Experimental Surgical Therapies for Huntington's Disease

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SUMMARY

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by abnormal movement, cognitive decline, and psychiatric disturbance. HD is caused by a trinucleotide repeat expansion in the *HTT* gene and a corresponding neurotoxic polyglutamine expansion in the huntingtin protein. There is currently no therapy to modify the progressive course of the disease, and symptomatic treatment options are limited. In this review we describe a diverse set of emerging experimental therapeutic strategies for HD: deep brain stimulation; delivery of neurotrophic factors; cell transplantation; *HTT* gene silencing using RNA interference or antisense oligonucleotides; and delivery of intrabodies. The common feature of these experimental therapies is that they all require a neurosurgical intervention, either for implantation of an electrode or for brain delivery of molecules, viruses or cells that do not cross the blood–brain barrier upon oral or intravenous administration. We summarize available data on the rationale, safety, efficacy, and intrinsic limitations of each of these approaches, focusing mainly on studies in HD patients and genetic animal models of HD. Although each of these strategies holds significant promise, their efficacy remains to be proven in HD patients.

Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a trinucleotide (CAG) repeat expansion in the *HTT* gene [1,2]. The clinical features of HD are variable, but usually consist of a combination of a movement disorder, cognitive dysfunction, and psychiatric problems. The movement disorder of HD comprises involuntary movements or dyskinesias (chorea, dystonia), but also bradykinesia, rigidity, and incoordination. Patients eventually become bedridden and unable to speak or swallow. Cognitive decline in HD usually begins with mild executive dysfunction and evolves into dementia. Psychiatric problems are manifold and can include depression, apathy, and disinhibition. Age at HD onset varies widely but is most often between 35 and 45 years. Death typically occurs approximately 20 years after onset.

The molecular pathogenesis of HD is only partially understood. The CAG repeat expansion in *HTT* results in an expanded polyglutamine tract in huntingtin, a protein of unknown function with abundant, ubiquitous expression in brain [1]. This expanded polyglutamine tract confers a neurotoxic gain of function to the mutant protein. There are probably multiple molecular mechanisms that mediate the toxicity of mutant huntingtin [3]. Mutant huntingtin tends to aggregate and interferes with cellular functions as diverse as gene transcription, energy metabolism, axonal transport, and synaptic transmission [3]. Disruption of mul-

tipple cellular processes eventually culminates in neuronal death. The most vulnerable cells in HD are the medium spiny neurons (MSNs) of the striatum, which are GABAergic projection neurons [4]. MSNs projecting to the globus pallidus externus (GPe) appear to be more vulnerable in HD than those projecting to the globus pallidus internus (GPi) [4,5]. Despite this preferential vulnerability, the disease process is by no means restricted to the striatum, but extends to many other brain regions such as the cerebral cortex, globus pallidus, thalamus, brainstem, and cerebellum [4,6,7].

Several animal models of HD have been developed. The earliest HD animal models relied on injection of excitotoxins [8] or 3-nitropropionic acid, a mitochondrial toxin [9]. Since the identification of the *HTT* gene, a number of valuable genetic (transgenic or knock-in) HD animal models have been generated that express mutant huntingtin or fragments of it [10]. A major advantage of the genetic animal models compared with the toxin models is that the causal link between mutant huntingtin and HD is certain, whereas the primary pathogenic role of excitotoxicity or mitochondrial dysfunction in HD remains more speculative.

There is as yet no curative or disease-modifying treatment for HD. Various options exist for pharmacological treatment of the motor and psychiatric symptoms of HD, but the evidence base for most of these therapies is weak [11,12]. So far the only compound shown to be effective in a randomized, double-blind, placebo-controlled clinical trial is tetrabenazine, a drug for relief of chorea [13].

Table 1 Overview of the surgical therapies explored in Huntington's disease (HD)

Surgical strategy	Studies in genetic HD animal models	Studies in HD patients	Evidence for efficacy in HD patients
Pallidotomy	–	+ [15]	–
DBS of GPi	–	+ [20–23]	Class IV
DBS of GPe	+ [26]	–	–
CNS infusion of NTFs	–	–	–
Viral delivery of NTFs to CNS	+ [30–33]	–	–
Ex vivo NTF delivery to CNS	+ [36]	+ [37]	–
Transplantation of primary fetal tissue	+ [40,62]	+ [41–55]	Class IV
Transplantation of stem cells	+ [66–68]	–	–
Silencing through RNAi	+ [75–82,89,90]	–	–
Silencing through ASOs	+ [97] ^a	–	–
Delivery of intrabodies to CNS	+ [100,101]	–	–

The plus and minus signs indicate the presence or absence, respectively, of published studies, irrespective of the outcome. The level of evidence was assigned using the American Academy of Neurology classification scheme [102]. DBS, deep brain stimulation; GPi, globus pallidus internus; GPe, globus pallidus externus; CNS, central nervous system; NTF, neurotrophic factor; RNAi, RNA interference; ASO, antisense oligonucleotides.

^aPublication in abstract form.

This overview gives an update on a diverse array of emerging experimental therapeutic strategies for HD (Table 1). The common feature of the experimental therapies covered in this review is that they all necessitate a neurosurgical intervention. Neurosurgery is required either for implantation of an electrode or for brain delivery of molecules, viruses, or cells that do not cross the blood–brain barrier upon oral or intravenous administration. This review will focus mainly on data from studies in HD patients and genetic rodent models of HD. We will describe the rationale, therapeutic potential, and intrinsic limitations of each of these approaches.

Ablative Surgery

Pallidotomy (surgical lesioning of the GPi) is an effective treatment for bradykinesia and dyskinesias in Parkinson's disease (PD) [14]. Cubo et al. performed bilateral stereotactic pallidotomy in a 13-year-old girl with disabling bradykinesia and dystonia after a 9-year history of HD [15]. However, postoperative assessment at 3 months showed only minimal improvement of dystonia and marked worsening of spasticity.

Deep Brain Stimulation

Ablative surgery in movement disorders has been largely abandoned in favor of high-frequency electrical stimulation via stereotactically implanted electrodes (deep brain stimulation or DBS). DBS tends to mimic the clinical effects of a lesion but has the advantage of greater reversibility and adjustability. DBS affects the local activity of the target structure but can also have distant effects through modulation of neuronal circuits.

DBS of the GPi is an effective treatment for levodopa-induced choreic and dystonic movements in PD [16,17] and for primary dystonia [18,19]. This has inspired attempts to alleviate chorea and dystonia with GPi DBS in HD patients as well.

Four case studies of GPi DBS in HD patients have been published [20–23]. Baseline patient characteristics in each of these

studies included severe chorea and absence of major psychiatric or cognitive problems. The Unified Huntington's Disease Rating Scale (UHDRS) [24] was the main assessment tool. Two of these reports [20,22] explicitly stated that the postoperative comparison of DBS-off and -on conditions was performed in a double-blind fashion. Moro et al. performed bilateral GPi DBS in a 43-year-old man with an 8-year history of HD [20]. Double-blind UHDRS motor assessment in DBS-on and -off conditions at 8 months showed that DBS clearly improved chorea and dystonia. This favorable effect on chorea and dystonia was observed with a stimulation frequency of 40 Hz as well as 130 Hz. However, 130 Hz stimulation slightly worsened bradykinesia, whereas 40 Hz did not. Activities of daily living at 8 months were also improved by 40 Hz stimulation. Hebb et al. used bilateral GPi DBS at 180 Hz in a 41-year-old man with a 13-year history of HD [21]. Comparison of DBS-on and -off conditions during the first postoperative year revealed significant improvement of chorea and total motor scores in DBS-on. Functional, cognitive, and behavioral scores did not change significantly. However, dysphagia and rigidity worsened at the end of the first postoperative year, leading to institutionalization despite sustained suppression of chorea. Fasano et al. described 1-year follow-up results of bilateral GPi DBS in a 72-year-old man with a 17-year HD history [22]. Stimulation at both 40 and 130 Hz improved chorea at 4 months. However, gait and cognitive functions substantially deteriorated in the first postoperative year. Finally, Biolsi et al. reported longer follow-up results of bilateral GPi DBS (130 Hz) in a 60-year-old man with a 10-year HD history [23]. GPi DBS substantially improved chorea, and this effect was maintained up to 4 years postoperatively. Apparently, cognitive dysfunction did not progress during this follow-up period. In summary, GPi DBS substantially reduced dyskinesias in each of these HD case studies, without any surgical complications. In two of the four cases, this favorable effect appeared to be overshadowed by worsening of axial motor features and/or cognitive deterioration in the first postoperative year. It is unclear whether this deterioration was a side-effect of stimulation or merely reflected the natural progression of HD.

The GPe has been proposed as an alternative target for DBS in HD [25,26]. As mentioned earlier, the GABAergic striatal projections to the GPe are more affected in HD than those to the GPi [5]. The direct pathway–indirect pathway model of basal ganglia functional organization predicts that the early preferential loss of striatal projections to the GPe (which are part of the indirect pathway) results in overactivity of the GPe, which in turn would lead to underactivity of the subthalamic nucleus, underactivity of the GPi, and overactivity of the thalamus, resulting in chorea [25,27,28]. The direct pathway from striatum to GPi degenerates later in the disease, which might account for the increasing rigidity and akinesia in advanced HD stages [27,28]. If this model is correct in predicting that the GPe is overactive and the GPi underactive in choreic HD patients, DBS of the GPe would be a more logical therapeutic strategy for HD chorea than GPi DBS, given the fact that DBS usually mimics the effect of a lesion. Temel et al. have explored this possibility in a transgenic rat model of HD [26]. DBS of the GP (the rat equivalent of the primate GPe) reduced choreiform movements and also improved performance in a cognitive task. Case studies of GPe DBS in HD patients have not been reported.

The human case reports cited earlier suggest that GPi DBS could be an effective treatment for HD chorea [20–23]. In the majority of HD patients chorea is not the most disabling symptom of the disease. Nevertheless, chorea can be extremely distressing and medication-resistant in some HD cases, and a surgical therapy for chorea could be a welcome option for such patients. The efficacy and safety of DBS in HD should therefore be tested in randomized controlled trials. As the DBS device can be reversibly switched on and off, DBS lends itself particularly well to a randomized, double-blind, sham-controlled trial design [19]. It will be crucial in such trials to measure not only severity of dyskinesias, but also other motor problems such as bradykinesia and axial symptoms, as well as cognitive, behavioral, and quality of life outcomes. A final note of caution is that DBS is unlikely to have a neuroprotective effect. There is as yet no evidence that DBS can slow down neurodegenerative processes. Any beneficial effects of DBS would probably be symptomatic in nature.

Delivery of Neurotrophic Factors

Neuronal survival depends on continuous trophic stimulation by neurotrophic factors (NTFs). NTFs are secreted by neurons themselves or by surrounding cells such as glia. Most NTFs belong to one of three families: (1) the neurotrophin family, mainly including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4); (2) the neurocytokine family, comprising, among others, ciliary neurotrophic factor (CNTF); and (3) the glial cell line-derived neurotrophic factor (GDNF) family, including, GDNF itself and neurturin (NTN).

In general, NTFs do not readily cross the blood–brain barrier. Several strategies have been used to deliver NTFs to the striatum of animal models of HD: intrastriatal or intraventricular infusion; gene delivery through stereotactic intrastriatal injection of NTF-encoding viral vectors, such as lentivirus (LV), adenovirus (AV), or adeno-associated virus (AAV), which do not destroy the infected

neurons because they are replication-deficient; and transplantation of cells that have been genetically modified to secrete NTFs (*ex vivo* delivery of NTFs).

So far, there have been no published clinical trials of intrastriatal or intraventricular infusion or viral vector-mediated delivery of NTFs in HD patients. Numerous preclinical studies in 3-NP or excitotoxic animal models of HD have shown some benefit of infusion or viral vector-mediated delivery of NGF, BDNF, NT-3, NT-4, CNTF, GDNF, and NTN [for review, see Ref. 29]. By comparison, there have been relatively few published studies on the effects of NTF delivery in genetic animal models of HD. Popovic et al. injected GDNF-encoding LV or control LV encoding green fluorescent protein (GFP) bilaterally into the striatum of the transgenic R6/2 mouse model of HD around 5 weeks of age [30]. GDNF gene delivery led to a dramatic increase in GDNF level in the striatum, but nevertheless failed to induce any favorable behavioral or neuropathological effect compared with control. By contrast, AAV-mediated intrastriatal GDNF delivery in 5-week-old N171-82Q transgenic HD mice was found to be beneficial compared with delivery of GFP [31]. This discrepancy may be attributable to the difference in the transgenic models. The N171-82Q mouse has a later disease onset and more protracted disease course than the R6/2 mouse. Thus, 5 weeks of age represents an earlier time point in the disease process in N171-82Q than in R6/2 mice, suggesting that GDNF therapy may be more effective when started early. Ramaswamy et al. compared AAV-mediated striatal delivery of NTN and GFP in 6-week-old N171-82Q mice [32]. AAV-NTN therapy delayed motor deficits and prevented striatal neuronal loss. Surprisingly, death of frontal cortical neurons was also prevented, in the absence of any detectable NTN delivery to the cortex [32]. Less promising results were obtained in a study of AAV-mediated striatal CNTF delivery in R6/1 transgenic HD mice [33]. R6/1 mice receiving AAV-CNTF showed more rapid weight loss and motor deterioration than untreated controls. Whether these adverse effects were caused by CNTF, the viral infection or the stereotactic procedure, was unclear, because there was no AAV-GFP or sham control arm.

An alternative strategy for NTF delivery is intrastriatal transplantation of cells that have first been genetically engineered to express and secrete NTFs. In contrast to restorative cell transplantation (cfr. *infra*), the transplanted cells do not need to integrate in the host neuronal circuitry, but merely need to secrete the desired protein. This may obviate the need for complicated infusion systems or for viral infection of the diseased striatum. Several groups have reported promising results of transplantation of NTF-secreting cells in excitotoxic or 3-NP animal models of HD [34; for review, see Ref. 35]. In addition, Ebert et al. recently reported favorable effects of *ex vivo* GDNF delivery on motor function and neuronal loss in the N171-82Q mouse model [36]. One study of *ex vivo* NTF delivery in HD patients has been published [37]. This phase I trial used a device formed by a semipermeable membrane encapsulating baby hamster kidney (BHK) cells engineered to synthesize CNTF. The device was implanted into the lateral ventricle and exchanged for a new one every 6 months over a period of 2 years. The semipermeable membrane allows the passage of oxygen and nutrients to the BHK cells and of secreted CNTF from the BHK cells to the brain. In addition, the BHK cells remain contained, thus reducing the risk of tumor formation and immune rejection.

This approach was demonstrated to be safe; however, the system needs further technical improvement in order to ensure sustained release of adequate CNTF levels [37].

More experiments in genetic animal models of HD are needed to compare the safety and efficacy profile of the different NTFs. A major challenge for the clinical application of NTF delivery for HD is the development of delivery systems that allow accurate control of NTF dosing and cessation of delivery in case of adverse effects. This seems more straightforward with NTF infusion systems than with virus-mediated NTF gene delivery, although more controllable viral vectors have been developed [38]. A final limitation is that intrastrially delivered NTFs usually do not reach extrastriatal brain areas. Thus, the effects will be limited in space, unless multiple delivery sites are used.

Cell Transplantation

Two forms of cell transplantation have been proposed as therapeutic strategies in HD. Cells can be transplanted to replace lost neurons (restorative cell transplantation). A different concept is transplantation of cells genetically modified to secrete NTFs (cf. supra).

The rationale behind restorative cell transplantation in HD is deceptively simple: neuronal cells lacking the HD mutation are transplanted into the striatum to replace the lost MSNs, repair the dysfunctional basal ganglia circuits and alleviate HD symptoms. For this approach to be effective, the transplanted cells need to survive after transplantation and form the proper afferent and efferent connections with the host tissue.

Transplantation of Primary Fetal Tissue

Animal studies have shown that donor neuronal tissue taken from fully differentiated adult brain does not survive transplantation, in contrast to developing neurons dissected from the fetal brain [39]. Extensive studies in excitotoxic and 3-NP animal models of HD have shown that cells dissected from the fetal ganglionic eminences and grafted into the lesioned adult striatum can survive, differentiate into MSNs, establish functional afferent and efferent connections with appropriate targets in the host brain and improve motor and cognitive deficits [35,39]. By comparison, surprisingly few fetal tissue transplantation studies have been performed in genetic HD animal models. To our knowledge, only one published study has investigated the effect of intrastriatal fetal tissue grafts in a genetic HD animal model (10-week-old R6/2 mice) [40]; this study found no meaningful behavioral effect.

Based on the encouraging results in excitotoxic and 3-NP models, a number of open-label pilot studies of stereotactic fetal tissue transplantation were performed in a total of at least 55 HD patients. With the exception of one porcine xenograft study [41], all of these used human allografts [42–55]. Various postoperative immunosuppressive regimens were applied in the different studies.

Several adverse events were reported. In the trial by Hauser et al. [47], three of the seven transplanted patients developed subdural hemorrhages (SDH) and two of these required surgical drainage. Subjects in this trial had more advanced HD than in

other transplantation studies, and it has been speculated that the more severe cerebral atrophy of these patients may have been a risk factor for SDH. Bachoud-Lévi et al. reported transient neurological symptoms (confusion, paresis, postural instability) in the immediate postoperative period in three of the five patients [48]. Poor compliance with immunosuppressive treatment was noted in three of the five patients, one of whom suffered a cyclosporine intoxication with full recovery [48]. In a subsequent phase II trial led by the same group, 1 of 13 transplanted patients exhibited general clinical deterioration 14 months after grafting, with evidence of graft rejection. The signs of rejection were reversed under aggressive immunosuppressive therapy [55]. Finally, development of mass lesions due to transplant overgrowth was recently reported in a patient who had received bilateral intrastriatal fetal transplants in combination with a unilateral putaminal autologous sural nerve graft [56].

Some of the pilot transplantation studies provide details on the postoperative evolution of HD features, and the clinical results appear to be mixed. Hauser et al. found no significant change in the primary outcome variable (the motor component of the UHDRS) at 12 months after transplantation in seven HD patients [47]. Much longer clinical follow-up data (6 years) were provided by Bachoud-Lévi et al. [49,50]. Three of the five transplanted patients showed motor, cognitive, and functional improvement or stabilization in the first two postoperative years, with subsequent deterioration of some of these parameters. The remaining two patients showed no clinical benefit and declined in the same way as a cohort of nongrafted patients. In a different study with 5 years of postoperative follow-up, one of the two transplanted patients showed dramatic functional, motor, behavioral and, to a lesser extent, cognitive improvement in the first 3 years and stabilization thereafter [52]. Interestingly, PET imaging of this patient revealed a postoperative increase in striatal availability of the D2 dopamine receptor, a marker of indirect pathway MSNs, suggesting long-term graft survival [52]. The other patient showed no clinical or imaging improvement [52]. The variability of the clinical results in the pilot studies may be due to differences in the many methodological variables of this multistep procedure: variability in fetal age, quality of the retrieved aborted tissue, dissected fetal brain region (lateral vs. whole ganglionic eminence), tissue preparation (solid tissue blocks versus dissociated cell suspensions), duration of the interval between abortion and transplantation, method of tissue preservation during this interval, patient selection, postoperative immunosuppressive regimen, etc. An additional source of variability may be placebo and investigator bias effects due to the open-label nature of these studies. Two large randomized, double-blind, sham surgery-controlled trials of fetal neurotransplantation were performed in PD patients and failed to show clinical benefit of transplantation in comparison with placebo with respect to the primary endpoint [57,58], despite the promising results of earlier open-label studies. This demonstrates the feasibility and importance of double-blind, sham-controlled trials for rigorous assessment of the efficacy of neurotransplantation in neurodegenerative diseases.

Postmortem histological findings after neural transplantation have been reported for eight HD patients [54,56,59–61]. Pathological data at 6 months to 6 years after transplantation were

consistent with graft survival and presence of neuronal and MSN markers [54,59,60]. However, neurons in the graft had an unhealthy appearance with signs of apoptosis in three patients who died 10 years after transplantation [61]. Intriguingly, MSNs in these 10-year-old grafts were preferentially affected in comparison with interneurons, suggesting that an HD-like degenerative process may spread from the host to the graft at long term [61]. However, none of these studies found huntingtin aggregates in the grafts. Keene et al. noted that graft-host connectivity was limited [60]. Although host-derived dopaminergic [54,59,61] and glutamatergic [61] fibers were observed in the grafts, none of the autopsy studies could demonstrate outgrowth of axons from the graft to the host.

Striatal fetal transplantation for HD has several intrinsic limitations. The degenerative process in HD is anatomically widespread, especially in later stages. Thus, additional transplantation into extrastriatal regions would be needed to repair other damaged circuits. Few studies have explored the possibility of fetal tissue transplantation into extrastriatal areas [62]. Another limitation of transplantation in neurodegenerative diseases is that there is no "off-switch." If the grafted tissue causes unwanted effects (e.g., graft-induced dyskinesias in PD) [57,58], the graft cannot be removed. Finally, ethical and logistic concerns are related to the dependence on primary fetal tissue. This has led to a search for renewable sources of transplant material, such as stem cells.

Transplantation of Stem Cells

Stem cells (SC) are defined by their strong capacity for self-renewal and their ability to differentiate into multiple mature cell types [63,64]. The classical SC is the embryonic stem cell (ESC) of the blastocyst, which can differentiate into every cell of the body. In addition, tissue-specific SCs with a more restricted differentiation potential, such as neural SCs (NSCs) and multipotent stromal cells (MSCs), can be found in tissues of more advanced developmental stages. Recently, it has even become possible to generate SCs from adult somatic cells such as skin fibroblasts by introduction of four embryogenesis-related genes [65]. As SCs can be expanded indefinitely *in vitro*, they could solve the logistic problems related to the use of primary fetal tissue. Intrastriatal transplantation of SC-derived neural progenitors committed to the MSN phenotype could thus result in replacement of lost MSNs and circuit reconstruction in HD.

There are currently no published clinical trials of transplantation of SC-derived cells in HD patients. Studies of transplantation of SC-derived cells at various stages of differentiation have been performed in excitotoxic and 3-NP animal models of HD [for review, see Ref. 64] and genetic HD mouse models [66–68], with some promising results. Recently, Aubry et al. have developed an *in vitro* protocol for differentiation of ESCs into postmitotic neurons exhibiting major phenotypic characteristics of MSNs, including expression of the classical MSN marker DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of 32 kDa) [69]. The same authors also demonstrated *in vivo* differentiation of ESC-derived striatal progenitors into DARPP-32-positive neurons after transplantation into an excitotoxic rat model of HD [69]. However, this study also revealed a potential roadblock on the way to the clinic,

because the grafts systematically overgrew in the host brain. This again highlights the need for an "off-switch" in case graft-induced side-effects occur.

Interestingly, beneficial effects of SC transplantation may occur even without long-term survival of the transplanted SCs. Snyder et al. transplanted MSCs into the striatum of the N171-82Q mouse model [68]. Although the transplanted MSCs all died within 15 days, striatal atrophy was significantly reduced 1 month after transplantation, apparently due to increased proliferation and differentiation of endogenous NSCs and increased neurotrophic signaling. Whether this had any favorable behavioral effects, was not reported.

Silencing the Expression of Mutant Huntingtin

Blocking the expression of the toxic mutant huntingtin protein would represent a definitive strategy to halt the pathogenesis of HD. Interestingly, work in a conditional transgenic mouse model of HD (using the tetracycline-responsive transgene system that allows mutant transgene expression to be turned off with oral administration of tetracycline analogs) has shown that shutting off mutant huntingtin expression after disease onset not only prevents further neuropathological and motor deterioration but even reverses existing deficits [70].

Several powerful technologies are emerging to suppress the expression of mutant huntingtin at the mRNA level *in vivo* by means of short single- or double-stranded RNA or DNA molecules, such as RNA interference (RNAi) and administration of antisense oligonucleotides (ASOs). Mutant huntingtin can also be silenced by increasing its degradation at the protein level, a mechanism that probably underlies the beneficial effects of intrabodies.

RNA Interference

RNAi is a physiological phenomenon by which RNA can repress gene expression at the posttranscriptional level. The genome encodes a large number of small RNAs (called microRNAs or miRNAs) that are not translated into protein but regulate the expression of specific genes [71,72]. A miRNA is transcribed as a long single-stranded precursor. This precursor is trimmed, exported from the nucleus and processed by cytosolic enzymes to a ~22-nucleotide duplex miRNA. After unwinding of this double-stranded structure, the guide strand of the miRNA is incorporated into the RNA-induced silencing complex (RISC), a multi-subunit protein complex, and binds to the target mRNA. The target mRNA is then silenced, either by degradation or by inhibition of its translation.

This naturally occurring system can be exploited to silence targets that are not normally subject to RNAi. Chemically synthesized ~22-nucleotide RNA duplexes with 2-nucleotide 3' end overhangs (small interfering RNAs or siRNAs) are structurally similar to mature, fully processed endogenous duplex miRNAs and can be introduced into cells by liposomal transfection. The siRNAs are then immediately available for unwinding, assembly with RISC and hybridization with the target mRNA. Essentially any gene can thus

be targeted based on sequence complementarity. A disadvantage of siRNAs is that their effects after a single administration are transient, as the siRNAs are degraded intracellularly over the course of days. Moreover, it is very difficult to transfect siRNAs into post-mitotic, nondividing cells such as neurons. To overcome these limitations, viral vectors have been developed that efficiently infect neurons and encode short hairpin RNAs (shRNAs) [73]. Upon neuronal infection, the shRNAs are transcribed in the nucleus, like naturally occurring miRNA precursors, exported to the cytosol and processed to produce functional siRNAs. Thus, a one-time administration of shRNA-encoding virus can result in enduring intra-neuronal production of shRNAs and the corresponding siRNAs. To bypass the blood–brain barrier, these viral vectors need to be injected stereotactically into the region of interest.

In a groundbreaking study in 2004, Xia et al. found that intracerebellar injection of recombinant AAV expressing shRNAs directed against mutant ataxin-1 suppressed neurodegeneration in a mouse model of spinocerebellar ataxia type 1, a polyglutamine disease closely related to HD [74]. Since then, a series of remarkable studies have provided similar proof of principle that viral vector-mediated RNAi of mutant huntingtin has beneficial effects on histological and/or motor abnormalities in rodent HD models expressing mutant huntingtin [75–82; for review, see Ref. 72].

Despite these promising results, several issues need to be addressed before RNAi can move from preclinical studies to clinical trials in HD patients. A first important question is whether simultaneous RNAi silencing of the normal *HTT* allele along with the mutant allele (i.e., non-allele-specific silencing) is deleterious. Although the normal function of huntingtin is not well defined, homozygous knockout of the mouse *Htt* gene (the homologue of the human *HTT* gene) causes early embryonic lethality, indicating that normal huntingtin is indispensable for embryonic development [83,84]. Homozygous brain-specific inactivation of *Htt* starting at postnatal day 5 was tolerated better, but still induced progressive neurodegeneration and shortened life span [85]. It is currently unclear whether initiation of normal *HTT* silencing in adult life would also be toxic. Most of the preclinical proof-of-principle RNAi studies cited earlier [75–79] used animal models that over-expressed human mutant *HTT* in the setting of two normal rodent *Htt* alleles. The RNAi strategy used in these studies took advantage of species-specific sequence differences to specifically suppress the mutant human allele without reducing endogenous normal *Htt* expression. However, three of the more recent RNAi studies took a non-allele-specific approach and found that 3- to 9-month suppression of normal *Htt* did not induce any major toxicity [80–82]. In contrast to genetic knockouts, RNAi suppresses expression of the target gene only partially, and the residual expression level of wild-type huntingtin may thus be sufficient to avoid adverse effects. It will be important to assess the safety of more long-term suppression of wild-type huntingtin in animals with longer life span such as monkeys.

If non-allele-specific silencing turns out to have adverse effects, allele-specific silencing (i.e., suppressing the mutant allele while leaving the normal allele unaffected) could provide a safer alternative. Unfortunately, siRNAs that target the CAG repeat cannot discriminate between mutant and normal alleles. However, one can exploit single nucleotide polymorphism (SNP) differences be-

tween the mutant and normal *HTT* alleles. If an siRNA is directed against an SNP isoform present in the mutant *HTT* allele but absent in the normal allele, it would fully match the mutant allele but have a single mismatch with the other allele. This single nucleotide mismatch could be sufficient to reduce the RNAi effect on the normal allele. A study by van Bilsen et al. in 2008 in cultured fibroblasts from an HD patient was the first to show that this allele-specific siRNA strategy can indeed successfully suppress endogenous mutant huntingtin without affecting the wild-type protein [86]. For this type of therapy to be clinically applicable, it is crucial to know whether there are enough heterozygous SNPs in the *HTT* gene in the HD population and which proportion of patients could be treated using the same siRNA molecules. Lombardi et al. and Pfister et al. genotyped SNP sites in the *HTT* gene in 327 and 109 HD patients, respectively, and suggested that a relatively small number of allele-specific siRNAs could provide effective RNAi for the majority (approximately three quarters) of European and US American HD patients [87,88].

In addition to the potential side-effects of non-allele-specific silencing, siRNAs can sometimes induce unexpected off-target effects. These can result from unintended binding of the siRNA to nontarget mRNAs. Off-target effects can also arise when exogenous shRNAs compete with endogenous miRNAs for interaction with the miRNA processing machinery. Flooding the cell with “therapeutic” shRNAs could thus prevent endogenous miRNAs from performing their physiological mRNA silencing roles. An important safety concern in RNAi therapy is therefore the necessity to have an “off switch” to shut down siRNA expression if adverse events occur. However, viral vector-mediated delivery of shRNAs is difficult to turn off once the virus has been injected. One solution could be the development of regulatable viral expression systems such as tetracycline-responsive vectors [81]. An alternative strategy to achieve more controlled delivery could be the use of nonviral siRNA systems. A single injection of liposome-encapsulated *HTT*-targeted siRNAs into the ventricles of early postnatal transgenic HD mice was reported to have surprisingly long-lasting beneficial effects on motor and histological read-outs [89]. Similarly, a single stereotactic intrastriatal injection of cholesterol-conjugated anti-*HTT* siRNAs led to significant siRNA uptake by surrounding tissue and favorable pathological and behavioral results [90]. A major challenge for RNAi therapy in HD is therefore the development of infusion systems for long-term, controlled, nonviral siRNA delivery.

A final limitation of RNAi therapy is that shRNAs expressed from injected viral vectors or directly injected siRNAs tend to remain confined to the brain region surrounding the injection site. Targeting mutant huntingtin in all affected brain areas would thus require multiple injection or infusion sites, increasing the invasiveness and risk of the surgical procedure.

Antisense Oligonucleotides

ASOs are short (15–25 nucleotides), synthetic oligomers. ASOs differ from siRNAs in several respects. They are DNA, not RNA; they are single-stranded, not double-stranded; and they induce intranuclear degradation of the target mRNA by RNase H, rather than intracytosolic degradation by RISC [91]. After entering the

cell nucleus the ASO hybridizes to the target mRNA, forming a DNA/RNA heteroduplex. Formation of the heteroduplex recruits RNase H, which degrades the target mRNA, leaving the ASO intact for binding to another target mRNA molecule [91].

Several studies have shown that ASOs can effectively downregulate expression of huntingtin protein in cultured cells [92–94]. However, an earlier *in vivo* study unsuccessfully attempted to reduce striatal huntingtin expression in the mouse by repeated intrastriatal ASO infusion [95]. Interest in ASOs as therapeutic agents for neurodegenerative diseases temporarily declined with the emergence of RNAi technology, but has recently resurged with the work of Cleveland et al. [96]. This group showed that ASOs, continuously infused intraventricularly, distributed surprisingly widely throughout the entire brain and spinal cord of rodents and primates [96]. The therapeutic potential of intraventricular ASO infusion was tested in a transgenic mouse model of amyotrophic lateral sclerosis (ALS). This ALS mouse overexpresses a toxic mutant form of the protein superoxide dismutase 1 (SOD1). Infusion of ASOs targeting SOD1 significantly slowed disease progression [96], suggesting that intraventricular ASO infusion could be an effective, regulatable method for treating neurodegenerative diseases caused by toxic proteins. Similar ASO experiments to suppress huntingtin expression in transgenic HD mice are ongoing [97].

Delivery of Intrabodies

Intrabodies are recombinant, intracellularly expressed, single-chain antibody fragments [98]. Intrabodies are highly specific and can in some cases discriminate between distinct conformations of the same protein. Intrabodies have been designed that preferentially bind to mutant huntingtin and increase its intracellular turnover [99,100]. Interestingly, stereotactic intrastriatal injection of viral vectors encoding intrabodies against mutant huntingtin

has been shown to ameliorate neurological symptoms in transgenic mouse models of HD [100,101]. No clinical trials with intrabodies in HD have been performed yet.

The main advantage of intrabody treatment compared with the siRNA strategy relates to the exquisite specificity of antibody–epitope interactions, resulting in a reduced risk, at least theoretically, of non-allele-specific and off-target effects.

Conclusions

The experimental strategies discussed in this review hold significant promise, but their efficacy remains to be further demonstrated in HD patients. While the aim of DBS is to relieve certain HD symptoms, NTF delivery, cell transplantation and silencing strategies have the additional and more ambitious goal of modifying the course of the disease. A limitation of surgical strategies for the treatment of a widespread brain disease like HD is that their effects tend to remain confined to the local brain region or circuit where the procedure was performed. With the exception of DBS and infusion strategies, most of these technologies currently also have the disadvantage that they are difficult to switch off in case of adverse effects. Given the intrinsic limitations of surgical strategies, the optimal HD therapy of the future will probably consist of a combination of surgical and nonsurgical (pharmacological) approaches.

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Conflicts of Interest

The authors declare no conflict of interests.

References

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1983;**72**:971–983.
2. Walker FO. Huntington's disease. *Lancet* 2007;**369**:218–228.
3. Roze E, Saudou F, Caboche J. Pathophysiology of Huntington's disease: From huntingtin functions to potential treatments. *Curr Opin Neurol* 2008;**21**:497–503.
4. Vonsattel JP, DiFiglia M. Huntington disease. *J Neuropathol Exp Neurol* 1998;**57**:369–384.
5. Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB. Differential loss of striatal projection neurons in Huntington disease. *Proc Natl Acad Sci USA* 1988;**85**:5733–5737.
6. Van Laere K, Casteels C, Dhollander I, Goffin K, Grachev I, Bormans G, Vandenberghe W. Widespread decrease of CB1 receptor availability in Huntington's disease in vivo. *J Nucl Med* 2010;**51**:1413–1417.
7. Rosas HD, Koroshetz WJ, Chen YI, et al. Evidence for more widespread cerebral pathology in early HD. An MRI-based morphometric analysis. *Neurology* 2003;**60**:1615–1620.
8. Coyle JT, Schwarcz R. Lesion of striatal neurons with kainic acid provides a model for Huntington's chorea. *Nature* 1976;**263**:244–246.
9. Beal MF, Brouillet E, Jenkins B, Henshaw R, Rosen B, Hyman BT. Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *J Neurochem* 1993;**61**:1147–1150.
10. Heng MY, Detloff PJ, Albin RL. Rodent genetic models of Huntington disease. *Neurobiol Dis* 2008;**32**:1–9.
11. Phillips W, Shannon KM, Barker RA. The current clinical management of Huntington's disease. *Mov Disord* 2008;**23**:1491–1504.
12. Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C. Therapeutic interventions for symptomatic treatment in Huntington's disease. *Cochrane Database Syst Rev* 2009;**8**:CD006456.
13. Huntington Study Group. Tetrabenazine as antichorea therapy in Huntington disease: A randomized controlled trial. *Neurology* 2006;**66**:366–372.
14. Fine J, Duff J, Chen R, Chir B, Hutchison W, Lozano AM, Lang AE. Long-term follow-up of unilateral pallidotomy in advanced Parkinson's disease. *N Engl J Med* 2000;**342**:1708–1714.
15. Cubo E, Shannon KM, Penn RD, Kroin JS. Internal globus pallidotomy in dystonia secondary to Huntington's disease. *Mov Disord* 2000;**15**:1248–1251.
16. Deep-Brain Stimulation for Parkinson's Disease Study Group. Deep-brain stimulation of the subthalamic nucleus or the pars interna of the globus pallidus in Parkinson's disease. *N Engl J Med* 2001;**345**:956–963.
17. Lohr TJ, Burgunder JM, Pohle T, Weber S, Sommerhalder R, Krauss JK. Long-term pallidal deep brain stimulation in patients with advanced Parkinson disease: 1-year follow-up study. *J Neurosurg* 2002;**96**:844–853.
18. Vidailhet M, Vercueil L, Houeto JL, et al. French Stimulation du Pallidum Interne dans la Dystonie (SPIDY) Study Group. Bilateral deep-brain stimulation of the globus pallidus in primary generalized dystonia. *N Engl J Med* 2005;**352**:459–467.
19. Kupsch A, Benecke R, Müller J, et al. Deep-brain stimulation for Dystonia Study Group. Pallidal deep-brain stimulation in primary generalized or segmental dystonia. *N Engl J Med* 2006;**355**:1978–1990.
20. Moro E, Lang A, Strafella A, et al. Bilateral globus pallidus stimulation for Huntington's disease. *Ann Neurol* 2004;**56**:290–294.
21. Hebb M, Garcia R, Gaudet P, Mendez I. Bilateral stimulation of the globus pallidus internus to treat choreoathetosis in Huntington's disease: Technical case report. *Neurosurgery* 2006;**58**:E383.
22. Fasano A, Mazzone P, Piano C, Quaranta D, Francesco S, Bentivoglio A. GPI-DBS in Huntington's disease: Results on motor function and cognition in a 72-year-old case. *Mov Disord* 2008;**23**:1289–1293.
23. Biolsi B, Cif L, El Ferit H, Robles S, Coubes P. Long-term follow-up of Huntington disease treated by deep brain

- stimulation of the internal globus pallidus. *J Neurosurg* 2008;**109**:130–132.
24. Huntington Study Group. Unified Huntington's disease rating scale: Reliability and consistency. *Mov Disord* 1996;**11**:136–142.
 25. Reiner A. Can lesions of GPe correct HD deficits? *Exp Neurol* 2004;**186**:1–5.
 26. Temel Y, Cao C, Vlamings R, et al. Motor and cognitive improvement by deep brain stimulation in a transgenic rat model of Huntington's disease. *Neurosci Lett* 2006;**406**:138–141.
 27. Albin RL, Young AB, Penney J. The functional anatomy of basal ganglia disorders. *Trends Neurosci* 1989;**12**:366–375.
 28. Albin RL, Reiner A, Anderson KD, Penney JB, Young AB. Striatal and nigral neuron subpopulations in rigid Huntington's disease: Implications for the functional anatomy of chorea and rigidity-akinesia. *Ann Neurol* 1990;**27**:357–365.
 29. Alberch J, Pérez-Navarro E, Canals JM. Neurotrophic factors in Huntington's disease. *Prog Brain Res* 2004;**146**:195–229.
 30. Popovic N, Maingay M, Kirik D, Brundin P. Lentiviral delivery of GDNF into the striatum of R6/2 Huntington mice fails to attenuate behavioural and neuropathological changes. *Exp Neurol* 2005;**193**:65–74.
 31. McBride JL, Ramaswamy S, Gasmi M, et al. Viral delivery of glial cell line-derived neurotrophic factor improves behavior and protects striatal neurons in a mouse model of Huntington's disease. *Proc Natl Acad Sci USA* 2006;**103**:9345–9350.
 32. Ramaswamy S, McBride JL, Han I, et al. Intrastriatal CERE-120 (AAV-Neurturin) protects striatal and cortical neurons and delays motor deficits in a transgenic mouse model of Huntington's disease. *Neurobiol Dis* 2009;**34**:40–50.
 33. Denovan-Wright EM, Attis M, Rodríguez-Lebron E, Mandel RJ. Sustained striatal ciliary neurotrophic factor expression negatively affects behavior and gene expression in normal and R6/1 mice. *J Neurosci Res* 2008;**86**:1748–1757.
 34. Giral A, Friedman HC, Caneda-Ferrón B, et al. BDNF regulation under GFAP promoter provides engineered astrocytes as a new approach for long-term protection in Huntington's disease. *Gene Ther* 2010;**17**:1294–1308.
 35. Dunnett SB, Rosser AE. Cell therapy in Huntington's disease. *NeuroRx* 2004;**1**:394–405.
 36. Ebert AD, Barber AE, Heins BM, Svendsen CN. Ex vivo delivery of GDNF maintains motor function and prevents neuronal loss in a transgenic mouse model of Huntington's disease. *Exp Neurol* 2010;**224**:155–162.
 37. Bloch J, Bachoud-Lévi AC, Déglon N, et al. Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: Results of a phase I study. *Hum Gene Ther* 2004;**15**:968–975.
 38. Régulier E, Pereira de Almeida L, Sommer B, Aebischer P, Déglon N. Dose-dependent neuroprotective effect of ciliary neurotrophic factor delivered via tetracycline-regulated lentiviral vectors in the quinolinic acid rat model of Huntington's disease. *Hum Gene Ther* 2002;**13**:1981–1990.
 39. Rosser AE, Dunnett SB. Neural transplantation in patients with Huntington's disease. *CNS Drugs* 2003;**17**:853–867.
 40. Dunnett SB, Carter RJ, Watts C, et al. Striatal transplantation in a transgenic mouse model of Huntington's disease. *Exp Neurol* 1998;**154**:31–40.
 41. Fink JS, Schumacher JM, Ellis SA, et al. Porcine xenografts in Parkinson's disease and Huntington's disease patients: Preliminary results. *Cell Transplant* 2000;**9**:273–278.
 42. Sramka M, Rattaj M, Molina H, Vojtassák J, Belan V, Ruzický E. Stereotactic technique and pathophysiological mechanisms of neurotransplantation in Huntington's chorea. *Stereotact Funct Neurosurg* 1992;**58**:79–83.
 43. Madrazo I, Franco-Bourland RE, Castrejón H, Cuevas C, Ostrosky-Solis F. Fetal striatal homotransplantation for Huntington's disease: First two case reports. *Neurol Res* 1995;**17**:312–315.
 44. Philpott LM, Kopyov OV, Lee AJ, et al. Neuropsychological functioning following fetal striatal transplantation in Huntington's chorea: Three case presentations. *Cell Transplant* 1997;**6**:203–212.
 45. Kopyov OV, Jacques S, Lieberman A, Duma CM, Eagle KS. Safety of intrastratial neurotransplantation for Huntington's disease patients. *Exp Neurol* 1998;**119**:97–108.
 46. Ross BD, Hoang TQ, Blüml S, et al. In vivo magnetic resonance spectroscopy of human fetal transplants. *NMR Biomed* 1999;**12**:221–236.
 47. Hauser RA, Furtado S, Cimoto CR, et al. Bilateral human fetal striatal transplantation in Huntington's disease. *Neurology* 2002;**58**:687–695.
 48. Bachoud-Lévi AC, Bourdet C, Brugières P, et al. Safety and tolerability assessment of intrastratial neural allografts in Huntington's disease patients. *Exp Neurol* 2000;**161**:194–202.
 49. Bachoud-Lévi AC, Rémy P, Nguyen JP, et al. Motor and cognitive symptoms in patients with Huntington's disease after neural transplantation. *Lancet* 2000;**356**:1975–1979.
 50. Bachoud-Lévi AC, Gaura V, Brugières P, et al. Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: A long-term follow-up study. *Lancet Neurol* 2006;**5**:303–309.
 51. Rosser AE, Barker RA, Harrower T, et al. NEST-UK. Unilateral transplantation of human primary fetal tissue in four patients with Huntington's disease: NEST-UK safety report ISRCTN no 36485475. *J Neurol Neurosurg Psychiatry* 2002;**73**:678–685.
 52. Reuter I, Tai YF, Pavese N, et al. Long-term clinical and positron emission tomography outcome of fetal striatal transplantation in Huntington's disease. *J Neurol Neurosurg Psychiatry* 2008;**79**:948–951.
 53. Gallina P, Paganini M, Lombardini L, et al. Human striatal neuroblasts develop and build a striatal-like structure into the brain of Huntington's disease patients after transplantation. *Exp Neurol* 2010;**222**:30–41.
 54. Capetian P, Knott R, Maciarczyk J, et al. Histological findings on fetal striatal grafts in a Huntington's disease patient early after transplantation. *Neuroscience* 2009;**160**:661–675.
 55. Krystkowiak P, Gaura V, Labalette M, Rialland A, Remy P, Peschanski M, Bachoud-Lévi AC. Alloimmunisation to donor antigens and immune rejection following foetal neural grafts to the brain in patients with Huntington's disease. *PLoS One* 2007;**2**:e166.
 56. Keene CD, Chang RC, Leverenz JB, et al. A patient with Huntington's disease and long-surviving fetal neural transplantation that developed mass lesions. *Acta Neuropathol* 2009;**117**:329–338.
 57. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;**344**:710–719.
 58. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 2003;**54**:403–414.
 59. Freeman T, Cicchetti F, Hauser R, et al. Transplanted fetal striatum in Huntington's disease: Phenotypic development and lack of pathology. *Proc Natl Acad Sci USA* 2000;**97**:13877–13882.
 60. Keene CD, Sonnen JA, Swanson PD, Kopyov O, Leverenz JB, Bird TD, Montine TJ. Neural transplantation in Huntington disease: Long-term grafts in two patients. *Neurology* 2007;**68**:2093–2098.
 61. Cicchetti F, Saporta S, Hauser RA, et al. Neural transplants in patients with Huntington's disease undergo disease-like neuronal degeneration. *Proc Natl Acad Sci USA* 2009;**106**:12483–12488.
 62. van Dellen A, Deacon R, York D, Blakemore C, Hannan AJ. Anterior cingulate cortical transplantation in transgenic Huntington's disease mice. *Brain Res Bull* 2001;**56**:313–318.
 63. Seaberg RM, van der Kooy D. Stem and progenitor cells: The premature desertion of rigorous definitions. *Trends Neurosci* 2003;**26**:125–131.
 64. Dunnett SB, Rosser AE. Stem cell transplantation for Huntington's disease. *Exp Neurol* 2007;**203**:279–292.
 65. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–872.
 66. Yang CR, Yu RK. Intracerebral transplantation of neural stem cells combined with trehalose ingestion alleviates pathology in a mouse model of Huntington's disease. *J Neurosci Res* 2009;**87**:26–33.
 67. Lee ST, Chu K, Jung KH, et al. Slowed progression in models of Huntington disease by adipose stem cell transplantation. *Ann Neurol* 2009;**66**:671–681.
 68. Snyder BR, Chiu AM, Prockop DJ, Chan AW. Human multipotent stromal cells (MSCs) increase neurogenesis and decrease atrophy of the striatum in a transgenic mouse model for Huntington's disease. *PLoS One* 2010;**5**:e9347.
 69. Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. *Proc Natl Acad Sci* 2008;**105**:16707–16712.
 70. Yamamoto A, Lucas JJ, Hen R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* 2000;**101**:57–66.
 71. Liu Q, Paroo Z. Biochemical principles of small RNA pathways. *Annu Rev Biochem* 2010;**79**:295–319.
 72. Harper SQ. Progress and challenges in RNA interference therapy for Huntington disease. *Arch Neurol* 2009;**66**:933–938.
 73. Davidson BL, Harper SQ. Viral delivery of recombinant short hairpin RNAs. *Methods Enzymol* 2005;**392**:145–173.
 74. Xia H, Mao Q, Eliason SL, et al. RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. *Nat Med* 2004;**10**:816–820.
 75. Harper SQ, Staber PD, He X, et al. RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. *Proc Natl Acad Sci USA* 2005;**102**:5820–5825.
 76. Rodríguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ. Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. *Mol Ther* 2005;**12**:618–633.
 77. Machida Y, Okada T, Kurosawa M, Oyama F, Ozawa K, Nukina N. rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. *Biochem Biophys Res Commun* 2006;**343**:190–197.
 78. Huang B, Schiefer J, Sass C, Landwehrmeyer GB, Kosinski CM, Kochanek S. High-capacity adenoviral vector-mediated reduction of huntingtin aggregate load in vitro and in vivo. *Hum Gene Ther* 2007;**18**:303–311.
 79. Franich NR, Fitzsimons HL, Fong DM, Klugmann M, During MJ, Young D. AAV vector-mediated RNAi of mutant huntingtin expression is neuroprotective in a novel genetic rat model of Huntington's disease. *Mol Ther* 2008;**16**:947–956.
 80. McBride JL, Boudreau RL, Harper SQ, et al. Artificial miRNAs mitigate shRNA mediated toxicity in the brain: Implications for the therapeutic development of RNAi. *Proc Natl Acad Sci USA* 2008;**105**:5868–5873.
 81. Drouot V, Perrin V, Hassig R, et al. Sustained effects of nonallele-specific huntingtin silencing. *Ann Neurol* 2009;**65**:276–285.
 82. Boudreau RL, McBride JL, Martins I, Shen S, Xing Y, Carter BJ, Davidson BL. Nonallele-specific silencing of

- mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. *Mol Ther* 2009;**17**:1053–1063.
83. Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, Efstratiadis A. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat Genet* 1995;**11**:155–163.
 84. Nasir J, Floresco SB, O'Kusky JR, et al. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioural and morphological changes in heterozygotes. *Cell* 1995;**81**:811–823.
 85. Dragatsis I, Levine MS, Zeitlin S. Inactivation of *Hdh* in the brain and testis results in progressive neurodegeneration and sterility in mice. *Nat Genet* 2000;**26**:300–306.
 86. van Bilsen PHJ, Jaspers L, Lombardi MS, Odekerken JCE, Burright, Kaemmerer WF. Identification and allele-specific silencing of the mutant huntingtin allele in Huntington's disease patient-derived fibroblasts. *Hum Gene Ther* 2008;**19**:710–719.
 87. Lombardi MS, Jaspers L, Spronkmans C, et al. A majority of Huntington's disease patients may be treatable by individualized allele-specific RNA interference. *Exp Neurol* 2009;**217**:312–319.
 88. Pfister EL, Kennington L, Straubhaar J, et al. Five siRNAs targeting three SNPs in Huntingtin may provide therapy for three-quarters of Huntington's disease patients. *Curr Biol* 2009;**19**:774–778.
 89. Wang YL, Liu W, Wada E, Murata M, Wada K, Kanazawa I. Clinico-pathological rescue of a model mouse of Huntington's disease by siRNA. *Neurosci Res* 2005;**53**:241–249.
 90. DiFiglia M, Sena-Estevés M, Chase K, et al. Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. *Proc Natl Acad Sci USA* 2007;**104**:17204–17209.
 91. Crooke ST. Progress in antisense technology. *Annu Rev Med* 2004;**55**:61–95.
 92. Nellenman C, Abell K, Nørremølle A, et al. Inhibition of huntingtin synthesis by antisense oligodeoxynucleotides. *Mol Cell Neurosci* 2000;**16**:313–323.
 93. Boado RJ, Kazantsev A, Apostol BL, Thompson LM, Pardridge WM. Antisense-mediated down-regulation of the human huntingtin gene. *J Pharmacol Exp Ther* 2000;**295**:239–243.
 94. Hasholt L, Abell K, Nørremølle A, Nellenman C, Fenger K, Sørensen SA. Antisense downregulation of mutant huntingtin in a cell model. *J Gene Med* 2003;**5**:528–538.
 95. Haque N, Isacson O. Antisense gene therapy for neurodegenerative disease?. *Exp Neurol* 1997;**144**:139–146.
 96. Smith RA, Miller TM, Yamanaka K, et al. Antisense oligonucleotides therapy for neurodegenerative disease. *J Clin Invest* 2006;**116**:2290–2296.
 97. Kordasiewicz HB, McAlonis-Downes M, Pytel KA, et al. Suppression of mutant and normal huntingtin with CNS or peripheral infusion of antisense oligonucleotides. *Clin Genet* 2009;**76**:24.
 98. Stocks M. Intrabodies as drug discovery tools and therapeutics. *Curr Opin Chem Biol* 2005;**9**:359–365.
 99. Southwell AL, Khoshnan A, Dunn DE, Bugg CW, Lo DC, Patterson PH. Intrabodies binding the proline-rich domains of mutant huntingtin increase its turnover and reduce neurotoxicity. *J Neurosci* 2008;**28**:9013–9020.
 100. Wang CE, Zhou H, McGuire JR, Cerullo V, Lee B, Li SH, Li XJ. Suppression of neuropil aggregates and neurological symptoms by an intracellular antibody implicates the cytoplasmic toxicity of mutant huntingtin. *J Cell Biol* 2008;**181**:803–816.
 101. Southwell AL, Ko J, Patterson PH. Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. *J Neurosci* 2009;**29**:13589–13602.
 102. French J, Gronseth G. Lost in a jungle of evidence: We need a compass. *Neurology* 2008;**71**:1634–1638.