No disease-slowing treatment exists for Huntington’s disease, but its monogenic inheritance makes it an appealing candidate for the development of therapies targeting processes close to its genetic cause. Huntington’s disease is caused by CAG repeat expansions in the \textit{HTT} gene, which encodes the huntingtin protein; development of therapies to target \textit{HTT} transcription and the translation of its mRNA is therefore an area of intense investigation. Huntington-inhibiting strategies include antisense oligonucleotides and RNA interference targeting mRNA, and zinc finger transcriptional repressors and CRISPR-Cas9 methods aiming to reduce transcription by targeting DNA. An intrathecally delivered antisense oligonucleotide that aims to lower huntingtin is now well into its first human clinical trial, with other antisense oligonucleotides expected to enter trials in the next 1–2 years and virally delivered RNA interference and zinc finger transcriptional repressors in advanced testing in animal models. Recent advances in the design and delivery of therapies to target \textit{HTT} RNA and DNA are expected to improve their efficacy, safety, tolerability, and duration of effect in future studies.

\textbf{Introduction}

All cases of Huntington’s disease are caused by the same mutation, so the pathogenic processes close to this genetic cause are ideal candidates for the development of therapies. The Huntington’s disease mutation—expansion of a CAG tract in the \textit{HTT} gene—results in ubiquitous expression of mutant huntingtin (HTT) protein, which is thought to be the predominant toxic agent. Individuals who will develop Huntington’s disease can be identified by genetic testing: so, in theory, intervention in the long premanifest phase could postpone or prevent symptom onset. Huntington’s disease is an inherited autosomal dominant disorder thought to be caused by toxic gains of function, thus, a reduction in mutant \textit{HTT} production should alleviate its pathogenesis. However, evidence also suggests mutant \textit{HTT} may have diminished function (ie, might cause haploinsufficiency), which might contribute to Huntington’s disease, and could be exacerbated by a reduction in \textit{HTT} production, so human trials should be designed to consider this possibility.

It is a pivotal time for therapies targeting disease-associated genes and proteins. The first clinical success for such a therapy in neurodegeneration was reported in 2016, when the US Food and Drug Administration (FDA) approved nusinersen, an antisense oligonucleotide (ASO) administered into the lumbar spinal cord, which extended survival in spinal muscular atrophy via targeted modulation of gene expression, increasing production of survival motor neuron 2 (SMN2) protein. This FDA-approved approach restored a missing protein, rather than suppressing the function of a toxic protein. Additionally, because spinal muscular atrophy is typically more aggressive, it might be easier to show a therapeutic effect in a short efficacy trial for this disease than for Huntington’s disease. Although one instance of clinical success cannot be generalised, the results of the spinal muscular atrophy trial provide evidence that neurodegeneration can be slowed in human beings by a targeted therapy modulating gene expression, which is encouraging for similar programmes in Huntington’s disease.

This Review focuses on novel therapeutic strategies targeting the mutant \textit{HTT} production pathway. Strategies designed to interact with \textit{HTT} mRNA include ASOs and RNA interference (RNAi) compounds that accelerate degradation of the transcript, and orally bioavailable small molecules that reduce expression of \textit{HTT} by altering mRNA splicing. Agents that directly interact with \textit{HTT} DNA include zinc finger transcriptional repressors and clustered regularly interspaced short palindromic repeats (CRISPR) and the accompanying CRISPR-associated system (Cas9) genome editing constructs (ie, CRISPR-Cas9). The term gene silencing is sometimes used for targeted reduction in gene expression, but could be interpreted as complete deactivation, which is probably neither desirable nor attainable; therefore, we prefer the term \textit{HTT} lowering when referring to reduction of \textit{HTT} expression. The term gene therapy is reserved for approaches in which the genetic material of living cells is modified, resulting in host cells manufacturing non-native mRNA and sometimes protein; these approaches have safety and regulatory implications.

\textbf{Huntingtin and the molecular pathology of Huntington’s disease}

The \textit{HTT} gene is highly conserved and its embryonic knockout in mice is lethal, suggesting the protein is essential for development. The function of wild-type \textit{HTT} is incompletely understood, but it is ubiquitously expressed and has many interaction partners. Wild-type \textit{HTT} is thought to have many roles: vesicular trafficking; mediation of endocytosis, vesicular recycling, and endosomal trafficking; coordination of cell division; and regulation of transcription and metabolism. The toxic effects of mutant \textit{HTT} are equally diverse, with many pathways disrupted in its presence. Despite many attempts, no approach designed to target these downstream pathways has slowed or halted progression of Huntington’s disease in human beings, emphasising the difficulty of designing therapies for diseases with pleiotropic cellular disruptions. This is perhaps one
reason for the emphasis of current research on the therapies that target the most proximal pathogenic events.

In carriers of the Huntington’s disease mutation, some protein functions might be impaired because mutant HTT functions less efficiently than wild-type HTT. For example, the overexpression of wild-type HTT stimulates axonal vesicular transport of BDNF in vitro, but overexpression of mutant HTT does not, suggesting HTT loss-of-function might contribute to the BDNF deficiency that has been observed in the striatum of patients with Huntington’s disease.\(^{10}\)

Lowering of HTT expression could exacerbate the putative pathogenic contributions from haploinsufficiency. This concern needs to be balanced against the detrimental effects of the persistent presence of mutant HTT. Complete inactivation of Htt in the adult rodent brain might cause a progressive neurological phenotype;\(^{11}\) however, partial reduction (ie, 50% or more) of HTT is well tolerated across model species, with the caveat that follow-up in these animal studies has generally been short.\(^{12-17}\) The longest primate study\(^{18}\) revealed no toxicity after 6 months of partial suppression in the striatum. Importantly, no HTT-lowering strategy currently proposed for human use would produce complete knockdown. Nonetheless, whether long-term partial suppression of HTT in human beings will prove safe is unknown, so human trials should be designed with long-term follow-up and safety measures sensitive enough to detect toxicity from this on-target mechanism. For now, these concerns also favour the prioritisation of agents with shorter half-lives, or that are reversible or can be inactivated if problems emerge. The suppression of mutant HTT alone (allele-selective HTT lowering) is both desirable and difficult, and efforts to develop allele-selective and non-selective strategies are both underway.

Although a CAG-expanded HTT gene is the proximal cause of Huntington’s disease, and mutant HTT is generally agreed to be harmful to neurons, recent years have seen debate about whether it is the sole pathogenic agent. For example, exon 1 of HTT contains the expanded polyglutamine tract, and is sufficient to cause pathology in the absence of the rest of the HTT protein.\(^{18}\) Cleavage of full-length mutant HTT is traditionally thought to be the source of this toxic fragment, but a variant of mutant HTT mRNA resulting from incomplete splicing was recently described.\(^{19}\) This transcript encodes a short exon 1 HTT protein, is formed more easily when Htt is CAG-expanded, and has been found post mortem in the brains of patients with Huntington’s disease but not controls.\(^{20}\) HTT mRNA has also been shown to be subject to repeat-associated non-ATG (RAN) translation, which can generate many unusual protein species from both sense and antisense strands, some of which might be toxic.\(^{21}\) Additionally, the CAG-expanded Htt RNA itself might be toxic.\(^{22}\) Figure 1 shows these proposed mechanisms alongside the conventional mutant-HTT-producing pathway and its toxic exon 1 fragment.

The contribution of each putative alternative mechanism is unclear, but their existence warrants consideration because the effect of each HTT-lowering approach on each pathogenic protein expression pathway will depend on precisely where the intervention is targeted (figure 1). The effects of these alternative mechanisms are more likely to be diminished by targeting of proximal events. This factor will need to be kept in mind, especially if an agent appears to have engaged with its target in a trial but fails to ameliorate clinical features.

**Therapies targeting RNA**

mRNA is accessible in the nucleus or cytosol and, by contrast to DNA, is unprotected by repair machinery. Thus, reduction of HTT mRNA translation should be easier than modulation of transcription or alteration of the gene itself. The three main methods to reduce HTT mRNA are ASOs, RNAi compounds, and small-molecule splicing modulators (table 1). ASOs and RNAi compounds are nucleotide-based therapeutic molecules that selectively bind to mRNA through Watson-Crick complementarity and trigger RNA degradation machinery to dispose of the transcript. ASOs are synthetic single-stranded DNA molecules that principally bind pre-mRNA in the nucleus and target it for degradation by RNase H.\(^{23}\) RNAi uses RNA-based therapeutic molecules, including short interfering RNA (siRNA), short hairpin RNA, and microRNA, which bind to mature spliced cytosolic mRNA and target it for removal by argonaute 2—the RNase enzyme within the RNA-induced silencing complex (RISC).\(^{24}\) Some differences between ASO and RNAi approaches are apparent from their sites of action (figure 1). By acting on pre-mRNA, ASOs can target exons and introns, permitting an increased choice of target sequences. ASOs should reduce the production of toxic mRNA and RAN proteins, but whether they can prevent the formation of exon 1 truncated mRNA is unclear, because it might be generated and released before the addition of the ASO-binding downstream pre-mRNA sequence. RNAi compounds act on spliced mRNA; thus, they can reduce the formation of exon 1 mRNA fragments only by directly targeting a sequence within that exon, and might not prevent the formation of toxic RNA or RAN proteins.

For reasons that remain unclear, single-stranded DNA diffuses well in the CNS and is taken up by neurons and other cells. Thus, the injection of ASOs into the CSF (intraventricular administration in mice or lumbar intrathecal administration in larger mammals) results in fairly widespread delivery of drug to the brain, and a corresponding reduction in mRNA and protein.\(^{25}\) By contrast, double-stranded RNA has low diffusion and cellular uptake in the CNS; therefore, enhanced delivery methods or viral vectors, such as adenov-associated virus (AAV), are needed to deliver these agents by injection.
into the brain parenchyma. Although more challenging, this mode of administration should permit lifelong treatment from a single dose.

HTT lowering with ASOs and RNA-based compounds has been successfully achieved in vitro and in animal models. Invariably, a reduction in HTT mRNA results in a concomitant reduction in HTT protein, which is usually accompanied by amelioration of pathology and improvements in neurological deficits when given to animal models of Huntington’s disease. When given to mutation-carrying animals before disease manifestation, onset of signs of disease is substantially delayed. Despite these encouraging results, the animal models have limitations. To date, no therapeutic success in an animal model of Huntington’s disease has predicted clinical benefit in a human trial. This issue is of particular concern for therapeutic agents administered directly into the CNS: a human brain is more than 3000 times larger than a mouse brain, and is likely to be vastly different in terms of diffusion through the CSF into the brain parenchyma and within cells.

Primate studies can indicate that a compound has broad distribution and is HTT lowering; however, these wild-type animals do not have mutant HTT, and are thus incomplete models for the assessment of potential toxicity and cannot inform us about the therapeutic effect of a compound. Large transgenic models, such as sheep and pigs, are beginning to be used in the study of HTT-lowering approaches, but the unparalleled complexity of the human brain and the highly variable, slowly progressive course of Huntington’s disease mean that no portfolio of animal testing can guarantee success. Combining multiple small and large animal models, with an awareness of the particular applicability and limitations of each model, should be paired with a cautious approach to clinical testing to maximise safety and the chance of successful translation.

Agents made from exogenous RNA and DNA have the potential for off-target effects. For example, some synthetic oligonucleotides are recognised by toll-like receptors on immune cells, increasing the risk of immunogenicity that might be difficult to predict from animal studies. Thrombocytopenia was observed in some human trials of ASOs administered systemically, and worsened peripheral neuropathy occurred in a clinical trial of an siRNA drug. Whether these are class effects is not clear. The immunological privilege of the brain and the smaller doses needed with direct CNS administration than with systemic administration might mitigate these risks in neurodegeneration, which appears to be the case for nusinersen. Such effects cannot be assumed for all new therapies: vigilance is essential.

ASO approaches
Several ASOs are being investigated in Huntington’s disease model systems, but we begin with a detailed discussion of the first targeted HTT-lowering compound to enter a human
Huntingtin-lowering programmes targeting mRNA, by class and mechanism

Table 1: Huntington-lowering programmes targeting mRNA, by class and mechanism

<table>
<thead>
<tr>
<th>Allele selectivity</th>
<th>Delivery</th>
<th>Vector</th>
<th>Sponsor</th>
<th>Key advantages</th>
<th>Key disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antisense oligonucleotides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-mRNA degradation</td>
<td>None</td>
<td>Intrathecal</td>
<td>None</td>
<td>Ionis Pharmaceuticals (Carlsbad, CA, USA)</td>
<td>Single drug for all carriers of the Huntington’s disease mutation</td>
<td>Theoretical risk from reducing wild-type HTT</td>
</tr>
<tr>
<td>Pre-mRNA degradation</td>
<td>SNP-targeted</td>
<td>Intrathecal</td>
<td>None</td>
<td>Wave Life Sciences (Cambridge, MA, USA)</td>
<td>Selective silencing of mutant allele</td>
<td>Several drugs required to treat most patients; SNP targeting limits choice of RNA-binding sequences</td>
</tr>
<tr>
<td>Pre-mRNA degradation</td>
<td>CAG repeat</td>
<td>Intrathecal</td>
<td>None</td>
<td>Biocarin (Leiden, Netherlands)</td>
<td>Selective silencing of mutant allele with a single drug for all mutation carriers</td>
<td>Reduced expression of other important CAG-containing genes, risking off-target effects</td>
</tr>
<tr>
<td><strong>RNA interference compounds</strong></td>
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<td></td>
<td></td>
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<tr>
<td>mRNA degradation</td>
<td>None</td>
<td>Intracranial</td>
<td>AAV2</td>
<td>Spark (Philadelphia, PA, USA)</td>
<td>Single treatment provides sustained HTT reduction</td>
<td>Invasive delivery; limited treatment volume; cannot be reversed if adverse events occur</td>
</tr>
<tr>
<td>mRNA degradation</td>
<td>None</td>
<td>Intracranial</td>
<td>AAV1</td>
<td>Voyager (Cambridge MA, USA)</td>
<td>As above</td>
<td>As above</td>
</tr>
<tr>
<td>mRNA degradation</td>
<td>None</td>
<td>Intracranial</td>
<td>AAV5</td>
<td>UniQure NV (Amsterdam, Netherlands)</td>
<td>As above</td>
<td>As above</td>
</tr>
<tr>
<td><strong>Small molecules</strong></td>
<td>Unknown</td>
<td>Potentially oral</td>
<td>None</td>
<td>CHDI Foundation (New York, NY, USA)</td>
<td>Potentially highly accessible route of delivery; potentially readily reversible</td>
<td>More difficult to achieve selectivity for HTT than with nucleotide approaches</td>
</tr>
</tbody>
</table>

SNP—single nucleotide polymorphism. AAV1, 2, and 3—adenovirus-associated virus 1, 2, and 3. HTT—huntingtin protein. *The mechanisms of action, route of delivery, and advantages and disadvantages of the small molecules being investigated by CHDI Foundation remain to be determined because the programme is currently at the phenotypic screen stage.

Table 1: Huntington-lowering programmes targeting mRNA, by class and mechanism.

In September 2015, the first patients were dosed with IONIS-HTTm, an ASO targeting human HTT, in a phase 1b/2a clinical trial (NCT02519036) at two sites: University College London, UK, and the University of British Columbia, Canada. Additional sites in Germany and the UK later joined the trial. IONIS-HTTm is a synthetic 20-nucleotide sequence, in which many of the non-bridging oxygen atoms in the phosphate backbone have been substituted for sulphur, to transform phosphodiester linkages to phosphorothioate. Additionally, the ASO has a DNA-like central region with 2′-O-methylxethyl modifications at each end; this combination is intended to optimise CNS distribution, half-life, cellular uptake, and RNAse activation (figure 2).

After 2 weeks of continuous intraventricular infusion into BACHD mice, which bear a full-length human mutant HTT gene, HTT mRNA expression was reduced by up to 80% and protein expression by around two-thirds—reductions that persisted for 4 months. The ASO was distributed throughout the brain, across several cell types, including neurons and glia. Disease phenotypes were ameliorated in three different mouse models of Huntington’s disease: near-complete restoration of motor deficits in young animals and partial improvement later in the disease course were observed in the slowly progressing YAC128 and BACHD models, and increased survival and reduced brain atrophy were observed in the rapidly progressing R6/2 model.

The beneficial histological and motor effects of bolus ASO treatment outlasted the presence of the drug and reduction in HTT, suggesting that cells given a brief respite from mutant HTT can regain lost ground in the battle between damage and repair—a phenomenon dubbed the huntingtin holiday. In wild-type rhesus monkeys, lumbar intrathecal infusion of a similar ASO, complementary to primate and human HTT mRNA, produced broad ASO distribution and HTT reduction in the cortex. This infusion was safe and well tolerated over a 3-month period.

After lumbar intrathecal injection, the flow of CSF from the spinal column to the external surface of the brain favours cortical, over striatal, distribution. However, a primate pharmacokinetic and pharmacodynamic study showed that lumbar intrathecal bolus administration of doses of IONIS-HTTm that produce about 50% reduction in cortical HTT are associated with 15–20% reductions in HTT in the striatum and were well tolerated in these animals, providing support for human dose escalation. Wang and colleagues showed that lowering mutant HTT expression in either the cortex or the striatum results in partial improvement in BACHD mice, and lowering expression in both regions produces a greater benefit than either alone. Lumbar intrathecal injection of IONIS-HTTm in Yucatan pigs, whose spinal column is roughly the same length as that of human beings, resulted in substantial distribution to brain tissues. In human beings, the striatum will probably receive a lower dose of the ASO than will the cortex with
intrathecal administration, but the amount of mutant HTT lowering that seems to be attainable might be sufficient to produce clinical benefit without resorting to dual intrathecal and intracranial administration—although this approach remains an option.

In the IONIS-HTT <sub>Rx</sub> trial, patients with early Huntington’s disease receive four lumbar intrathecal bolus doses of drug or placebo (randomisation ratio 3:1) at monthly intervals. Several dose escalations have taken place within the multiple ascending dose design (NCT02519036), and an open-label extension study was recently announced. 6 In addition to safety and tolerability, the trial seeks to study the pharmacokinetics of IONIS-HTT <sub>Rx</sub> in the human CNS, and will examine target engagement biomarkers, 7 including the concentration of mutant HTT in the CSF, which can be reliably quantified with a novel immunoassay. 8

Progress in other neurodegenerative diseases is encouraging for the future of intrathecally delivered ASOs in the treatment of Huntington’s disease. A safety trial 9 of an ASO in patients with superoxide-dismutase 1 (SOD1) mutation-associated amyotrophic lateral sclerosis revealed no concerns and another is underway with a more potent ASO (NCT02623699). The efficacy of nusinersen in spinal muscular atrophy is evidence of disease modification by an ASO in a previously unalterable human neurodegenerative condition. Notably, post-mortem brain tissue from the trial 9 showed the ASO in neuronal and non-neuronal cells in the cortex and brainstem, and increased expression of SMN2 in multiple brain areas—the first evidence that lumbar intrathecal bolus injection of an ASO can achieve brain penetration and target engagement in human beings. 6

**Allele-selective ASOs**

IONIS-HTT <sub>Rx</sub> is expected to reduce expression of both mutant and wild-type HTT alleles equally. It might be many years before we know whether lowering of wildtype HTT is safe in human beings, especially for long-term use in symptom-free mutant HTT gene carriers. Therefore, allele-selective methods for the reduction of mutant HTT by targeting the CAG tract or heterozygous single-nucleotide polymorphisms (SNPs) are also under investigation.

A long CAG tract is not unique to HTT: many human genes contain CAG repeat stretches. 9 Thus, an ASO or RNAi drug targeting the causative mutation is likely to downregulate other genes, which might produce off-target, adverse effects. Several genes that include CAG repeats encode transcription factors, and thus these off-target effects could be profound. Nonetheless, this approach appears at least feasible. An ASO targeting the mRNA equivalent of seven CAG repeats, called (CUG), produced an 83% reduction in the mutant HTT mRNA transcript in fibroblasts derived from Huntington’s disease patients, and a 43% reduction in the wild-type transcript. It also reduced the transcripts of other genes that include CAG repeats including ATXN3, ATXN1, and ATN1. 10 This finding suggests an ASO targeting CAG repeats could be of value in other CAG-expansion diseases. Despite its selectivity, partial reductions in wild-type HTT persist with this approach, and might produce long-term risks. Combined with suppression of other genes containing CAG repeats, this restricts the potential of an ASO targeting CAG repeats as a candidate treatment. Nonetheless, Datson and colleagues 10 have administered (CUG), to R6/2 and Q175 knockin mice, producing potent lowering of mutant HTT and neurological benefit.

**HTT** has many common SNPs. 12 Specialist sequencing techniques, known as phasing, can identify the allele in

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**Figure 2:** First-generation, second-generation, and third-generation ASOs and native DNA

Each generation might contain several variations around the characteristic that has been altered (shown in red). 15 IONIS-HTT <sub>Rx</sub> combines a phosphorothioate backbone (first-generation) with 2′-O-methoxyethyl modification (second-generation) and is considered a second-generation antisense oligonucleotide (ASO) overall. 25

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Representative structure</th>
<th>Aim of modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Native nucleic acid</td>
<td></td>
</tr>
</tbody>
</table>

First generation

- Non-bridging oxygen in phosphodiester bond replaced by sulphur
- Higher resistance to nuclease degradation

Second generation

- 2′-Alkyl ribose modifications
- 2′-O-Methoxyethyl (MOE)
- Higher nuclease resistance and mRNA-binding affinity

Third generation

- Furanoce ring modifications
- Locked nucleic acid
- Further increases in nuclease resistance and mRNA-binding affinity
which an SNP occurs. Thanks to haplotypes, particular SNPs frequently accompany the Huntington’s disease CAG mutation; drugs targeting the three most common SNPs are estimated to be sufficient to treat about 80% of the population who carry the HTT mutation—with the caveat that these studies were largely based on populations of European descent (Huntington’s disease is more prevalent in European populations than in others, but is present in all ethnicities and regions studied to date). Each SNP-targeting candidate must be developed and trialled separately—a considerable undertaking. The success of this approach depends on several incompletely understood factors: the true frequency of the SNPs in the population seeking treatment might differ from that in the prevalence studies; the procedure for phasing the SNP to the mutant allele is crucial (accidental selective silencing of wild-type HTT, leaving mutant HTT untouched, might cause considerable harm); allele specificity is relative, not absolute (the real-world selectivity of a compound for mutant over wild type might be less than expected, given the close sequence similarity between the two alleles); and the need to target SNP-containing regions dramatically reduces the repertoire of candidate sequences, limiting the ability to select a potent drug candidate with minimal off-target effects. Nonetheless, patients with a suitable heterozygous SNP might benefit from the availability of such compounds, especially if liabilities emerge from non-selective lowering of HTT expression.

In 2017, plans to initiate parallel trials (NCT03225833, NCT03225846) of two ASOs targeting SNP-containing regions of HTT mRNA were announced. Potential participants will be screened for the presence of these SNPs within the mutant but not wild-type allele before allocation to groups that will receive one ASO compound or the other. The ASOs were selected on the basis of not only nucleotide sequence but also the stereochemistry of the backbone, which might enable greater selectivity between alleles and optimisation of other aspects of the pharmacokinetics.

Peptide-conjugated ASOs
A potentially important advance is the development of peptide-conjugated ASOs, which, after intravenous injection, produced broad CNS distribution and dramatically extended survival in a mouse model of spinal muscular atrophy. This technology warrants investigation for use in Huntington’s disease.

RNAi approaches
Although no RNA-based nucleotide agents have reached human trials, work in animal models and planning for human trials are underway. The absence of human trials reflects the delivery and distribution challenges associated with RNAi: RNA does not distribute well throughout brain tissue even after intraventricular administration, so stereotactic surgery is needed to deliver the agent directly to the brain parenchyma by a viral vector to encourage spread and longevity. Virally delivered RNA therapeutic agents permanently transduce CNS cells, effectively turning them into factories making drugs that suppress mutant HTT. Thus, these approaches are gene therapy. Virally delivered gene therapy has been attempted in neurodegeneration; for example, in a phase 2 trial of AAV2-encapsulated nerve growth factor RNA in patients with Alzheimer’s disease (NCT00087789). The technique was safe and well tolerated, albeit without apparent clinical benefit.

**siRNA in animal models of Huntington’s disease**
siRNA approaches have been successful in multiple animal systems. The most detailed published work has been led by Beverly Davidson who, having established safety, potency of HTT reduction, and phenotypic benefits from AAV-delivered siRNA in several Huntington’s disease rodent models, went on to deliver a microRNA, encapsulated in an AAV2 vector and targeting the human and primate HTT transcript, by injection into the putamen of wild-type rhesus macaques. A fluorescent reporter indicated successful transfection of various cell types in a small volume of tissue (approximately 0.1 cm³) around the injection site, accompanied by a halving of the expression of HTT transcripts. No serious adverse effects were detected after comprehensive histological, biochemical, and clinical assessment after 6 weeks. However, the development of dystonia in one sham-injected animal highlights the risk associated with any neurosurgical delivery technique.

**Enhanced delivery methods**
Stiles and colleagues used convection-enhanced delivery to infuse radiolabelled naked siRNA (not packaged in a viral vector), under constant pressure, into the primate putamen continuously for several days or weeks, producing siRNA distribution and HTT suppression over the entire striatum. Other non-viral approaches have been proposed to improve CNS delivery and distribution of RNAi compounds. Single-stranded RNA molecules distribute well throughout brain parenchyma and into cells, and exosomes or other Trojan horse methods might eventually permit intravenous dosing.

Even with enhanced delivery methods, limited tissue distribution remains a great challenge for RNAi-based compounds, which necessitates consideration of either multiple injection sites or prioritisation of one region over others. Although the striatum is known to be disproportionately affected in prodromal and early Huntington’s disease, the cause (intrinsic mutant HTT production vs loss of trophic support from the cortex) is debated. Either way, Huntington’s disease is undoubtedly a whole-brain condition. Although the cortex and striatum are each desirable targets, whether mutant HTT lowering in either brain region is necessary or sufficient to produce clinical benefit in patients is unknown. Acting on both,
and indeed beyond, would be preferable, if possible. Any neurosurgical procedure carries risk, so early work will most likely err on the side of caution, and perhaps expand to more regions of the brain in time.

**Novel viral vectors**

Recent years have seen progress in the development of viral vectors for CNS delivery. Many AAV serotypes exist, each of which might be combined with different cargoes, enhancers, and promoters. In 2015, Stanek reported success using an AAV1 under control of a hybrid cytomegalovirus enhancer and chicken beta actin promoter, which produced widespread transduction of cells in the cortex, thalamus, and hippocampus when injected into primate striatum. Miniariyova and colleagues showed efficacy of an optimised AAV5-encapsulated microRNA in rodents, which is now being readied for use in clinical trials.

Deverman and colleagues made a striking development when they used Cre recombination-based AAV-targeted evolution (CREATE) to develop AAV strains with novel capsids and selected them for their ability to transduce neuronal tissue. One variant, AAV-PHP.B, showed improvement in efficiency at least 40 times that of AAV9, as measured by the number of viral genomes in all brain regions studied, and produced widespread distribution and transduction in the rodent brain even after intravenous administration; however, there was also transfection of liver, heart, and muscle. This systemic transfection opens up the possibility of peripherally mediated side-effects, and whether the virus transfects a sufficient diversity of CNS cell types to produce a meaningful reduction in HTT remains to be seen. Nonetheless, this novel AAV, and the methods used to develop it, are of interest to researchers in the field of CNS gene therapy.

Such progress is a double-edged sword: researchers must decide whether to proceed to clinical trials with familiar but less widely distributed vectors, or await trial-readiness of improved viruses and surgical techniques. An incremental process is likely to be adopted. The immunogenicity of AAVs in the human brain, especially with repeated administration, is also unclear. Although repeated dosing with a particular agent will probably not be needed, patients who volunteer for early trials might later find themselves unable to receive more advanced treatments because of the risk of immunogenicity from receiving multiple different AAV constructs.

**Small molecule approaches**

A brain-penetrant, orally bioavailable small molecule acting on protein manufacture to lower HTT expression is desirable. Naryshkin and colleagues used a screening process to identify small molecules that alter the splicing of SMN2 pre-mRNA, favouring the production of a functional SMN protein (a mechanism proven effective by nusinersen). In patient fibroblasts, these small molecules increased SMN concentration in a dose-dependent manner, and improved outcomes in a mouse model of spinal muscular atrophy—albeit through uncertain mechanisms. A phase 1b/2a clinical trial (NCT02240355) in spinal muscular atrophy of the lead compound, RG7800, was terminated after ocular complications emerged in ongoing animal studies, perhaps highlighting an increased risk of off-target effects with protein-modulating compounds that do not possess the specificity conferred by nucleotide base-pairing. Nonetheless, a phase 1 study of a second compound, RG7916, was recently completed (NCT02633709). Similar work is now underway to identify small molecule agents to lower mutant HTT expression.

**Therapies targeting DNA**

The design and implementation of a therapeutic construct that interacts directly with DNA to reduce transcription of the mutant HTT gene brings both different challenges and the potential for greater rewards than targeting of RNA. DNA-targeting therapeutic agents would be expected to ameliorate all aspects of Huntington's disease, including those mediated by alternative splicing, non-RAN translation, or any other mechanism we might postulate. Moreover, DNA editing opens up the future prospect of germ-line treatment in living patient tissue, which could benefit future offspring in addition to the person carrying the mutation; however, this, of course, brings its own ethical issues. The two DNA-targeting gene therapies currently under investigation are zinc finger proteins and CRISPR-Cas9 (table 2). Each uses a protein-coding sequence encapsulated in a viral vector, injected intracranially, which transduces cells, causing them to produce a functional, non-native therapeutic protein.

**Zinc finger proteins**

Zinc fingers are naturally occurring structural motifs that can bind specific DNA sequences; they can be generated synthetically and used as DNA-targeting therapeutic compounds. Zinc finger proteins designed for therapeutic use typically contain a zinc finger array specific to the DNA sequence of interest—one finger per three bases—fused to a functional domain intended to act on the DNA. Examples include zinc finger nucleases to cleave DNA and zinc finger transcription factors to modulate gene expression.

Although zinc finger nucleases are theoretically capable of targeted genome editing, the process is not sufficiently precise or predictable for therapeutic application in postmitotic patient brain cells. The CAG repeat stretch is particularly undesirable as a target for zinc finger nuclease-mediated cleavage. However, zinc fingers are sufficiently reliable to contemplate clinical development of therapeutic zinc finger transcription factors. To bring the transcriptional repressor into
proximity with the HTT promoter, the zinc finger array must bind a sequence near the 3’ end of the DNA sense strand. This approach limits the selection of target sequences and makes it difficult to avoid unwanted binding to other genes.

Fortuitously, although many genes contain CAG repeat stretches, in HTT this tract is closer to the 3’ end than in other genes, so a CAG-targeting zinc finger transcription factor will have selectivity for HTT over other genes, and for the mutant over the wild-type allele. Such constructs have been developed by two groups. Both have shown early promise in animal models, reducing mutant HTT protein expression in neurons without adversely affecting the expression of other genes or wild-type HTT.66,67 To our knowledge, this is the only allele-selective HTT-lowering approach currently being readied for use in clinical trials in which a single agent should selectively suppress mutant HTT long term in all mutation carriers.

One limitation of zinc finger therapeutic agents is the production of non-native proteins that can trigger inflammatory and immune reactions, which results in neuronal death and limited duration of effect. Agustín-Pavón and colleagues68 combined a zinc finger transcription factor targeting CAG repeats with a non-viral promoter and a novel repressor element redesigned to be homologous to the host (mouse) protein. This construct produced more sustained silencing than others with non-native protein sequences, although mutant HTT expression gradually rebounded over 6 months. Such optimisations to prevent inflammatory and immune reactions could make a difference to the duration and safety of huntingtin across the lifespan of a person carrying the Huntington’s disease mutation, especially because the ultimate goal is to treat once, well before symptoms begin, to prevent the onset of the disease.

CRISPR-Cas9
Together, CRISPR and Cas form the basis of a prokaryote immune system that recognises and destroys foreign DNA. Cas9 nuclease can be combined with synthetic guide RNA to produce a construct that can cut DNA with high precision at any chosen site. The use of such CRISPR-Cas9 complexes for targeted genome editing is a rapidly evolving field with huge potential for the study and treatment of diseases, including Huntington’s disease.72 The possibilities for HTT-directed CRISPR-Cas9 therapeutic strategies are great—from excision of CAG repeats to form harmless alleles, to inactivation of the mutant allele by the insertion of stop codons or missense mutations (perhaps, in tandem, upregulating the wild-type allele by the insertion of stop codons or missense mutations).73 Investigations of the use of CRISPR-Cas9 in Huntington’s disease are in the early stages. The method was first used to inactivate the mutant HTT allele permanently and selectively in patient-derived fibroblasts, with two constructs to excise a larger region of HTT DNA, resulting in near-total reduction in both RNA and mutant HTT protein.67 In 2017, the method was successfully tested in a rodent model of Huntington’s disease, producing selective mutant HTT reduction, attenuation of pathology, and improved motor function, but not extended survival, after treatment of the striatum with a CRISPR-Cas9 construct that deleted the region containing the CAG repeat in the human HTT gene of Q140 knockin mice.73 This finding affirms the feasibility of this approach, but much work is needed to bring these rapidly evolving technologies to the clinic.

### Table 2: Huntingtin-lowering programmes targeting DNA, by class and mechanisms

<table>
<thead>
<tr>
<th>Allele selectivity</th>
<th>Delivery</th>
<th>Vector</th>
<th>Sponsor</th>
<th>Key advantages</th>
<th>Key disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zinc finger transcription factor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcriptional repression</td>
<td>CAG repeat</td>
<td>Intracranial</td>
<td>AAV</td>
<td>Single drug for all carriers of the Huntington’s disease mutation; single administration to provide long-term treatment; targeting transcription should ameliorate all pathogenic pathways</td>
<td>Invasive; cannot be deactivated; small treatment volumes; risk of inflammation from non-host repressor proteins</td>
<td>Zeitler, 201466</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Use of human proteins in clinical candidate compound might limit utility of animal work</td>
<td>Garriga-Canut, 2012,69 Agustín-Pavón, 201646</td>
<td></td>
</tr>
<tr>
<td><strong>CRISPR-Cas9</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Genome editing</td>
<td>SNP-targeted</td>
<td>Direct to fibroblasts</td>
<td>None</td>
<td>Permanent removal of genetic cause; highly specific and targeted</td>
<td>Very early work in model systems only; irreversible; shares delivery problems with other virally delivered approaches; ethical concerns for germline manipulation; bacterial proteins might be immunogenic</td>
<td>Shin, 201669</td>
</tr>
<tr>
<td></td>
<td>Nonselective HTT depletion by polyglutamine domain deletion</td>
<td>Intracranial</td>
<td>AAV</td>
<td>As above</td>
<td>As above</td>
<td>Yang, 201771</td>
</tr>
</tbody>
</table>

AAV=adeno-associated virus. CRISPR-Cas9=clustered regularly interspaced short palindromic repeats-CRISPR-associated system. SNP=single nucleotide polymorphism.
Conclusions and future directions

Huntington’s disease is particularly suited to experimental therapies that target DNA and RNA to modulate protein expression. The first human trial of an ASO that seeks to reduce the manufacture of mutant HTT protein by targeting HTT mRNA is underway, and future ASO trials are planned, including those of agents that seek to lower mutant HTT selectively. RNA-based HTT-lowering agents require viral delivery, bringing the key advantage of potential lifelong treatment from a single dose at the cost of increased invasiveness, the challenge of brain penetration, and risks associated with long-term toxicity; nonetheless, several programmes are nearing the clinical trial stage. Small molecule RNA-targeting compounds are appealing in terms of delivery, have been shown to be attainable in other conditions, and are being investigated for Huntington’s disease. Zinc finger transcriptional repressors might reduce HTT by targeting DNA without altering it, whereas CRISPR-Cas9 therapies bring the promise of permanently correcting the CAG expansion mutation that causes Huntington’s disease—but these approaches share the challenges of viral delivery and have liabilities of their own, such as immunogenicity and off-target effects, so are far from human trials.

This is a time of great progress for molecular therapies targeting HTT expression. The coming years will certainly bring more trials of novel agents, which are likely to be periodically boosted by technological improvements in CNS delivery and distribution. The first few efficacy trials will focus on patients with early manifest disease, in whom both benefit and harm can be studied, in a situation in which clinical progression is rapid and predictable, enabling more reliable detection of benefit, and function is still preserved, improving the prospect of meeting meaningful endpoints. The selection of suitable outcome measures and the design of trials capable of identifying meaningful clinical benefit are a focus of current work.

If efficacy can be shown in manifest Huntington’s disease, the next hurdle will be the leap to premanifest mutation carriers. This effort will need to be supported d by a battery of clinical and imaging biomarkers that have been under concerted development for this purpose, plus novel biochemical markers of target engagement and biological effect. Cost, availability, and the infrastructure required for the delivery of invasive treatments are legitimate concerns, but first we need to establish that disease modification is possible.

The Huntington’s disease research community is distinguished by its trial-readiness, with global registries of well characterised potential participants and strong networks of health-care professionals, researchers, and Huntington’s disease family members. Although success is far from guaranteed, we are perhaps better placed than ever to make a meaningful impact on the treatment and ultimately prevention of Huntington’s disease.

Search strategy and selection criteria

References for this Review were identified by searches of PubMed up to July 6, 2017, and references from relevant articles. The search terms “Huntington’s disease”[MeSH term], “gene silencing”, “huntingtin lowering”, “antisense oligonucleotide”, “RNA interference”, “siRNA”, “miRNA”, “zinc finger”, “CRISPR”, “Cas9”, and “AAV” were used. There were no language restrictions. The final reference list was generated on the basis of relevance to the topics covered in this Review.

Contributors

EJW did the literature search, created the figures, and drafted the Review. SJT designed the Review, provided critical review, and finalised the work with EJW. Both authors approved the final version.

Declaration of interests

EJW has participated in scientific advisory boards with Hoffmann-La Roche Ltd, Ionis Pharmaceuticals, Shire, Novartis, and Wave Life Sciences and is an investigator on the IONIS-HTTRx trial. SJT has been on scientific advisory boards with Hoffmann-La Roche Ltd, Ionis Pharmaceuticals, Shire, Teva Pharmaceuticals, GSK, Takeda Pharmaceuticals, and Heptares Therapeutics and is the global principal investigator on the IONIS-HTTRx trial, for which she receives no personal salary or fees. All honoraria for these advisory boards were paid through University College London (UCL) Consultants Ltd—a wholly owned subsidiary of UCL. The authors’ host clinical institution, UCL Hospitals NHS Foundation Trust, receives funds as compensation for conducting clinical trials for Ionis Pharmaceuticals, Pfizer, and Teva Pharmaceuticals.

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References