Huntington’s disease: progress and potential in the field

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1. Background

Huntington’s disease (HD) is an inherited autosomal dominant disorder of the CNS. Roughly 250,000 Americans are affected by or are at risk of inheriting this fatal disease. Clinically, HD is characterized by cognitive and memory impairments, heightened irritability, depression, weight loss and choreic motor abnormalities [1]. Interestingly, subtle behavioral alterations occur prior to clinical diagnosis [2,3], consistent with evidence of altered neuropathology before manifest disease [4]. Once manifest disease occurs, however, the duration of HD is ~ 15 – 20 years, with clinical symptoms becoming increasingly disabling prior to death [1]. Despite great progress, a direct causative pathway from the HD gene mutation to neuronal death has not yet been established. As there is no proven therapy to delay or slow the progression of HD, there is a growing demand on improved clinical management for affected individuals and their families.

While other brain regions are altered in HD, the most prominent neuropathological feature is marked gross atrophy of the neostriatum with concomitant neuronal degeneration within the caudate and putamen. There is a topographic progression of neuronal loss and astrogliosis first observed in the dorso-medial aspect of the striatum and progressing ventro-laterally, with relative sparing of the...
ventral striatum [5]. Not all neurons are affected equally within the neostriatum [6]. While large cholinergic neurons and medium-sized NADPH-diaphorase aspiny neurons remain relatively spared in HD, medium-sized spiny GABAergic projection neurons of the striatum, which make up ~95% of striatal neuron content, are disproportionately affected early and most severely [6-9]. There is also a reduction in striatal neurochemicals that parallels striatal neurodegeneration in HD [10,11], with substance P-expressing striatal projection neurons appearing more vulnerable [12]. In addition, striatal medium spiny neurons show both proliferative and degenerative changes leading to neuronal dysfunction and cell death [13,14].

HD is caused by an expanded trinucleotide CAG repeat in the gene coding for the protein, huntingtin (Htt) [15]. In HD patients, the CAG repeat is expanded beyond the normal repeat range, >38 repeats, with an inverse relationship between the CAG repeat number and the age of onset, with higher repeats associated with younger age [16]. Expression of Htt is observed throughout the brain within the nucleus, cytoplasm, axons and dendrites of neurons [17-19]. While the function of normal Htt is not known, it is believed to play a role in cellular transport mechanisms [17,20]. Cleaved during proteolysis, mutant Htt (mHtt) releases a persistent N-terminal fragment that contains the expanded polyglutamine amino acid sequence. This fragment, which is responsible for cellular toxicity, forms aggregates with itself and other proteins seen in both the cytoplasm and nucleus [18]. The aberrant protein interactions of mHtt and formation of mHtt aggregates may lead to neuronal toxicity through a toxic gain of function mechanism. Observations where nuclear aggregates have been seen in interneuron populations known to be resistant to HD-induced neurodegeneration [19], suggest that soluble mHtt is the harbinger of neurodegeneration. In contrast, additional evidence suggests that the aggregated form of mHtt is toxic and can induce neurodegeneration [21].

2. Existing clinical management and need

Existing care in HD is focused primarily on symptom management. As such, a number of drug agents have been used to treat the motor and behavioral changes found in HD. Both amantadine [22] and memantine [23], glutamatergic N-methyl-D-aspartate (NMDA) antagonists, have been used to treat chorea. While haloperidol has also been used in the management of dystonia and psychosis in HD, treatment is complicated by extrapyramidal side effects [24]. More recent data regarding the type 2 vesicular monoamine transporter inhibitor, tetrabenazine, which selectively blocks dopamine release, demonstrates significant improvement in chorea in ambulatory HD patients [25]. Clinical practice in HD also employs a number of antipsychotic agents for the management of depression, anxiety and other psychiatric disturbances. These include the selective serotonin uptake inhibitors sertraline and fluoxetine and/or benzodiazepines, such as clonazepam, diazepam, risperidone or sulpiride [26]. There is no significant evidence that any given antipsychotic drug is better than another in the treatment of symptoms.

3. Mechanisms of cell death and potential therapeutic targets in Huntington’s disease

3.1 Mutant Huntingtin aggregation (mHtt)

While the gene responsible for HD was discovered almost 15 years ago [15], the relationship between mHtt and the multiple molecular pathways that appear to mediate neuronal death in HD is still not well understood. Transglutaminase activity has been proposed to mediate mHtt aggregation [27]. There is ample evidence regarding transglutaminase expression in HD [28-30] and a role for transglutaminase in HD pathogenesis is now well accepted. Interestingly, proteins containing polyglutamine expansions, such as mHtt, are degraded in a limited context by the ubiquitin–proteosome system (UPS) [31]. However, recent data suggests that the proteasome may not cleave polyglutamine sequences within the mutant protein [31]. Ongoing debate continues, as with other neurodegenerative disorders in which protein aggregates are a hallmark of disease, questioning whether inclusions formed by the aggregated N-terminal truncation of mHtt cause neuronal death through alterations of nuclear transport or DNA arrangements affecting transcription. Recent studies have suggested a protective role for aggregation [19,32,33]. Through the use of an automated microscopy technique to assess the time frame in which neurons expressing mHtt expire, improved survival of neurons that contained mHtt aggregates has been shown [32]. Whether the formation of mHtt aggregates is protective or toxic, it is clear that coincident with mHtt aggregation, there are additional pathogenic cascades at work in HD.

3.2 Oxidative stress and mitochondrial dysfunction

Several lines of evidence lend support for mitochondrial dysfunction and increased oxidative stress in HD [34]. There is a general reduction in striatal glucose utilization in both human and transgenic mouse HD brain that precedes tissue loss [35,36], as well as a reduction in the activity of several mitochondrial complexes [37,38]. Lactate levels are elevated in the striatum and cortex, with the increase correlated with the CAG repeat size [39]. Biomarkers of oxidative stress are also elevated in human and transgenic mouse HD brain and serum, such as DNA oxidative modifications and strand breaks [40-42] and deletions in mitochondrial DNA [43].

Recent evidence suggests that mHtt interacts directly with mitochondria in HD [44]. This interaction causes an alteration in mitochondrial calcium buffering, leading to mitochondrial dysfunction. In addition, it has been reported that mHtt represses peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) [45]. PGC-1α is a
transcriptional co-activator regulating a number of genes and metabolic processes that protect against reactive oxygen species and is important in the mitochondrial regulation of ATP [46]. Reduced levels of PGC-1α result in striatal neurodegeneration and motor abnormalities in HD mice, along with increased sensitivity to oxidative stressors. Importantly, delivery of lentiviral-mediated PGC-1α expression into the striatum of R6/2 mice significantly improved the pathological phenotype.

3.3 Transcriptional dysregulation
Another profound aspect of disease pathology in HD is the alteration in gene transcription [47]. While the precise molecular basis for transcriptional dysregulation is unknown, abundant evidence suggests a direct interaction between the mHtt protein and transcription factors [48,49]. Through sequestration of transcription factors into mHtt aggregates, it is thought mHtt brings about alterations in gene expression as observed in both human HD and murine models of HD [50-52]. Importantly, transcriptional alterations associated with mHtt appear presymptomatically, suggesting such dysregulation is not an epiphenomenon. As such, there is now strong evidence that transcriptional dysfunction is related to histone hypoacetylation and hypermethylation in HD [53,54]. Experimental studies in murine models have demonstrated significant hypoacetylation of histone H4 [55-57], while hypermethylation of histone H3 is observed in human HD patients and HD mice [56-58].

3.4 Apoptosis
Pro-apoptotic signaling cascades initiated by mHtt likely play a role in mHtt-induced striatal neurodegeneration. In apoptotic-induced cell death, signaling cascades activate multiple proteases that destroy proteins essential for neuronal survival, along with a concurrent activation of genes involved in cell suicide [59]. The primary constituents of the apoptotic cascade are the cytokine proteases known as caspases. There are at least four initiator caspases and at least three effector caspases, including caspase-3, -6 and -7 [60].

Expanded polyglutamine stretches have been shown to sequentially activate the initiator caspases [60]. There is increasing evidence implicating apoptosis-mediated cell death in the pathogenesis of neurodegenerative diseases. One important event in the apoptotic cascade is the release of cytochrome C by mitochondria into the cytoplasm, activating caspase-9 and leading to the subsequent activation of downstream executioner caspases [61]. Given apoptotic activity in HD, pharmacologic inhibition of proteins involved in various levels of the signaling cascade may represent a potential beneficial therapeutic strategy to treat HD.

3.5 Excitotoxicity
In HD, excessive glutamatergic input to the striatum is hypothesized to contribute to the striatal neurodegeneration observed. Evidence supporting the excitotoxic hypothesis stems from observations of similarities between kainic, glutamic and quinolinic acid lesions and the striatal pathology observed in rodent and primate models of HD [62-65]. Increases in striatal glutamate in the brains of HD patients [66], as well as alterations in presynaptic glutamate receptors in the R6/2 murine model of HD [52], lend additional support to the role of aberrant glutamate excitotoxicity in HD pathogenesis. Given the fact that increased levels of excitatory amino acids are not elevated in HD, the concept of slow excitotoxicity in HD was suggested by Albin and Greenamyer and Beal as an alternative excitotoxic hypothesis in which normal circulating levels of glutamate could result in neuronal dysfunction and death [67,68].

Given the multiple mechanisms driving HD neuropathology, therapeutic intervention at any number of accessible points should prove beneficial for treating HD (Figure 1 and Figure 2). Importantly, targeting multiple pathogenic mechanisms may hold the greatest potential to ameliorate or prevent disease progression in HD. Notwithstanding, recent therapeutic trials in murine models of HD have provided preclinical proof-of-principle and rationales for clinical trials in human HD, with some success in HD patients.

4. Therapeutic candidates

4.1 Mitochondrial dysfunction and oxidative stress
Given the role of oxidative stress associated with mitochondrial dysfunction, several preclinical antioxidant strategies have been employed with promising success. First among these is the guanidine compound creatine, which, while produced endogenously, is also obtained from the diet (see Table 1 for a compound list) [69]. In addition to its antioxidant capacity, creatine also buffers intracellular energy reserves, stabilizes intracellular calcium and inhibits activation of the mitochondrial transition pore [70]. In neurons, creatine can exist as free substrate or phosphocreatine (PCr). According to the PCr shuttle hypothesis, sites of energy production are connected with sites of energy consumption when creatine kinase mediates the transfer of a phosphoryl group from PCr to ADP, creating ATP [71]. In HD, there is a significant shift in the ratio of PCr to phosphate [39]. Thus, creatine administration may be able to restore normal metabolic activity. To this end, several preclinical studies have provided ample evidence of the neuroprotective benefit of creatine in chemical and animal models of neurodegenerative disease, including HD [72-78].

In the R6/2 mouse, creatine significantly improved survival and motor performance, ameliorated brain and striatal atrophy and reduced striatal mHtt aggregation in a dose-dependent manner. Oral creatine administration also increased brain levels of creatine. This effect has been confirmed in another animal model of HD [72], suggesting the significant promise of creatine administration in the treatment of HD.
Several clinical trials show safe and tolerable doses of creatine in HD patients in the range of 5 – 10 g/d [41,79-81]. Creatine treatment in human HD resulted in a significant reduction in brain glutamate [79] and oxidative stress, as measured by 8-hydroxy-2′-deoxyguanosine (8OH2′dG) [41]. The 8OH2′dG findings are the first instance of parallel efficacy using a common peripheral biomarker in the administration of a therapeutic agent in HD mice and HD patients. However, no studies have been sufficiently powered to detect a significant slowing of progression or improvement in clinical measures. Although in a 1-year open-label pilot study, creatine (10 g/d) administered for 12 months resulted in unchanged Unified Huntington’s disease Rating Scale (UHDRS) scores, suggesting that creatine may be effective in stabilizing disease progression [82]. Although the optimal dose of creatine is not yet certain, it is possible that the dose of creatine supplementation in the above studies may have been underestimated.

Recently, the authors examined the effects of a high dose creatine administration in multiple murine models of HD [83]. High-dose creatine administration was well tolerated by both R6/2 and CAG140 mice and, at dosages > 200% of previously successful preclinical dosing strategies [74], significant improvement in survival and motor performance was demonstrated. Further analysis revealed significant improvements in striatal neuropathology, with concomitant reductions in both mHtt aggregation and 8OH2′dG levels. In addition, there was a significant creatine-mediated improvement in striatal ATP levels. While drug trials in mice confirm therapeutic direction, the challenge is in determining what dose might be of value in patients, as the pharmacokinetics of mice and humans is dissimilar. As such, a much higher dose may be feasible for humans. In this regard, a dose escalation study up to 40 g/d to determine whether there is a maximally tolerated dose in HD, as well as whether there are doses at which serum and brain levels of creatine are maximized, have been initiated. Preliminary results suggest that creatine (20 – 30 g/d) is effective in slowing disease progression.

Another antioxidant compound that has demonstrated preclinical efficacy in multiple murine models of HD is coenzyme Q10 (CoQ10) [84-87]. CoQ10, also known as ubiquinone, is a lipid-soluble benzoquinone that possesses significant antioxidant properties when reduced to ubiquinol, or through a CoQ10-induced increase in α-tocopherol [88]. It is located in the inner mitochondrial membrane and is essential for Complex I and II electron transfer activities during oxidative phosphorylation [89], playing a vital role in ATP production. Importantly, CoQ10 administration has been demonstrated to significantly increase brain mitochondrial CoQ10 concentrations [90].

Initial preclinical therapeutic trials using CoQ10 in a striatal lesion model of HD demonstrated significant neuroprotection [84]. Malonate-induced lesions within the striatum were significantly reduced by CoQ10. Expanding these results, the authors and others conducted preclinical therapeutic trials using CoQ10 in murine models of HD [85,86]. CoQ10 treatment significantly extends survival and delays the typical decline in weight loss and motor performance as assessed on the rotarod. In addition, CoQ10 administration significantly attenuates brain weight loss, gross brain atrophy and ventricular enlargement and striatal neuron atrophy. These data have given way to several human safety and tolerability trials using CoQ10 [39,91,92].

In all instances, CoQ10 has been found to be both safe and tolerable in HD patients. CoQ10 treatment has resulted in a significant decrease in cortical lactate [39], as well as a non-significant trend towards slowing in total functional capacity decline over 30 months [92]. In addition, there were significant beneficial effects on cognitive function, including Stroop color naming and word reading tasks [92]. However, as the single target dose did not provide significance in the specified primary outcome of the trial, it remains unclear whether a higher CoQ10 dose would provide greater efficacy in HD patients. A number of studies in other neurodegenerative diseases suggest that a higher CoQ10 dose is possible. A double-blind, randomized, controlled trial in Parkinson’s disease (PD) patients, using CoQ10 at 1200 mg/d, slowed the rate of deterioration in the Unified PD Rating Scale score [93]. Follow-up studies in both PD and amyotrophic...
lateral sclerosis patients have demonstrated safe and tolerable doses up to 3000 mg/d [94,95].

Addressing this, the authors conducted a high dose trial using CoQ10 in R6/2 mice [96], with dosages 10 times those previously reported [84,85]. High dose CoQ10 treatment in R6/2 resulted in significant survival extension with significant improvements in motor performance. As with previous preclinical trials using CoQ10, high dose CoQ10 treatment in R6/2 resulted in improved neuropathology, with marked reductions in striatal mHtt aggregation. Coupled with significant improvement in brain ATP levels and a reduction in brain 8OH2’dG, these results demonstrate the pluripotent efficacy of high dose CoQ10 in the treatment of HD. A multicenter Phase II – III clinical trial using high-dose CoQ10 has been initiated.

Therapies targeting alternative aspects of mitochondrial function may also be effective. In this regard, the n-3 fatty acid eicosapentaenoic acid (EPA) possesses hypo-triglyceridermic activity, shown to occur through EPA interactions with mitochondria [97], acting as a mitochondrial proliferator. EPA-induced hippocampal neuroprotection has been observed in rats treated with whole body γ-irradiation [98] by significantly reducing reactive oxygen species, cytochrome C translocation and caspase-3 activation. Importantly, mitochondrial dysfunction in HD mediated by mHtt has been shown to promote altered calcium permeability and associated cytochrome C release [99]. The ability of EPA to interact with and promote mitochondrial fitness has stimulated interest in EPA as a potential therapy for the treatment of HD.

Using a purified derivative of EPA known as ethyl-EPA, an animal trial in the R6/1 murine model of HD showed significant improvements in multiple motor and behavioral abnormalities [100]. A subsequent 6-month clinical trial using ethyl-EPA in advanced HD patients demonstrated significant improvement in several orofacial aspects of the UHDRS [101]. The improvements in the UHDRS were concomitant with improved neuropathology, assessed through MRI. More recently, however, ethyl-EPA treatment in HD patients was reported to have no effect on the UHDRS [102]. While secondary analysis revealed ethyl-EPA-induced improvements in motor function, further studies will
### Table 1. Emerging therapeutic compounds for Huntington's disease.

<table>
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<tr>
<th>Compound</th>
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<tr>
<td><strong>Mitochondrial dysfunction and oxidative stress</strong></td>
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<tr>
<td>Creatine</td>
<td>C₄H₉N₃&lt;sub&gt;2&lt;/sub&gt; N-Aminosarcosine; 2-imino-N-methylhydantoin</td>
<td>Mitochondrial Supplement; inhibitor of mitochondrial pore transition; antioxidant</td>
<td>Increases brain levels of phosphocreatine</td>
<td>R6/2</td>
<td>17.4% extension in survival; improved rotarod performance</td>
<td>Human HD trials Phase III</td>
</tr>
<tr>
<td>Coenzyme Q₁₀</td>
<td>C₃₀H₄₆O₄(C₄H₆)n Ubiquinone; 2,3-dimethibenzoquinone</td>
<td>Antioxidant; supports ATP production as an electron carrier; stabilizes slow Ca²⁺ channels</td>
<td>Increases brain levels of Coenzyme Q₁₀</td>
<td>R6/2</td>
<td>14.5% extension in survival; improved rotarod performance</td>
<td>Human HD trials Phase III</td>
</tr>
<tr>
<td>Ethyl-EPA</td>
<td>5,8,11,14,17-Eicosapentaenoic acid</td>
<td>That targets mitochondrial and interacts with peroxisome proliferator activated receptors</td>
<td></td>
<td>R6/1</td>
<td>Improved limb clasping, stride length, and locomotion</td>
<td>Human HD trials Phase III</td>
</tr>
<tr>
<td>Dimebolin</td>
<td>C₁₂H₁₄N₂ 2,3,4,5-Tetrahydro-2,8-dimethyl-5-(2-(6-methyl-3-pyridyl)ethyl)-1H-pyrido(4,3-b) indole</td>
<td>An antihistamine that interacts with mitochondria and limits the mitochondrial pore transition</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>Human HD trials Phase II</td>
</tr>
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EPA: Eicosapentaenoic acid; HD: Huntington's disease; mHtt: Mutant Huntington gene; mTor: Mammalian target of rapamycin; SAHA: Suberoylanilide hydroxamic acid; SMER: Small molecule enhancer.
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<tr>
<td>Sodium butyrate</td>
<td>C₄H₇O₂NaCOONa</td>
<td>An inhibitor of histone deacetylase, transcriptional modifier</td>
<td>R6/2</td>
<td>20.8% extension in survival; improved rotarod performance</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>SAHA</td>
<td>C₁₄H₂₀O₃N₂</td>
<td>Suberoylanilide hydroxamic acid</td>
<td>An inhibitor of histone deacetylase, transcriptional modifier</td>
<td>R6/2</td>
<td>Improved rotarod performance</td>
<td>N/A</td>
</tr>
<tr>
<td>Phenylbutyrate</td>
<td>C₉H₁₀O₂COOH</td>
<td>A histone deacetylase inhibitor that acts as a transcriptional modifier</td>
<td>Penetrates CNS. High bioavailability in brain</td>
<td></td>
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<tr>
<td>Mithramycin</td>
<td>C₅₂H₇₀O₂₄</td>
<td>An inhibitor of neuronal apoptosis, can also bind DNA and alter epigenetic histone modifications to influence transcription</td>
<td>Penetrates CNS. Concentration in brain persists longer than in other tissues</td>
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Table 1. Emerging therapeutic compounds for Huntington’s disease (continued).

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<tr>
<td>Chromomycin</td>
<td>C₅₂H₈₂O₂₆</td>
<td>An inhibitor of neuronal apoptosis, can also bind DNA and alter epigenetic histone modifications to influence transcription</td>
<td>Persists CNS. Concentration in brain persists longer than in other tissues</td>
<td></td>
<td></td>
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<tr>
<td>Rapamycin</td>
<td>C₅₁H₉₀N₁₃</td>
<td>Inhibitor of mTor, a protein kinase that regulates autophagy</td>
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<tbody>
<tr>
<td>CCI-779, temsirolimus</td>
<td>C_{26}H_{36}NO_{16} 42-[3-Hydroxy-2-(hydroxymethyl)-2-methylpropanoate] rapamycin</td>
<td>Rapamycin ester Inhibitor of mTor</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SMER 10</td>
<td>C_{10}H_{17}N_{2}O_{1}</td>
<td>Small molecule enhancer of autophagy. Mechanism unknown</td>
<td></td>
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<tr>
<td>Cystamine</td>
<td>C_{2}H_{12}N_{2}S_{2} 2-2′-Dithiobistannamine</td>
<td>Inhibitor of mHtt aggregation, through inhibition of transglutaminase. Also possesses antioxidant properties</td>
<td></td>
<td></td>
<td>Penetrates CNS</td>
<td></td>
</tr>
<tr>
<td>Cysteamine</td>
<td>C_{2}H_{3}NS 2-Aminoethanol 2-Mercaptethylamine</td>
<td>Inhibitor of mHtt aggregation, through inhibition of transglutaminase</td>
<td></td>
<td></td>
<td>Penetrates CNS</td>
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<tbody>
<tr>
<td>Apoptosis</td>
<td>Minocycline</td>
<td>A tetracycline antibiotic that inhibits apoptosis through inhibition of caspase-3</td>
<td>Penetrates CNS</td>
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<tr>
<td></td>
<td>M826</td>
<td>An inhibitor of apoptosis via reversible inhibition</td>
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<tr>
<td>Excitotoxicity</td>
<td>Riluzole</td>
<td>An inhibitor of voltage-dependent sodium channels, resulting in attenuation of glutamate release</td>
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</table>
be required to determine the therapeutic potential of ethyl-EPA.

The antihistamine dimebolin (2,3,4,5-tetrahydro-2,8-dimethyl-5-(2-(6-methyl-3-pyridyl)ethyl)-1H-pyrido[4,3-b]indole), is an orally active small molecule that has multiple mechanisms of action. It may exert a neuroprotective effect by interacting with the mitochondrial permeability transition pore and preventing the calcium-induced opening of the pore [108]. Studies of dimebolin in animal models of Alzheimer’s disease have showed improved cognitive ability, while inhibiting β-amyloid [104]. Preliminary results in Alzheimer’s disease patients have also been promising; for example, dimebolin may also regulate calcium homeostasis and reduce the excitotoxicity, thus there may be potential benefit in administering dimebolin to HD patients. As such, a Phase II clinical trial examining safety and tolerability of dimebolin is presently recruiting HD patients.

4.2 Transcriptional dysregulation

The transcriptional repression observed in HD likely results from alterations in chromatin packaging associated with epigenetic modifications of histone proteins. In general, therapeutic manipulation of transcription may offer significant benefit in treating HD, as well as other neurodegenerative disorders. In particular, pharmacological targeting of histone methylation and acetylation status may be a unique method by which to achieve transcriptional homeostasis and, extension, neuroprotection in HD. Several preclinical trials with compounds directed toward altered histone profiles in HD have been performed. One strategy has been to target histone acetylation by administering histone deacetylase inhibitors (HDACi) [55,105].

The HDACi’s sodium butyrate or suberoylanilide hydroxamic acid (SAHA) in a Drosophila model of HD, provides significant neuroprotection [105]. These results were supported by in vitro analyses that demonstrated mHtt-induced inhibition of the histone acetyltransferase proteins CBP and p300, and improved acetylation profile of histone H4 after sodium butyrate. Sodium butyrate and SAHA were also shown to offer neuroprotection in the R6/2 murine model of HD [55,106]. Sodium butyrate and SAHA improved motor performance and, while not reported for SAHA, sodium butyrate significantly improved survival [55]. Both compounds also markedly improved striatal morphology. Importantly, both sodium butyrate and SAHA improved acetylation of histone H4. Improvements in H4 acetylation mediated by sodium butyrate were concomitant with transcriptional improvements in R6/2 striatum, assessed by microarray gene profiling, resulting in improved mRNA expression [55].

Confirming and expanding these data, administration of the HDACi phenylbutyrate in the N171-82Q murine model of HD resulted in significant neuroprotection as well [107]. Of interest, in addition to phenylbutyrate-mediated improvements in H4 acetylation, there was also a significant reduction in methylation of H3 within the striatum. Together, these data clearly link HDACi treatment with improved transcription. With the prospect of HDACi compounds offering therapeutic benefit in the treatment of HD, a dose-finding study using sodium phenylbutyrate recently demonstrated doses in the range of 12 – 15 g/d were safe and well-tolerated [108]. The authors’ own preliminary data using 15 g/d in a safety and tolerability trial in HD patients confirms the above study and, in addition, shows that sodium phenylbutyrate was therapeutically salient in significantly improving hypoacetylation levels in blood buffy-coat specimens.

In addition to compounds that directly interact with HDACs, another class of potential HD therapeutics exist that interact directly with DNA and that may be able to influence transcriptional activity in HD. Two such compounds are mithramycin and chromomycin, anthracycline antibiotics that act through modulating gene transcription. By binding to guanine-cytosine-rich regions within gene promoters, anthracyclines displace transcriptional elements that activate and repress transcription [109]. Importantly, anthracyclines have been reported to interact directly with histones H3 and H4 [110]. This has led to several preclinical studies investigating the potential utility of mithramycin or chromomycin in murine models of HD [56-58].

Mithramycin administration in R6/2 HD mice resulted in the largest significant extension in survival (29.1%) compared with any other preclinical therapeutic trial in HD to date [56]. The mithramycin-mediated improvement in survival was concomitant with significant improvements in motor performance and striatal morphology. Notably, mithramycin induced a significant decrease in methylated H3. In a follow-up study, the authors demonstrated mithramycin-mediated improvements in the methylation and acetylation profile of H3 and H4 within the striatum of N171-82Q mice [57]. Treatment with chromomycin or mithramycin in R6/2 and N171-82Q HD mice significantly increased acetylation of H4 and significantly reduced trimethylation of H3 at lysine 9. Additional analyses of H3 revealed an anthracycline-mediated shift toward greater acetylation and reduced methylation compared with untreated controls. This latter finding may be of particular importance given that methylation of H3 at lysine 9 is thought to be a dominant marker of transcriptional repression and the balance between methylation and acetylation of H3 at lysine 9 is believed to play an important role in the transcriptional disruption observed in HD [58].

While it may seem incongruous that cytotoxic antitumor compounds may play a positive role in neurodegenerative disorders, parallels between cancer and neurodegenerative disorders have been suggested. However, if cellular stresses and transcriptional signals elicit different responses in dividing cells versus cells that are terminally differentiated (leading to oncogenesis in the former and neurodegeneration in the latter), then different pathogenic mechanisms may underlie each. Importantly, previous clinical use of these
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Among antiapoptotic drug candidates, the tetracycline antibiotic minocycline has emerged as a potentially beneficial compound has been associated with negative side effects, including fever, nausea or vomiting, fatigue and depression. Both agents cross the blood–brain barrier and mithramycin has been used chronically in a number of human conditions. The preclinical mithramycin and chromomycin data provide a rationale for clinical trials of these clinically approved anthracyclines to test for efficacy in the treatment of HD.

4.3 The ubiquitin–proteosome system, autophagy and the aggregation of mutant Htt

The degradation of mHtt is another potential therapeutic target in HD. In normal neurons, organelle and protein turnover is a critical feature that promotes health and function. Altered proteolysis may result in aberrant protein changes in denaturation or misfolding. Two distinct routes mediating proteolysis in neurons are the ubiquitin–proteosomal pathway and the lysosomal pathway. Proteins destined for degradation mediated by the UPS must first be tagged for degradation. In general, the UPS is responsible for the degradation of transiently expressed proteins [111]. Importantly, proteins destined for proteosomal degradation must first be sufficiently unfolded to fit through the narrow opening of the proteosome [112].

In addition, degradation of proteins and organelles in bulk is accomplished through the lysosomal pathway, in a process termed autophagy [113]. Through this pathway, cellular components destined for degradation are enveloped in double membrane bound vesicles, called autophagosomes, which fuse with lysosomes. Once fused, hydrolytic lysosomal enzymes degrade the contents. While the mechanisms regulating autophagy are not completely characterized, it is a process regulated by protein kinases, including the well characterized mammalian target of rapamycin (mTOR) [114].

Phosphorylated mTOR is linked with protein synthesis, whereas dephosphorylation of mTOR induces autophagy [115]. Furthermore, mTOR mediated autophagy has been linked to glucose levels, with increased glucose stimulating autophagy and enhanced mHtt clearance through reduced mTOR phosphorylation [116]. It is worth noting, however, that autophagy can be induced through activity of the insulin receptor substrate-2, independent of mTOR activity [117]. This results in a significant reduction of mHtt aggregation in vitro and is dependent on normal autophagosome formation mediated by Beclin1 and hVps34.

Notwithstanding, with the importance of mTOR in autophagy and the role of autophagy in HD, compounds that can interact with mTOR to promote autophagy may prove exceptionally beneficial in HD. Using the specific inhibitors of autophagy 3-methyladenine or N6,N6-dimethyladenosine, the number and size of mHtt aggregates increases [118]. In contrast, induction of autophagy by rapamycin resulted in a significant reduction in mHtt aggregation. Rapamycin, a macrolide antibiotic, is approved for use in human patients. Employed in several clinical contexts, recent effectiveness has been demonstrated in cancer chemotherapy [119]. More recent evidence demonstrated that rapamycin significantly improved neuronal survival, compared with wild type flies. In addition, the rapamycin ester CCI-779 significantly improved motor performance and striatal neuropathology in the N171-82Q murine model of HD [120].

A subsequent screen of small molecules capable of modulating autophagy independent of mTOR was performed [121]. Of ~ 100 compounds screened, there were a dozen small molecule enhancers (SMERs) of autophagy, from which three positive hits (SMER 10, 18 and 28) were shown to reduce mHtt aggregation in vitro. Further analysis demonstrated significant protection against mHtt toxicity in an in vivo model of HD. While the precise therapeutic mechanism of SMERs remains elusive, analysis of phosphorylation status in targets of mTOR showed no SMER-induced effects, suggesting the SMERs act downstream of mTOR to induce autophagy.

An additional study of potential inducers of autophagy as a treatment for HD, which operate independently of mTOR, has identified lithium as a potential therapeutic compound for HD. Lithium significantly reduces clearance of mHtt and mHtt-induced cell death in vitro [122]. The similar mood stabilizing drug carbamazepine also reduced mHtt-induced cell death and mHtt aggregation in vitro. The in vitro lithium-induced clearance of mHtt mediated by autophagy is independent of mTOR activity and dependent on inositol monophosphatase 1 (IMPase) activity and the stimulatory effect was blocked with subsequent addition of inositol triphosphate. Interestingly, combined inhibition of mTOR and IMPase by rapamycin and lithium, respectively, resulted in additive clearance of mHtt in vitro [122].

While strategies targeting enhanced clearance may promote improved neuronal survival, therapeutic attenuation of mutant protein aggregation may also prove therapeutically valuable in treating HD. In this regard, cystamine may hold significant promise. Cystamine is a disulfide-containing compound that possesses multiple modes of action, from antioxidant properties [123] to inhibition of transglutaminase [124]. Indeed, recent preclinical data from multiple laboratories has demonstrated the potential therapeutic benefit of cystamine in treating polyglutamine disorders, including HD [125-127]. More recently, the dimer of cystamine, mercaptamine, a product of cystamine reduction, has completed Phase I human trials determining maximum dose tolerability and safety [128]. These data, in concert with previous clinical use of mercaptamine for treatment of cystinosis [129], demonstrate the unique potential of cystamine and its analogs in the treatment of HD.

4.4 Apoptosis

Among antiapoptotic drug candidates, the tetracycline antibiotic minocycline has emerged as a potentially beneficial
therapeutic intervention for treatment in HD. Minocycline possesses potent antiapoptotic capacity through inhibitory effects on caspase-1 and -3. In addition, minocycline also attenuates disruptions in mitochondrial function, including the release of cytochrome C [61,130]. Importantly, minocycline also readily crosses the blood–brain barrier. Clinically, chronic administration of minocycline has yielded a good safety record [131]. From a therapeutic standpoint, minocycline has shown significant improvement in multiple models of neurodegeneration, including brain trauma, spinal cord injury, PD and HD [61,87,132-135].

In preclinical trials using minocycline in murine models of HD, several studies have demonstrated a significant neuroprotective effect. Minocycline significantly inhibited caspase-1 and caspase-3 activation in R6/2 mice [61]. Minocycline also significantly reduced mHtt cleavage. In addition to their role in apoptotic signaling cascades, caspases also play a role in cleaving mHtt, yielding the toxic fragment [136]. Inhibition of caspase activity was associated with improved survival and motor behavior in R6/2 mice. Extending these findings, minocycline has been shown to significantly inhibit both initiator and effector caspases, including caspase-1, -3, -8 and -9, as well as the pro-apoptotic BID cleavage [134]. In addition, minocycline also inhibited both the release of cytochrome C and Smac/Diablo from mitochondria in R6/2 mice, indicating that mitochondria are a direct target of minocycline-mediated neuroprotection [130,133,134]. These preclinical minocycline studies have given way to pilot clinical trials assessing safety and tolerability in human HD. At doses of 100 and 200 mg/d, minocycline was found to be well tolerated by patients [137]. In terms of cognitive outcomes, there were no clinically relevant differences in cognition assessed by UHDRS. Similar results in pilot trials using minocycline at 100 mg/d over 6 months have been reported [138,139]. Excellent safety and tolerability data for minocycline treatment in HD patients has led the Huntington Study Group to conduct a Phase II trial that is presently underway. Of interest is a recent clinical trial in amyotrophic lateral sclerosis using 400 mg/d that showed no efficacy [140]. It has been suggested that the target dose was too great, resulting in the negative findings.

In addition to minocycline-mediated caspase inhibition, a recent report demonstrated a novel reversible inhibitor of caspase-3 that was shown to provide significant neuroprotection in a chemical rat model of HD [141]. In a preclinical proof-of-principle trial, M826, a pyrazinone mono-amide, demonstrated significant protection against malonate lesions, with a pharmacokinetic profile indicating the ability of M826 to inhibit caspase-3 6 h postadministration. Striatal lesion volumes were significantly reduced following M826 administration and the number of neurons expressing active caspase-3 was also significantly reduced. While these results demonstrate significant neuroprotective potential, the route of administration (intracerebral injection) will require additional study to improve and assess both M826 solubility and brain penetration in vivo [141,142].

4.5 Excitotoxicity
Glutamate excitotoxicity is also thought to play a role in HD pathogenesis. Given extensive evidence in support of an excitotoxic hypothesis for HD, compounds that counter excessive glutamate release may, therefore, be candidates for therapeutic intervention in HD. One such FDA-approved compound is riluzole (2-amino-6-trifluoromethoxy benzothiazole), a potent antiglutamatergic agent, which attenuates glutamate release through its ability to inhibit voltage-dependent sodium channels [143]. In HD, the potential benefit of riluzole was first suggested by preclinical studies in rats and non-human primates, using the 3-nitropropionic (3-NP) chemical model of HD [144,145]. In the 3-NP model of HD, riluzole offers significant improvements in motor performance, with significant neuroprotection observed. Expanding these findings, riluzole was found to significantly increase survival in R6/2 mice concomitant with significant improvements in motor behavior [146]. There was also a marked riluzole-mediated reduction in ubiquitin-positive mHtt aggregates within the striatum. Riluzole was also found to protect medium spiny neurons against glutamate-induced apoptosis in vitro [147]. The aberration in corticostratial function resulting in excessive glutamate release is widely thought to contribute to the selective striatal pathology observed in HD [60,148]. Interestingly, riluzole administration significantly reduces aberrant excitatory postsynaptic currents in R6/2 mice, lending further support for riluzole therapy in HD [149].

To that end, several clinical trials in human HD have been conducted. In a 6-week safety and tolerability trial with riluzole that assessed motor performance and brain lactate levels [150], riluzole was found safe and well tolerated, with a non-significant trend toward lower basal ganglia lactate levels. Analysis of motor function demonstrated a significant decrease in chorea, as measured via the UHDRS. Further clinical evaluations in HD patients confirmed riluzole’s efficacy [151,152]. Interestingly, the efficacy of other glutamatergic NMDA antagonists, amantadine [22] and memantine [23], has had mixed results [153-156]. Each of these drug agents is associated with significant side effects.

4.6 Supplementary therapeutic strategies
In addition to potential therapies as described above, there are several other therapeutic approaches that may prove beneficial in treating HD and, thus, deserve mention here.

RNA interference (RNAs) is one such therapy that takes advantage of a functionally conserved pathway present in all eukaryotes [157]. The molecular machinery mediating RNAi activity includes both micro RNA (miRNA) and short interfering RNA (siRNA). Through associations between various proteins, including Argonaute-2 and individual
RNAi molecules, a functional complex is formed that can then target homologous mRNA. Once bound to the homologous mRNA species, Argonaute-2 cleaves, and thus inactivates, the homologous mRNA [158]. Both miRNA and siRNA can prevent translation of homologous mRNA when each possesses a limited number of mismatches [159]. Through this mechanism, RNAi could be manipulated to reduce expression of protein products known to cause disease. In the case of HD, the ability to effectively target and downregulate mHtt expression may hold significant promise.

In this regard, several preclinical studies have shown the potential promise of RNAi therapy in HD. Using an adeno-associated viral vector (AAV) expressing a short hairpin RNA precursor targeting the Htt gene, mHtt expression is reduced in the striatum of N171-82Q mice [160]. RNAi targeting mHtt also improved motor behavior, with improved gait and rotarod performance. Similar results were also obtained in the R6/1 murine model of HD [161].

Through elimination of the toxic mHtt via specific targeting of the mHtt protein, these preclinical studies demonstrate the promise of RNAi-based therapies for the treatment of HD. However, it is important to note that such therapeutic intervention in HD may yet be many years off. While intracerebral infusion of AAV containing RNAi may be suitable in animal studies, safe and effective delivery of RNAi molecules to humans has yet to be firmly established.

Striatal neuron transplant, via striatal tissue graft or dissociated striatal suspension, has also been suggested to hold promise as a therapeutic intervention in HD. The rationale for neural transplantation arises from the fact that neurodegeneration eliminates specific neuronal populations, which can theoretically be replaced with the addition of new neurons, akin to organ transplant. In this system, it is proposed that transplanted neuronal tissue would re-establish the anatomical and functional aspects of the damaged and lost neurons [162]. Several preclinical and clinical studies in PD have provided proof-of-principle data suggesting the potential benefit of transplantation in the treatment of PD [163,164].

Initial studies using rodent chemical lesion models of HD have demonstrated successful striatal transplantation survival, including dopamine terminal innervation of the transplant and a recovery of striatal choline acetyltransferase and glutamic acid decarboxylase [165]. Subsequent studies have demonstrated functional recovery of motor behaviors after striatal transplantation [166]. Similar studies in a non-human primate chemical lesion model of HD have demonstrated successful stereotaxic implantation of cross-species striatal neuronal grafts (rat to baboon) into the caudate-putamen [167]. Post-transplant analyses revealed graft survival, with expression of striatal markers evident. Additional studies in non-human primates confirmed that grafting of striatal tissue into lesioned caudate ameliorated motor and behavioral alterations, demonstrating improved functional capacity [168].

Using recommendations for trial criteria from the Core Assessment Program for Intracerebral Transplantation in HD [169], an initial pilot grafting paradigm was employed where three HD patients received bilateral transplantation of fetal striatal tissue into the caudate and putamen [170]. Graft survival was determined through comparison of pre- and 1 year postsurgical MRI, with marked improvement in signal, consistent with graft survival. In all three patients, striatal tissue transplantation resulted in improved motor behavior, as assessed by the UHDRS.

In a subsequent safety and tolerability trial employing fetal striatal tissue transplants into the caudate and putamen [171], several HD patients showed marked improvement in UHDRS scores. However, complications in the use of immunosuppressant therapies postoperatively were observed, making analyses impossible. Additional trials with cross-species striatal transplantation were performed using porcine fetal tissues in human HD patients [172]. Even with therapy to suppress immunological xenograft rejection, no surviving striatal transplants were observed and no functional improvements noted. Together, these latter trials represent the difficulties of treating human HD with clinical striatal transplants.

While completed clinical trials demonstrate safety and tolerability, with adequate surgical procedures to perform the tissue transplants, certain aspects of tissue or cell preparation and delivery for surgical implantation remain unresolved. Furthermore, given ethical concerns regarding the use of fetal tissue or the use of alternative cells such as porcine fetal grafts, issues arise regarding immunological function and management of tissue rejections [173]. Finally, while striatal cell transplant may hold promise for the treatment of HD, one caveat that remains is the significant gross neuropathology observed as disease progresses, with extra-striatal neuropathology present. Indeed, cortical neuropathology in HD likely contributes to many of the behavioral and cognitive disruptions associated with advanced disease. As previous studies have not examined behavioral and motor performance beyond 1 year postoperatively, it remains to be seen whether striatal transplantation will have a long-term, broad therapeutic benefit.

5. Conclusion

The search for effective strategies aimed at halting or reversing the insidious march of HD will require extensive preclinical and clinical validation to provide the necessary safety and tolerability data for effective clinical use. All of the compounds described in this review are at some stage of this process. Many have demonstrated significant potential in preclinical trials involving mice. The development of genetic models has greatly expanded our understanding of HD pathogenesis. These models also provide complex,
yet accessible, biological systems with which candidate therapeutic compounds can be tested for efficacy and mode of action. While such models greatly enhance the discovery potential, it is important to understand the difficulty inherent in predicting the transference of success from mouse to man. Interestingly, recent evidence shows a parallel in efficacy in both HD patients and murine models of HD using antioxidant therapies in reducing peripheral oxidative stress levels of 8OH2’dG [41,96]. Difficulties notwithstanding, preclinical therapeutic trials with murine models provide perhaps the best foundation on which to base human clinical trials. Importantly, data from preclinical trials using multiple models is likely to be most informative when assessing potential benefit in human HD.

6. Expert opinion

A major goal of existing clinical research in HD is to improve early detection of disease and premanifest detection of neuronal dysfunction with translation to therapeutic trials. Biomarkers are urgently needed for diagnosis, disease progression and for potential disease-modifying therapies that are being developed and evaluated in clinical trials, especially at the preclinical stage. The development of early premanifest biomarkers is of great importance, as these may improve the power and cost-effectiveness of drug trials. While many different approaches have been undertaken to identify biomarkers, profiling objective biomarker measurements of HD has proven difficult at the present time. The optimal biomarker should be easily quantified and measured, reproducible and not subject to wide variation. In therapeutic evaluation, linear change with disease progression that closely correlates with clinico-pathological assessments of the disease is critical. As there may be a prolonged period of time in which neurons become dysfunctional before clinical expression of disease, preclinical detection of biomarkers offers the promise of administering disease-modifying medications during the premanifest period, further delaying or ameliorating disease symptoms.

The early identification of premanifest biomarkers in HD that correspond to disease activity, disease progression and disease response to therapy would greatly facilitate the accurate evaluation of the effectiveness of new therapies and improve the safety and efficiency of clinical trials.

While successful preclinical trials demonstrating improved phenotype in HD transgenic mice have yet to be fully validated in HD patients, this may be the consequence of underpowered clinical trials in humans, preventing a comparison of therapeutic efficacy between mouse and human. Alternatively, optimal therapeutic dosing may be underestimated. The reliance on human equivalent dose extrapolation measurements derived from body surface area criteria in animals may be deficient [174], as it likely does not accurately predict the maximum recommended safe dose in humans. This is evident in human trials where human equivalency dosing of bioenergetic agents based on preclinical murine trials in mice has not demonstrated similar efficacy in patients. However, while dose extrapolation may not be straightforward, it is well accepted that the phenotypes from mouse models of neurological diseases closely correlate with human diseases, thus providing a system with which to validate known CNS drug targets. As drug trials in mice can confirm therapeutic potential, the challenge is to identify an efficacious dose in humans.

An additional factor to consider when assessing potential clinical benefit is that most compounds described above are already available for use in humans. This is likely to accelerate clinical validation. However, an important consideration that must be addressed is the myriad of mechanisms underlying HD pathogenesis. It is likely that mHtt induces disruptions in transcriptional activity that ultimately results in neurodegeneration. Due to the regional specificity of neurodegeneration, there are likely other important mechanisms, such as mitochondrial dysfunction and associated oxidative stress, which play an important role in HD pathogenesis. Given the multiple pathogenic mechanisms active in HD, combinatorial treatment paradigms will be essential for treatment of HD. In this regard, it is important to acknowledge the multiple therapeutic mechanisms inherent in certain compounds, such as cystamine. Given that cystamine acts both as a tissue transglutaminase inhibitor and a potent antioxidant, its potential benefit, when paired with the transcriptional modulator chromomycin, may yield synergistic results enhancing the therapeutic benefit of each. While the precise combination that may yield the most significant benefit is not yet known, it is likely that a combinatorial paradigm will prove most suitable for effectively treating HD. Given the relative safety of creatine and CoQ10, it is likely that either high-dose CoQ10 or creatine, or both agents, will represent a cornerstone defense in ameliorating the progression of HD.

Declaration of interest

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Bibliography


65. MCCGEEF EG, MCCGEE PI: Duplication of biochemical changes of Huntington's chorea by intrastriatal

Stack & Ferrante


99. CHOO YS, JOHNSON GV, MACDONALD M, DETLOFF PJ, LESORT M: Mutant huntingtin directly


Stack & Ferrante