkeys were given bilateral putaminal injections of AAV vectors expressing fake miRNAs (McBride et al., 2011), and a 45% decrease in HTT levels did not cause any behavioral signs or neuropathology to be seen. Six months following treatment, sustained knockdown remained visible with no negative side effects.

Preparations are being made for miRNA treatment clinical studies in HD (Table 1). In patients with Alzheimer's disease, a phase 2 experiment using stereo tactically guided intracerebral injections of AAV2-encapsulated nerve growth factor RNA demonstrated the safety and potential well-tolerance of virally delivered gene therapy. In the coming years, a clinical trial with AAV1-delivered anti-HTT miRNA—which has demonstrated potential in non-human primates (NHPs)—sponsored by Spark Therapeutics is scheduled to begin (McBride et al., 2011).

The predictive validity of these studies is further enhanced by recent research demonstrating that AAV miRNAs targeting HTT can be safely delivered to the brain of non-human primates

using an FDA-approved intra-MRI delivery platform that is comparable to the neurosurgical platform that can be used in human patients (McBride, 2018, CHDI Foundation, conference). According to Evers et al. (2018), intracranial delivery of AAV5-delivered anti-HTT miRNA into a transgenic HD minipig model decreased mHTT levels in many brain regions. UniQure NV intends to submit an investigational new drug (IND) application for this later in 2018. Similar research is being conducted by Voyager Therapeutics for an AAV-delivered anti-HTT RNAi medication called VY-HTT01, which has been demonstrated recently to decrease HTT mRNA in non-human monkey brains after intracranial delivery and to be well tolerated.

Unintended results of administering protein-lowering medicines are known as off-target effects. According to Jackson and Linsley (2010), RNAi effectors have the capacity to bind mRNA sequences that are homologous with the intended target, inadvertently downregulating unrelated proteins.

The majority of manufactured miRNAs are made to have complete homology with their target, which lessens the possibility that endogenous miRNAs will attach their targets imperfectly through base-pairing complementarity.

Mammalian cells are capable of mounting an immunological defense against species of double-stranded RNA, such as siRNAs, which are often misinterpreted for lingering virus particles. According to Sledz et al. (2003), siRNAs have been shown to bind to specific Toll-like receptors (TLRs) and increase the expression of genes that are stimulated by interferon (IFN). Pro-inflammatory cytokines are released as a result (Sioud, 2005; Hornung et al., 2005; Kariko´ et al., 2004). From time to time, careful engineering can result in reduced immunogenicity, increased stability, and high complementarity, all of which can mitigate these effects.

Exogenous shRNAs that overload exportin-5 may overwhelm the endogenous RNAi system in cells (Barik, 2006; Martin et al., 2011). This can have further deleterious effects, such as liver damage (Grimm et al., 2006; Borel et al., 2011). This can be assisted by choosing weaker promoters for shRNAs or co-expressing recombinant exportin-5 (Grimm et al., 2006; Yi et al., 2005). According to Boudreau et al., 2009a; McBride et al., 2008, synthetic siRNAs can completely avoid the nuclear processing phase and artificial miRNAs are more well tolerated.

ASOs. Compared to RNAi effectors, ASOs have a higher upstream location of action. Their synthetic, short, single-stranded oligonucleotide analogs have a base count of 16–22. They bind to complementary pre-mRNA targets in the nucleus by Watson–Crick base pairing, which can influence gene expression via a variety of possible mechanisms. The recruitment of RNase H1 is one such route. An RNA-DNA hybrid is created when ASO binds, and this substrate is used by RNase H1 to hydrolyse and destroy the target mRNA.

The cytoplasm and nucleus then employ regular cellular processes to eliminate the cleavage products.

In a phase 1/2a clinical trial supported by Ionis Pharmaceuticals, it was recently demonstrated that the HTT-targeting ASO IONIS-HTTRx (RG6042) lowers the levels of mHTT protein in the cerebrospinal fluid (CSF) of individuals with early-stage HD (Tabrizi et al., 2018). This has raised a great deal of hope that ASOs will soon result in a workable HD disease-modifying treatment.

It is believed that the HTT-Rx (RG6042) ASO targets both wild-type and mutant HTT levels, resulting in non-specific HTT reduction, via the RNase H1 pathway. Preclinical investigations with HTT-targeting ASOs given to BACHD and YAC128 mice models with HD symptoms have demonstrated amelioration of important striatal gene expression alterations as well as reversal or improvement of motor abnormalities. According to Lu and Yang (2012), phenotypic improvements were most pronounced when treatment was started earlier in the course of the illness and continued for several months after treatment ended. In these mice, a single ASO injection resulted in a 12-week suppression of the target mRNA itself (Kordasiewicz et al., 2012). This affects when an intervention should be started and how often HD patients need to take their doses. It has also been demonstrated that lumbar intrathecal infusion of a comparable ASO into non-human primates significantly lowers HTT in numerous brain regions targeted by HD. The administration of the IONIS-HTTRx (RG6042) ASO intrathecally through lumbar puncture into the CSF has demonstrated safety and good toleration in HD patients. Its impact on disease modification will shortly be evaluated as a component of a larger phase 3 trial funded by Roche. Furthermore, as Table 1 illustrates, allele-selective ASOs that target single nucleotide polymorphisms linked to the CAG expansion have been created (Skotte et al., 2014), and Wave Life Sciences is currently conducting a clinical trial (Hersch et al., 2017). Additionally, according to Dawson et al.

(2017), Biomarin is developing a pre-clinical allele-selective ASO that targets the enlarged CAG repeat directly.

The position of ASO binding to the target mRNA determines other mechanisms of ASO activity. Since the long-term effects of complete HTT removal in humans are unclear, ASOs binding to the AUG translation start site may result in steric obstruction of the ribosomal machinery and complete translational inhibition, which is not desirable in the case of HD. By disrupting splice sites or by attracting or repressing splicing factors, ASO binding to intronexon junctions may modify splicing. Targeting the synthesis of the neurotoxic mutant exon 1 HTT protein produced by alternative splicing could be a beneficial use for this kind of ASO. After hybridization, ASOs can also be chemically changed to break the target mRNA directly by adding intrinsic catalytic nucleic acids like DNAzymes and ribozymes.

To increase their suitability as therapeutic agents, ASOs have undergone a number of chemical changes since their creation. Endonucleases could tear down the initial phosphoribose backbone very quickly. Sulphur was substituted for non-bridging oxygen atoms to create a phosphorothioate backbone, which enhanced protein interaction, caused nuclease resistance, and lengthened the half-life. Furthermore, several modifications made to the ribose sugar moiety at position 20 have enhanced the safety and effectiveness of ASOs by increasing their binding affinity to the target mRNA, increasing their resistance to nucleases, and lowering their immunogenicity (Rinaldi and Wood, 2018).

Both phosphorothioate and 20-O-methoxyethyl (sugar) modifications are incorporated into the IONIS-HTTRx.

For a number of neurodegenerative diseases, ASOs have demonstrated promising results in human trials; however, these studies were neonates and their mode of action differed from that of the HTT ASOs that are currently in HD clinical trials. Children with spinal muscular atrophy (SMA) type 1 exhibited a significant improvement following lumbar intrathecal bolus injections of nusinersen. ASO modified with methoxyethyl (MOE) changes the mRNA splicing mechanism of the SMN2 gene, resulting in the production of functional SMN protein, which is absent in people with SMN1 gene mutations. The medicine, Spinraza, produced compelling results that led to the early end of this experiment and expedited the FDA's approval for the treatment of all forms of SMA. Mercuri et al. (2018) report that patients with SMA that manifested later in life also shown remarkably positive outcomes. It's

important to remember that SMA is a rapidly progressing, aggressive illness; hence, clinical trials may reveal therapeutic improvements sooner rather than later. Furthermore, it has been demonstrated that an ASO targeting SOD1 for the treatment of SOD1-positive amyotrophic lateral sclerosis (ALS) is safe and well tolerated after lumbar intrathecal infusion (Miller et al., 2013). As a result, Ionis Pharmaceuticals and Biogen are preparing to enter a phase 1/2a clinical trial (https://clinicaltrials.gov/ct2/show/NCT02623699) for a stronger ASO called ISIS-SOD1Rx.

As distinct target sequences that do not occur anywhere else in the genome can be carefully chosen with ASOs, the unintentional binding of mRNA sequences with shared homology to the target is less of a problem than with RNAi. Because ASOs bind to pre-mRNA rather than mature transcripts, they can be engineered to target both intronic and exonic areas.

More RNA "real estate" is available, making it easier to choose the best possible treatment candidate. ASOs also have the benefit of having distinct, dose-dependent, and reversible actions without overwhelming any natural cell pathways. To maintain therapeutic results, frequent administration of ASOs will probably be necessary.

Techniques Using Small Molecules to Lower HTT. A much more enticing treatment for HD involves an ingestible, brain-transmitting small molecule. The process of intrathecal administration can be a little intrusive, particularly when done frequently. Using phenotypic screening, it has been possible to identify tiny drugs that change the quantities of HTT (or mutant HTT) protein in ESCs and iPSCs produced from HD patients (Doherty, 2017, CHDI Foundation, conference). PTC Therapeutics is investigating the optimization of medications that alter pre-mRNA splicing and reduce HTT concentrations.

With tiny compounds, the specificity that Watson-Crick base-pairing provides is crucial for RNAi effectors and ASOs, increasing the likelihood of off-target effects. Tiny screening revealed compounds that modify SMN2 pre-mRNA splicing, which when added to severe SMA animal models resulted in increased motor function and longer lifespans (Naryshkin et al., 2014). However, ocular difficulties in ongoing monkey research led to the termination of the phase 1b/2a clinical trial (https://clinicaltrials.gov/ct2/show/NCT02240355) testing the lead chemical RG7800.

However, RG7916, a different medication, just finished a phase 1 study (https://clinicaltrials.gov/ct2/show/NCT02633709), and three phase 2 studies including adults and children with various types of SMA are currently underway. According to preliminary data, RG7916 increases SMN protein in patients with SMA types 2 and 3. This suggests that small-molecule splicing modifiers may be able to theoretically achieve target engagement comparable to that of ASOs (PTC Therapeutics, 2017).

Nuredis has created a potentially effective innovative method for preventing the transcription of mHTT mRNA. Certain transcription elongation cofactors must bind in order for transcription to proceed across DNA areas containing long extended CAG repeats (Liu et al., 2012). In the R6/2 mouse model of HD, genetic interference in this pathway preferentially reduced mHTT levels but not wild-type HTT levels in HD patient lymphoblastoid cells, leading to enhanced rotarod performance and longer longevity (Liu et al., 2012). Pre-clinical work is presently underway for small-molecule inhibitors of this pathway.

Approaches to Target Alternative Toxic Species in HD

Current RNAi or ASO medications being studied in the clinic may not lower the exon 1 HTT protein that results from faulty splicing of mutant HTT mRNA (Sathasivam et al., 2013). These treatments operate on locations downstream from the activated cryptic polyA signals. It is unclear how this will affect the overall therapeutic and clinical benefit. On the other hand, complementary RNAi or ASOs to HTT exon 1 mRNA might still be useful. In order to selectively target this HTT exon 1 mRNA, efforts are currently being made to investigate biologic and small chemical approaches (Gillian Bates, personal communication).

RNA interference (RNAi) may not completely target hazardous RAN proteins that have been previously discovered (Baṇ ez-Coronel et al., 2015), especially if those proteins originate from the antisense strand. However, ASOs operating at the premRNA level would probably result in a decrease in all RAN proteins as well as a reduction in the cytosol's quantity of potentially hazardous CAG-expanded mRNA (Rue´ et al., 2016). Figure 1 summarizes the production and targeting of alternative hazardous species. It is clear that treatments at the HTT DNA level would prevent the formation of all of these downstream products.

Management

A multidisciplinary strategy combining doctors, nurses, physiotherapists, speech and language therapists, dieticians, and other healthcare experts is necessary for the best

management of HD. Optimizing quality of life and anticipating the patient's evolving demands as the illness worsens are the goals. Usually, a mix of non-pharmacological and pharmaceutical therapies are used for this. Decisions about pharmacological therapies are frequently relied on professional judgment and clinical experience because the evidence foundation is often weak.

Motor symptoms

One of the most noticeable signs of HD is cholerea, which appears early in the illness. Tetrabenazine 44 is the only medication that is specifically approved to treat chorea. At daily dosages between 50 and 75 mg, this inhibitor of synaptic vesicular amine transport has a long-lasting anti-choreic action. Sleep issues, melancholy, anxiety, and restlessness are side effects.

A modified form of tetrabenazine containing deuterium molecules is called deutetrabenazine. Longer half-lives and less metabolic variability are the outcomes of this. Although head-to-head studies comparing tetrabenazine and deutetrabenazine have not been conducted, there is evidence that deutetrabenazine may cause fewer side effects, such as depression and somnolence, than tetrabenazine. The FIRST-HD study found that deutetrabenzine significantly reduces chorea 46 when compared to placebo.

In a randomized controlled trial, the neuroleptic sulpiride has proven effective in treating chorea (RCT). Other neuroleptics, such as olanzapine, respiridone, and quetiapine, are also often used in clinical practice; the most frequent adverse effects include sleepiness and weight gain. Physiotherapy is a typical treatment for other motor complaints, such as irregular gait, poor balance, and frequent falls.

Psychiatric symptoms

Since there is little data to support the treatment of mental symptoms in HD, clinical judgment and expert opinion are used to make therapy decisions. Non-pharmacological therapies like cognitive behavioural therapy or psychodynamic therapy can be used to treat depression, anxiety, obsessive compulsive disorder, and irritability; however, their applicability may be restricted in cases of cognitive impairment. Pharmaceutical treatments include the selective serotonin uptake inhibitors (citalopram, fluoxetine, paroxetine, and sertraline) as well as the noradrenergic and serotonergic-acting medications mirtazapine and venlafaxine. Neuroleptics are helpful in the treatment of psychosis and violence. Many drugs,

such as bupropion, atomoxetine, amantadine, bromocriptine, methylphenidate, and modafinil, have been used to treat apathy; however, no RCTs have been carried out.

Cognitive symptoms

Anticholinesterase inhibitor use for cognition in HD has been evaluated in two RCTs, however the number of participants was limited and the findings were inconsistent (49). Citalopram had no effect on cognitive performance 50, according to another RCT. Coping mechanisms for cognitive impairments can be helpful; these include asking employers to modify the nature of work or the workspace, setting up a quiet place to work, or switching to a task that doesn't require as much multitasking.

CONCLUSION

HTT reduction techniques have advanced from pre-clinical development to clinical use as a result of international cooperation. Phase 3 clinical trials for an ASO that lowers HTT are set to start, and trials for RNA interference and zinc finger nucleators will soon follow. The difficulties associated with drug delivery and distribution have been thoroughly studied and, for the most part, resolved; intrathecal delivery of ASOs has been shown to be a workable technique, and intracranial delivery of viral vectors for RNA interference has shown promise in people and animal models.

Work on Huntington's disease (HD) may provide insights into other neurodegenerative illnesses for which a known toxic species or causative mutation exists. DeVos et al. (2017) have highlighted the possibility of using tau reduction as a therapeutic intervention for Alzheimer's disease and other tauopathies. Currently, clinical trials including ASOs that target MAPT mRNA are being conducted.

It has become possible to identify objective biomarkers that precisely follow the development of HD. When combined with the availability of predictive genetic testing for HD, this offers a chance to start treatment just before symptoms appear and stop neurodegeneration. There's a good chance that HD will soon have a disease-modifying treatment, and the field of HTT-lowering medicines will continue to grow in the coming years.

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