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REVIEW ARTICLE

Are immunotherapies for Huntington's disease a realistic option?

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Abstract
There is compelling evidence that the pathophysiology of many neurodegenerative diseases includes dysregulation of the immune system, with some elements that precede disease onset. However, if these alterations are prominent, why have clinical trials targeting this system failed to translate into long-lasting meaningful benefits for patients? This review focuses on Huntington's disease, a genetic disorder marked by notable cerebral and peripheral inflammation. We summarize ongoing and completed clinical trials that have involved pharmacological approaches to inhibit various components of the immune system and their pre-clinical correlates. We then discuss new putative treatment strategies using more targeted immunotherapies such as vaccination and intrabodies and how these may offer new hope in the treatment of Huntington's disease as well as other neurodegenerative diseases.

Introduction

Research over the last decades has provided compelling evidence for a role of the immune system in neurodegenerative diseases. However, immunotherapies have met with only minimal efficacy when tested in these conditions. This may relate to the aggressiveness of the treatment regime and the nature of the agent being tested [1] as well as the relevance of such approach in disorders where immune changes are likely to not be a central feature of the condition. Such disorders include Huntington's disease (HD), an inherited condition in which an expansion of a CAG repeat (>37 CAG) in the coding sequence of the huntingtin protein result in an abnormally long and pathogenic polyglutamine (polyQ) tract [2], leading to huntingtin protein misfolding and subsequent cellular pathology. The main pathological hallmarks of HD include severe neuronal loss in distinct brain structures [2] as well as microglial activation, which precedes, by many years in some cases, disease onset [3]. However, the mutated form of the huntingtin protein (mHTT)—the gene product of HD—is expressed ubiquitously, and thus all tissues could be involved in the disease process and this includes circulating immune cells. Evidence for this comes from studies that have shown significant increases in the concentration of circulating pro-inflammatory molecules, which, if replicated and shown to be causally linked to disease progression, could offer an alternative treatment target for HD [3]. In this regard, a number of trials have already been initiated to test anti-inflammatory or antibiotic agents in HD such as laquinimob, anti-semaphorin-4D, cannabinoid agonists and minocycline. In pre-clinical studies, a plethora of additional approaches have been identified including active immunization, where the patient's own antibodies are produced in response to vaccination against mHTT, or passive immunization regimes using infusions of anti-mHTT antibodies.

In this review, we not only summarize the ongoing clinical trials using anti-inflammatory strategies in HD but we also discuss compounds in the pipeline or identified in pre-clinical studies that are likely to enter the clinic in the near future.

HD is associated with early and prominent abnormalities within the immune system

Years before the typical psychiatric and motor features of HD are perceptible, both cerebral and peripheral

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inflammatory responses can be detected [3]: in the brain, with the presence of activated astrocytes and microglia and in the periphery, with increased levels of various cytokines in the plasma. What suggests that inflammation contributes to HD pathology is that the increased microglial activation correlates with disease progression and can predict disease onset in pre-symptomatic gene carriers [4]. Furthermore, several pro-inflammatory cytokines—such as interleukin (IL-6, IL-8), and tumor necrosis factor alpha (TNF-α)—are present in the cerebrospinal fluid of HD patients, whereas in the blood, multiple chemokines (such as chemokine ligands: CCL2, CCL4, CCL11, CCL13, CCL26), matrix metallopeptidase-9, vascular endothelial growth factor (VEGF), and transforming growth factor beta 1 are abnormally elevated from early in the disease process [5–7]. All of this suggests that indeed, inflammation could contribute to disease onset as well as disease evolution [8].

It has been further demonstrated that monocytes derived from HD patients and which are stimulated by lipopolysaccharide (LPS) produce greater concentrations of cytokines [5] as well as impaired migration *in vitro* [5, 9, 10]. It has been further suggested that mHTT expression within peripheral immune cells impairs gene expression and intracellular mechanisms leading to this abnormal immune response [3], as demonstrated by the elevated production of pro-inflammatory cytokines as well as impairments in the nuclear factor-kappa B (NF-κB) pathway, a critical player in the activation of an inflammatory response within innate immune cells [11]. Furthermore, it has been demonstrated that HD mice (R6/1 and zQ175) immunized with peptides, proteins or plasmids to produce antibodies against the pathogenic protein, are characterized by a different transcriptome profile when compared to immunized wild-type (WT) mice [12]. More specifically, a differential activation of genes associated with the innate immune response, an absence of negative feedback control of gene expression and a transcriptional repression of genes associated with memory T-cell responses, have all been observed. In addition, HD mice (BACHD and YAC128) transplanted with bone marrow from WT mice show a partial decrease of circulating pro-inflammatory cytokines (IL-6, CXCL1, IFN-γ, and IL-10) associated with moderate improvements in total open field activity, but not in rotarod performance [13]. Despite the modest benefits, these results suggest that the immune system is involved, at least in part, in the disease pathophysiology.

This pre-clinical evidence has led to a number of clinical initiatives with the goal being to modify this abnormal immune response and therefore treat, if not prevent or slow down, the disease process in HD patients.

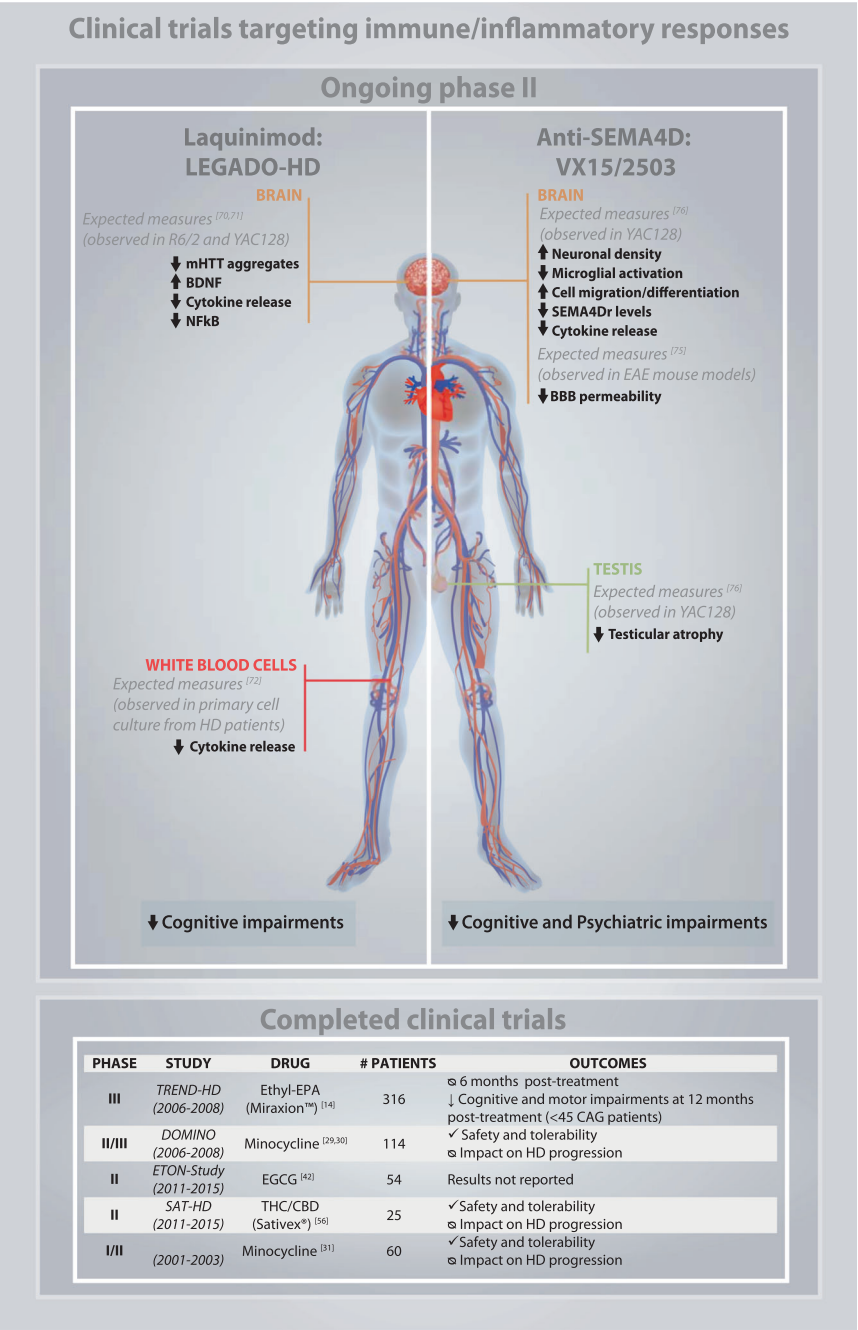
Immune therapy trials in patients with HD

Eicosapentaenoic acid (Ethyl-EPA) (Miraxion™): TREND-HD–Amarin Neuroscience Ltd and Huntington Study Group

The first phase III trial undertaken in the realm of immunotherapies was conducted in 2005. It consisted of a multicenter randomized double blind study to test the efficacy of the Ethyl-EPA compound *Miraxion*™ (NCT00146211) [14], a ω-3 fatty acid that has been shown to interfere with apoptosis [15], inflammation [16], and mitochondrial dysfunction [17], all of which are abnormal in HD. The trial enrolled 316 adults with mild-to-moderate HD (Fig. 1). Participants were randomized and received either Ethyl-EPA or placebo. At the conclusion of the study, it was shown that Ethyl-EPA failed to improve scores on any of the clinical scales used when all patients were assessed as a group (treated vs. placebo). However, patients with a CAG repeat length shorter than 45 showed benefits in (1) the total motor score-IV, (2) the total chorea score, (3) the total motor score, and (4) the clinical global impression score. The authors concluded on these potentially beneficial effects of *Miraxion*™ at 12 months post-treatment, although emphasizing that this finding required confirmation in longer placebo-controlled studies.

EPA targets mitochondrial function and effects gene expression by acting on transcription factors such as peroxisome proliferator-activated receptors (PPARs) [18], but it also acts on the c-Jun N-terminal kinases and NF-κB pathways [19–21]. It has further been shown to inhibit TNF-α mRNA expression in LPS-stimulated murine macrophages and LPS-stimulated human monocytes [22, 23].

EPA administered orally, from conception throughout life, was shown to ameliorate HD-related phenotypes in the R6/1 transgenic mouse model [24], whereas YAC128 transgenic HD mice fed Ethyl-EPA demonstrated significantly better performances on the rotarod as well as increased activity in the open field in comparison with placebo-treated mice [25]. In HD patients, the first open label study testing EPA revealed beneficial effects on motor function [26]. This was the first time that a significant improvement was reported in a randomized trial in HD. This pilot trial included 19 patients who took 1000 mg capsules of EPA twice daily for two years. This result was further supported by a small placebo-controlled trial in late-stage HD undertaken in 2002 [27]. In 2008, 34 stage 1 or 2 HD patients taking two 500 mg capsules twice daily for one year also showed by high-resolution magnetic resonance imaging a significant reduction in brain atrophy, particularly of the caudate nucleus and thalamus [28]. All of this lent support to the undertaking of a double blind placebo-controlled study.



PHASE	STUDY	DRUG	# PATIENTS	OUTCOMES
III	*TREND-HD (2006-2008)*	Ethyl-EPA (Miraxion™) [14]	316	⦸ 6 months post-treatment ↓ Cognitive and motor impairments at 12 months post-treatment (<45 CAG patients)
II/III	*DOMINO (2006-2008)*	Minocycline [29,30]	114	✓ Safety and tolerability ⦸ Impact on HD progression
II	*ETON-Study (2011-2015)*	EGCG [42]	54	Results not reported
II	*SAT-HD (2011-2015)*	THC/CBD (Sativex®) [56]	25	✓Safety and tolerability ⦸ Impact on HD progression
I/II	*(2001-2003)*	Minocycline [31]	60	✓Safety and tolerability ⦸ Impact on HD progression

Fig. 1 Clinical trials targeting immune/inflammatory responses. Upper panel: Illustration of the two ongoing clinical trials targeting components of the immune/inflammatory responses in HD. Lower panel: Summary table of completed clinical trials targeting components of the immune/inflammatory responses in HD. Abbreviations: Anti-SEMA4D: anti-semaphorin 4D; BBB: blood–brain barrier; BDNF: brain-derived neurotrophic factor; EAE: autoimmune encephalomyelitis; EGCG: epigallocatechin gallate; Ethyl-EPA: ethyl-eicosapentaenoic acid; HD: Huntington's disease; NF-kB: nuclear factor-kappa B; mHTT: mutant huntingtin protein; SEMA4Dr: Semaphorin 4D receptor; TFC: total functional capacity; THC/CBD: delta-9-tetrahydrocannabinol and cannabidiol

Completed phase II/III clinical trials

Minocycline: DOMINO - Huntington Study Group

Between April 2006 and November 2008, 114 subjects were randomized at 12 Huntington Study Group clinical sites (NCT00277355) [29] to receive 100 mg of minocycline twice daily. The goal of this trial was to determine the safety and tolerability of minocycline and to establish the impact of this drug on the progression of HD, as measured by a change in total functional capacity (TFC) score from the Unified Huntington's Disease Rating Scale (UHDRS) at baseline and 18 months

following treatment (Fig. 1). The authors reported a significant decline in TFC scores in treated patients, translating to a worsening of the disease [30], leading to trial completion for futility. Earlier Phase I/II trial had allowed the investigators to determine that daily doses of 100 and 200 mg of minocycline administered to HD patients over a period of eight weeks was well tolerated and safe (NCT00029874) [31], results which were in line with an independent pilot study conducted by Thomas et al. [32].

The pre-clinical work that led to this DOMINO trial suggested that minocycline delayed disease progression and mortality in the transgenic R6/2 mouse model of HD [33–35]. The efficacy of minocycline as a neuroprotective agent was attributed, in part, to its capacity to block the release of pro-apoptotic mitochondrial factors and caspase activation [34, 36] and/or to inhibit the inflammatory response [32, 37]. However, potential detrimental effects of minocycline had also been identified in animal models of HD [38–40] and Parkinson's disease (PD) [39], although this had been claimed to relate to dosing, with low doses being beneficial and safe [41]. Even in these pre-clinical studies it was clear that minocycline had variable benefits in different models of neurological disorders.

Completed phase II clinical trials

Epigallocatechin gallate (EGCG) (Sunphenon®): ETON-Study–Charité University, Berlin, Germany

Between 2011 and 2015, the efficacy and tolerability of EGCG, a green tea polyphenol, was tested in 54 HD patients (NCT01357681). The investigators hypothesized that *Sunphenon®* administered at a maximal daily dose of 1200 mg would improve cognition [42]. The results of this trial have unfortunately never been published (Fig. 1).

The large family of polyphenolic compounds exerts its anti-inflammatory effects by modulating pro-inflammatory gene expression such as lipoxygenase, cyclooxygenase (COX), nitric oxide synthases (NOS), critical cytokines as well as by inhibiting the NF-κB pathway but there are several studies and disease contexts that have revealed similar effects of EGCG, in particular [42–44]. For example, EGCG has been shown to reduce glutamate-induced oxidative cytotoxicity via the inactivation of the NF-κB-signaling pathway in a mouse hippocampal neuronal cell line [45]. Moreover, EGCG has shown neuroprotective effects in an amyotrophic lateral sclerosis (ALS) transgenic mouse model (SOD1-G93A mice) where the number of motor neurons was greater compared with mice which did not receive ECGC, diminishing the microglial activation and reducing NF-κB protein levels [46]. During neuronal injury, EGCG can mediate inflammation by suppressing microglial activation [47]. EGCG has also been shown to inhibit the NF-κB pathway and suppress TNFα, IL-1β, IL-6 as well as inducible NOS in Amyloid-β stimulated EOC13.31 microglial cells [48]. In traumatic spinal cord injury, EGCG promoted neuroprotection by reducing lipid peroxidation, apoptosis, attenuation of pro-inflammatory cytokine production with improved locomotor recovery [49]. In LPS-activated murine macrophage cell lines, EGCG further prevented the production of IL-12 by down-regulating mRNA transcription [50]. Finally, even in carcinogenesis studies using different human and mouse cell lines, EGCG has been shown to have an anti-inflammatory effect via COX-1/COX-2 inhibition [51].

Previous research on yeast and fly models of HD have also indicated that EGCG is a potent inhibitor of mHTT exon 1 protein aggregation *in vitro* and that it interfered with protein misfolding as well as the assembly of oligomers in cell-free assays, reducing both toxicity and aggregate formation [42]. This was subsequently confirmed for other proteins such as tau (which is also found in HD brains [52–54]), as EGCG could prevent the conversion of the protein into toxic oligomers in Alzheimer's disease (AD) mouse models [55].

Cannabinoids: SAT-HD (Sativex®)–GW Pharmaceuticals Ltd

In 2016, a phase II trial confirmed the safety and tolerability of *Sativex®*, a botanical extract with an equimolecular combination of delta-9-tetrahydrocannabinol and cannabidiol (THC/CBD) (NCT01502046) (Fig. 1). The agent was administered at a dose of 2.7 mg of THC/2.5 mg of CBD Spray, one spray per day, up to a maximum of 12 sprays daily and compared to placebo [56]. Clinical improvements were not recorded for motor, cognitive, behavioural or functional scores (functional UHDRS).

Pre-clinical studies looking at *Sativex®* as a neuroprotective agent had shown that it delayed signs of disease progression in malonate-lesioned rats, a toxin model of HD, and that these effects were mediated through cannabinoid receptor type 1 (CB1r) and cannabinoid receptor type 2 (CB2r) [57]. The evidence that these receptors are relevant to HD rather than being a peculiarity of this model comes from several sources.

CB1r expression has been noted to be lower in the basal ganglia of both HD patients and in the R6/2 mouse model and that this correlates with chorea and cognitive deficits [58]. Furthermore, activation of these receptors protect striatal cells from excitotoxic death via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin complex 1 pathway, which, in turn, induces the secretion of brain-derived neurotrophic factor (BDNF) [59], which is reduced in HD patients [60]. In studies using mouse striatal neurons derived cell line (STHdhQ7/Q7 and STHdhQ111/Q111), THC or THC/CBD (1:1) impaired cellular function

and the viability of cells expressing mHTT [58]. Likewise, treatment of R6/1 and R6/2 mouse models with 10 mg THC was associated with worsening of their HD signs [61]. However, others reported improvements in motor control and reduced striatal atrophy in R6/1 and R6/2 HD treated for six weeks with 2 mg/kg THC beginning at four weeks of age [59], suggesting a dose- and time-dependent effect. A significant increase in the number of ubiquitinated striatal aggregates was further observed with HU210 treatment (a cannabinoid agonist), indicating an influence of CB1 on the disease process [58]. Adeno-associated viral vector (AAV) delivery of the CB1r in the dorsolateral striatum of R6/2 mice induced increased expression of BDNF and rescued the neuropathological deficits seen in these animals [57].

CB2r have been shown to be upregulated in microglial cells of both HD patients and genetic murine models of the disease (e.g., R6/1 and R6/2) [62]. More specifically, increased levels of CB2r have been found in both striatal activated astrocytes and reactive microglial cells in a model of HD generated by an intrastriatal lesion using malonate [63]. However, a recent study has pointed out that the upregulation of these receptors is within vascular cells, and not in activated glial cells [64]. Interestingly, the genetic deletion of CB2r in a slowly progressing HD mouse model (BACHD; CB2−/− mice) accelerates the onset of motor deficits and increases disease severity [65]. On the other hand, treatment with a CB2r agonist (GW405833) extends lifespan and suppresses motor deficits, synapse loss as well as central nervous system (CNS) and peripheral inflammation in R6/2 mice [65] (Fig. 2).

In summary, there are two major cannabinoid receptor types: the CB1r, which is widely distributed throughout several regions of the CNS, particularly in the basal ganglia, cortex, hippocampus and cerebellum, and the CB2r, which is primarily associated to the immune system [56]. Prior to motor symptom onset or neuronal cell loss in HD, levels of CB1r decrease in the basal ganglia and are strongly correlated with chorea and cognitive deficits [58]. Given that CB2r signalling also regulates immune cell migration, which is impaired in HD, the protective effects of CB2r agonists may be mediated by effects on immune cell migration coincident with dampening of pro-inflammatory cytokine production [65]. Taken together, CB receptor signalling in peripheral immune cells and in CNS has an important role in HD.

Ongoing phase II clinical trials

Laquinimod: LEGADO-HD–TEVA pharmaceuticals

TEVA initiated a phase II clinical trial referred to as LEGATO-HD where Laquinimod's efficacy and safety is being tested in HD patients (NCT02215616). The study, which began in November of 2014, recruited 400 patients and is expected to come to completion by August 2018 (Fig. 1).

Laquinimod is a compound that was originally developed for the treatment of relapsing-remitting multiple sclerosis and demonstrated a striking impact on brain atrophy in this condition [66, 67]. Although the mechanisms of action of laquinimod are still unclear, its beneficial effects appear to be related to anti-inflammatory actions and may not be restricted to multiple sclerosis as this molecule is able to cross the blood–brain barrier [68, 69]. In this regard, increased levels of BDNF and anti-apoptotic effects were observed following laquinimod treatment in animal models of HD (Fig. 1). Indeed, the therapeutic potential of laquinimod has been reported in both the YAC128 and R6/2 mouse models of HD with respect to motor function, behavior, weight loss, longevity, and various histopathological measures such as an increased number of DARPP-32 positive cells, the percentage of cells expressing mHTT and BDNF mRNA levels [70, 71]. Laquinimod has also been shown to dampen hyperactive cytokine production in primary human monocyte and macrophage cultures derived from HD patients and healthy volunteers [72].

Anti-semaphorin 4D: VX15/2503-N-131–Vaccinex

Semaphorin 4D (SEMA4D) is a protein expressed on infiltrating immune cells and oligodendrocyte precursor, whereas its receptor is localized on neurons, oligodendrocytes, and endothelial cells [73]. In 2000, Vaccinex undertook the development of a monoclonal anti-SEMA4D antibody (VX15/2503) to dampen neuroinflammation and neurodegeneration in various disease contexts. Based on this and the fact that elevated expression of SEMA4D, and its receptor Plexin-B1, has been reported in the striatum and cortex of HD patients [74], Vaccinex received, in 2015, clearance from the FDA to proceed with a Phase II clinical trial in HD (NCT02481674) (Fig. 1). The primary objective of VX15/2503-N-131 is to evaluate the safety and tolerability of monthly intravenous administration of a single dosage of VX15/2503. The secondary objectives include determining the effect of VX15/2503 on (1) brain volumes, (2) brain inflammation measures, and on (3) clinical features of HD. Enrolment is set to 116 individuals with late prodromal or early manifest HD and the first set of results are expected in March 2020.

Early pre-clinical studies demonstrated improvements in various rodent models of autoimmune encephalomyelitis through increased oligodendrocyte precursor cell survival, migration, and maturation along with re-myelination and restored blood–brain barrier integrity with improved clinical scores [75]. VX15/2503 treatment was further observed to

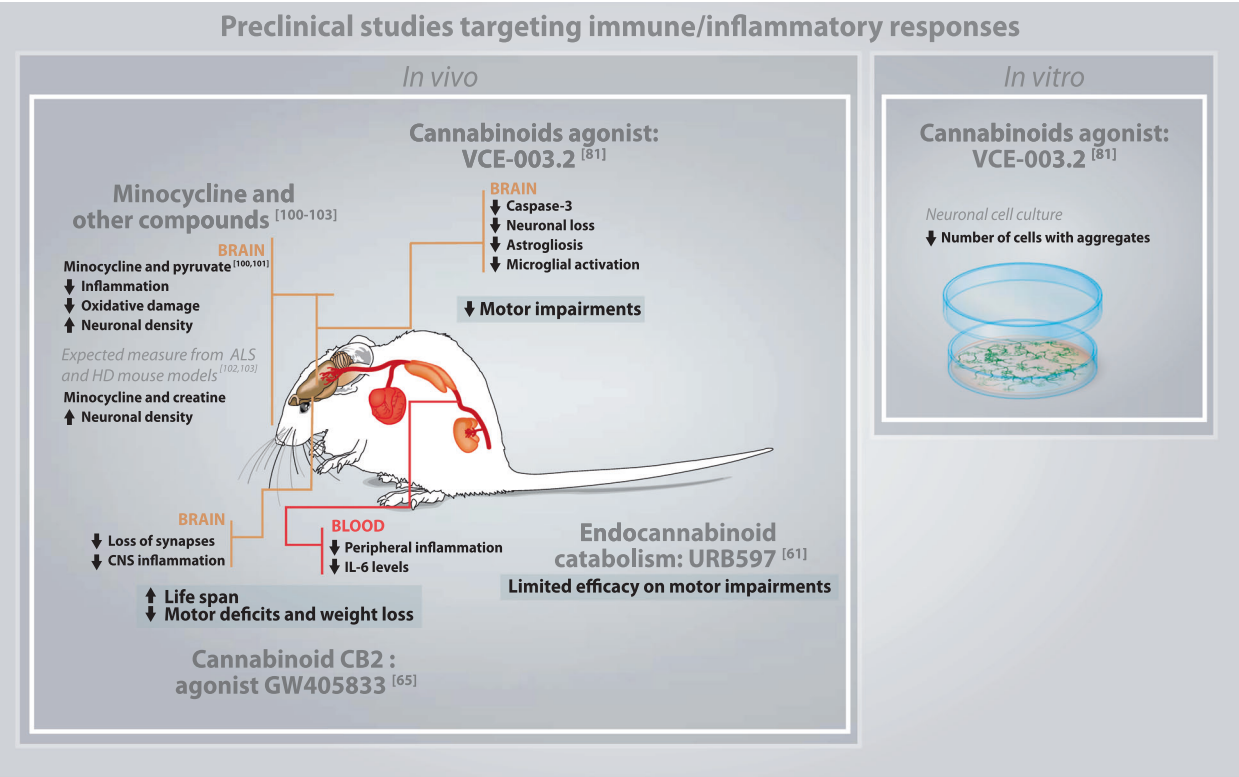


Fig. 2 Pre-clinical studies targeting immune/inflammatory responses. Summary of the major findings in various pre-clinical studies, including both small animals and *in vitro* systems, targeting components of the immune/inflammatory responses. Abbreviations: ALS: Amyotrophic Lateral Sclerosis; CNS: central nervous system; IL-6: Interleukin 6

ameliorate behavioural deficits, rescue testicular atrophy as well as neuropathological phenotypes in the transgenic YAC128 mouse model of HD [76] (Fig. 1).

Although the results of completed trials have been met with uncertainty regarding what they are showing, ongoing clinical trials seem to hold some promise, the results of which will therefore be critical to the advancement of the field. The following section reviews all pre-clinical studies that have been dedicated to identifying potential candidate compounds that target the immune system and that could be used in HD. The main findings have been summarized in Figs. 2 and 3.

Compound identified in pre-clinical studies

Additional cannabinoids-related compounds

Cannabinoid agonist: VCE-003.2–VivaCell Biotechnology España S.L

VCE-003.2 has been identified as an immunosuppressant that exerts a protective action in models of multiple sclerosis through a PPAR-γ dependent pathway (associated to chemokines) [77, 78], which is dysfunctional in HD [79, 80]. The beneficial effects of this novel cannabigerol derivative were first demonstrated in the quinolinic acid-lesioned model of HD [81]. The benefits seemingly resulted from a decrease in inflammatory-related genes (caspases), neuronal loss, astrogliosis, and microglial activation, all of which had positive consequences on motor impairments in this mouse model (Fig. 2). VCE-003.2 was also shown to be neuroprotective by reducing the number of cells expressing mHTT aggregates in *in vitro* transfected striatal precursors cells, which were transfected with an exon 1 mHTT expression vector encoding 94 expanded polyQ repeats [81] (Fig. 2).

Targeting endocannabinoid catabolism: URB597

URB597 is a fatty-acid amide hydrolase that has the capacity to inhibit endocannabinoid catabolism. Only a single study has reported the effects of this compound in HD, with minimal efficacy recorded on motor deficits in R6/2 mice [61] (Fig. 2). However, URB597 is currently in clinical trials for schizophrenia (NCT00916201), so could in theory be easily moved to trials in HD.

Tropomyosin receptor kinase B (TrkB) ligand: LM22A-4

TrkB is the main receptor of BDNF, which is involved in neuronal/glial development and survival. BDNF is also

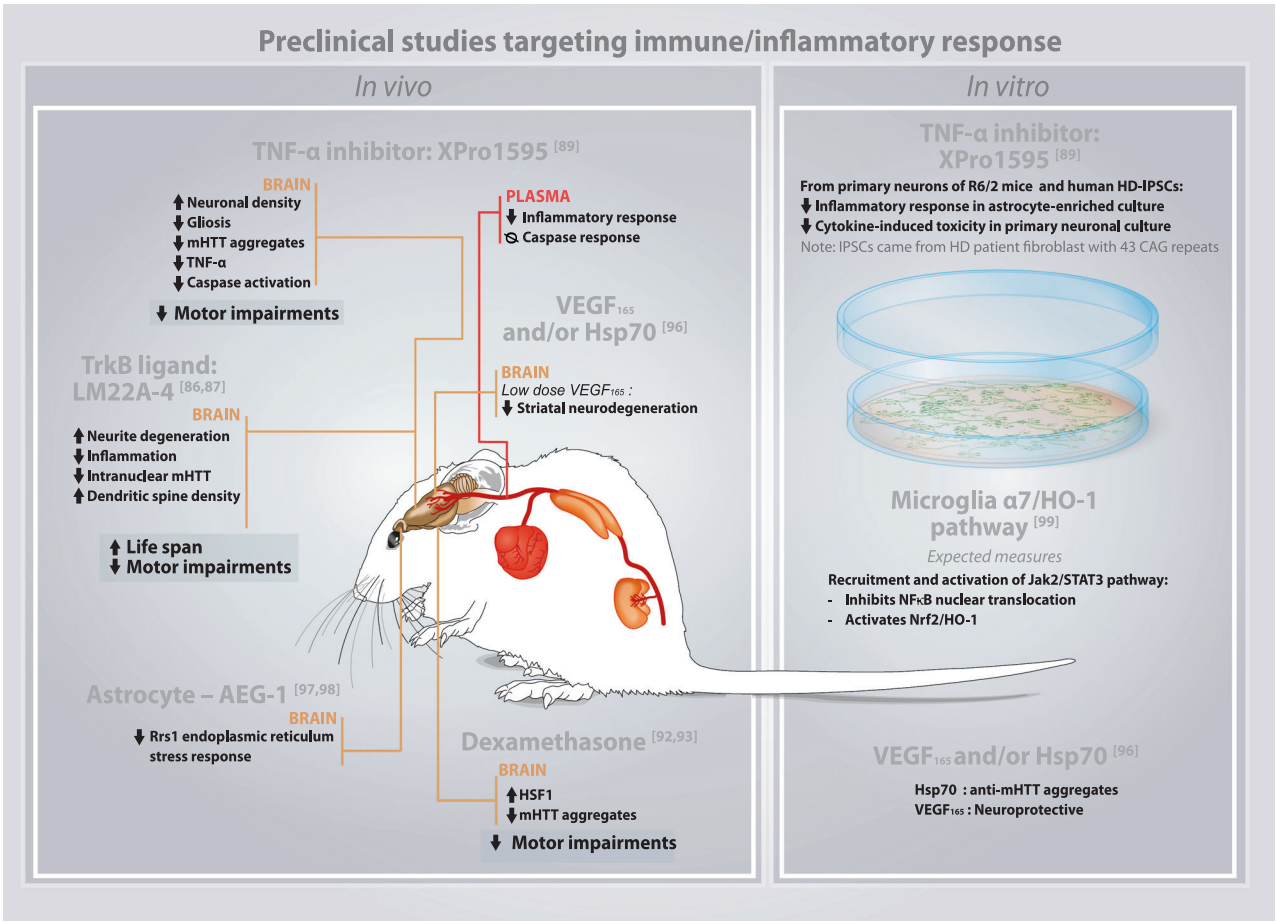


Fig. 3 Pre-clinical studies targeting immune/inflammatory responses. Summary of the major findings in various pre-clinical studies, in both small animals and *in vitro* systems, targeting components of the immune/inflammatory responses. Abbreviations: AEG-1: Astrocyte elevated gene-1; DARPP-32: dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32,000; HSF1: Heat shock factor 1; Hsp70: heat shock 70 kDa; Jak2/STAT3: Janus kinase 2/Signal transducer and activator of transcription 3; mHTT: mutant huntingtin protein; NF-kB: nuclear factor-kappa B; Nrf2/HO-1: nuclear factor [erythroid-derived 2]-like 2/heme-oxigenase-1; TNFα: tumor necrosis factor alpha; Rrs1: ribosome biogenesis regulator homolog; VEGF165: Vascular endothelial growth factor 165; α7/HO-1: Nicotinic acetylcholine receptor alpha 7/heme-oxigenase-1

expressed in T cells, B cells and activated monocytes/macrophages [82, 83]. Although the reported anti-inflammatory effects of TrkB on immunity are rather indirect, it has recently been shown that mice infected with *Streptococcus pneumoniae* to induce meningitis have decreases in pro-inflammatory factors (TNF-α, IL-1β, and IL-6) as well as increases in IL-10, a pro-inflammatory factor with neuroprotective effects, after pretreatment with BDNF [84]. All of these responses can be reversed when a TrkB inhibitor (k252a) is applied, suggesting that the interaction between TrkB and BDNF can indeed provoke a direct anti-inflammatory effect [84].

LM22A-4 is a BDNF mimetic and partial agonist of TrkB [85, 86]. It has been demonstrated to alleviate HD-related pathology in R6/2 and BACHD mouse models [86, 87]. Indeed, LM22A-4-treated mice were characterized by a decrease in neurite degeneration and reduced number of striatal microglia, which prevented the loss of dendritic spines in medium spiny neurons and intranuclear mHTT aggregates as well as in cortical cells. Notably, mice administered with LM22A-4 demonstrated improved motor impairments and longevity (Fig. 3).

TNF-α inhibitor: XPro1595

Specific AAVs have been designed to target soluble TNF-α through the delivery of a dominant-negative inhibitor of TNF-α (DN-TNF-α) [88]. Results using this approach have shown that efficient transduction of AAV-DN-TNF-α can be achieved *in vitro* in HEK293 cells, which led to the inhibition of NF-kB. However, bilateral striatal injections in YAC128 mice showed limited AAV-DN-TNF-α spread *in vivo*, which may very well explain the absence of any beneficial effects of this agent on neuroinflammation, medium spiny neuronal loss, and locomotor deficits [88]. In parallel, the soluble form of DN-TNF-α (XPro1595) was directly injected into R6/2 mice [89] and was reported to improve neuronal density as well as to decrease TNF-α rate,

caspase activity, cerebral mHTT aggregates and gliosis compared to mice that did not receive this treatment. However, reducing the peripheral inflammatory response by systemic injections of XPro1595 only improved motor functions in R6/2 mice (Fig. 3). *In vitro*, it has been reported that XPro1595 effectively suppresses inflammation in primary astrocyte-enriched cultures isolated from the R6/2 mouse model as well as in human astrocytes-enriched cultures derived from induced pluripotent stem cells (iPSCs) of HD patients [89]. Moreover, XPro1595 protected the cytokine-induced toxicity of primary R6/2 neurons and human neurons derived from HD iPSCs [89].

Taken together, XPro1595 exhibits anti-inflammatory and neuroprotective effects in addition to delaying disease progression in HD. Anti-inflammatory treatment targeting abnormal soluble TNF-mediated brain inflammatory responses, via XPro1595, may therefore be a valuable therapeutic strategy for this disease.

Dexamethasone

Dexamethasone binds to the glucocorticoid receptor (GR) and thereof mimics the natural glucocorticoid cortisol release [90]. The glucocorticoid-GR complex then moves to the nucleus where it can activate the transcription of anti-inflammatory genes. In addition to its interactions with various transcription factors, dexamethasone can suppress COX-2, an important element of the inflammatory pathway, as shown, for example, in adenocarcinomic human alveolar basal epithelial cells exposed to pro-inflammatory cytokines [90]. Of interest, when either HEK293 cells and mouse nerve cells are transfected with plasmids containing hemagglutinin-tagged htt exon 1 fragment from a HD patient with 62 CAG repeats, diminished mHTT protein aggregation can be observed [91].

More recently, Heat shock factor 1 (HSF1) was shown to be downregulated in murine and fly models of HD after the administration of dexamethasone. This treatment also significantly decreased aggregate load and provoked transient recovery of HD-related behavioural phenotypes in both disease models [92, 93] (Fig. 3). Heat shock proteins (HSPs) are important to the cell's response to stress and play a role in cellular homeostatic functions such as protein synthesis and protein transport across membranes. HSPs participate in cytokine signal transduction and in the control of cytokine gene expression [94]. It has been reported that the HSF1 protein as well as its mRNA are decreased in HD models and that dysfunction of HSF1 correlates with the onset of aggregate formation in R6/2 mice (at four or five weeks of age), suggesting a prominent role of HSF1 in the early stages of disease pathogenesis [92].

Interestingly, dexamethasone treatment (4 mg daily for 20 days followed by 8 mg daily for an additional 20 days) in six female HD patients demonstrated improvements of abnormal involuntary movements and manual dexterity, with no signs of side-effects [95]. However, glucocorticoid therapy normally causes serious systemic side-effects, especially in chronic or high dose application. It is therefore very important to consider this in the development of steroid therapies.

A few other compounds targeting VEGF165 [96], astrocytes [97, 98], microglia [99], or used in combination with minocycline [100–103] have been tried and are summarized in Figs. 2 and 3 but will not be discussed here as they have only been superficially studied or investigated in other disease contexts.

Targeting the huntingtin protein with immunotherapies

Active vaccination

Immunotherapies using vaccination against the HTT/mHTT protein have also been tested in HD. However, only two such studies have been reported in the literature [12, 104]. In the first study [104], Miller et al. vaccinated R6/2 mice with a plasmid encoding an N-terminal fragment of the protein composed of the first 17 amino acid followed by 103 glutamine residues. In this study, the authors reported that vaccination protected R6/2 mice against the development of the diabetic phenotype with no sign of benefits of vaccination on pathological features *per se*. More recently [12], active vaccination approaches have been tested in R6/1 and zQ175 mice using peptide, protein, and plasmid immunization approaches. These studies have highlighted the safety of active immunization against HD but there have been no reports on the effect of this treatment on the pathology. Active vaccination has only been superficially investigated and therefore further research should be conducted to assess the potential of such methodologies.

Targeting mHTT within cells using intrabodies

Intrabodies, or intracellular antibodies, are designed to target specific proteins inside cells. This maneuver is technically challenging and as a result intrabodies are most often delivered using gene therapy approaches [105]. Generally, expression is restricted to the antigen-binding fragment of the antibody; i.e., single-chain Fv fragment (scFv) (Fig. 4). Given that mHTT is found within cellular elements, using intrabodies could, in theory, prevent aggregate formation and consequently disease manifestation. In this context, intrabodies have been developed and tested in *in vitro* and *in vivo* models of HD. The first logical target was the expanded polyQ tract, which is responsible for the disease

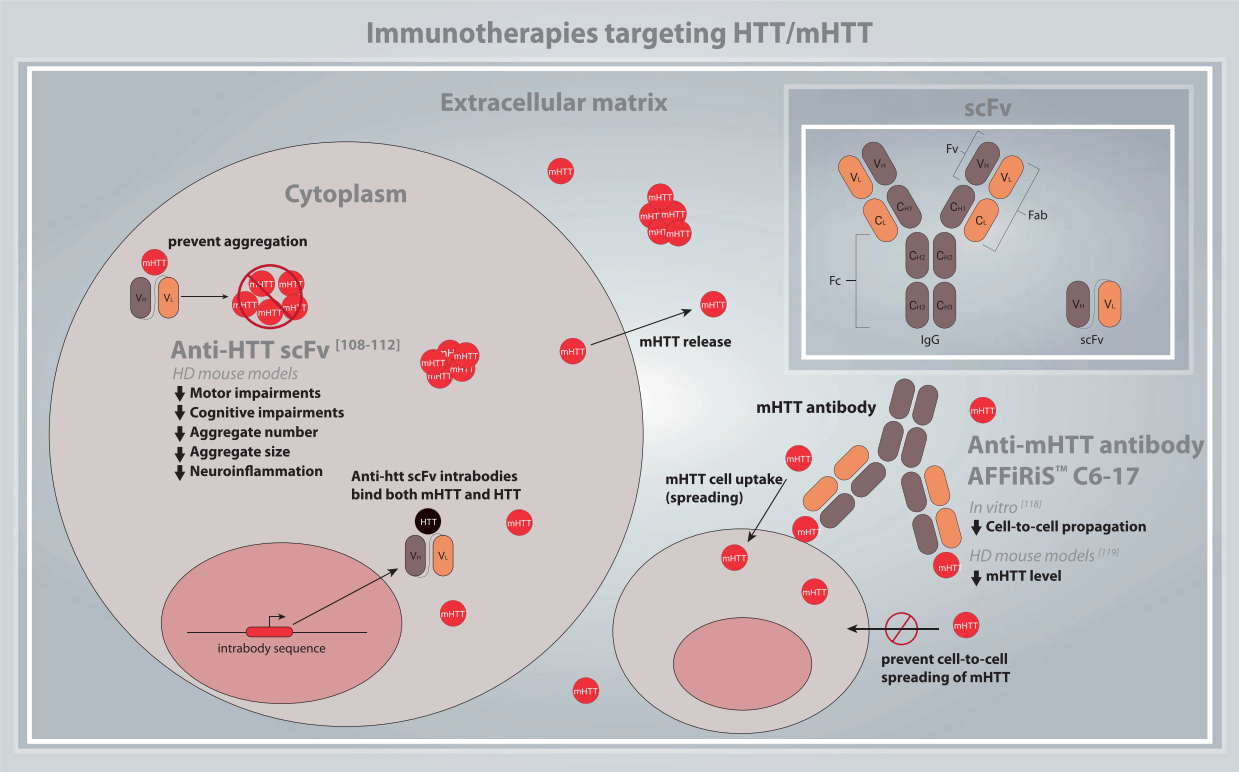


Fig. 4 Immunotherapies targeting mHTT. Illustration of the mechanisms of action of antibodies and intrabodies targeting HTT and mHTT. Abbreviations: HD: Huntington's disease; HTT: Huntingtin protein; mHTT: mutant huntingtin protein; scFv: single-chain variable fragment; IgG: Immunoglobulin G; Fc: Fragment crystallisable region; Fv: variable domain fragment; Fab: Fragment antigen-binding; V_L: light chain variable domain; V_H: heavy chain variable domain; C_L: light chain constant domain; C_H: heavy chain constant domain

state, but experiments using polyQ-specific intrabodies led to accelerated cell death and aggregate formation in various cell culture systems [106]. Based on these findings, other targets were selected including the N-terminal part of the HTT protein, which showed a good capacity to inhibit aggregate formation and reduce cell toxicity in cultures [107]. Anti-N-terminal HTT intrabodies have also been shown to reduce the size and frequency of aggregates in striatal neurons in the R6/1 mouse model [108]. Another intrabody, which targets the proline-rich domain of the HTT protein, has been tested in several HD mouse models and has led to reductions in number of mHTT aggregates in the cytoplasm and nucleus of striatal neurons with improvements in several motor and cognitive tests [109]. In a recent study [110], a scFv intrabody (W20) targeting oligomeric epitopes such as β-amyloid, α-synuclein, and polyQ have been used in BACHD mice. The treatment with this W20 antibody reduced brain mHTT aggregates, ameliorated motor and memory impairments and attenuated gliosis and neuroinflammation. Even if intrabodies drastically reduce the presence of mHTT aggregates, it does not entirely prevent aggregate formation but only slows down the process [108]. Indeed, it is possible that a small fraction of the HTT/mHTT protein escapes intrabody interaction, even for a short period of time, and then aggregate [111]. To counteract this, an anti-N-terminal HTT intrabody fused with a proteosomal PEST signal to direct the protein into the proteasome system for degradation has been developed [112]. This modified intrabody leads to significant turnover of antigen by allowing mHTT to enter the proteasome and then be degraded. Attempts to use an anti-fibrillar scFv fused with a PEST signal failed to eliminate already aggregated proteins [112].

Targeting free mHTT

To develop efficient therapeutic strategies for HD, we believe that the following very important observations must be considered: (1) mHTT is expressed outside the CNS, leading to peripheral manifestations [1, 113] and (2) mHTT is not always found inside cellular compartments, but is also present in the cerebrospinal fluid, plasma and within the extracellular matrix [114–117]. This extracellular pool of mHTT represents a very attractive target given its tractability for therapeutic as well as biomarker development. AFFiRiS™, a biopharmaceutical company, which has

developed peptide-based vaccines for the treatment of neurodegenerative diseases with candidates already in clinical trials for AD and PD, has made significant strides in the field and developed an antibody (C6–17) intended for passive immunization treatment that targets the caspase-6 cleavage region (aa 586) of HTT [118]. The strategy for using this agent is therefore to block cell-to-cell propagation and neutralize the extracellular pool of mHTT, preventing disease progression. This antibody has shown efficacy in inhibiting cell-to-cell propagation of mHTT *in vitro* with a decrease in mHTT levels in YAC128 mice treated with C6–17 antibodies [119].

Discussion

A role for the immune system in neurodegenerative disorders cannot be denied and should certainly not be ignored. Several immune mechanisms are dysregulated in HD, as in other neurodegenerative disorders and in particular there is consistent data showing that both elevated blood circulating pro-inflammatory cytokines and microglial activation in premanifest phases of the disease, supporting the idea that inflammation is not just a consequence of disease but an active, early participant. Drugs intended to restore the normal function of these pathways should, in theory, have the potential to slow down the disease and improve clinical features.

Some of the treatments discussed herein have shown promising results in HD animal models but have failed to produce equivalent efficacy in clinical trials. Of course, extrapolation from rodents to humans is challenging and the choice of animal models to test a drug can introduce an enormous source of variability. For example, the choice of the promoter that drives the expression of mHTT, the number of CAG repeats and the form of mHTT expressed (only exon 1 or full mHTT) varies between HD mouse models. In parallel, disease course/evolution is unique to each patient and is not only determined by the CAG repeat length but by environmental/epigenetic factors as well. When envisioning immunotherapy, we must also take into account that each individual presents with a unique immune system that can drive different disease evolution patterns. During the entire lifespan, the immune system is exposed to a plethora of antigens that will determine its singular profile. However, experimental mice are less subject to such environmental variability, further complicating the extrapolation of findingsfrom mice and human patients. To better understand the participation of the immune system in HD, it will still be critical to study the impact of the environment on the immune response as well as the impact of the microbiome. Indeed, we are now aware of a close relationship between the microbiome and the brain, via the "gut–brain axis" and its implication in some neurodegenerative disorders such as PD and AD [120]. Unfortunately, the relationship between the microbiome and the development of HD has hardly been studied [121]. Finally, it remains difficult to determine when anti-inflammatory agents should be tested in the disease course and at what dose and how target engagement can be confirmed upon administration.

Another aspect that is largely overlooked in HD is the fact that mHTT is also found extracellularly. Targeting free/extracellular mHTT is the prime objective of vaccine-based therapy, an aspect that is certainly not addressed by other therapeutic strategies being developed (e.g., gene silencing, antisense oligonucleotide—ASO). Another advantage of antibody-based therapies is that they could easily and synergistically be combined with other experimental therapies such as ASO, conjointly targeting intra- and extracellular mHTT. The safety of passive immunization has been widely studied and represents a straightforward approach that can be rapidly translated from bench to bedside. Combining immunotherapy with other types of approaches, such as gene silencing, could be a better approach and ultimately may be the best way by which to trial these therapeutic approaches in HD.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Soulet D, Cicchetti F. The role of immunity in Huntington's disease. Mol Psychiatry. 2011;16:889–902.
2. Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, et al. Huntington disease. Nat Rev Dis Prim. 2015;1:10155.
3. Crotti A, Glass CK. The choreography of neuroinflammation in Huntington's disease. Trends Immunol. 2015;36:364–73.
4. Politis M, Pavese N, Tai YF, Kiferle L, Mason SL, Brooks DJ, et al. Microglial activation in regions related to cognitive function predicts disease onset in Huntington's disease: a multimodal imaging study. Hum Brain Mapp. 2011;32:258–70.
5. Björkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, et al. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med. 2008;205:1869–77.
6. Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Björkqvist M, Petersén A, et al. Proteomic profiling of plasma in Huntington's

disease reveals neuroinflammatory activation and biomarker candidates. J Proteome Res. 2007;6:2833–40.
7. Wild E, Magnusson A, Lahiri N, Krus U, Orth M, Tabrizi SJ, et al. Abnormal peripheral chemokine profile in Huntington's disease. PLoS Curr. 2011;3:RRN1231
8. Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 2007;130:1759–66.
9. Kwan W, Träger U, Davalos D, Chou A, Bouchard J, Andre R, et al. Mutant huntingtin impairs immune cell migration in Huntington disease. J Clin Invest. 2012;122:4737–47.
10. van der Burg JMM, Björkqvist M, Brundin P. Beyond the brain: widespread pathology in Huntington's disease. Lancet Neurol. 2009;8:765–74.
11. Träger U, Andre R, Lahiri N, Magnusson-Lind A, Weiss A, Grueninger S, et al. HTT-lowering reverses Huntington's disease immune dysfunction caused by NFκB pathway dysregulation. Brain. 2014;137:819–33.
12. Ramsingh AI, Manley K, Rong Y, Reilly A, Messer A. Transcriptional dysregulation of inflammatory/immune pathways after active vaccination against Huntington′s disease. Hum Mol Genet. 2015;24:6186–97.
13. Kwan W, Magnusson A, Chou A, Adame A, Carson MJ, Kohsaka S, et al. Bone marrow transplantation confers modest benefits in Mouse models of Huntington's disease. J Neurosci. 2012;32:133–42.
14. Huntington Study Group TREND-HD Investigators. Randomized controlled trial of ethyl-eicosapentaenoic acid in Huntington disease: the TREND-HD study. Arch Neurol. 2008;65:1582–9.
15. Hickey MA, Chesselet M-F. Apoptosis in Huntington's disease. Prog Neuropsychopharmacol Biol Psychiatry. 2003;27:255–65.
16. Singhrao SK, Neal JW, Morgan BP, Gasque P. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Exp Neurol. 1999;159:362–76.
17. Panov AV, Gutekunst C-A, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci. 2002;5:731–6.
18. Jump DB. The biochemistry of n-3 polyunsaturated fatty acids. J Biol Chem. 2002;277:8755–8.
19. Murck H, Manku M. Ethyl-EPA in Huntington disease: potentially relevant mechanism of action. Brain Res Bull. 2007;72:159–64.
20. Moon D-O, Kim K-C, Jin C-Y, Han M-H, Park C, Lee K-J, et al. Inhibitory effects of eicosapentaenoic acid on lipopolysaccharide-induced activation in BV2 microglia. Int Immunopharmacol. 2007;7:222–9.
21. Minogue AM, Lynch AM, Loane DJ, Herron CE, Lynch MA. Modulation of amyloid-beta-induced and age-associated changes in rat hippocampus by eicosapentaenoic acid. J Neurochem. 2007;103:914–26.
22. Lo CJ, Chiu KC, Fu M, Lo R, Helton S. Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF kappa B activity. J Surg Res. 1999;82:216–21.
23. Zhao Y, Joshi-Barve S, Barve S, Chen LH. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. J Am Coll Nutr. 2004;23:71–8.
24. Clifford JJ, Drago J, Natoli AL, Wong JYF, Kinsella A, Waddington JL, et al. Essential fatty acids given from conception prevent topographies of motor deficit in a transgenic model of Huntington's disease. Neuroscience. 2002;109:81–8.
25. Van Raamsdonk JM, Pearson J, Rogers DA, Lu G, Barakauskas VE, Barr AM, et al. Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Exp Neurol. 2005;196:266–72.
26. Vaddadi KS, Soosai E, Chiu E, Dingjan P. A randomised, placebo-controlled, double blind study of treatment of Huntington's disease with unsaturated fatty acids. Neuroreport. 2002;13:29–33.
27. Puri BK, Bydder GM, Counsell SJ, Corridan BJ, Richardson AJ, Hajnal JV, et al. MRI and neuropsychological improvement in Huntington disease following ethyl-EPA treatment. Neuroreport. 2002;13:123–6.
28. Puri BK, Bydder GM, Manku MS, Clarke A, Waldman AD, Beckmann CF. Reduction in cerebral atrophy associated with ethyl-eicosapentaenoic acid treatment in patients with Huntington's disease. J Int Med Res. 2008;36:896–905.
29. M.E. Cudkowicz, M. McDermott, R. Doolan, F. Marshall, K. Kieburtz, HSG. A phase 2 trial of minocycline in Huntington's disease. *Mov Disord.* 2009;24:S1–653.
30. Huntington Study Group DOMINO Investigators. A futility study of minocycline in Huntington's disease. Mov Disord. 2010;25:2219–24.
31. Huntington Study Group. Minocycline safety and tolerability in Huntington disease. Neurology. 2004;63:547–9.
32. Thomas M, Ashizawa T, Jankovic J. Minocycline in Huntington's disease: a pilot study. Mov Disord. 2004;19:692–5.
33. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, et al. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. Nat Med. 2000;6:797–801.
34. Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, et al. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proc Natl Acad Sci USA. 2003;100:10483–7.
35. Stack EC, Smith KM, Ryu H, Cormier K, Chen M, Hagerty SW, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. Biochim Biophys Acta. 2006;1762:373–80.
36. Zhu S, Stavrovskaya IG, Drozda M, Kim BYS, Ona V, Li M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature. 2002;417:74–8.
37. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, et al. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. J Neurosci. 2002;22:1763–71.
38. Diguet E, Rouland R, Tison F. Minocycline is not beneficial in a phenotypic mouse model of Huntington's disease. Ann Neurol. 2003;54:841–2.
39. Diguet E, Fernagut P-O, Wei X, Du Y, Rouland R, Gross C, et al. Deleterious effects of minocycline in animal models of Parkinson's disease and Huntington's disease. Eur J Neurosci. 2004;19:3266–76.
40. Smith DL, Woodman B, Mahal A, Sathasivam K, Ghazi-Noori S, Lowden PAS, et al. Minocycline and doxycycline are not beneficial in a model of Huntington's disease. Ann Neurol. 2003;54:186–96.
41. Bantubungi K, Jacquard C, Greco A, Pintor A, Chtarto A, Tai K, et al. Minocycline in phenotypic models of Huntington's disease. Neurobiol Dis. 2005;18:206–17.
42. Ehrnhoefer DE, Duennwald M, Markovic P, Wacker JL, Engemann S, Roark M, et al. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum Mol Genet. 2006;15:2743–51.
43. Santangelo C, Varì R, Scazzocchio B, Di Benedetto R, Filesi C, Masella R. Polyphenols, intracellular signalling and inflammation. Ann Ist Super Sanita. 2007;43:394–405.

44. Relja B, Töttel E, Breig L, Henrich D, Schneider H, Marzi I, et al. Plant polyphenols attenuate hepatic injury after hemorrhage/resuscitation by inhibition of apoptosis, oxidative stress, and inflammation via NF-kappaB in rats. Eur J Nutr. 2012;51:311–21.
45. Kang KS, Wen Y, Yamabe N, Fukui M, Bishop SC, Zhu BT. Dual beneficial effects of (-)-epigallocatechin-3-gallate on levodopa methylation and hippocampal neurodegeneration: in vitro and in vivo studies. PLoS ONE. 2010;5:e11951.
46. Xu Z, Chen S, Li X, Luo G, Li L, Le W. Neuroprotective effects of (-)-epigallocatechin-3-gallate in a transgenic mouse model of amyotrophic lateral sclerosis. Neurochem Res. 2006;31:1263–9.
47. Li R, Huang Y-G, Fang D, Le W-D. (-)-Epigallocatechin gallate inhibits lipopolysaccharide-induced microglial activation and protects against inflammation-mediated dopaminergic neuronal injury. J Neurosci Res. 2004;78:723–31.
48. Cheng-Chung Wei J, Huang H-C, Chen W-J, Huang C-N, Peng C-H, Lin C-L. Epigallocatechin gallate attenuates amyloid β-induced inflammation and neurotoxicity in EOC 13.31 microglia. Eur J Pharmacol. 2016;770:16–24.
49. Boadas-Vaello P, Verdú E. Epigallocatechin-3-gallate treatment to promote neuroprotection and functional recovery after nervous system injury. Neural Regen Res. 2015;10:1390–2.
50. Ichikawa D, Matsui A, Imai M, Sonoda Y, Kasahara T. Effect of various catechins on the IL-12p40 production by murine peritoneal macrophages and a macrophage cell line, J774.1. Biol Pharm Bull. 2004;27:1353–8.
51. Kundu JK, Na H-K, Chun K-S, Kim Y-K, Lee SJ, Lee SS, et al. Inhibition of phorbol ester-induced COX-2 expression by epigallocatechin gallate in mouse skin and cultured human mammary epithelial cells. J Nutr. 2003;133:3805S–3810S.
52. Gratuze M, Noël A, Julien C, Cisbani G, Milot-Rousseau P, Morin F, et al. Tau hyperphosphorylation and deregulation of calcineurin in mouse models of Huntington's disease. Hum Mol Genet. 2015;24:86–99.
53. Vuono R, Winder-Rhodes S, de Silva R, Cisbani G, Drouin-Ouellet J, REGISTRY Investigators of the European Huntington's Disease Network. et al. The role of tau in the pathological process and clinical expression of Huntington's disease. Brain. 2015;138:1907–18.
54. Cisbani G, Maxan A, Kordower JH, Planel E, Freeman TB, Cicchetti F. Presence of tau pathology within fetal neural allografts in patients with Huntington's and Parkinson's disease. Brain. 2017;140:2982–92.
55. Wobst HJ, Sharma A, Diamond MI, Wanker EE, Bieschke J. The green tea polyphenol (−)-epigallocatechin gallate prevents the aggregation of tau protein into toxic oligomers at substoichiometric ratios. FEBS Lett. 2015;589:77–83.
56. López-Sendón Moreno JL, García Caldentey J, Trigo Cubillo P, Ruiz Romero C, García Ribas G, Alonso Arias MAA, et al. A double-blind, randomized, cross-over, placebo-controlled, pilot trial with Sativex in Huntington's disease. J Neurol. 2016;263:1390–400.
57. Valdeolivas S, Satta V, Pertwee RG, Fernández-Ruiz J, Sagredo O. Sativex-like combination of phytocannabinoids is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease: role of CB1 and CB2 receptors. ACS Chem Neurosci. 2012;3:400–6.
58. Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM. Biased type 1 cannabinoid receptor signaling influences neuronal viability in a cell culture model of Huntington disease. Mol Pharmacol. 2016;89:364–75.
59. Blázquez C, Chiarlone A, Bellocchio L, Resel E, Pruunsild P, García-Rincón D, et al. The CB_1 cannabinoid receptor signals striatal neuroprotection via a PI3K/Akt/mTORC1/BDNF pathway. Cell Death Differ. 2015;22:1618–29.
60. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science. 2001;293:493–8.
61. Dowie MJ, Howard ML, Nicholson LFB, Faull RLM, Hannan AJ, Glass M. Behavioural and molecular consequences of chronic cannabinoid treatment in Huntington's disease transgenic mice. Neuroscience. 2010;170:324–36.
62. Navarro G, Morales P, Rodríguez-Cueto C, Fernández-Ruiz J, Jagerovic N, Franco R. Targeting cannabinoid CB2 receptors in the central nervous system. medicinal chemistry approaches with focus on neurodegenerative disorders. Front Neurosci. 2016;10:406
63. Sagredo O, González S, Aroyo I, Pazos MR, Benito C, Lastres-Becker I, et al. Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. Glia. 2009;57:1154–67.
64. Dowie MJ, Grimsey NL, Hoffman T, Faull RLM, Glass M. Cannabinoid receptor CB2 is expressed on vascular cells, but not astroglial cells in the post-mortem human Huntington's disease brain. J Chem Neuroanat. 2014;59–60:62–71.
65. Bouchard J, Truong J, Bouchard K, Dunkelberger D, Desrayaud S, Moussaoui S, et al. Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of Huntington's disease. J Neurosci. 2012;32:18259–68.
66. Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet. 2003;35:76–83.
67. Comi G, Jeffery D, Kappos L, Montalban X, Boyko A, Rocca MA, et al. Placebo-controlled trial of oral laquinimod for multiple sclerosis. N Engl J Med. 2012;366:1000–9.
68. Brück W, Wegner C. Insight into the mechanism of laquinimod action. J Neurol Sci. 2011;306:173–9.
69. Thöne J, Gold R. Laquinimod: a promising oral medication for the treatment of relapsing-remitting multiple sclerosis. Expert Opin Drug Metab Toxicol. 2011;7:365–70.
70. Garcia-Miralles M, Hong X, Tan LJ, Caron NS, Huang Y, To XV, et al. Laquinimod rescues striatal, cortical and white matter pathology and results in modest behavioural improvements in the YAC128 model of Huntington disease. Sci Rep. 2016;6:31652
71. Ellrichmann G, Blusch A, Fatoba O, Brunner J, Hayardeny L, Hayden M, et al. Laquinimod treatment in the R6/2 mouse model. Sci Rep. 2017;7:4947.
72. Dobson L, Träger U, Farmer R, Hayardeny L, Loupe P, Hayden MR, et al. Laquinimod dampens hyperactive cytokine production in Huntington's disease patient myeloid cells. J Neurochem. 2016;137:782–94.
73. Okuno T, Nakatsuji Y, Moriya M, Takamatsu H, Nojima S, Takegahara N, et al. Roles of Sema4D-plexin-B1 interactions in the central nervous system for pathogenesis of experimental autoimmune encephalomyelitis. J Immunol. 2010;184:1499–506.
74. Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, et al. Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet. 2006;15:965–77.
75. Smith ES, Jonason A, Reilly C, Veeraraghavan J, Fisher T, Doherty M, et al. SEMA4D compromises blood–brain barrier, activates microglia, and inhibits remyelination in neurodegenerative disease. Neurobiol Dis. 2015;73:254–68.
76. Southwell AL, Franciosi S, Villanueva EB, Xie Y, Winter LA, Veeraraghavan J, et al. Anti-semaphorin 4D immunotherapy ameliorates neuropathology and some cognitive impairment in

the YAC128 mouse model of Huntington disease. Neurobiol Dis. 2015;76:46–56.
77. Granja AG, Carrillo-Salinas F, Pagani A, Gómez-Cañas M, Negri R, Navarrete C, et al. A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis. J NeuroImmune Pharmacol. 2012;7:1002–16.
78. Carrillo-Salinas FJ, Navarrete C, Mecha M, Feliú A, Collado JA, Cantarero I, et al. A cannabigerol derivative suppresses immune responses and protects mice from experimental autoimmune encephalomyelitis. PLoS ONE. 2014;9:e94733.
79. Dickey AS, Pineda VV, Tsunemi T, Liu PP, Miranda HC, Gilmore-Hall SK, et al. PPAR-δ is repressed in Huntington's disease, is required for normal neuronal function and can be targeted therapeutically. Nat Med. 2016;22:37–45.
80. Corona JC, Duchen MR. PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. Free Radic Biol Med. 2016;100:153–63.
81. Díaz-Alonso J, Paraíso-Luna J, Navarrete C, Del Río C, Cantarero I, Palomares B, et al. VCE-003.2, a novel cannabigerol derivative, enhances neuronal progenitor cell survival and alleviates symptomatology in murine models of Huntington's disease. Sci Rep. 2016;6:29789.
82. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WEF, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? J Exp Med. 1999;189:865–70.
83. Kruse N, Cetin S, Chan A, Gold R, Lühder F. Differential expression of BDNF mRNA splice variants in mouse brain and immune cells. J Neuroimmunol. 2007;182:13–21.
84. Xu D, Lian D, Wu J, Liu Y, Zhu M, Sun J, et al. Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis. J Neuroinflamm. 2017;14:156
85. Massa SM, Yang T, Xie Y, Shi J, Bilgen M, Joyce JN, et al. Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. J Clin Invest. 2010;120:1774–85.
86. Longo FM, Massa SM. Small-molecule modulation of neurotrophin receptors: a strategy for the treatment of neurological disease. Nat Rev Drug Discov. 2013;12:507–25.
87. Simmons DA, Belichenko NP, Yang T, Condon C, Monbureau M, Shamloo M, et al. A small molecule TrkB ligand reduces motor impairment and neuropathology in R6/2 and BACHD mouse models of Huntington's disease. J Neurosci. 2013;33:18712–27.
88. Alto LT, Chen X, Ruhn KA, Treviño I, Tansey MG. AAV-dominant negative tumor necrosis factor (DN-TNF) gene transfer to the striatum does not rescue medium spiny neurons in the YAC128 mouse model of Huntington's disease. PLoS ONE. 2014;9:e96544.
89. Hsiao H-Y, Chiu F-L, Chen C-M, Wu Y-R, Chen H-M, Chen Y-C, et al. Inhibition of soluble tumor necrosis factor is therapeutic in Huntington's disease. Hum Mol Genet. 2014;23:4328–44.
90. Newton R, Seybold J, Kuitert LME, Bergmann M, Barnes PJ. Repression of cyclooxygenase-2 and prostaglandin E2release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA. J Biol Chem. 1998;273:32312–21.
91. Diamond MI, Robinson MR, Yamamoto KR. Regulation of expanded polyglutamine protein aggregation and nuclear localization by the glucocorticoid receptor. Proc Natl Acad Sci. 2000;97:657–61.
92. Maheshwari M, Bhutani S, Das A, Mukherjee R, Sharma A, Kino Y, et al. Dexamethasone induces heat shock response and slows down disease progression in mouse and fly models of Huntington's disease. Hum Mol Genet. 2014;23:2737–51.
93. Chafekar SM, Duennwald ML. Impaired heat shock response in cells expressing full-length polyglutamine-expanded huntingtin. PLoS ONE. 2012;7:e37929.
94. Moseley PL. Heat shock proteins and the inflammatory response. Ann N Y Acad Sci. 1998;856:206–13.
95. Nuti A, Maremmani C, Ceravolo R, Pavese N, Bonuccelli U, Muratorio A. [Dexamethasone therapy in Huntington chorea: preliminary results]. Riv Neurol. 1991;61:225–7.
96. Ellison SM, Trabalza A, Tisato V, Pazarentzos E, Lee S, Papadaki V, et al. Dose-dependent neuroprotection of VEGF165 in Huntington's disease striatum. Mol Ther. 2013;21:1862–75.
97. Fossale E, Wheeler VC, Vrbanac V, Lebel L-A, Teed A, Mysore JS, et al. Identification of a presymptomatic molecular phenotype in Hdh CAG knock-in mice. Hum Mol Genet. 2002;11:2233–41.
98. Carnemolla A, Fossale E, Agostoni E, Michelazzi S, Calligaris R, De Maso L, et al. Rrs1 is involved in endoplasmic reticulum stress response in Huntington disease. J Biol Chem. 2009;284:18167–73.
99. Egea J, Buendia I, Parada E, Navarro E, León R, Lopez MG. Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. Biochem Pharmacol. 2015;97:463–72.
100. Ryu JK, Kim SU, McLarnon JG. Blockade of quinolinic acid-induced neurotoxicity by pyruvate is associated with inhibition of glial activation in a model of Huntington's disease. Exp Neurol. 2004;187:150–9.
101. Ryu JK, Choi HB, McLarnon JG. Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage, and neuronal loss in an excitotoxic animal model of Huntington's disease. Neuroscience. 2006;141:1835–48.
102. Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, et al. Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. J Neurosci. 2000;20:4389–97.
103. Zhang W, Narayanan M, Friedlander RM. Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. Ann Neurol. 2003;53:267–70.
104. Miller TW, Shirley TL, Wolfgang WJ, Kang X, Messer A. DNA vaccination against mutant huntingtin ameliorates the HDR6/2 diabetic phenotype. Mol Ther. 2003;7:572–9.
105. Marschall ALJ, Dübel S. Antibodies inside of a cell can change its outside: can intrabodies provide a new therapeutic paradigm? Comput Struct Biotechnol J. 2016;14:304–8.
106. Khoshnan A, Ko J, Patterson PH. Effects of intracellular expression of anti-huntingtin antibodies of various specificities on mutant huntingtin aggregation and toxicity. Proc Natl Acad Sci USA. 2002;99:1002–7.
107. Lecerf J-M, Shirley TL, Zhu Q, Kazantsev A, Amersdorfer P, Housman DE, et al. Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci USA. 2001;98:4764–9.
108. Snyder-Keller A, McLear JA, Hathorn T, Messer A. Early or late-stage anti-N-terminal Huntingtin intrabody gene therapy reduces pathological features in B6.HDR6/1 mice. J Neuropathol Exp Neurol. 2010;69:1078–85.
109. Southwell AL, Ko J, Patterson PH. Intrabody gene therapy ameliorates motor, cognitive and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci. 2009;29:13589.
110. Zha J, Liu X-M, Zhu J, Liu S-Y, Lu S, Xu P-X, et al. A scFv antibody targeting common oligomeric epitope has potential for treating several amyloidoses. Sci Rep. 2016;6:36631.
111. Messer A, Joshi SN. Intrabodies as neuroprotective therapeutics. Neurotherapeutics.2013;10:447–58.
112. Butler DC, Messer A. Bifunctional anti-Huntingtin proteasome-directed intrabodies mediate efficient degradation of mutant

Huntingtin exon 1 protein fragments. PLoS ONE. 2011;6: e29199.
113. Carroll JB, Bates GP, Steffan J, Saft C, Tabrizi SJ. Treating the whole body in Huntington's disease. Lancet Neurol. 2015;14:1135–42.
114. Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, Bae J, et al. Human-to-mouse prion-like propagation of mutant Huntingtin protein. Acta Neuropathol (Berl). 2016;132:577–92.
115. Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, et al. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci. 1999;19:2522–34.
116. Liu X, Valentine SJ, Plasencia MD, Trimpin S, Naylor S, Clemmer DE. Mapping the human plasma proteome by SCX-LC-IMS-MS. J Am Soc Mass Spectrom. 2007;18: 1249–64.
117. Wild EJ, Boggio R, Langbehn D, Robertson N, Haider S, Miller JRC, et al. Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients. J Clin Invest. 2015;125:1979–86.
118. Bartl S, Southwell AL, Parth M, Salhat N, Burkert M, Siddu A, et al. Antibody-based targeting of mutant Huntingtin for the treatment of Huntington's disease. Abstract, 12th Annual Huntington's Disease Therapeutics Conference, CHDI Foundation April 2017.
119. OW Smrzka, Southwell AL, Bartl S, Parth M, Burkert M, Villanueva EB, et al. Passive treatment with huntingtin (HTT)-specific mAb's lowers HTT levels and improves motor performance in YAC128 mice. Abstract, 11th Annual Huntington's Disease Therapeutics Conference, CHDI Foundation February 2017.
120. Main BS, Minter MR. Microbial immuno-communication in neurodegenerative diseases. Front Neurosci. 2017;11:151
121. Rosas HD, Doros G, Bhasin S, Thomas B, Gevorkian S, Malarick K, et al. A systems-level "misunderstanding": the plasma metabolome in Huntington's disease. Ann Clin Transl Neurol. 2015;2:756–68.