

Neuroprotection for Huntington's Disease: Ready, Set, Slow

Steven M. Hersch* and H. Diana Rosas†

*MassGeneral Institute for Neurodegenerative Disease and †Center for Neuroimaging of Aging and Neurodegenerative Diseases, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129-4404

Summary: The ultimate goal for Huntington's disease (HD) therapeutics is to develop disease-modifying neuroprotective therapies that can delay or prevent illness in those who are at genetic risk and can slow progression in those who are affected clinically. Neuroprotection is the preservation of neuronal structure, function, and viability, and neuroprotective therapy is thus targeted at the underlying pathology of HD, rather than at its specific symptoms. Preclinical target discovery research in HD is identifying numerous distinct targets, along with options for modulating them, with some

proceeding into large-scale efficacy studies in early symptomatic HD subjects. The first pilot studies of neuroprotective compounds in premanifest HD are also soon to begin. This review discusses the opportunities for neuroprotection in HD, clinical methodology in premanifest and manifest HD, the clinical assessment of neuroprotection, molecular targets and therapeutic leads, and the current state of clinical development. **Key Words:** Huntington's disease, neuroprotection, disease modification, neurogenetics, genetic testing, clinical trials, presymptomatic, premanifest.

INTRODUCTION

The ultimate goal for Huntington's disease (HD) therapeutics is to develop disease-modifying neuroprotective therapies capable of delaying or preventing clinical illness in those who are at genetic risk, and capable of slowing progression and permitting some recovery in those who have clinical illness. Neuroprotection can be defined quite literally as the preservation of neuronal structure, function, and viability, or more generically as the slowing or prevention of neurodegeneration. Neuroprotective therapy is thus targeted at the underlying pathology of HD, rather than at its specific symptoms.

The assumption we make is that selecting treatments based on their ability to limit neuropathology will be complementary to, and ultimately more fundamentally beneficial than, selecting treatments based on their ability to suppress symptoms. Basic and translational research in HD is creating an expanding pipeline of candidate neuroprotective therapies that are beginning to be tested in early- and late-phase clinical trials. Candidate therapies are generally filtered through genetic mouse models

of HD, and when supportive preclinical data demonstrate significant neuroprotection, a compound is considered for advancement to clinical trials.

The therapies already on this pathway are existing compounds that were selected by a candidate approach connecting hypotheses about mechanisms of disease and existing medications that might modulate them. Commercial and academic efforts that screen compound libraries against elements of the HD phenotype suitable for *in vitro* or cell-based assays are also bringing forward families of novel compounds to be sorted for efficacy, toxicity, and drugability (i.e., the potential for pharmaceutical delivery of the compound).^{1,2} These approaches are expanding the preclinical segment of the pipeline of potential neuroprotective treatments and are beginning to provide new compounds suitable for early-phase clinical testing.

This review presents a conceptual framework for the clinical development of neuroprotective therapies for HD and an overview of possible neuroprotective targets and compounds.

CLINICAL FEATURES AND THERAPEUTIC OPPORTUNITIES

Huntington's disease is an autosomal, dominant-inheritance neurodegenerative disorder that is characterized by progressive motor dysfunction, emotional disturbances, de-

Address correspondence and reprint requests to: Steven M. Hersch, M.D., Ph.D., MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, MGH East, Bldg. 114, Room 205, 114 16th Street, Charlestown, MA 02129-4404. E-mail: hersch@helix.mgh.harvard.edu.

mentia, and weight loss. The disease occurs worldwide, in all races and ethnic groups.³ Its prevalence is 5 to 10 cases per 100,000, and there is a new mutation rate as high as 1% to 3%.^{4,5} There are about 30,000 affected individuals in North America, and another 150,000 have a genetic risk for developing the disease. The incidence of being gene mutation-positive in the at-risk population is about 40% (60,000 individuals), because of individuals being diagnosed and depleting the pool of potential gene-positive individuals. The average age of clinical onset is about 37 years of age; however, the range is from infancy into the 80s.

Affected individuals are disabled by early functional decline and require increasing levels of care and support, usually with residential long-term care,⁶ and survive for about 15 to 25 years from the time of diagnosis before succumbing to the effects of severe physical and mental deterioration. Symptomatic therapies are few and have limited effect.^{7,8} There is no therapy proven to delay onset or slow progression,^{9,10} and the best current medical care has a positive impact by focusing multidisciplinary attention on symptom management and caregiver support and by maximizing function and quality of life.¹¹⁻¹⁴ Because of the early functional decline, the chronic and increasingly intensive care required, and the profound multigenerational impact on entire families,¹⁵ HD disproportionately consumes medical, social, and family resources. The principal target populations for neuroprotective therapies are those who are premanifest (not yet symptomatic but known to possess a huntingtin gene with the causative CAG expansion) as well as those who are manifest (overtly symptomatic), but not yet so advanced that there is a vastly diminished quality of life to preserve.

NEURODEGENERATION

Huntington's disease is caused by the expression of the aberrant huntingtin protein, which contains an abnormally expanded polyglutamine tract near its *N*-terminus.¹⁶ The presence of the abnormal huntingtin protein in cells sets off a complex and poorly understood series of deleterious and progressive biochemical events leading in neurons to stress, physiological dysfunction, compensatory responses, neurodegeneration, and eventually cell death. Neurodegeneration appears to be quite a prolonged process, as evidenced by signs of chronic neuroplasticity^{17,18} and of the slow involution of neurons with gradual loss of synapses, dendritic spines, dendritic branches, axonal segments, and supportive cytoplasmic resources such as mitochondria and organelles involved in the biosynthesis, modification, transport, and degradation of cellular molecules. The process of neurodegeneration ends in cell death, and dead and dying neurons *in situ* can cause local inflammatory responses and possibly worsen conditions in their immediate vicinity. Be-

cause neurons exist to influence one another, as well as the periphery, the neurodegenerative process can profoundly affect networks of interconnected neurons and the neurochemical, electrophysiological, and trophic lines of communication and regulation that underlie neuronal and neurological function. The progressive symptoms of HD clearly are caused by the functional network effects of neurodegeneration and neuronal death in many brain regions and the depletion of the brain's reserve capacity to compensate.

Location confers both function and dysfunction in the brain, and one neuropathologic hallmark of the neurodegeneration and cell death in HD is regional and cellular selectivity.¹⁹ The earliest and most aggressive neuropathologic changes are found in the neostriatum. As a result, there is a long tradition of ascribing the broad range of neurologic symptoms that occur in HD to basal ganglia circuitry and sometimes an excessive conviction that protecting the striatum is the principal goal of neuroprotective therapy. Striatal neurodegeneration undoubtedly provides a significant component of the early HD phenotype; marked neuronal loss has, however, been identified in many other regions of the brain,¹⁹ and phenotypes associated with damage to them are significant in HD. For example, the involvement and clinical contributions of cerebral cortical neurodegeneration have recently become more fully appreciated. Neuroimaging techniques are showing that cortical neurodegeneration is early, heterogeneous, progressive, and correlates strongly with HD symptoms.^{20,21} It seems likely that cortical dysfunction and degeneration play significant roles in the motor control, cognitive, and psychiatric symptoms of HD and in the heterogeneous evolution of clinical symptoms as neurodegeneration plays out and involves more and more of the cerebral cortex. An important implication is that cell replacement, trophic support, RNA interference, or other potential neuroprotective treatments that might be focally applied to the striatum may have very limited usefulness.

NEUROPROTECTION

Neuroprotection in premanifest HD

Individuals destined to develop HD are born with the HD genetic mutation. Because the abnormal huntingtin protein is present, it is possible that there are biochemical or other abnormalities present from birth. All existing information, however, suggests that most of these individuals experience a period of apparent clinical and biological normalcy before entering a prodromal period in which there is active underlying disease without any evident clinical or functional consequences. Current evidence indicates that the causative processes involved in HD are present for at least 10 to 20 years before HD can

be diagnosed clinically^{21–28} on the basis of the unequivocal presence of the movement disorder.

The transition from HD prodrome to manifest HD (an expression of diagnostic certainty made by the assessing physician) is termed *phenoconversion*.²⁹ Progressive atrophy of the striatum and cerebral cortex has been well documented to occur in the premanifest prodrome period, indicating that the neurodegenerative process is ongoing.^{21,28,30} Subtle cognitive, motor, psychiatric,^{31–33} and metabolic abnormalities^{34,35} are detectable in premanifest HD, and biochemical alterations are beginning to be detected in blood.^{24,36} It is unknown whether the HD prodrome represents a stable condition until some decompensation causes manifest HD to emerge, or whether it is a continuum in which clinically silent neurodegeneration gradually accumulates sufficiently to cause unequivocal symptoms. The pace of the disease process can certainly vary over time, or can be accelerated by other stresses. For example, we have observed clinical onset to be hastened by significant traumatic brain injury.

Regardless, it seems desirable to begin a neuroprotective therapy before or during the prodrome, with the aim of delaying onset, as well as slowing progression of the underlying pathologic processes while they are still subclinical. This should not be considered prevention, however, because the treatment is aimed at active disease. Because there are about five times as many at-risk as symptomatic individuals, an even larger ratio of potential years of neuroprotective treatment, and probably less to gain from treating the most advanced individuals, the premanifest population represents the largest therapeutic opportunity for HD for neuroprotection. In addition, because symptomatic individuals generally become unable to work within a few years, delaying their disability and dependence would have great economic and social benefits.

Two large ongoing observational trials (PHAROS, PREDICT-HD)^{29,37} conducted by the Huntington Study Group and conceived to help design and power efficacy trials in this population will be completed soon. These studies will help determine how to power a neuroprotection study seeking to delay onset based on an expected rate or time of phenoconversion.^{38,39} In addition, there is active research examining possible biomarkers that could provide endpoints for detecting and monitoring progression during the HD prodrome. The first pilot interventional studies in presymptomatic individuals using putative neuroprotective compounds will be underway during 2008.

Because premanifest individuals are healthy and fully functional, the period of neuroprotective treatment for these individuals could be for decades. Therefore, the ideal intervention requires a very high level of safety and tolerability. Accordingly, CoQ₁₀ and creatine, which are capable of

ameliorating energy depletion and whose efficacy in manifest HD will be known in a few years, have been suggested by SET-HD (Systematic Evaluation of Treatments for Huntington's Disease: an independent program to identify, systematically assess, and prioritize experimental therapies for HD; <http://www.huntingtonproject.org>) to be appropriate for examination in premanifest HD. These compounds will be tested first in separate studies, supported by the U.S. National Institutes of Health, to examine safety, tolerability, dosing, and biomarkers.

An important issue in designing a neuroprotection trial in the premanifest population is the fact that the vast majority of these individuals (>95%) have not desired genetic testing.⁴⁰ Some reasons for this include fear of genetic discrimination,⁴¹ the lack of effective treatment, and concern about the negative consequences of genetic testing.^{42,43} Focus groups with at-risk individuals have revealed that many would be adverse to taking part in clinical trials if informative genetic testing is required. Similarly, many would willingly take an experimental medication and risk side effects in a clinical trial without genetic testing, understanding that there would be about a 60% chance of not having the gene mutation.

Performing a clinical trial only in subjects who have had genetic testing is feasible for smaller scale, early-phase studies. Without a dramatic upsurge in genetic testing, however, it is difficult to imagine sufficient subjects being available for most efficacy designs seeking evidence for neuroprotection. Furthermore, the population of available premanifest subjects who have had testing may not be fully representative of the at-risk population.

Requiring genetic testing for entry into a desirable clinical trial raises an important ethical concern about creating a coercive incentive for genetic testing, along with its negative consequences in subjects wishing to participate. To allay this concern and also to have normal controls for tolerability and biomarker measures, an option is to allow the enrollment of individuals who either (a) are at risk for HD by virtue of having a first-degree relative with HD or (b) have tested positive for the HD gene mutation. Approximately 60% of the former would be gene negative, and all of the latter would be gene positive. This novel design would greatly neutralize any incentive for genetic testing.

If a high level of safety and tolerability can be expected from the intervention, treating gene-negative individuals is preferable to coercing genetic testing, which carries its own risks.⁴⁴ A design to perform double-blinded genetic testing and provide placebo to gene-negative individuals and active compound to gene-positive individuals might also be feasible, although it would be complicated to administer. These will be important considerations in designing clinical trials in premanifest HD—something for which there is no road map.

Neuroprotection in manifest HD

Once diagnosed clinically, individuals with HD have a highly variable phenotype. Different affected individuals can have predominantly motor (chorea, dystonia, bradykinesia), predominantly cognitive (executive dysfunction), or predominantly psychiatric (depression, emotional dyscontrol, psychosis, obsessive–compulsive symptoms) presentation. Symptom severity can be modulated by many temporary factors such as mood, nutrition, medications, and sleep disturbances. Accordingly, treatments benefiting symptoms may or may not do so on the basis of neuroprotection. Additionally, each symptom in HD has its own temporal course of onset, progression, plateau, and waning—most likely because the neurodegeneration in the brain that underlies the symptoms reaches some point at which its clinical expression has little room to progress further. For example, chorea may be present early, worsen for some years, stabilize, and wane as it is supplanted by dystonia and spasticity. Regional cerebral cortical neurodegeneration in HD is heterogeneous in time and location, which may explain some of the differences between patients, as well as the evolution of symptoms in time, despite the early and stereotypical decimation of the striatum.²⁰

Despite the great day-to-day variability in symptoms and their uneven evolution over time, the overall clinical progression of HD is slow, as assessed by an integrated measure of functional capacity (e.g., the Total Functional Capacity scale, or TFC⁴⁵). Clinical trials in early symptomatic HD include subjects with a TFC of about 7 or greater. This encompasses a broad phenotypic range: from normal to impaired but functional at home and work, to unable to work but independent at home, to needing some assistance at home but still ambulatory, independent for ADLs, and competent to direct their activities. These subjects retain a high quality of life, and slowing their decline would delay loss of independence and the need for an escalation in care resources, including residential long-term care. From the time an individual is diagnosed as having unequivocal clinical symptoms, it takes about 5 to 10 years for the TFC to decline to 7 from a normal score of 13. There is sufficient phenotypic range in this population to observe both progression and its slowing by a disease-modifying treatment, and power calculations are currently based on this information.

NEUROPROTECTION AND TREATMENT APPROVAL

Measurement of neuroprotection

Neuroprotection can be defined as a beneficial treatment effect on a biological process that contributes to neurodegeneration in HD and thus to clinical progression. Many possibilities for measuring such effects must be considered carefully when designing a clinical trial. In early-phase clinical trials, evidence for potential efficacy

may be of secondary interest if examining dosing, pharmacokinetics, pharmacodynamics, safety, and tolerability are the principal objectives. Preliminary evidence of potential neuroprotective efficacy from early-phase studies, however, can be vital for decision-making about whether to continue the development of the treatment into large and expensive phase III studies. A great need for HD has been clinical or other outcome measures that can provide that preliminary evidence. Indeed, without such signals it is also very difficult to stop development of a compound short of its failure in a large-scale study.

Clinical measures, such as those contained in the Unified Huntington's Disease Rating Scale (UHDRS), can reveal an effect on HD symptoms, although symptomatic effects (whether better or worse) and neuroprotective effects cannot be considered to be synonymous. Global clinical measures that correspond to disease progression, such as the TFC or other indicators of functional decline, are insensitive measures in typical early-phase studies (involving anywhere from a few to 100 subjects, with durations measured in weeks to months).

There are refined, often quantitative, clinical measures of motor and cognitive dysfunction that can be sensitively measured in HD subjects, including premanifest subjects. Some of these could be surrogates for measuring progression of the underlying disease and serve as indicators of the disease-modifying potential of a treatment. Because it is difficult to relate response magnitudes for such measures to clinically significant benefits, they are not yet useable as primary endpoints for assessing efficacy. Even more promising are biomarkers from neuroimaging or from biological samples or fluids ('wet' biomarkers) that could provide measures of disease activity or progression.

To the extent that such biomarkers suggest a neuroprotective effect, they can be supportive of decisions about whether a compound should continue in development. Biomarkers are especially promising for this purpose, because neuroimaging showing slowed regional or whole-brain atrophy or 'wet' biomarkers showing a pharmacodynamic response are especially close to the biology of neurodegeneration can be revealing in premanifest or manifest HD, and may have sufficient sensitivity in small sample size studies. Keep in mind also that brain volumes could be affected by a treatment without an accompanying effect on neurodegeneration, and that 'wet' biomarkers may capture only limited aspects of the entire biochemistry underlying neurodegeneration and thus may not be predictive of a significant clinical response later. Regardless, such indicators of potential benefits that could best be explained by disease modification should increase the likelihood of demonstrating efficacy in a phase III study.

At the same time, negative findings can help in decision-making about whether the likelihood of a neuropro-

tective effect from a potential treatment is too low to justify further investment. In the absence of accepted biomarkers, an alternative for seeking preliminary evidence of efficacy is the futility design.⁴⁶ With a futility design, the TFC can still be used as the primary outcome measure, but the prespecified indicator is sufficient divergence between it and a standard derived from historical or limited placebo controls such that the probability of the two groups being different crosses a threshold indicating that some minimal difference is likely. An ongoing futility study examining minocycline in HD will enroll 124 subjects, who will be followed for 18 months. Such studies are still relatively large, and (despite the investment) a finding of nonfutility will not normally count as a demonstration of efficacy for regulatory purposes.

The U.S. Food and Drug Administration (FDA) considers for approval treatments that are sufficiently demonstrated as providing a significant clinical benefit. Slowing of the decline of the TFC has been acceptable to the FDA as the primary outcome measure in efficacy studies designed to determine if a treatment slows functional decline, an integrated clinical reflection of neuroprotection. Currently, efficacy studies in symptomatic patients with HD using the TFC as the primary outcome require about 650 subjects and 3 years of follow-up to detect a 25% slowing of decline (1:1 randomized placebo controlled trial, and assuming 10% to 15% dropout). Other global clinical measures, such as quality of life scales or functional disability measures, could likewise serve as outcome measures reflecting disease modification or neuroprotection during manifest HD, and measures with greater power than the TFC would enable testing more treatments.

The expense, time, and magnitude of effort needed to test efficacy means that few interventions can be tested. Furthermore, having large numbers of subjects on placebo treatment for years in these trials is an unfortunate necessity. Biomarkers of disease biological activity can be used to help answer whether treatments have the desired pharmacologic effects, and whether there are potential explanations for response heterogeneity. Biomarkers corresponding to disease progression that could help assess efficacy could thus supplement the TFC or other clinical measures as secondary endpoints and, if qualified, may ultimately serve as surrogate endpoints for regulatory purposes, enabling the testing of disease modification in fewer subjects more quickly. These would have to first be qualified in successful neuroprotection trials, but they could enable more efficient testing of more potential neuroprotective therapies in the future. It is a high priority to take every available opportunity to study possible biomarkers in therapeutic trials to bring that day closer.

No measures of clinical effect suitable for regulatory approval exist for premanifest HD, because these indi-

viduals have no disability to measure, even if they have measurable symptoms or signs.⁴⁷ At present, an efficacy study in premanifest HD would have to be designed to slow the rate of phenoconversion to meet a regulatory standard of demonstrating a clinical benefit. To examine whether a treatment delays the onset of clinical symptoms in premanifest individuals with the HD genetic mutation, it has been estimated that a daunting 1000 to 3000 subjects and 3 to 6 years of follow-up would be necessary to detect even a large 30% to 40% decline in the frequency of symptom onset.

A regulatory approval for a neuroprotective treatment based on studies in manifest HD could be applicable to premanifest HD, because there is no reason to think premanifest and manifest HD differ biologically. Regulators could, however, view concerns about safety differently in premanifest and manifest individuals, such that toxicity acceptable in symptomatic individuals might not be considered acceptable in asymptomatic individuals. There might also be additional concerns about treating healthy individuals for many years before symptoms occur, because of uncertainty about long-term toxicity and also uncertainty about identifying the period in which it is necessary to treat for the clinical benefit of delayed onset. Thus, a treatment could potentially be approved with an indication for symptomatic individuals specifically, although once it is approved the treatment would be in the hands of prescribers.

Biomarkers could provide pharmacodynamic indicators of disease slowing in premanifest HD in the absence of clinical symptoms and provide evidence of disease modification in smaller-scale trials. Ideally, biomarkers indicating subclinical disease activity or clinical predictors of future disease onset could eventually help decision-making in practice about when to start a neuroprotective treatment in premanifest HD. A potential strategy to include premanifest individuals in clinical research and in the approval process is to perform pivotal phase III efficacy studies in early manifest HD and supplement these with limited studies examining safety, tolerability, and biomarkers of disease progression in premanifest HD. Should a treatment prove efficacious in manifest HD and similarly affect biomarkers of disease progression in premanifest and manifest HD, it may be possible to build a rationale for not excluding premanifest individuals in the treatment label. There is as yet no clear road map for regulatory approaches for an indication to treat premanifest HD.

The role of preclinical genetic mouse models in initiating neuroprotection trials

Candidate neuroprotective treatments for HD can be tested in proof-of-concept studies using genetic mouse models of HD, either transgenic mice expressing part or all of an exogenous mutant huntingtin or knockin mice in which the polyglutamine tract in the HD gene is patho-

logically expanded. Positive results in these models have promoted go-ahead decisions in industry and academia for clinical trials in HD subjects. The predictive value of the mouse models, though logical, has not yet been validated, because there have not yet been sufficient efficacy studies in humans testing compounds effective in mice. There is also a history of disappointment with predictability from animal models of other neurologic diseases, such as stroke and amyotrophic lateral sclerosis. We think mouse models of HD may be more representative of the disease than in those other cases, given their underlying genetic fidelity. There are other reasons, however, apart from model accuracy, why mouse data may not scale to humans, such as inherent biological differences and bioequivalence differences with the treatment. Currently, it is fair to state that positive mouse data do not necessarily predict efficacy and negative mouse data do not necessarily predict failure. Nevertheless, positive data in mouse models can be supportive when considering all of the factors around the potential rationale for testing a putative neuroprotective treatment in human HD.

Several categories of data that can be obtained in pre-clinical models can be helpful. The least helpful are positive or negative behavioral or motor performance data, because the outcome measures can be influenced pharmacologically without affecting neuroprotection (preservation of neuronal structure, function, and viability). Because neurodegeneration is defined neuropathologically, neuropathologic indicators of neuroprotection provide the most useful proof-of-concept information. These include measures such as brain weight, atrophy, ventricular size, neuronal size, and neuronal number, and there are established means for measuring each of these. Modulation of the presence of huntingtin protein aggregates in brain does not correlate strictly to toxicity or to neuroprotection, but can provide information about how the treatment affects huntingtin itself. Behavioral data have much more meaning in the context of the underlying neuropathology, which enables interpreting the data in relation to neuroprotection.

Other types of studies in HD mice can help by filling in understanding about how neuroprotection might occur (or fail) and by modeling potential biomarkers. Despite the uncertain predictive value of positive data in HD mouse models, there are some principles for assessing the usefulness of the data, especially in the setting of a HD research community with extensive experience focused on such studies. At the simplest level, any positive evidence for neuroprotection (neuropathology) is better than none.

Most negative data are not as compelling as positive data, because there are many more alternative explanations for achieving the former than the latter. Negative studies in HD mouse models are quite easily achieved and rarely published, and there are many reasons why

these may not reflect the neuroprotective potential of a compound. These can include the fidelity of the model, genotype or strain effects, experimental conditions, the many variables that relate to optimal brain bioavailability, and methodological errors. For positive results, the more rigor the better in terms of methodology, limitation of bias, replication, controls, and exposure to peer review. Replication is especially powerful for confirming a result, and this could be in different cohorts, in different labs, in alternative mouse models, or by using distinct but related compounds. Ideally, positive evidence for neuroprotection from more than one laboratory, with more than one mouse model, using neuropathology outcome measures, and using more than one compound provides the maximal preclinical proof-of-concept support. Lesser levels of evidence than this ideal can certainly be supportive, with their limitations understood, when formulating how the mouse data contribute to decision-making about translation to human testing, where many other considerations come into play.

CANDIDATE TARGETS AND THERAPIES

Candidate targets for neuroprotection

Huntington's disease is caused by the expression of the abnormal huntingtin protein which contains an expanded polyglutamine tract near its *N*-terminal. Although many leads have been uncovered, a stepwise pathway from huntingtin to neuronal dysfunction and death has not been established. Huntingtin is a widely expressed, predominantly cytoplasmic, protein of unknown function found heterogeneously in neurons throughout the brain and widely in the body.⁴⁸⁻⁵² In HD, both normal and mutant alleles are expressed, and both gain of function alterations (in which mutant huntingtin is toxic) and loss of function alterations⁵³⁻⁵⁵ (in which suppression of normal huntingtin functions might also be toxic) have been identified.

Proteolysis of mutant huntingtin, whereby abnormal and ultimately toxic *N*-terminus fragments of huntingtin are released,⁵⁶⁻⁵⁹ seems to play a dominant role in causing disease.⁶⁰ Most evidence points to a proximal toxicity residing in mutant huntingtin or its proteolytic fragments and their soluble interactions with other proteins, including huntingtin itself or the hundreds of other proteins that have been demonstrated to associate with huntingtin.⁶¹ Proximal events mediated by mutant huntingtin in turn trigger cascades of both damaging and compensatory molecular processes and genetic programs. These events and sequelae include mitochondrial dysfunction, energy depletion,⁶²⁻⁶⁴ oxidative stress,^{65,66} DNA damage,⁶⁷ synaptic stress,⁶⁸ disordered neurophysiology,⁶⁹ proapoptotic signals,⁷⁰ protein aggregation,⁷¹⁻⁷³ malfunctioning proteolysis,⁷⁴ autophagy,⁷⁵⁻⁷⁹ ubiquitin/proteosomal dysfunction,⁸⁰⁻⁸² neurotrophin deficiency,⁸³⁻⁸⁵

and disrupted intracellular transport⁸⁶—all of which might play a role in neuronal death.⁸⁷ The presence of huntingtin or its fragments in the nucleus seems to particularly drive pathology.^{88,89} This is likely due to interactions in the nucleus with a variety of transcription factors and regulators leading to complex and multifaceted transcriptional alterations that also seem important to pathogenesis and reverberate through cellular biochemistry.^{90–101}

These various complex processes ultimately lead to increasingly fragile, atrophic, dysfunctional neurons that ultimately die. These processes have suggested many potential therapeutic targets:

- Huntingtin production
- Huntingtin cleavage into toxic fragments
- Nuclear transport of huntingtin
- Huntingtin misfolding
- Huntingtin clearance
- Huntingtin posttranslational modifications
- Protein aggregation
- Transcription factor/complex function
- Chromatin regulation
- Energetic abnormalities
- Oxidative stress
- Synaptic stress
- Cell death signaling
- Autophagy
- Proteasome dysfunction
- Neurotrophin deficiencies

Some of these therapeutic targets have had some pre-clinical validation through studies in HD transgenic mice and are represented in the increasing pipeline of possible disease-modifying therapies. What is uncertain is the relative importance and interdependence of each, although reducing levels of mutant huntingtin (e.g., by RNA interference^{102–106} or suppressing its cleavage,⁶⁰ improving its misfolding,¹⁰⁷ or reducing its nuclear transport) might be the most fundamental (i.e., proximal).

Candidate therapies for Huntington's disease

Not all of the potential neuroprotective targets just listed have been subjected to proof-of-concept studies in HD mouse models; however, preclinical studies in HD transgenic mice have provided a basis for testing a growing list of compounds in HD. Included are compounds that enhance mitochondrial function or suppress oxidative injury, such as coenzyme Q10,¹⁰⁸ creatine,^{109,110} dichloroacetate,¹¹¹ α -lipoic acid,¹¹² the antiapoptotic antibiotic minocycline,¹¹³ the transglutaminase inhibitor

cystamine,^{114,115} metal chelators,^{116,117} glutamate antagonists and other neurotransmitter modulators such as remacemide, riluzole,^{118,119} and paroxetine¹²⁰; transcriptionally active compounds such as histone deacetylase inhibitors, DNA intercalating agents,^{121–124} and agents, such as RNA interference, for blocking the translation of huntingtin protein itself;^{102–106} and tauroursodeoxycholic acid (TUDCA)¹²⁵ or other agents that might act by increasing levels of brain-derived neurotrophic factor (BDNF), which has been implicated repeatedly in HD,¹²⁶ although its potential as a therapeutic target remains untested. There are so many potential disease-modifying therapies that they are not reviewed exhaustively here. The reader is instead referred to the SET-HD website (Systematic Evaluation of Treatments for Huntington's Disease; <http://www.huntingtonproject.org>).

Most clinical trials to date in humans with HD have focused on preventing oxidative and glutamatergic stress. These studies, many of which were multicenter trials organized by the Huntington Study Group, included the antioxidants idebenone, vitamin E,^{127–129} creatine,^{130,131} coenzyme Q₁₀ (CoQ₁₀),¹³² the antioxidant transglutaminase inhibitor cysteamine,¹³³ ethyl-eicosapentaenoate,¹³⁴ and the glutamate antagonists lamotrigine,¹³⁵ remacemide,¹³² and riluzole.^{119,136,137} These studies have not demonstrated slowing of functional decline, but most have been phase II studies that were not powered to test efficacy.

In the CARE-HD (CoQ₁₀ and Remacemide Evaluation in Huntington's Disease) study completed by the Huntington Study Group, a trend toward slowed progression was observed with CoQ₁₀, whereas remacemide, although not affecting progression, appeared to improve chorea.¹³² We note that the rates of slowed progression in CARE-HD and survival prolongation in the mice treated with CoQ₁₀ were the same (~14%). Remacemide was neuroprotective in mice, but not in CARE-HD; however, the maximally tolerated doses used were much greater in mice than in humans. Thus, there is some evidence that preclinical results in mice may be predictive of therapeutic responses in humans. Given the encouraging preliminary results in CARE-HD, a series of studies testing the pharmacokinetics, safety, and efficacy of higher doses of CoQ₁₀ is ongoing.

Additional active or planned therapeutic clinical trials for HD in manifest individuals using potential neuroprotective treatments include a high-dose phase III creatine trial expected to start in 2008, a phase II trial of phenylbutyrate (the results of are to be reported in 2008), and a futility study using the antiapoptotic antibiotic minocycline. The incorporation of biomarkers and exploratory clinical outcome measures in these studies should facilitate and improve future neuroprotection trials.

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