Therapeutic strategies for Huntington's disease

Carlos Estevez-Fraga[a,b], *Michael D. Flower*[a,b,c], *and Sarah J. Tabrizi*[a,b,c]

Purpose of review
Huntington's disease is a fatal autosomal dominant neurodegenerative disorder caused by a trinucleotide expansion in the *HTT* gene, and current therapies focus on symptomatic treatment. This review explores therapeutic approaches that directly target the pathogenic mutation, disrupt *HTT* mRNA or its translation.

Recent findings
Zinc-finger transcription repressors and CRISPR-Cas9 therapies target *HTT* DNA, thereby preventing all downstream pathogenic mechanisms. These therapies, together with RNA interference (RNAi), require intraparenchymal delivery to the brain in viral vectors, with only a single delivery potentially required, though they may carry the risk of irreversible side-effects.
Along with RNAi, antisense oligonucleotides (ASOs) target mRNA, but are delivered periodically and intrathecally. ASOs have safely decreased mutant huntingtin protein (mHTT) levels in the central nervous system of patients, and a phase 3 clinical trial is currently underway.
Finally, orally available small molecules, acting on splicing or posttranslational modification, have recently been shown to decrease mHTT in animal models.

Summary
Huntingtin-lowering approaches act upstream of pathogenic mechanisms and therefore have a high *a priori* likelihood of modifying disease course. ASOs are already in late-stage clinical development, whereas other strategies are progressing rapidly toward human studies.

Keywords
antisense oligonucleotides, gene therapy, Huntington's disease, RNA interference, small molecules

INTRODUCTION

Huntington's disease is an autosomal dominant neurodegenerative disorder caused by CAG trinucleotide repeat expansion in the *HTT* gene, which is fully penetrant when the number of CAG repeats exceeds 39. The mutation leads to the production of the mutant huntingtin protein (mHTT). Clinically, Huntington's disease is characterized by adult-onset, progressive cognitive, motor and neuropsychiatric symptoms, ultimately leading to death around two decades later [1▪].

Biology of wild-type huntingtin

Huntingtin (HTT) is ubiquitously expressed in the adult, taking part in numerous cellular processes such as vesicle trafficking, cell division, ciliogenesis, transcription regulation, production of brain-derived neurotrophic factor and autophagy. It is present in both the cytoplasm and the nucleus and interacts with a large number of proteins and genes [2]. During embryogenesis, it is essential for neurogenesis and neuronal migration.

It has long been known that *Htt* knockout in mice is embryonically lethal [3], and its inactivation in the early postnatal period causes progressive neurodegeneration, suggesting HTT is essential for neurodevelopment [4]. Loss of *Htt* in young mice causes pancreatitis, but no neurodegeneration, and has no detectable effect over four months of age [5].

Several studies have evaluated the effects of partial lowering of wild-type huntingtin (wtHTT) in the larger brains of adult nonhuman primates (NHP). Protein reduction of 45% in the striatum of the adult rhesus monkey has been shown to be

[a]University College London (UCL), Huntington's Disease Centre, [b]Department of Neurodegenerative Disease, Queen Square Institute of Neurology, UCL and [c]UK Dementia Research Institute at UCL, London, UK

Correspondence to Sarah J, Tabrizi, MD, University College London (UCL), Huntington's Disease Centre, 2nd Floor Russell Square House, 10–12 Russell Square, WC1B 5EH London, UK.
Tel: + 44 20 3456 7890; e-mail: s.tabrizi@ucl.ac.uk

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KEY POINTS

- Following promising results in Huntington's disease animal models, several therapies targeting the causative mutation or its mRNA transcript are in clinical development.
- CRISPR-Cas9, ZFP and RNAi require one-off intraparenchymal delivery, with potentially long-term effects.
- Potential side-effects from total HTT lowering include the knockdown of the wild-type allele.
- ASOs have produced a dose-dependent HTT reduction in Huntington's disease patients' CNS, and a large phase 3 clinical trial is currently underway to determine whether this modifies disease course.

clinically safe [6] with another study achieving similar levels of wtHTT lowering also in the basal ganglia not revealing side-effects or neuropathological changes after 6 months [7]. Moreover, wtHTT reduction in cortical areas and spinal cord of adult NHP was not associated with adverse histological findings [8,9].

These differential effects of *Htt* deletion during different stages and throughout animal models suggest it is safe to knockdown HTT in adult patients, though may have implications for how young we are able to intervene.

Pathogenesis in Huntington's disease

The chronic expression of mHTT causes protein aggregation in the nucleus and the cytosol, leading to inclusions that are the pathological hallmark of the disease. Neuronal death can also result from oligomeric stages of aggregation and there is evidence suggesting the formation of inclusions may even be protective [10].

Many factors contribute to disease pathogenesis in Huntington's disease. The expanded CAG repeat can alter splicing, generating a small exon 1 HTT protein that is highly toxic. Furthermore, cell to cell spread of the protein, inflammation, mitochondrial dysfunction, RNA toxicity, alteration of DNA repair mechanisms and somatic instability also contribute to molecular pathogenesis [11].

HUNTINGTIN-LOWERING THERAPIES

Huntington's disease is most likely caused by a toxic gain-of-function mechanism [2], so decreasing CNS mHTT expression is expected to mitigate pathology and improve symptoms [12]. As neurodegeneration in Huntington's disease follows a topographically specific pattern [13], regional mHTT lowering may have distinct effects on pathology and phenotype. This was investigated by Wang *et al.* [14] who showed that reduction of mHTT in the cortex improved behaviour, whereas reduction in striatal neurons slowed brain atrophy rates. However, knockdown in both had synergistic effects, suggesting more widespread lowering is beneficial [14].

HTT-lowering therapies can be classified as non-allele-specific, reducing levels of both mHTT and wtHTT, or allele-specific, selectively lowering the mutant allele.

In this review, we focus on therapeutic reduction of HTT expression through a range of different approaches, including those targeting RNA, such as RNA interference (RNAi), antisense oligonucleotides (ASOs) or small molecules, and those acting on DNA, such as zinc finger proteins (ZFP) and the 'clustered regularly interspaced short palindromic repeats associated caspase 9' (CRISPR-Cas9) system (Table 1; Fig. 1).

THERAPIES TARGETING DNA

These treatments either edit the *HTT* gene or alter its transcription. Targeting the pathogenic mutation itself has the potential to prevent all downstream mechanisms [15,16]. These compounds tend to be formed of two constituents: a DNA-binding element that targets the *HTT* locus, and an effector that edits the genome or modulates expression.

Zinc-finger proteins

Zinc-finger domains are one of the most frequent DNA-binding motifs found in eukaryotic transcription factors [17]. In zinc-finger domains, a zinc ion acts as structural stabilizer [18], allowing the molecule to bind a three-to-five base pair DNA array, whereas the effector element can be modified to repress transcription [17]. This approach has a risk of production of nonhuman proteins prompting an immunogenic response. Furthermore, ZFP DNA binding may not completely accurate, resulting in potential off-target binding [19]. ZFPs need to be integrated into an adeno-associated virus (AAV) or lentiviral vector and delivered intraparenchymally to provide stable expression. These vectors have gained increased popularity because of their long-term effects, low immunogenicity, and inability to replicate [20].

ZFPs, delivered by intrastriatal injection of AAV-ZFP in a Huntington's disease mouse model, achieved 98% mHTT protein and 78% mRNA reduction, without lowering wtHTT [21]. Another study by Zeitler *et al.* [22▪▪] evaluated an AAV-ZFP targeting the *HTT* CAG repeat in cell and mouse models. Following intrastriatal delivery, the ZFP transfected

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Table 1. Huntingtin-lowering therapies targeting DNA and RNA

Target	Drug class	Delivery	Sponsor	Mechanism	Allele selectivity	Stage	Advantages	Disadvantages	References
DNA	ZFP	Intracranial (AAV)	Imperial College London	Repression of transcription	Yes, CAG repeat	Preclinical	One drug for all Huntington's disease patients Long-term effects after single infusion Decreasing all toxic species	Invasive Risk of persistent side-effects Risk of off-target binding Risk of production of nonhuman proteins	[8,15]
DNA	ZFP	Intracranial (AAV)	Sangamo Therapeutics/ Takeda	Repression of transcription	Yes, CAG repeat	Preclinical	Same as above	Same as above	[16▪▪,17]
DNA	CRISPR-Cas9	Intracranial (AAV)	Harvard University/ University of Pennsylvania	Genome editing	Yes, SNP	Preclinical	Accurate binding to targeted DNA region Long-term effects after single infusion Still in early stages of development Decreasing all toxic species	Immunogenicity derived from bacterial proteins Risk of persistent side-effects Risk of production of nonhuman proteins	[18–20]
DNA	CRISPR-Cas9	Intracranial (AAV)	Emory University/ University of California	Genome editing	Not allele selective, eliminates CAG expansion	Preclinical	Same as above	Same as above	[21▪,22]
RNA	ASO	Intrathecal	Ionis pharmaceuticals/ Hoffmann-La Roche	Pre-mRNA degradation	Not allele selective	Phase 3	Reversible, titratable One drug for all Huntington's disease patients	Requires repeated administration Risks of reducing wild-type protein	[8,23,24▪▪]
RNA	ASO	Intrathecal	Wave Life Sciences	Pre-mRNA degradation	Yes, SNP	Phase 1b/2a	Reversible, titratable	Requires repeated administration Does not target all Huntington's disease population with one drug	[25▪,26]
RNA	ASO	Intrathecal	Biomarin	Pre-mRNA degradation	Yes, CAG repeat	Preclinical	Reversible, titratable One drug for all Huntington's disease population	Requires repeated administration May target other CAG containing genes	[27]

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Table 1 (*Continued*)

Target	Drug class	Delivery	Sponsor	Mechanism	Allele selectivity	Stage	Advantages	Disadvantages	References
RNA	RNAi	Intracranial (AAV)	UniQure	mRNA degradation	Not allele selective	Phase 1/2	One drug for all Huntington's disease patients Long-term effects after single infusion Limited volume distribution	Invasive Risk of persistent side-effects	[28,29▪▪, 30,31▪, 32,33]
RNA	RNAi	Intracranial (AAV)	Genzyme/Voyager therapeutics	mRNA degradation	Not allele selective	Preclinical	Same as above	Same as above	[34▪,35]
RNA	RNAi	Intracranial (AAV)	Spark therapeutics	mRNA degradation	Not allele selective	Preclinical	Same as above	Same as above	[36▪]
RNA	Small molecules	Oral	PTC therapeutics	Splicing modification	Unknown	Preclinical	Orally available	Unknown	[37▪,38]

AAV, Adeno-associated virus; ASO, antisense oligonucleotides; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats associated caspase 9; RNAi, RNA interference; ZFP, zinc-finger proteins. Adapted from [12] with permission.

50–70% of the striatum and achieved dose-dependent mHTT knockdown, which in turn significantly reduced mHTT aggregates, particularly if administered early in disease course. There was mild improvement in behavioural phenotypes, though there was no significant change in brain volumes, weight loss or survival. Importantly, allele specificity was only achieved when there were large differences between the number of repeats on each allele [22▪▪].

CRISPR-Cas9

CRISPR-Cas9 technology is based on the bacterial analogue of an immune system, which protects against viral infections. It is composed of two elements: a guide RNA that is complementary to the target DNA, and a Cas9 nuclease that cleaves DNA at that location, introducing a strand break which is then repaired by endogenous mechanisms [23]. There are several potential approaches in Huntington's disease, including blocking *HTT* transcription, excising CAG repeats, or targeting associated SNPs. It has been used to successfully lower mHTT in patient-derived cells [23,24], and following intrastriatal delivery in Huntington's disease mouse models, the mHTT allele has been excised, thereby reducing expression, improving neuropathology, motor function and prolonging survival [25,26,27▪,28▪].

However, CRISPR-Cas9 has several significant disadvantages. Repair mechanisms are not entirely precise, so mutations can be introduced. There is also risk of off-target effects at similar sequences elsewhere in the genome and finally, its bacterial origin entails a risk of immune response in the human brain [23,24]. All these drawbacks will have to be assessed before administering CRISPR-Cas9 to Huntington's disease patients in the context of clinical trials.

THERAPIES TARGETING RNA

There are three main approaches being investigated, each one acting at different stages of mRNA maturation: ASOs trigger the degradation of pre-mRNA in the nucleus, RNA interference (RNAi) binds to mature mRNA in the cytosol, and small molecules can alter pre-mRNA splicing to encode a protein that is not viable.

Antisense oligonucleotides

ASOs are synthetic oligomers that bind to pre-mRNA through Watson–Crick base pairing [29]. Their effects are reversible, which is advantageous in case

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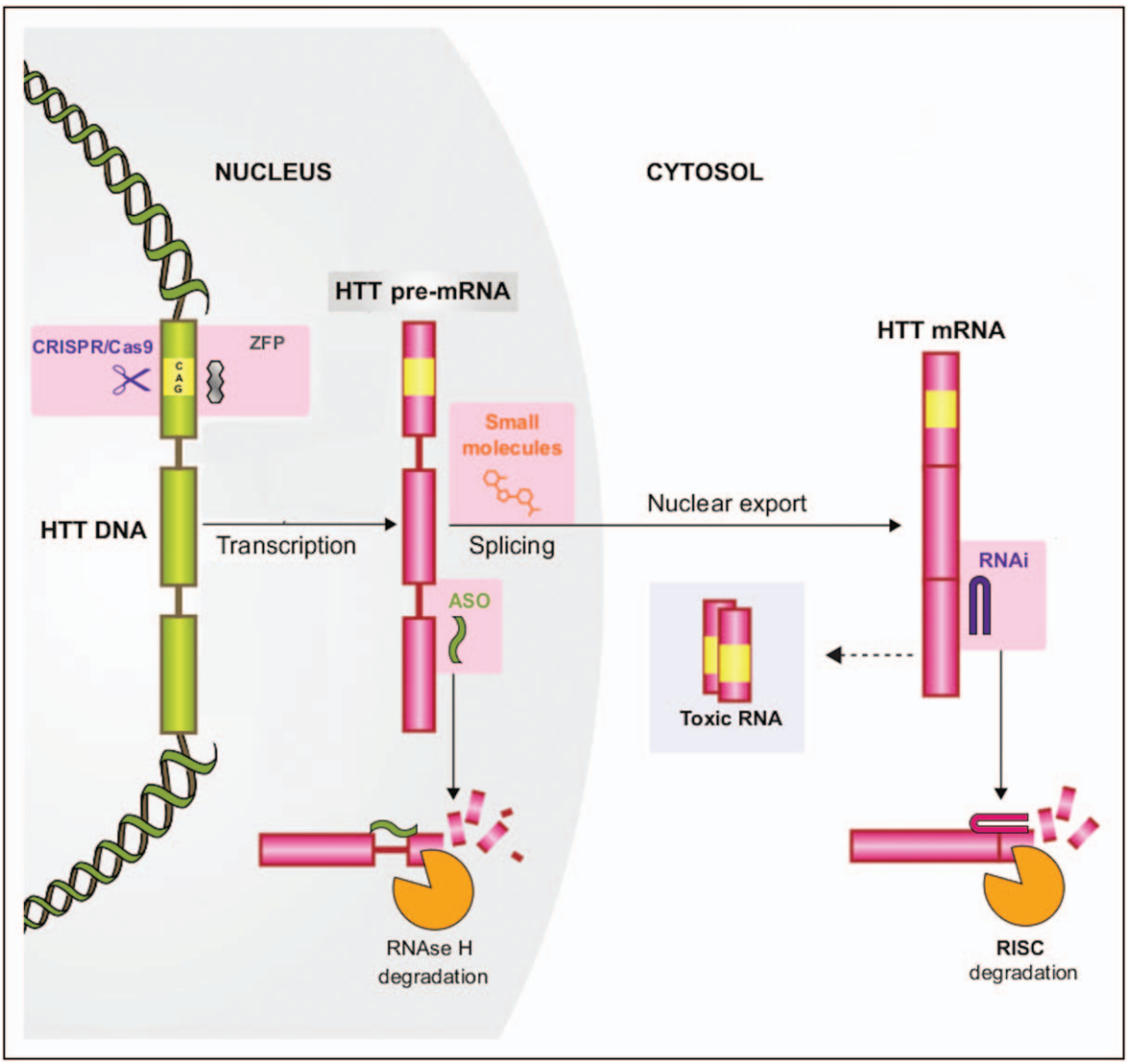

FIGURE 1. mHTT-lowering mechanisms. Yellow sections of DNA, RNA, and protein represent the pathogenic expanded CAG tract. Therapeutic compounds are represented in pink. ZFP, Zinc-finger protein; ASO, antisense oligonucleotide; RISC, RNA-induced silencing complex. Adapted with permission from [19].

of side-effects, but they require repetitive administration. They freely enter cells, rather than requiring a viral vector, but cannot cross the blood–brain barrier (BBB), so have thus far been delivered by intrathecal injection [12].

Non allele-specific ASOs

Kordasiewicz *et al.* [8] showed that intraventricular infusion of a nonallele-specific *HTT* ASO in Huntington's disease mouse models persistently lowered HTT by 66%, restored motor function and increased survival, particularly if administered early. Furthermore, good distribution was shown in the brain of NHP [8].

HTT_{Rx} (also known as ISIS443139 and RG6042) is an ASO that targets a nucleotide sequence common to both the mutant and the wild-type allele. Tabrizi *et al.* recently published the results of the first in-human phase 1–2a study evaluating the safety and tolerability of HTT_{Rx} [30▪▪]. It included 46 patients with stage 1 Huntington's disease, randomized to receive four infusions of active drug at escalating doses, or placebo. There were no significant safety concerns. Importantly, Cerebrospinal Fluid (CSF) mHTT levels showed a dose-dependent decrease by up to 40%. Though this study was not powered to detect changes in clinical outcomes (Fig. 2), a posthoc analysis showed a correlation between CSF mHTT lowering and a composite functional, cognitive and motor score [30▪▪,31▪,32].

CSF levels of neurofilament light chain (NfL), a marker of neuroaxonal damage, showed an increase

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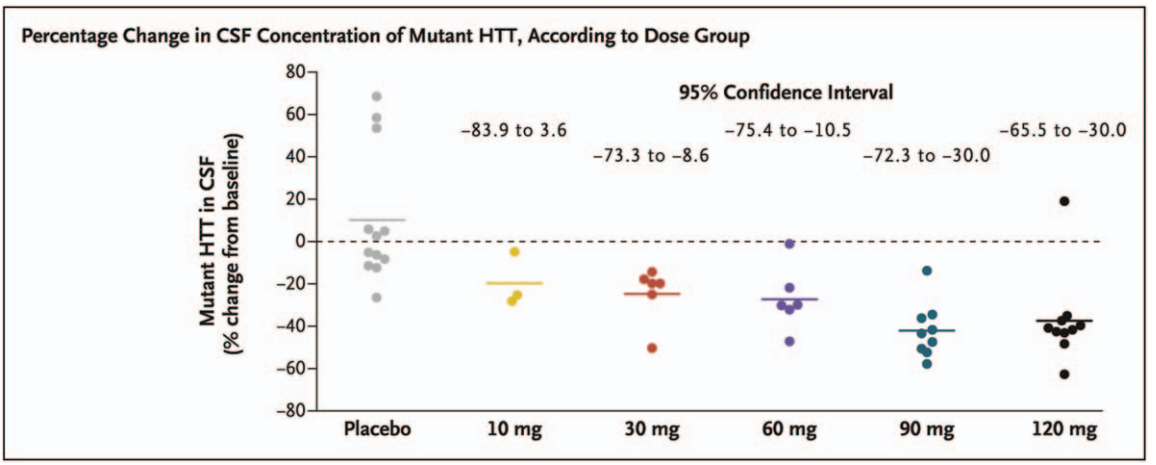

FIGURE 2. CSF mHTT reduction in the ASO HTTRx phase 1–2a trial. Percentage change in the concentration of mutant HTT in CSF, by dose group, from baseline (dotted line) to final time point, 28 days after the previous dose. Circles indicate individual patients, and horizontal lines indicate group means; 95% confidence intervals are also shown for the active-agent dose groups. Reproduced with permission from [30▪▪].

at the final visit of the phase 1–2a study which resolved at the beginning of the open-label extension (OLE). In the OLE, this increase improved by nine months, despite continued dosing [31▪]. This finding is as yet unexplained, and it remains to be seen whether levels will ultimately fall below baseline, or levels expected accounting for progression. However, resolution despite continued treatment suggests it is not an adverse effect of total HTT lowering [33]. Ventricular volumes increased in those treated with higher doses, without parallel decreases in whole brain volume, which may reflect the resolution of disease-associated inflammation, or increased CSF outflow because of the removal of mHTT from neurons [30▪▪]. In 2019, a large phase 3 study evaluating HTT_{Rx} commenced, recruiting 801 patients at over 90 sites worldwide, to be dosed for 24 months; this study is sufficiently powered to assess the drug's efficacy in symptomatic patients [34].

Allele-specific antisense oligonucleotides

A molecule that selectively targets the mutant allele would not carry the theoretical risks of lowering wtHTT. Allele-specificity can be achieved by either targeting the CAG repeat itself, or genetic variants inherited along with it [12].

Targeting the CAG repeat carries a substantial risk of binding other CAG containing genes, with undesirable side-effects [12]. However, this approach was successfully tested in two different mouse models showing a significant decrease in mHTT mRNA, together with improved performance, decreased atrophy and reduced mHTT aggregates [35].

Although targeting linked variants is potentially safer, several ASOs would need to be independently developed to be able to treat the majority of Huntington's disease patients [36]. Skotte *et al.* [37] showed that two ASOs could potentially be used in all Huntington's disease patients, though with allele specificity in only 50%.

Two phase 1b/2a human studies, PRECISION-HD1 and PRECISION-HD2, have already commenced, targeting two SNPs enriched on the mutant allele. Each study has enrolled 48 patients with early Huntington's disease for intrathecal injection of escalating doses of active drug, or placebo, with completion planned in 2020 [38▪,39,40].

RNA interference

RNA interference (RNAi) is a natural process by which RNA molecules target mRNA for degradation by the RNA-induced silencing complex reducing protein expression. They act further downstream in mRNA processing than ASOs, degrading mature mRNA in the cytosol. Similar to ZFPs, they require lentiviral or AAVs vectors and intrastriatal infusion to provide stable expression of the drug [41] but are expected to provide long-term benefit after a single delivery.

Miniarikova *et al.* [41] effectively suppressed HTT using microRNA (miRNA) in a rat model. The same group also injected AAV-miHTT into the thalamus and striatum of minipigs, achieving widespread distribution. After three months mHTT levels were halved in the striatum, and decreased by 21.2% in the cortex, though there was a paradoxical increase in CSF mHTT after infusion and an inflammatory response, with the production of cytokines [42▪▪].

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UniQure recently administered their AAV-miHTT (AMT-130) by striatal injection to NHPs. They saw no neurological side-effects, and identified the dose that would be required to achieve knockdown in the human brain [43]. The FDA recently granted it a Fast-Track designation, permitting the initiation of the first-in-human phase 1/2 trial of AAV gene therapy in Huntington's disease targeting the caudate and putamen in early manifest Huntington's disease [44,45▪].

Spark therapeutics employed a nonallele selective approach, with intraputaminal infusion of an AAV-miHTT that reduced mHTT by 45% in the putamen, without significant side-effects [46▪]. Voyager Therapeutics, used a similar method in the rhesus macaque, achieving good distribution and 50% improvement in motor function [47▪,48].

In summary, RNAi is an attractive approach for mHTT lowering, and the first human studies are planned. The main challenge is delivery, as they require the intraparenchymal injection of viral vectors into relatively small parts of the brain, which may be immunogenic and are potentially irreversible [20].

Small molecules

A small molecule drug is a compound with a low molecular weight that regulates a biological process. They are of significant interest in neurology because they can be administered orally and cross the BBB. The main drawback is their lack of target specificity [49▪].

Recently, PTC therapeutics [50] identified a small molecule that lowers both cortical and striatal mHTT in Huntington's disease mice; it acts through the inclusion of a poison exon that leads to the degradation of *HTT* mRNA [51▪].

Another study by Li *et al.* [52▪▪] has identified compounds that interact with both the mHTT protein and the autophagosome protein microtubule-associated protein 1A/1B light chain 3. These substances targeted mHTT for allele-specific autophagy, resulting in mHTT lowering and improved phenotypes in animal models [52▪▪].

CHALLENGES FOR HUNTINGTIN-LOWERING THERAPIES

Delivery and distribution

Delivery is a major challenge facing all HTT-lowering therapies and may significantly influence each methods' therapeutic potential (Fig. 3). Current ASOs have to be administered intrathecally and would require repeated dosing throughout life. Following CSF dosing, ASO concentration is expected to be higher in cortex than striatum, though encouragingly postmortem studies in spinal muscular atrophy (SMA) show they can distribute more widely [53]. Tabrizi *et al.* [30▪▪] achieved 40–60% CSF HTT-lowering, which exceeds the lowering that improved phenotype in animal models, and reflects around 20–50% reduction in striatum lumbar puncture were generally well tolerated [31▪], though new delivery methods, including intraventricular and subcutaneous catheters could potentially improve safety, tolerability and efficacy [54▪,55,56]. Following CSF delivery, parenchymal distribution is limited, so approaches such as convection-enhanced delivery [56], transient BBB disruption and focused ultrasound coupled with microbubbles, are gaining interest [57]. Such requirements could be overcome by small molecules that freely cross the BBB following oral intake.

ZFP, CRISPR-Cas9 and miRNA require a viral vector to achieve long-term transduction. These have relatively limited distribution to isolated brain regions, could potentially disrupt the host genome, and may provoke immunogenicity or neutralising antibodies [28▪,42▪▪,58] but have the advantage of potentially only needing a single injection.

Off-target binding

The DNA binding specificity of each approach varies but is critical to their success. Off-target activity could disrupt other critical genes and transcripts, with potentially deleterious consequences [59]. Trials of the small-molecule of the small molecule risdiplam in spinal muscular atrophy, for example, were stopped because of retinal abnormalities in animal models linked to off-target binding [60].

ASOs have good target affinity, and their reversibility is beneficial, in case of side-effects. RNAi, ZFP and CRISPR-Cas9, still remain in preclinical stages of development and bear a significant risk of off-target editing and knockdown, though methods are continually developing to avoid these risks [14,61].

Lowering of wild-type huntingtin

Preclinical animal studies suggest that HTT lowering in adults is safe and well tolerated, though it will be important to monitor for side-effects in human trials from long-term total HTT lowering [62]. It is important to note that HTT-lowering strategies, such as ASOs, reduce, but do not completely deplete

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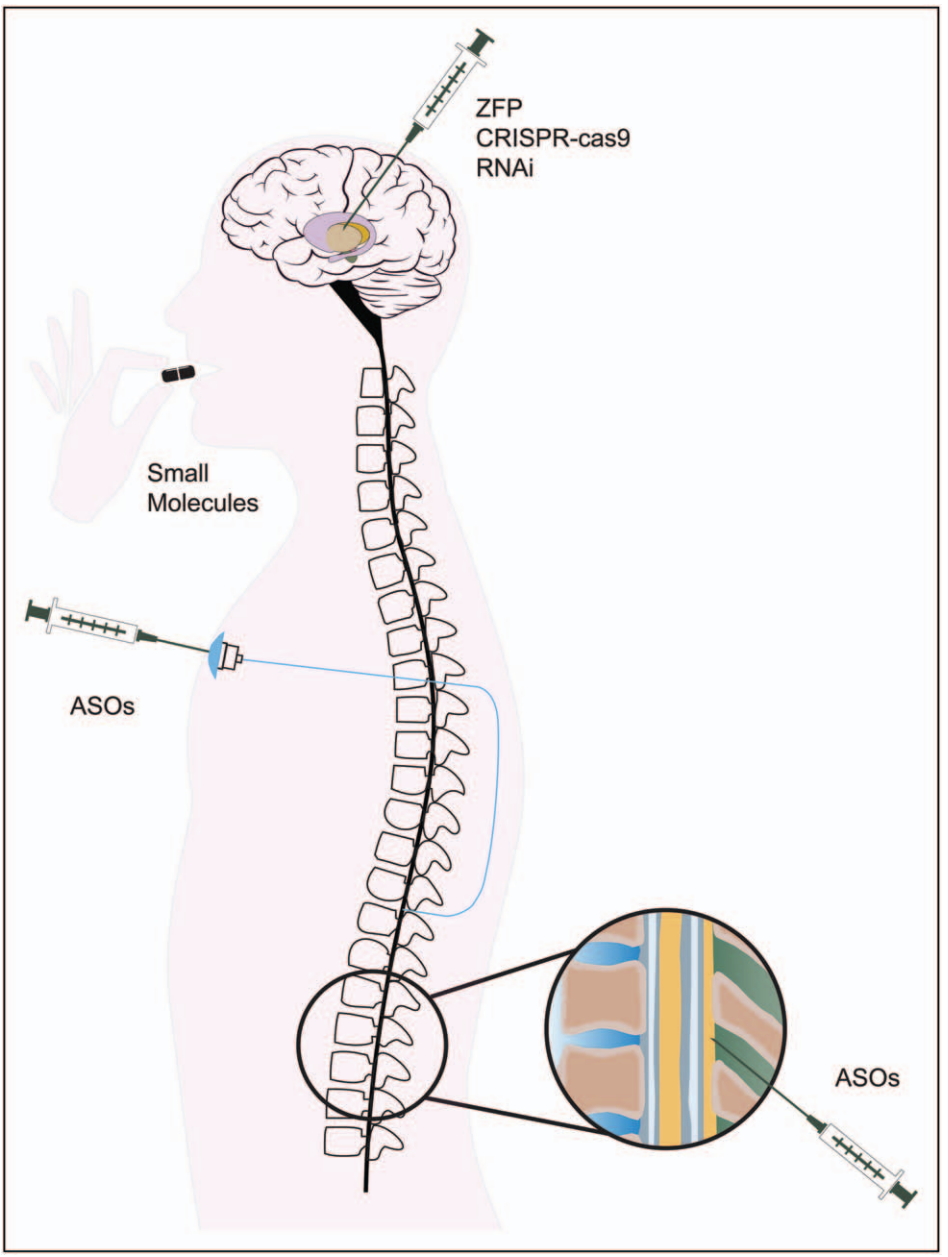

FIGURE 3. Delivery methods for different mHTT therapies. ZFP, CRISPR-Cas9 and RNAi are integrated into viral vectors and administered intraparenchymally. ASOs have been administered intrathecally by Lumbar puncture and could potentially be delivered by Portacath or Ommaya reservoir via an intrathecal or intraventricular catheter. ZFP, zinc-finger protein; ASO, antisense oligonucleotides.

HTT. Therefore, the physiological roles of wtHTT may be preserved [19].

Clinical endpoints and when to start treatment

There is little point in developing therapies for Huntington's disease if we lack the sensitivity to measure whether they modify disease course. As such, the availability of reliable biomarkers is critical. Huntington's disease slowly progresses over two decades; a timeframe which is far too long for clinical trials [1▪]. However, imaging studies have shown regional brain atrophy precedes and then parallels symptomatic onset and disease progression [63], and CSF biomarkers, rise with disease progression [64].

The fact that imaging and CSF biomarkers are abnormal many years before clinical onset suggests intervention in the premanifest period may be

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necessary to prevent neurodegeneration. To this end, the Huntington's Disease Young Adult Study is currently investigating the earliest detectable changes in premanifest patients far from onset [65].

CONCLUSION

The history of therapeutic trials in Huntington's disease is beset with failures, largely because of the difficulty in influencing a complex web of downstream pathogenic mechanisms. Novel therapeutic approaches target the RNA or the pathogenic mutation itself and have the potential to alleviate all of these. Cell and animal studies have produced encouraging results, and human trials of HTT-lowering approaches, including ASOs and RNAi, are underway. Each method faces its own challenges, though delivery to the most affected brain regions is common to all.

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Conflicts of interest

C.E.F. is a subinvestigator in the active HTT_{Rx} trials. M.F. was a subinvestigator in the Phase 1–2a study with HTT_{Rx}. S.J.T. 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 HTT_{Rx} trials, 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 Hoffmann-La Roche, Ionis Pharmaceuticals, Pfizer, and Teva Pharmaceuticals.

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