Potential disease modifying therapies for Huntington's disease, lessons learned and future opportunities

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Abstract

Huntington's disease is the most frequent autosomal dominant neurodegenerative disorder, for which we have no approved disease-modifying treatments. The molecular pathogenesis of Huntington's disease is complex, with toxicity arising from full length expanded huntingtin and N-terminal fragments of huntingtin, which are both prone to misfolding due to proteolysis, from aberrant intron-1 splicing, and from somatic expansion of the CAG repeat in the *HTT* gene. Potential therapies for Huntington's disease include those targeting huntingtin DNA and RNA, clearance of huntingtin protein, DNA repair pathways, and other treatment strategies targeting inflammation and cell replacement. The early termination of the tominersen antisense oligonucleotide trials sent a strong signal that it is timely to reflect on lessons learned, where the field stands now, and our challenges and opportunities for the future.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative condition caused by a CAG repeat expansion in the first exon of the *HTT* gene, encoding for a mutant huntingtin (mHTT) protein[1]. Symptoms involve motor, cognitive and psychiatric areas, generally appearing during mid-life with slowly progressive decline over the course of two decades[2]. There is currently no approved disease-modifying treatment for HD[3].

In this Review, we initially explore the effects of the *HTT* mutation and toxic species in HD pathogenesis relevant for the development of disease-modifying treatments. Next, we describe the potential implications derived from impairing the function of the wild-type protein through non-allele selective compared to allele-selective approaches targeting the disease-causing mutation or its by-products.

We review therapies targeting *HTT* DNA or RNA including recent results of trials using antisense oligonucleotides (ASO) followed by strategies targeting other disease mechanisms such as DNA repair, or downstream effects of mHTT protein. Subsequently, we focus on restoration of neuronal death through cell replacement. Finally, we will comment on methods for efficient delivery of these therapies to the brain, enrichment and target engagement/response biomarkers and on aspects of clinical trial design that should be considered in future HD trials.

HD pathogenesis

HD is primarily thought to arise predominantly by a toxic gain-of-function of the mHTT protein[4,1] (Figure 1). The CAG repeat expansion encodes for an enlarged polyglutamine (polyQ) tract in the N-terminal fragment of the protein[3] causing mHTT to fold abnormally and accumulate in brain cells[2]. The expression of mHTT leads to progressive disruption of neuronal physiology. Subsequently, there is detectable atrophy in the basal ganglia nuclei, including the caudate, putamen, globus pallidus and subthalamic nuclei as well as in subcortical white matter before the emergence of motor symptoms. As the disease progresses, significant

neuronal loss in cortical, thalamic and hypothalamic areas and eventually throughout the entire brain is also observed[5] (Figure 1).

The underlying HD disease mechanism is complex and widespread and includes deregulation of the ubiquitin/proteasome and autophagy systems contributing to toxic protein accumulation[2,6]. There is also oxidative stress with peripheral and central inflammation from the earliest stages of the disease [7].

In addition, mHTT affects transcriptional regulation[8] and axonal transport resulting in decreased signalling mediated by brain-derived neurotrophic factor (BDNF)[2]. Moreover, there are alterations in cortical glutamatergic and striatal dopaminergic neurotransmission as well as evidence for synaptic dysfunction in HD[2].

Genome-wide association (GWA) studies have shown that age at onset and disease progression in HD is associated with loci in DNA repair genes[9] whose proteins are known to influence the extent of somatic expansion of the CAG repeat. Consistently, extreme increases in CAG repeat length (up to 1,000 CAG repeats) are present in the striatum of post-mortem HD brains[10].

HTT lowering as a therapeutic approach

In contrast to most neurodegenerative conditions, in HD a single mutation is responsible for all cases of the disease[1]. In addition, there is substantial evidence supporting the toxic effects of mHTT, and CSF mHTT levels are correlated with disease stage and function in HD gene-carriers[11].

Animal models carrying the HD mutation show protein inclusions and brain atrophy alongside progressive neurological phenotypes[12]. Consistently, numerous studies using different approaches in multiple mouse models to decrease mHTT have shown that this strategy is well-tolerated and effective. Mutant HTT-lowering improves motor performance[13,14,15,16,17] and cognitive function[17]. There is increased survival and decreased weight loss in response to HTT-lowering therapies[15]. Moreover, biochemical deficits[13], synaptic

dysfunction[16,13,18] and transcriptional dysregulation can be also ameliorated through mHTT-lowering, together with reduced atrophy rates[16,17] and decreased intraneuronal protein aggregates[13,14].

Exon 1 toxic species

A highly toxic HTT exon 1 protein containing the polyQ tract is present exclusively in mutation carriers[19]. Incomplete splicing of *HTT* mRNA generates a short polyadenylated *HTT* mRNA comprising exon 1 and the 5' part of intron 1, leading to production of the exon 1 fragment[19]. This exon 1 protein is constantly generated and accumulates in striatal neurons[20], is associated with faster neurological phenotypes in HD mouse models, and has also been detected in juvenile onset HD post-mortem brains[19]. These findings suggest that a highly toxic exon 1 protein may be a primary pathogenic species. However, the presence of this toxic fragment could pose a challenge to some HTT-lowering therapies. It may not be lowered by splicing modulators (branaplam, PTC518), existing ASOs (tominersen, WVE-12010, WVE-120102) or RNA interference (RNAi) (VY-HTT01, AMT-130) agents in clinical development, as these target *HTT* downstream from exon 1 *HTT* mRNA. Alternatively, therapies targeting DNA or HTT exon 1 mRNA may be effective in decreasing the exon 1 protein (Table S1)

Panel 1 - Challenges of delivery and distribution to the HD brain

The need to deliver molecules or cells directly to the brain is driven by protective properties of the blood-brain barrier (BBB), and by the desirability of targeting some therapies to specific brain regions (see Figure S1 and Table S1). The GENERATION HD1 study (NCT03761849) developed by Roche delivered tominersen, a non-allele selective ASO targeting *HTT* mRNA via lumbar intrathecal injection; advantages of this route included widespread clinical familiarity and availability of the approach, and disadvantages included unequal distribution of the ASO predominantly to cortical over deeper brain structures and the potential discomfort of repeated injections[21]. Intrathecal infusion catheters could circumvent the latter, but not the former and carry appreciable rates of complications[22]. Intraventricular catheters, inserted using contemporary navigation methods, have decreased long-term complications and are better positioned for efficient delivery

to the striatum, although distribution, tolerability and safety should be determined empirically for individual therapeutic agents[23]. Direct intraparenchymal delivery of molecules to the striatum has benefitted from iterative improvements of convection-enhanced delivery techniques, especially within oncology, with a number of delivery systems in use for a variety of neurological applications[24], whilst development of optimised devices for delivering cells has lagged behind[25] and needs further attention. Stereotactic neurosurgery carries potential challenges, including risks of surgery (infection, bleeding, death), establishing appropriate technical expertise, intervention fidelity, and regulatory considerations,[24,26] some of which may be addressable in the future through non-surgical approaches [27].

The challenge of allele versus non allele selectivity in HTT lowering

HTT-lowering therapies can be classified as non-allele selective (total HTT lowering), reducing the levels of both wild-type HTT (wtHTT) and mHTT; or allele-selective, if only mHTT is decreased[4] (See Figure 2 and Table S1).

Depletion of wtHTT shows age-dependent pathological phenotypes in mice. Knockout of the *Htt* gene in rodents is embryonically lethal[28] consistent with an essential role in neurodevelopment[29]. Inactivation on day five during the postnatal period in mice leads to progressive neurological deterioration[1,30]. In contrast wtHTT ablation at two months of age causes pancreatitis but does not appear to be deleterious in the adult mouse brain[15,31]. Importantly, CAG expansions do not alter the transcription of the wild-type allele[8]. Consequently, the expression of the wild-type allele is not expected to be affected by the presence of the mutation.

In human case studies (Table S2) a woman with a balanced translocation in *HTT* resulting in one intact and one disrupted gene that at best could produce a partial protein, displayed a normal phenotype at 46 years[32]. Conversely, compound heterozygous variants in *HTT,* expected to lead to decreased transcription of normal HTT below 50% have been associated with a Rett-like phenotype[33,34]. Similarly, HD individuals with

decreased transcription of the wild-type allele, had earlier age at onset[35] while rare cases with homozygous expansions in *HTT* have similar development and age at onset compared to heterozygous carriers[36–39].

In summary, animal studies and case reports suggest that partial lowering of wtHTT (~50%) in the healthy adult brain is likely safe[1]. However, the long-term effects of decreasing total HTT levels (both wtHTT and mHTT) in the brains of HD patients are unknown[3].

In contrast, allele-selective approaches lack the potential side effects from impairing the physiological function of the wild-type protein. However, there are some significant limitations with current allele-selective approaches, such as their inability to target the entire HD population with SNP-related approaches[3], or decreasing the transcription of other genes sharing similar sequences with some CAG-targeting therapies[3].

Nucleic acid therapeutics

Two programs of nucleic acid-based approaches for HD using gapmer ASOs to lower huntingtin have recently reported preliminary results. Gapmer ASOs are a string of nucleotides with unmodified nucleotides in the central region flanked by modified nucleotides, to increase durability of the response. After binding of the ASO to the target mRNA, the unmodified central region can recruit RNAse H enzyme leading to degradation of the transcript (Figure 2).

The Ionis/Roche ASO program uses tominersen, an ASO targeting exon 36 of human *HTT* mRNA. Binding of the ASO results in RNase H degradation of both the mutant and wild-type *HTT* transcripts[15]. Results from a phase 1/2a study were promising (NCT02519036) with bolus intrathecal administrations every 4 weeks at ascending dose levels from 10 to 120 mg showing no serious adverse events and around 40% reduction of mHTT in CSF after four doses[21] of tominersen, being above the degree of mHTT lowering showing phenotypic improvement in animal studies[21]. There were dose-dependent and time-dependent increases in ventricular volume in participants receiving tominersen compared to the placebo group, without corresponding changes in caudate or

whole-brain volume. Similarly, cerebrospinal fluid (CSF) neurofilament light (NFL) protein concentrations also showed time-dependent and dose-dependent increases in treated participants compared with those on placebo. Participants in the phase 1/2a study were offered to take part in the open-label extension (OLE) (NCT03342053) clinical trial with tominersen, where they received the 120 mg of the ASO every four or eight weeks during a period of 15 months. Preliminary results from the OLE study, showed increases in CSF NFL concentrations at day 141 that decreased thereafter despite continued dosing with tominersen[40,41].

Based on these promising phase 1/2a data, a large multinational phase 3 study, GENERATION HD1 (NCT03761849), was initiated with intrathecal dosing of tominersen every four or eight weeks. However, based on results from the OLE study (NCT03342053) showing greater than expected CSF huntingtin-lowering, together with reduced tolerability and increased adverse events in the four-weekly dosed arms[41] the phase 3 protocol was amended to two less frequent dosing frequencies: 120mg tominersen every eight or 16 weeks (NCT03761849). In March 2021, dosing in this trial was halted based on the independent Data Monitoring Committee's recommendation. Although there were persistent dose-dependent decreases in CSF mHTT, the group receiving tominersen every eight weeks performed worse on clinical rating scales and had higher frequencies of serious adverse events compared to placebo. There were no statistically significant differences between placebo-treated participants and the 16-weekly group regarding the clinical endpoints. Similarly, participants receiving the drug every eight weeks had significant increases in CSF NFL at week 21, decreasing thereafter despite continued dosing, while only mild CSF NFL increases were found in the 16-weekly cohort, before decreasing back to baseline. *Post hoc* exploratory subgroup analysis of the phase 3 study with tominersen investigated the association between disease burden and ASO exposure. The data generated by this analysis suggests that younger participants with a lower disease burden might derive benefit from less frequent or lower dose treatment with tominersen in contrast to the other subgroups. However, these analysis are preliminary and have not yet been published in a peer reviewed journal. Following these results, Roche will continue the tominersen program with a new phase 2 clinical trial exploring different doses of the ASO in younger patients with lower disease burden[42–44].

The second ASO phase 1/2 program in HD used a potentially allele-specific approach to HTT-lowering developed by Wave Life Sciences. WVE-120101 (NCT03225833) and WVE-120102 (NCT03225846) are proprietary stereopure gapmer ASOs directed against SNPs unique to the mutant *HTT* transcript[45]. With this approach, m*HTT* mRNA is selectively targeted over wt*HTT* mRNA. ASOs were administered monthly via intrathecal injection with the highest dose of 32 mg. Both trials reported a lack of target engagement, with no significant mHTT lowering in the CSF at any dose[43].

What can we learn from these recent ASO clinical trials? For the WVE-120101/2 trial there are no published animal studies, partly due to a lack of animal models with the relevant SNPs at the time these ASOs were developed. Failing to show any target engagement with their first two SNP-targeting ASOs, Wave is moving forward with a novel ASO based a modified chemistry, targeting a third SNP (WVE-003)(NCT05032196). In contrast to the WVE-120101/2 trials, tominersen effectively targeted the *HTT* mRNA based on the CSF decreases in mHTT protein. Although, the GENERATION HD1 phase 3 trial was a large study, following a phase 1/2a study and open-label phase 2 extension study, adequate data on absorption and distribution in humans was lacking. However, the transient rise in CSF NFL found with the highest ASO doses in the OLE and GENERATION HD1 studies was, in retrospect, a portent of the negative outcome of the initial tominersen studies [21,41].

The transient increase in CSF NFL before full suppression of CSF mHTT suggest an off-target inflammatory effect of the ASO damaging susceptible neurons. Furthermore, peak-dose toxicity is possible, and rodent studies have shown an initial high retention of signal in the meninges covering the spinal cord and base of the brain during one to eight hours, followed by diffuse ASO immunostaining throughout the spinal cord and brain[46]. However, the decrease in CSF NFL in the face of continuing dosing[41] in the eight weekly cohorts suggests the potential for benefit if the initial ASO adverse effects may be reduced with a lower dose of tominersen in more resilient, younger brains with lower disease burden. However, sufficient mHTT lowering in target structures such as the caudate and putamen nuclei, will need to be achieved in the brains of HD patients.

The CSF white cell count and protein rises in the OLE study with tominersen[41] also suggest an inflammatory reaction possibly due to ASO-exposure related toxicities in association with the high 120mg dose chosen to maximise ASO concentrations in the striatum after intrathecal delivery[42]. Patients in the phase 3 trial developed time and dose-dependent ventricular volume increases without matched brain atrophy[42]. In contrast to CSF NFL, the ventricular increases do not recede and could be secondary to increases in CSF white cells and proteins. Interestingly, cases of hydrocephalus have been reported in infants with spinal muscular atrophy receiving nusinersen, a non-gapmer ASO[47].

However, deleterious effects of too much wild-type HTT lowering with the high exposure dose of tominersen and the relatively late stages of the study participants are also important to consider, and the analyses of this invaluable dataset will inform all future HTT lowering programmes. The new dose-finding phase 2 clinical trial will determine the optimum and safest dose of tominersen to possibly change the course of the disease in younger participants with lower disease burden.

In retrospect, a full phase 2b study of tominersen would have been helpful to prepare for the phase 3 efficacy trial, a lesson that the HD field should learn from in the future.

Nucleic acid therapeutics in animal and *in vitro* stages of development

Allele-specific ASOs targeting SNPs are being developed by Ionis[17,48] as well as ASOs targeting the CAG repeat by VICO Therapeutics[49]. Benefits of targeting the CAG repeat would be that a single ASO is applicable to multiple polyQ disorders[50], however they risk off-target effects through binding to other CAG repeat containing RNAs.

RNAi approaches include, among others, short-interfering RNA (siRNA) or microRNA (miRNA). These molecules target mature mRNA in the cytosol triggering degradation through the RNA-induced silencing complex, eventually reducing protein expression (Figure 2) [3,51]. Conjugated siRNA can be internalised into neurons, being also in development for HD. Alnylam Pharmaceuticals together with Regeneron have a programme with ALN-HTT[51] targeting exon 1. In a rapidly progressing HD mouse model the siRNA was

infused into the striatum with an implantable infusion system and ameliorated the molecular and motor dysfunction in this mouse model[51].

Atalanta Therapeutics, together with Biogen, are developing a novel chemically-synthesized branched siRNA[52], that shows widespread distribution throughout the brain after a single administration into the CSF, with promising results in lowering mHTT levels[53,54].

Future perspectives for nucleic acid therapies

Challenges for the field in nucleic acid therapies lie mainly in improving safety, toxicity and delivery. New developments ensure that nucleic acid molecules still hold promise for treating HD. Tricyclo-DNA ASOs are thought to cross the BBB[55]. Neubase Therapeutics are developing nucleic acid ligands that target CAG-repeat expansion in the mRNA and are in early stages of development[56].

What can the HD field learn from the approved RNA-targeting ASOs for neurological disorders such as nusinersen (for spinal muscular atrophy) and milasen (Batten's disease)? Importantly, ASOs can be successful therapies. The major differences to the HD ASOs are the much lower dose requirements (12 mg for nusinersen and 63 mg for milasen[57,58]) and the ASOs restored wild-type protein expression instead of lowering expression of a mutant (and a wild-type) protein. Counteracting neurodegeneration by downregulating a toxic protein is much less straightforward than restoring protein expression, for which small increases of the missing protein have proven to be beneficial. In contrast, tofersen, an ASO degrading *SOD1* mRNA tested in SOD1-amyotrophic lateral sclerosis at similar doses (100mg) to those used in the tominersen phase 3 clinical trial, was associated with increased CSF protein and CSF pleocytosis but not with NFL increases, while there were no imaging data[59]. However, concentrations of ASOs are lower in the basal ganglia and higher in cortical regions and the spinal cord after intrathecal administration, being advantageous for amyotrophic lateral sclerosis or spinal muscular atrophy, while HD requires targeting deep grey matter nuclei [60].

Gene therapy approaches

Gene therapy uses genes (transgenes) expressing a therapeutic principle to treat and prevent disease[61]. A major hurdle facing many current HTT-lowering therapies based on RNAi, zinc-finger proteins (ZFP) and genome editing (CRISPR) is efficient delivery of these therapeutic agents to the critical target regions in the central nervous system. Un-modified constructs do not readily cross the BBB, nor are they taken up by neurons, and currently recombinant adeno-associated viral (AAV) vectors are the most commonly used delivery method for effective cell transduction and, eventually, achieving stable expression of the transgene. Recombinant AAV vectors have a number of advantages: they are non-pathogenic, do not replicate in the host, and do not integrate into the host genome[62]. Delivery of viral vectors to specific brain regions can be achieved by bilateral intraparenchymal infusion. The striatum (caudate/putamen) is the most severely affected brain region in HD and is the most common target for AAV-mediated delivery, but HD affects the entire brain and effective therapies will likely require delivery to the cortex and other affected brain regions (Panel 1). Despite the potential utility, there are significant limitations to AAV delivery. Immune responses to the viral vector occur and repeated administration is not usually possible. Viral delivery is a single shot on goal, it is irreversible and thus dose correction in an individual is not possible. Tissue distribution is highly variable; decreasing as distance from the injection site increases[63].

AAV RNAi Studies in HD models

In vivo animal studies of AAV-mediated RNAi therapies for HD have provided evidence for target engagement (HTT-lowering) and efficacy in transgenic mice, minipigs and non-human primate models. Early studies of AAV-mediated RNAi therapies in transgenic mouse models of HD found that 40-60% lowering of total HTT (both wtHTT and mHTT) was safe, extended lifespan, and decreased motor symptoms even when initiated in symptomatic mice[64]. Intracranial administration of AAV5-delivered anti-HTT miRNA into a transgenic HD minipig model reduced mHTT levels in widespread brain regions[65] and resulted in sustained mHTT lowering for up to a year after injection[63]. These results suggested that this approach could be effective in patients with HD and support the first human trial of an AAV-mediated therapy in HD (NCT04120493). Partial lowering

of wtHTT by RNAi was also safe and well-tolerated in NHPs. Reduction of HTT levels by 45% did not result in abnormal neuropathology or adverse symptoms[66] and sustained total huntingtin-lowering in NHPs had no observed toxicity even 6 months post-injection[67].

Clinical trials of AAV-miRNA

Clinical trials of miRNA therapy in HD are already underway (Table S1). The uniQure AMT-130 trial (NCT04120493) is evaluating the safety, tolerability, and effect on CSF biomarkers of bilateral intrastriatal injections of AAV5-miHTT in early HD. AAV5-miHTT is an engineered AAV5 vector expressing miRNA targeting total huntingtin, injected using MRI-guided convection-enhanced delivery, and a new open-label clinical trial with AMT-130 (NCT05243017) started in 2021. Voyager Therapeutics had a similar program under development for AAV-delivered anti-HTT RNAi therapy (VY-HTT01). This programme is currently on hold pending development of more efficient viral delivery (Table S1). A third AAV clinical trial in HD sponsored by Spark Therapeutics is currently being planned to evaluate an AAV1-delivered anti-HTT miRNA that has shown promise in NHPs[66].

DNA targeted approaches

Therapeutic approaches directly targeting the DNA of the *HTT* gene have exceptional promise and correction of the underlying genetic defect should prevent all associated pathogenicity in HD (Figure 2). Zinc finger proteins (ZFPs) consist of an active effector element such as a transcriptional repressor protein or nuclease, bound to a DNA-binding element composed of multiple zinc finger peptides that enable selective targeting to specific DNA sequences[68]. ZFPs have been shown to selectively lower mHTT expression in cell lines without significantly altering wtHTT expression. AAV-ZFP infusions reduced mHTT levels and improved some HD-like behavioural phenotypes in HD mice[13].

CRISPR (clustered regularly interspaced short palindromic repeats)-based systems have emerged as potentially versatile, specific, and efficient gene-editing technologies with potential to treat HD and other neurodegenerative disorders. CRISPR gene editing can selectively inactivate the mutant *HTT* gene in patient-derived fibroblasts[69], in HD mice[70] and in human differentiated induced pluripotent stem cells[71]. Animal and

in vitro studies support the general feasibility of gene editing approaches in HD, but no clinical trials are yet underway. However, further testing in HD animal models and advances in both viral and non-viral delivery methods in large animals will be essential before DNA targeting CRISPR approaches are ready for testing in HD patients.

Splicing modulators

The latest therapeutic agents to emerge in HD are a new class of oral, brain-penetrant, small molecule agents targeting HTT expression, the first of which was initially identified in a phenotypic screen targeting expression levels of the spinal motor neuron-2 gene, as a treatment for spinal muscular atrophy. Branaplam (LMI070) is in phase 2 for spinal muscular atrophy (NCT02268552). Selectivity analysis showed that branaplam can decrease the expression of both copies of the HTT protein via splicing modulation. The mechanism of action seems to be mis-splicing of exon-49/50 leading to decay of the *HTT* mRNA and decreasing protein levels[72] (Figure 3). Novartis received US FDA Orphan Drug Designation for branaplam in HD, and a phase 2b trial (NCT05111249) started enrolling in December 2021.

PTC therapeutics developed a drug discovery splicing platform to identify small molecules that target the same mechanism as branaplam, finding compounds that successfully decrease the *HTT* mRNA and HTT protein in the brain and the periphery[73]. Subsequently, PTC therapeutics is conducting a phase 1 trial of PTC518, to evaluate the safety, pharmacokinetics and pharmacodynamic properties in healthy volunteers, with a phase 2 trial starting in 2022[74,75].

DNA repair therapeutics

Repetitive DNA is inherently unstable, and over a HD patient's lifetime the expanded CAG repeat in the *HTT* gene tends to gain additional repeats, a process known as somatic instability. The speed of expansion differs between tissues and cell types, more prominent in neurons than glia, with fast expansion in striatal and

cortical cells, which are most prone to degeneration in HD[10]. Patients with more somatic instability, as measured in blood, have earlier disease onset and faster progression, establishing somatic instability as a rate driver of HD[9]. The SHIELD HD study (NCT04406636) is currently underway to study the natural history of somatic instability in different biofluids of pre- and early manifest HD patients to inform future therapeutics targeting repeat expansion.

In the last few years GWA studies in thousands of HD patients have found DNA repair to be the main modifier of HD disease course[9,76], and that the same genes also affect other trinucleotide repeat diseases[77]. One of the strongest modifier genes was *FAN1*, that codes for a nuclease that cuts DNA during repair of crosslinks between DNA strands[9]. FAN1 is protective in HD, likely through accurate repair of loopouts that form at CAG repeats[78] (Figure 4) and its depletion in animal models and HD patient neurons accelerates repeat expansion[78]. Genetic variants decreasing FAN1 function advance onset by over 5 years, whereas those that increase its expression can delay onset over a year[9].

Mismatch repair is the most significant DNA repair pathway driving HD pathogenesis. It is initiated by the recognition of base mismatches or single strand loopouts by a MutS complex. This involves recruitment of a MutL nuclease complex to nick DNA, digestion of the error-containing strand, re-synthesis using the remaining template strand, and finally sealing the ends of the newly synthesised strand[79] (Figure 4). Several components of this mismatch repair pathway influence HD onset, including MSH3, MLH1, PMS1, PMS2 and LIG1[9,76]. Studies in HD mice suggest that MSH3, PMS1 and MLH3 drive expansion, whereas MSH6 and PMS2 may protect against it[80].

Our understanding of the role that DNA repair mechanisms play in trinucleotide repeat expansion is crystalising, with opposing forces battling to either drive or prevent it, and this is already translating into new therapeutics targeting the mutation itself. Whilst MSH2, MSH6 or MLH1 depletion is associated with cancer in humans[79], MSH3 is tolerant of loss of function and deletion of MSH3 in mice does not affect lifespan or cause cancer[79]. Triplet therapeutics plans to begin a phase 1/2a clinical trial of the MSH3 ASO TTX-3360

in 2022, and one can envisage other strategies, including small molecule MSH3 inhibitors, acting for example through ATPase inhibition, or promotors of FAN1 activity as potential therapies for HD.

Other therapeutic approaches – pridopidine, C1q antibody

Pridopidine

Pridopidine (formerly ACR16) is a sigma-1 receptor agonist[81]. Pridopidine rescued mHTT-induced cell death in HD mouse neurons and human derived induced pluripotent stem cells, and restored homeostatic synaptic plasticity and calcium signalling alterations in HD neurons; responsiveness to the compound was abolished in sigma-1 receptor knockout neurons[82], in HD patient lymphoblasts[83], and human HD derived induced pluripotent stem cells [84]. In an HD mouse model, pridopidine led to improvements in motor coordination, and ameliorated a subset of gene expression changes[83].

Studies using [18F] fluspidine (an sigma-1 receptor ligand) and [18F] fallypride (a D2/D3R ligand), in healthy volunteers and in a small number of patients with HD[81], suggest that at the doses tested clinically (90 mg/day), pridopidine is saturating for sigma-1 receptors and does not occupy D2/D3Rs[81].

Pridopidine has been investigated as a treatment for HD in three randomized, double-blind, placebo controlled clinical trials: HART (NCT00724048, NCT01306929), MermaiHD (NCT00665223), and PRIDE-HD (NCT02006472, NCT02494778). Initial analysis suggested a small effect on motor endpoints in the HART and MermaiHD studies. PRIDE-HD[85] failed its primary endpoint analysis but showed a small beneficial effect on Unified Huntington's Disease Rating Scale (UHDRS) Total Functional Capacity (TFC) at week 52 which was more pronounced for early HD participants and supported by quantitative motor assessments. These observations led to a phase 3 study, PROOF-HD (NCT04556656), currently enrolling using the UHDRS-TFC score as the primary endpoint.

Complement (C1q-neutralizing antibody) directed therapeutics

Synaptic elimination can be mediated by components of the classical complement pathway. For example, synapses marked with activated C1q protein are subsequently eliminated by activated astrocytes. Dysregulation of this mechanism has been associated with excessive synaptic loss in affected circuits in neurodegenerative disorders including HD[86] and complement proteins were elevated in a small sample of HD brains[87]. Annexon has developed the anti-C1q monoclonal antibody ANX-005, currently enrolling for a phase 2 open label study in HD (NCT04514367) with the aim of evaluating the pharmacokinetics and pharmacodynamic effects of this agent.

Cell replacement therapy

The underpinning concept of cell therapy is restoration of structure and function. This can be achieved through implanting cells designed to adopt the function of cells lost to the disease process, or through cellular secretion of neuroprotective or disease-modifying molecules. For many years, the focus of cell therapy in HD was to replace degenerated medium-spiny neurons (MSNs) using cells procured from normal developing foetal striatum (Figure S1). Foetal grafts can restore synaptic connectivity and function in HD animal models[88]. Human studies have demonstrated safety and feasibility, with some proof-of-concept evidence to suggest that foetal grafts can improve function, although solid evidence of efficacy is still required. The practical limitations of foetal-derived cells for human use has encouraged research using MSN-like neurons derived from pluripotent stem cell sources, and provided evidence of functional improvements with a view to future clinical translation[88]. There is significant interest in the restorative value of non-MSN cell types, including cortical-like cells and glial progenitors. The latter population has been employed in rodent models of HD and shown to confer significant phenotypic benefit, restoring deficient glial signalling, both in astrocytes and oligodendrocytes[89].

Panel 2 - Biomarkers for clinical trials and the state of the field

Critical to the success of clinical trials is the ability to reliably measure the impact of treatment on the disease. Effects can be assessed at the cellular, macrostructural and behavioural levels[60,90–92] (Table S3) and biomarkers (target engagement, response to intervention) are being employed in ongoing clinical trials.

Biofluids

Two particularly useful protein biomarkers have emerged recently: mHTT itself – the pathogenic agent; and NFL – released from damaged axons. Both increase with disease progression[93,94], but NFL has the greater prognostic potential[11], while mHTT has demonstrated specific value as an indicator of target engagement by HTT-lowering drugs[3]. Dose-ranging studies of future HTT-lowering therapeutics may try to use mHTT or NFL or both to define biochemically the lower and upper bounds, respectively, of a therapeutic window. There are still challenges to this approach namely because there is large inter-individual variability for mHTT.

Biomarkers reflecting specific pathways such as YKL40[95] and IL-6[96] (neuroinflammation), tau (microtubular damage)[97] or with some region-specificity like the striatal marker proenkephalin[98] have shown potential in HD but have yet to be evaluated to the same extent as mHTT and NFL.

CSF huntingtin quantification remains limited by the sensitivity of current mHTT assays – many far from clinical motor onset individuals have unquantifiably low levels[11,99], making therapeutic lowering difficult to assess; and the difficulty of quantifying wtHTT either independently or through inference from the total HTT level based on current assays[100]. More broadly, the HD biomarker field lacks well-powered replication studies, but the HDClarity bioresource has a large and expanding CSF and plasma collection for this purpose[101].

MRI

Structural MRI is a well-established technique which has already been employed in clinical trials[21,102]. Studies have highlighted disease-related atrophy centred in the striatum and throughout the white matter in HD gene carriers at least 15 years before onset[103] with progressive involvement of the cortex as symptom onset

approaches[104]. Reduced striatal volume in gene carriers compared with controls is even evident as early as 24 years prior to expected symptom onset[99] although it is not clear whether this is a developmental or neurodegenerative effect. White matter microstructure can be interrogated with diffusion MRI techniques which have shown sensitivity before clinical motor onset[105].

PET

PET is generally able to provide more specific information about pathological processes in HD, although is invasive, more expensive and standardisation across multiple sites may be more difficult. Glucose metabolism is reduced, likely preceding atrophy[106], and predicts clinical motor onset[107]. PET has also shown disease-related decreases of dopamine receptors and increased microglial activation[108] as well as decreased PDE10 binding[109]. Novel PET ligands targeting aggregated mHTT itself have recently shown promise in animal studies[110] and phase 1/2 studies in humans are ongoing.

Digital biomarkers

The UHDRS Total Motor Score (TMS) is well-established as a clinical measure to assess movement disability[2], yet digital biomarkers aim to capture these motor deficits in a more objective and sensitive way. Unbiased quantitative assessment of motor symptoms using force transducers with the Q-motor suite have been used in multicentric clinical trials, being more sensitive than the UHDRS-TMS prior to clinical motor onset[111]. In addition, digital measures of daytime motor activity[112,113], finger tapping[103,114], chorea[115], gait[116], circadian rhythm, speech[117] and sleep disturbance[118] have all shown sensitivity to disease effects and have great potential for clinical trials[119]. Cognitive function can be assessed by the HD-CAB, a computerised assessment of multiple cognitive domains, which has been specifically developed for use in clinical trials, showing decline in HD participants after clinical motor onset, alongside acceptable reliability[120]. Recent developments in wearable/portable devices show great promise for capturing such data non-invasively, but more work is required to standardise devices and harmonize protocols before they are widely accepted as endpoints for clinical trials[92].

Conclusions and future directions

Therapeutic approaches under development have the potential to deliver several licensable medicinal products, but uncertainties remain. Genetically validated targets have an overall higher development success rate[121]; therefore, both HTT and genetic modifiers targets are the best positioned. Besides target validation, drug development success depends on effective delivery to the brain and the benefit-risk relationship. There are three main approaches in the run namely ASOs, gene therapy and small molecules. Gene therapy may see revolutionary changes with the development of novel vectors (viral and non-viral) and gene editing approaches. The concept of aiming at multiple targets simultaneously deserves stronger consideration.

The pathophysiology of HD is complex (Figure 1) and the wild-type protein has important functions[122]. The correction of such complex mechanisms is less straightforward than correcting mutations that cause loss of protein expression for which small increases of the missing protein have proven to be beneficial[57]. Those characteristics of the HD mutation partially explain why the targeted interventions currently in development are not tailored to halt the disease or revert the prior pathogenic processes. Intervening when the degree of neurodegenerative changes is marked and there are already clinical manifestations may prove to be a critical limitation, although the recently halted and ongoing trials will be an invaluable dataset to inform future programmes.

Other HD features pose challenges for designing efficient clinical trials: Huntington's Disease in adults is phenotypically indistinguishable from controls in established biomarkers or clinical readouts on average at least until 20 years of age[99]; the pathogenic process evolves very slowly, spanning more than 4 decades[2], making the detection of an intervention effect particularly difficult. These issues may be addressed by conducting clinical trials using biomarkers (single or signature) (Panel 2) as primary endpoints in the very early phases of the disease, before the emergence of detectable clinical symptoms (Stage 0 and 1 in the Huntington Disease Integrated Staging System-HD-ISS[123]). Regulatory authorities consider such designs possible[124], but the actual roadmap is still unclear, despite important inroads in form of guidance

documents[125] and fruitful collaborations between the academia and industry[102]. Even if traditional methods for demonstration of a clinically meaningful effect will not be applicable at those early stages, we need a mechanism to show that treatments are of value for far-from-clinical motor onset individuals[126]. Evidence of disease-modifying benefit will justify the putative health risks, long term compliance, and the financial effort of treating early in the course of the disease. Novel research in this area is critically needed. Achieving a meaningful reduction in the progression rate might require combination therapy (aiming at complementary targets) either in the same construct or by combining independent constructs. Such combination treatments have specific regulatory constraints that need a swift solution. The ongoing technological and regulatory progress allows optimism about the development of impactful therapies for HD in a reasonable timeframe.

Search Strategy and Selection Criteria

A literature search was done using the PubMed and Scopus electronic databases for articles published in English from 1st Jan 2016 to 10th February 2022. The search terms "Huntington's disease", "gene silencing", "huntingtin lowering", "antisense oligonucleotide", "RNA interference", "siRNA", "miRNA", "zinc finger", "CRISPR", "Cas9", "AAV", "somatic instability", "biomarker", "pridopidine", "complement", "c1q", "cell therapy" and "cell replacement" were used. Additional articles were identified by reviewing the reference lists from relevant articles. We also included references from conference presentations, publicly available webinars and clinicaltrials.gov. We prioritised material published between 2016 and 2022, including older references only if they were essential to the field. There were no language restrictions. The final references were chosen on the basis of relevance to the topics covered in this Review.

Contributors

Declaration of Interests

Acknowledgements

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