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# Pepinemab antibody blockade of SEMA4D in early Huntington's disease: a randomized, placebo-controlled, phase 2 trial

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**SIGNAL is a multicenter, randomized, double-blind, placebo-controlled phase 2 study (no. NCT02481674) established to evaluate pepinemab, a semaphorin 4D (SEMA4D)-blocking antibody, for treatment of Huntington's disease (HD). The trial enrolled a total of 265 HD gene expansion carriers with either early manifest (EM,  $n = 179$ ) or late prodromal (LP,  $n = 86$ ) HD, randomized (1:1) to receive 18 monthly infusions of pepinemab ( $n = 91$  EM, 41 LP) or placebo ( $n = 88$  EM, 45 LP). Pepinemab was generally well tolerated, with a relatively low frequency of serious treatment-emergent adverse events of 5% with pepinemab compared to 9% with placebo, including both EM and LP participants. Coprimary efficacy outcome measures consisted of assessments within the EM cohort of (1) a two-item HD cognitive assessment family comprising one-touch stockings of Cambridge (OTS) and paced tapping (PTAP) and (2) clinical global impression of change (CGIC). The differences between pepinemab and placebo in mean change (95% confidence interval) from baseline at month 17 for OTS were  $-1.98$  ( $-4.00, 0.05$ ) (one-sided  $P = 0.028$ ), and for PTAP  $1.43$  ( $-0.37, 3.23$ ) (one-sided  $P = 0.06$ ). Similarly, because a significant treatment effect was not observed for CGIC, the coprimary endpoint, the study did not meet its prespecified primary outcomes. Nevertheless, a number of other positive outcomes and post hoc subgroup analyses—including additional cognitive measures and volumetric magnetic resonance imaging and fluorodeoxyglucose-positron-emission tomography imaging assessments—provide rationale and direction for the design of a phase 3 study and encourage the continued development of pepinemab in patients diagnosed with EM HD.**

Huntington's disease is a progressive fatal neurodegenerative disease that affects between five and ten per 100,000 people of European descent, but is less prevalent in Asian and African countries. More than 30,000 Americans are diagnosed with manifest disease and approximately another 150,000 are at risk of having inherited the dominant mutation, with approximately equal prevalence in Europe and Australia. To date, no potential disease-modifying therapy has demonstrated efficacy. Currently available treatments focus on managing psychiatric and motor symptoms such as chorea, but may be limited by poor tolerability and short duration and they may not produce meaningful functional benefits. Patients and caregivers have identified cognitive and emotional impairment as the most substantial issues impacting their lives<sup>1,2</sup>. Neuropathologic changes associated with toxic aggregates of mutant huntingtin protein are characterized by brain atrophy, neuronal loss and dysfunction. The most pronounced changes occur in striatal medium spiny neurons but spread to other brain regions (for example, cortex and white matter)<sup>3</sup>. Evidence from imaging studies in patients with HD and animal models suggests that neuronal dysfunction is accompanied by major changes in glial cells, including loss of normal functions and gain of reactive neurotoxic and neuroinflammatory responses. These changes occur early in disease progression, before substantial neuronal loss and the onset of motor, cognitive and behavioral deficits<sup>4</sup>.

SEMA4D plays a critical role in regulating the transition between homeostatic and reactive states of glial cells. SEMA4D binds to its plexin receptors (plexins B1 and B2) expressed on glial cells, including astrocytes, microglia, and glial progenitor cells, as well as endothelial cells, and signals through small-membrane Rho GTPases<sup>5-7</sup> to regulate the actin cytoskeleton and activate NF $\kappa$ B, a master regulator of inflammatory cytokines<sup>8-12</sup>. In the central nervous system, SEMA4D is upregulated in stressed or damaged neurons and signals through plexin-B1/B2 receptors to (1) activate glial cells<sup>13,14</sup>; (2) disrupt normal astrocyte metabolic and synaptic functions, including glucose and glutamate transport (E.E.E. et al., in press, *J Neuroinflammation*, SEMA4D is upregulated in neurons of diseased brains and triggers astrocyte reactivity); (3) inhibit migration and differentiation of glial progenitor cells that can replace damaged oligodendrocytes and replenish astrocytes<sup>5,6,15,16</sup>; and (4) disrupt endothelial tight junctions that are required for the integrity of the blood-brain barrier (BBB)<sup>13</sup>. Glial cells are increasingly recognized for their important contribution to the onset and progression of neurodegenerative diseases<sup>17</sup>. Given the multifaceted mechanism of action of SEMA4D and the crosstalk between affected glial cells, blockade of this pathway may prevent loss of normal astrocytic functions, reprogram microglia, promote myelin repair and restore vascular changes associated with neuronal dysfunction and degeneration in HD<sup>4</sup>. We previously

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**Table 1 | Overview of TEAEs for the safety analysis period**

Category	Cohort B1, <i>n</i> = 179		Cohort B2, <i>n</i> = 86		Cohort B, overall <i>n</i> = 265	
	PBO, <i>n</i> = 87	PEPI, <i>n</i> = 92	PBO, <i>n</i> = 45	PEPI, <i>n</i> = 41	PBO, <i>n</i> = 132	PEPI, <i>n</i> = 133
Subjects with $\geq 1$ TEAE, <i>n</i> (%) <sup>a</sup>	82 (94%)	84 (91%)	41 (91%)	41 (100%)	123 (93%)	125 (94%)
Probably or definitely related <sup>b</sup>	10 (11%)	16 (17%)	9 (20%)	17 (41%)	19 (14%)	33 (25%)
Study drug discontinuation	2 (2%)	5 (5%)	1 (2%)	0	3 (2%)	5 (4%)
Serious TEAE	8 (9%)	4 (4%)	4 (9%)	2 (5%)	12 (9%)	6 (5%)
Probably/definitely related <sup>b</sup>	0	0	0	0	0	0
Grade $\geq 3$ TEAE <sup>c</sup>	14 (16%)	17 (18%)	6 (13%)	8 (20%)	20 (15%)	25 (19%)

Column header counts are the number of subjects in the safety population; TEAEs are those that occurred during the safety analysis period with onset on or after the date of the first exposure to study drug (PEPI or PBO). <sup>a</sup>Subjects are counted at most once in each cell, regardless of the number of events they may have had; denominators are the number of subjects in the safety population, unless otherwise specified. <sup>b</sup>Relatedness to study drug is defined as any of attribution of probable or definite as reported by the investigator. <sup>c</sup>Grade  $\geq 3$  refers to any severe, life-threatening or fatal event.

reported that an anti-SEMA4D antibody ameliorated neuropathological brain atrophy and reduced anxiety-like behavior and cognitive deficits in YAC128 HD knock-in mice<sup>10</sup>.

Based on the mechanism of action, activity in preclinical models of HD and other neurological diseases<sup>10,13</sup> and a favorable safety profile in previous clinical studies<sup>18–20</sup>, the SIGNAL study was initiated to evaluate prespecified measures of safety, tolerability and potential efficacy of pepinemab as a potential therapy for HD. Efficacy endpoints were selected based on previous natural history data from the PREDICT-HD and TRACK-HD studies<sup>21–23</sup>, as well as results of a completed randomized pilot study in HD (cohort A, 15 EM and 21 LP participants). Magnetic resonance imaging (MRI) has been used extensively as a measure of brain atrophy in HD<sup>24</sup>. Fluorodeoxyglucose–positron-emission tomography (FDG–PET) detects changes in glucose transport and is a measure of overall brain metabolic activity. Decline in FDG–PET standardized uptake value ratio (SUVR) has previously been shown to correlate with cognitive and functional decline during HD disease progression<sup>25–27</sup>, and correlates with cognitive decline and clinical progression in other neurodegenerative diseases<sup>28,29</sup>.

Herein we report the results of the SIGNAL cohort B study in 265 participants known to have  $\geq 36$  cytosine-adenine-guanine repeats in one huntingtin gene and total functional capacity (TFC) 11–13, with HD diagnostic confidence level (DCL) 4 (EM) or DCL 2–3 (LP). DCL is determined by clinician assessment of motor function on the Unified Huntington's Disease Rating Scale (UHDRS): DCL4 is defined as motor abnormalities that are unequivocal signs of HD while DCL2 and 3 are defined, respectively, as motor signs that may be (50–89% confident) or are probable (90–98% confident) signs of HD. Although the study did not meet its coprimarily clinical efficacy endpoints, a favorable safety profile, significant changes in imaging measures and multiple prespecified exploratory assessments supporting cognitive benefit to patients encourage further development. The organization of data presented here is, first, to focus on prespecified endpoints stipulated in the hierarchical testing plan and, second, to report prespecified endpoints of special interest, including imaging measures of brain atrophy and metabolic activity and, given that cognitive decline is of particular concern to patients and their families, the HD–cognitive assessment battery (HD–CAB) Index. Finally, because of its importance in guiding the design of a subsequent phase 3 study, we present key post hoc subgroup analyses in support of the hypothesis that patients at a mildly advanced stage of disease, as defined below, may be more responsive to treatment with pepinemab.

## Results

**Study design and baseline demographics.** The SIGNAL phase 2 trial was designed to determine whether treatment with a SEMA4D-blocking antibody, pepinemab, is safe and well-tolerated in participants with EM or LP HD, and to assess efficacy in the

EM cohort, as determined by cognitive, global (CGIC), functional and neuroimaging measures. The SIGNAL cohort B phase 2 study enrolled 179 EM participants (cohort B1) with diagnosed HD (DCL4) and with minimal to modest functional impairment (TFC 11–13), and 86 LP participants (cohort B2) with DCL2 or 3 and cytosine-adenine-guanine (CAG) repeat/age product (CAP) score  $\geq 200$ . Cohorts B1 and B2 were independently randomized 1:1 to either pepinemab (PEPI), administered intravenously (i.v.) every four weeks at a dose of 20 mg kg<sup>-1</sup>, or placebo (PBO) for 18 months of treatment followed by ~3 months of safety follow-up. Participants were enrolled at 30 clinical sites in the United States and Canada from 28 December 2015 to 31 December 2018. Participants, caregivers and clinician raters were blinded to treatment. Database lock and unblinding occurred in September 2020.

Study completion was high, with only 13/265 (*n* = 9 PBO, *n* = 4 PEPI) study discontinuations, as shown in the CONSORT diagram (Extended Data Fig. 1). There was limited impact of the Covid-19 pandemic, which occurred late in the study and that, as described in greater detail in Methods, was accommodated by allowing some flexibility in data collection at clinical study sites. Demographics and baseline characteristics were generally balanced between the treatment groups with respect to age, age of onset and other clinical measures obtained at screening or baseline (Extended Data Table 1 and Supplementary Fig. 1).

**Safety and tolerability.** Table 1 is a summary of treatment-emergent adverse events (TEAEs). No clinically significant group differences were observed in the frequency of participants experiencing  $\geq 1$  TEAE (*P* = 0.57, 0.12 and 0.81 for B1, B2 and B overall, respectively), TEAEs of grade  $\geq 3$  (*P* = 0.7, 0.57 and 0.52) or serious adverse events (*P* = 0.24, 0.68 and 0.16). During the safety analysis period (up to 18 months of treatment), fewer subjects overall had at least one TEAE considered probably or definitely related to study drug in the placebo group compared with pepinemab (14% versus 25%, respectively). This difference was predominantly in certain mild (grades 1 and 2) TEAEs in pepinemab-treated subjects, among which headache was most frequent. One suicide death was observed in an individual with EM disease following pepinemab treatment, but was deemed unrelated to treatment.

Extended Data Table 2 shows the percentage of most common TEAEs (>10%). Most TEAEs were considered mild to moderate in severity by the investigator. The percentage of subjects with a TEAE considered severe (Common Terminology Criteria for Adverse Events (CTCAE) grade  $\geq 3$ ) was marginally higher for pepinemab than placebo-treated subjects (19% versus 15%, *P* = 0.52). For each treatment group, the frequency of severe TEAEs was  $\leq 5\%$  within each system organ class. Results were consistent in a subset of patients evaluated for a total of 36 months of treatment and safety follow-up (Extended Data Table 2).

**Table 2 | Results from hierarchical testing of coprimary and secondary endpoints**

Population <sup>a</sup>	Endpoint analysis <sup>b</sup>		PBO, <i>n</i>	PEPI, <i>n</i>	LS mean diff. (95% CI) PEPI versus PBO	One-sided <i>P</i> value	Favors PEPI <sup>c</sup>
<b>Coprimary efficacy endpoints</b>							
Cohort B1 mITT	Two-item cognitive family	OTS (s) <sup>d</sup>	88	90	−1.98 (−4.00, 0.05)	0.028	(+)
		PTAP (1 s <sup>−1</sup> ) <sup>e</sup>	87	89	1.43 (−0.37, 3.23)	0.060	(+)
Cohort B1 CGIC	CGIC <sup>f</sup>		83	84	0.06 (−0.24, 0.37)	0.35	(+)
<b>Secondary efficacy endpoints</b>							
Cohort B1 mITT	Q-motor tap speed IOI duration (ms) <sup>g</sup>		87	89	−1.44 (−24.26, 21.38)	0.46	(+)
	UHDRS-TFC <sup>h</sup>		88	88	−0.29 (−0.81, 0.23)	0.87	(−)

<sup>a</sup>The cohort B1 mITT population comprised 178 participants (PBO, 88; PEPI, 90) while the CGIC population comprised 169 participants (PBO, 83; PEPI, 86). The number of subjects included in an analysis may differ from the full analysis population (for example, mITT or CGIC) size if any subjects do not have both baseline (if applicable) and post-baseline observation. <sup>b</sup>Analysis results are from an MMRM of change from baseline values unless otherwise specified, with estimation of the difference in means between groups at month 17. <sup>c</sup>The sign next to the *P* value indicates whether the direction of the estimated difference indicates better (+) or worse (−) performance for PEPI relative to PBO. <sup>d</sup>Time to a correct response (averaged over all trials per visit); lower values indicate better performance. <sup>e</sup>Tapping consistency measured as the reciprocal of the average standard deviation of inter-tap interval (ITI) durations (over all trials per visit); higher scores indicate better performance. <sup>f</sup>A seven-point Likert scale, ranging from very much worse (−3) to very much improved (+3); values are set to −3 following deaths adjudged by a blinded data review committee to be related to HD. <sup>g</sup>IOI duration in the more-affected hand at baseline (averaged over all trials per visit); lower values indicate better performance. The more affected hand at baseline is defined for each outcome as the side with the worse score at baseline; if both hands have the same baseline score, the more affected hand will be the self-reported dominant hand; if the dominant hand is mixed, the right hand will be reported. If a subject has a baseline score for only one hand, that hand will be the more affected hand. <sup>h</sup>Score ranging from 0 to 13; higher scores indicate better functioning.

Immunogenicity was evaluated by measuring the presence of anti-drug antibodies (ADA). Only eight subjects ( $n=0$  PBO,  $n=8$  PEPI) were confirmed positive for ADA, and all responses were transient and of low titer (<20). No relationship between immunogenicity status and safety outcomes was observed. No subject exhibited symptoms of cytokine release syndrome during the study. Three pepinemab- and two placebo-treated subjects had six TEAEs of infusion site reaction or infusion-related reaction, but none were considered serious, led to permanent discontinuation of the study drug, nor resulted in early study withdrawal. There were no clinically meaningful changes in any hematology, serum chemistry, urinalysis, vital signs or electrocardiogram (ECG) parameters over time. No clinically notable differences in HADS anxiety or suicidal ideation (Columbia–Suicide Severity Rating Scale) were observed between pepinemab- and placebo-treated subjects.

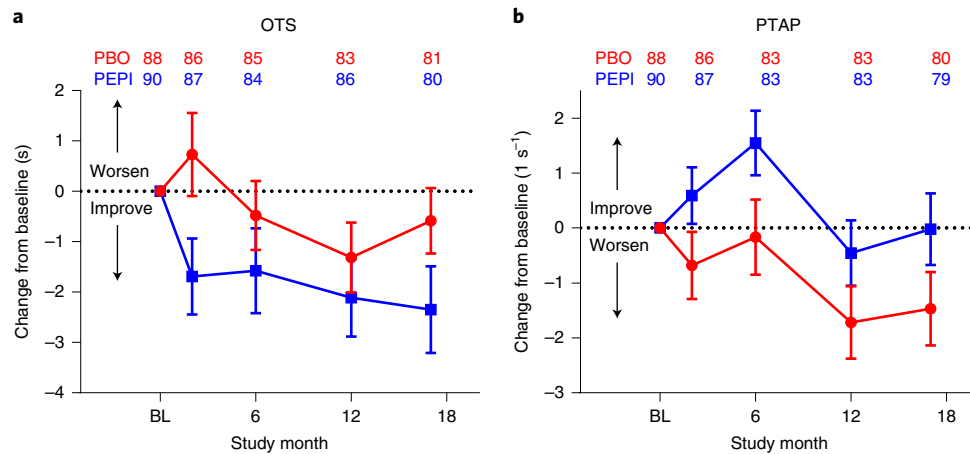
**Efficacy.** The coprimary efficacy measures for EM cohort B1 were a cognitive assessment family consisting of OTS and PTAP, two of the six components of the HD–CAB index, and the CGIC score evaluated by the treating physician. A summary of results from prespecified hierarchical testing of coprimary and secondary endpoints for cohort B1 is shown in Table 2. We had planned to analyze other UHDRS performance scales, as well as efficacy analysis of the combined B1 and B2 population (B-pooled) if the coprimary endpoints were successful. Since that was not the case, results for B-pooled are presented primarily for safety parameters in this report.

**Efficacy: cognition.** The HD–CAB is designed specifically for use in clinical studies of individuals with early HD<sup>30</sup> and offers sufficient range for detection of both symptomatic improvement and slowing of decline. The HD–CAB consists of six performance measures of different cognitive domains, including executive function, attention, memory, visuospatial processing, timing and emotion processing: OTS<sup>31</sup>, PTAP<sup>32</sup>, the symbol digit modalities test (SDMT)<sup>33</sup>, the emotion recognition test (EMO)<sup>34,35</sup>, the Hopkins verbal learning test–revised (HVLTR)<sup>36</sup> and the trail-making test–part B (TMT-B)<sup>37</sup>. Although the HD–CAB index derived from the average *z*-scores of the six components above was determined to be the most sensitive cognitive endpoint based on analysis of the pilot cohort A data, US regulators did not favor composite scores and recommended that we instead employ a smaller cognitive family evaluated without resorting to the use of *z*-scores. Results of the

pilot cohort A study indicated that OTS and PTAP had the highest effect sizes (0.43 and 0.38, respectively) of the six HD–CAB components. This was consistent with the relative HD–CAB component effect sizes reported by Stout et al.<sup>30</sup> Further, OTS and PTAP correlated with HD–CAB index (Pearson correlation coefficient for the change from baseline at 18 months,  $r = -0.24$ ,  $P = 0.034$  and  $r = 0.57$ ,  $P < 0.001$ , respectively). We therefore selected a two-member cognitive family consisting of OTS and PTAP as a prespecified coprimary endpoint for cohort B1 evaluated using the Hochberg procedure<sup>38</sup> and, as discussed below, retained the HD–CAB index as an exploratory endpoint.

In the two-member cognitive family, OTS, a test of executive function, assesses both spatial planning and working memory<sup>39</sup> while PTAP, an assessment of timing and psychomotor coordination, assesses the ability to maintain a preset rhythm by consistent inter-tap intervals in the absence of continuing aural cues. EM individuals treated with pepinemab demonstrated, on average, improved performance in OTS over the 18-month treatment duration (Fig. 1a and Table 2). Improvement is reflected in lower scores in this assessment (reduced latency to correct answer in seconds), and the change from baseline at month 17 resulted in least-squares (LS) mean difference (95% confidence interval (CI)) = −1.98 (−4.00, 0.05) (one-sided  $P = 0.028$ ). Similarly, change from baseline at month 17 in PTAP (improvement reflected in higher scores) resulted in LS mean difference (95% CI) = 1.43 (−0.37, 3.23) (one-sided  $P = 0.06$ ; Fig. 1b and Table 2). Because neither OTS nor PTAP attained the significance threshold of one-sided  $P = 0.025$ , and neither member independently met the higher standard of  $P = 0.0125$  required by the prespecified Hochberg analysis<sup>38</sup>, the results did not meet the prespecified coprimary hypothesis that pepinemab is superior to placebo in EM cohort B1 for this two-member cognitive family.

**Efficacy: clinical global impression of change.** The second coprimary hypothesis was that pepinemab would be superior to placebo in EM cohort B1 with respect to CGIC score at month 17 relative to baseline. CGIC is considered meaningful because it is a generic global measure<sup>40</sup> consisting of a single-item assessment of the individual's overall disease status from the clinician's perspective based on a seven-point Likert scale. This coprimary endpoint was not met because the overall treatment effect did not reach the critical one-sided *P* value (Table 2).



**Fig. 1 | Effects of pepinemb treatment on primary cognitive assessments in EM cohort B1. a,b**, Observed mean changes from baseline (BL) by treatment group over time for the mITT sample of EM cohort B1. **a**, OTS measures time to a correct response (averaged over all trials per visit). **b**, PTAP measures tapping consistency as the reciprocal of the average standard deviation of inter-tap interval durations following cessation of aural cues (over all trials per visit). **a,b**, Error bars show one standard error on either side of the mean, with sample sizes at each time point for each group listed above the profile lines.

**UHDRS scales and motor activity.** As shown in Table 2 and further detailed in Extended Data Table 3, treatment effects on UHDRS–total functional capacity (TFC) and measures of motor activity, including UHDRS–total motor score (TMS) and multiple separate quantitative measures of motor activity (Q-motor)<sup>41</sup>, were not significant.

**MRI measures of brain atrophy.** Volumetric MRI (vMRI) as a measure of brain atrophy was evaluated as a key secondary objective. Changes in brain vMRI (ml, %) detected by boundary shift integral (BSI)<sup>42</sup>, an analytic method employed in other HD studies<sup>21–23,43</sup>, showed a nominally significant 26% reduction in caudate atrophy for EM subjects (PEPI,  $n=90$ ; PBO,  $n=88$ ) treated with pepinemb relative to placebo, and the percentage change from baseline at month 17 resulted in LS mean difference (95% CI) =  $-1.54$  ( $-2.79$ ,  $-0.29$ ) (two-sided  $P=0.017$ ; Fig. 2a and Extended Data Table 4). During that same period, the percentage change in ventricular volume BSI resulted in LS mean difference (95% CI) =  $-2.47$  ( $-5.04$ ,  $0.10$ ) (two-sided  $P=0.06$ ; Fig. 2b). Changes in the same direction were observed in EM subjects for reduction in white matter volume (Fig. 2c) and whole-brain atrophy (Fig. 2d) (Extended Data Table 4) but were not significant. LP participants (cohort B2: PEPI,  $n=41$ ; PBO,  $n=45$ ) did not demonstrate meaningful differences between drug and placebo in vMRI during the 18-month treatment duration in any of these same brain regions (Extended Data Table 4).

**Pepinemb treatment may reverse decline in FDG–PET SUVR.** Change from baseline to month 17 in FDG–PET SUVR was assessed as a prespecified secondary endpoint in 23 brain regions of interest (ROIs), plus total white matter and two composite regions of extended frontal and additional cortical brain regions (ROIs and composite regions listed in Methods). Subsets of EM subjects ( $n=40$  PEPI,  $n=36$  PBO) and LP subjects ( $n=22$  PEPI,  $n=29$  PBO) were analyzed for FDG–PET imaging, dependent on the ability to perform imaging at each site and subsequent imaging quality control. The longitudinal change in SUVR was calculated for each EM subject in each ROI, and group mean changes are plotted in Fig. 2e. As expected from previous natural history studies, SUVR declined across all ROIs in the placebo group and this is also reflected in the composite regions. In striking contrast, an increase in SUVR was observed in almost all ROIs following treatment with pepinemb, with the exception of regions of the basal ganglia including striatum

(caudate, putamen) and globus pallidus. The difference in mean percentage change in SUVR between pepinemb and placebo is plotted by rank order in Fig. 2f and listed alphabetically in Extended Data Table 5a. The estimate of the difference between pepinemb and placebo in percentage change from baseline in FDG–PET SUVR was quantitatively beneficial for pepinemb over placebo for all 26 regions in EM cohort B1, and significant for 15 regions ( $P\leq 0.05$ ). These data suggest that, after 18 months of treatment in EM subjects, pepinemb prevents and/or reverses the decline in metabolic activity in the majority of brain regions evaluated, particularly in the frontal and cortical regions.

**Exploratory cognitive measures.** Given the importance attached by patients and their families to cognitive decline during disease progression<sup>12</sup>, we investigated whether the HD–CAB index, a prespecified exploratory endpoint previously employed in observational studies, could provide additional information regarding cognitive decline. Each of the six HD–CAB components individually showed change in the direction of benefit favoring pepinemb over placebo for EM cohort B1 (Extended Data Table 6) and, under these conditions, the mean difference in HD–CAB index between drug and placebo, which is an average of the individual z-scores, can be substantially more significant than that of the individual component scores due to reduced standard error. The HD–CAB index change from baseline at month 17 (95% CI) resulted in LS mean difference (95% CI) =  $0.13$  ( $0.03$ ,  $0.23$ ) (one-sided nominal  $P=0.007$ ; Fig. 3 and Extended Data Table 6). In a post hoc sensitivity analysis of EM cohort B1, the same analyses were repeated on HD–CAB index modified by omission of OTS, resulting in LS mean difference (95% CI) =  $0.12$  ( $0.00$ ,  $0.23$ ) (nominal one-sided  $P=0.023$ ), omission of PTAP (LS mean difference (95% CI) =  $0.09$  ( $0.01$ ,  $0.18$ ) nominal (one-sided  $P=0.019$ ) or omission of both OTS and PTAP (LS mean difference (95% CI) =  $0.10$  ( $-0.03$ ,  $0.22$ ) (nominal one-sided  $P=0.064$ )). These data highlight that the HD–CAB index captures the trend of change in HD–CAB assessments beyond the significance of just the one or two leading individual assessments.

An important consideration for evaluation of cognitive performance is the role of ‘learning effects’ during early evaluation sessions. It has previously been reported for Alzheimer’s disease that reduced learning effects are associated with increased levels of biomarker burden and greater cognitive decline<sup>44,45</sup>. A comparison of change in HD–CAB index for EM cohort B1 and LP cohort B2



subjects treated with placebo only (Fig. 3a) suggests that there is a greater learning effect for LP than for EM subjects. Importantly, pepinemab treatment preserves this learning ability in EM subjects (Fig. 3b,c). Because the ability to learn is itself an important cognitive function, this suggests that improvement in the HD-CAB index of EM subjects during the first months of treatment with pepinemab may be due to preservation of learning effects that are otherwise lost in EM patients. Treatment effects do, however, appear to continue through 18 months of exposure as detected by longitudinal changes in MRI (Fig. 2a–d), and are also suggested by increasing separation in HD-CAB index between drug and placebo for months 12–17 (Fig. 3b,d).

Finally, in regard to other changes related to cognition, the apathy severity subscore of the problem behaviors assessment (PBA-s) has previously been reported to correlate with cognitive deficits during early HD progression<sup>46</sup>. In a post hoc analysis of the PBA-s apathy severity subscore for EM cohort B1, the proportion of subjects with increased apathy severity at month 17 was nominally significantly lower in the pepinemab group compared with placebo (23% PEPI versus 40% PBO), with an odds ratio (95% CI): 0.46 (0.22, 0.94), one-sided  $P=0.017$ . Subscores for the three other most prevalent problematic behaviors in HD—depressed mood, anxiety and irritability—did not show significant change from baseline at month 17 (Extended Data Table 7). Treatment-related changes in PBA-s subscores including apathy were not evident in LP cohort B2.

**Imaging and cognitive effects in LP cohort B2.** The SIGNAL study tested a clinical intervention in both a prodromal and a manifest HD population. Cohort B2 enrolled LP subjects to explore which, if any, outcomes might indicate efficacy signals in this population. As reported in Extended Data Table 4, there was no evidence of a significant treatment effect on caudate atrophy ( $n=78$ ) or ventricular volume ( $n=75$ ) in LP subjects. The magnitude of changes in FDG-PET SUVR for LP cohort B2 (Extended Data Table 5b) was much reduced compared with EM cohort B1 (Extended Data Table 5a). The average percentage change between baseline and month 17 across all 26 indicated brain ROIs for the LP B2 placebo group was  $-0.21\%$ , and for B1 was  $-1.79\%$ . Further, no significant treatment effect was observed in any brain ROI for B2 (Extended Data Table 5b) whereas  $P\leq 0.05$  for B1 in 15 of 26 ROIs (Fig. 2f and Extended Data Table 5a). Similarly, for LP cohort B2, treatment-related changes in individual-component HD-CAB cognitive assessments were not consistent in either magnitude or direction, and the difference in HD-CAB index between drug and placebo was not significant for this prodromal population, with LS mean difference (95% CI) =  $-0.11$  ( $-0.27, 0.06$ ) (nominal one-sided  $P=0.90$ ; Extended Data Table 6 and Fig. 3c). We discuss below how this may be consistent with our understanding of a principal mechanism of action of SEMA4D blockade on glial cell reactivity.

**Post hoc analysis: contribution of baseline disease state.** It became evident in the analysis of treatment outcomes that some

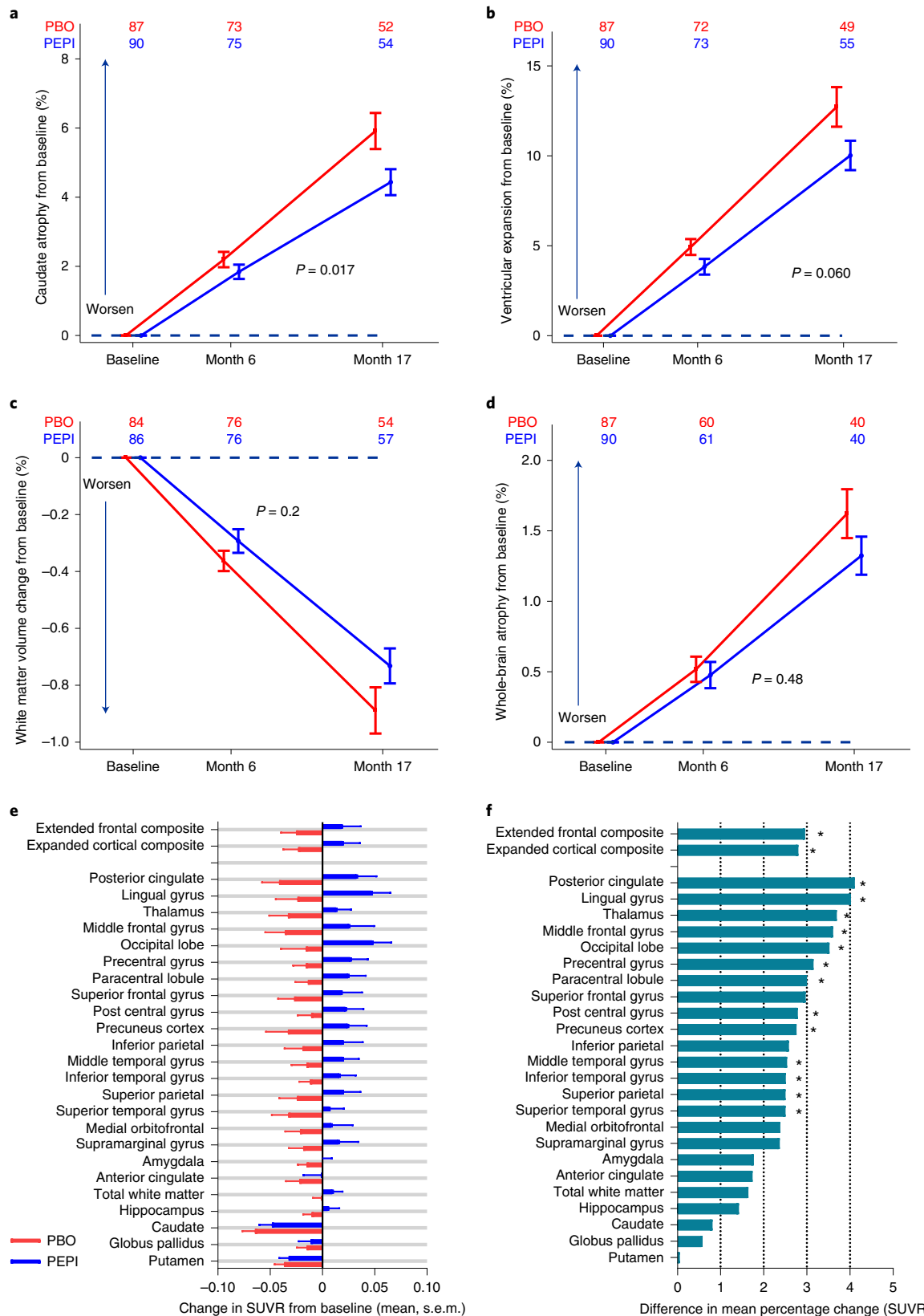
effects of pepinemab treatment were detectable in EM but not in LP subjects. We therefore tested the hypothesis that the ability to detect change over the 18-month study duration might be improved in participants already showing deficits at baseline: an important consideration for clinical trial design. We first investigated whether cognitive change in HD-CAB is differentially detectable in subjects with early signs of cognitive decline. The Montreal Cognitive Assessment (MoCA) is a validated rapid-screening instrument for assessment of cognitive dysfunction, and was administered to determine subject eligibility based on an exclusion criterion of MoCA score  $\leq 22$ . The maximum possible MoCA score is 30 points; a score of  $\geq 26$  is considered normal and a score  $< 26$  indicates some degree of cognitive deficit. Using MoCA as an independent measure of cognition, we performed post hoc analyses to stratify outcomes of HD-CAB by baseline score of MoCA  $< 26$  or  $\geq 26$ . Results for the HD-CAB index, PTAP and OTS are shown in Fig. 3d–f, and for the other HD-CAB components in Supplementary Fig. 2. The LS mean difference between treatment arms for HD-CAB index in the MoCA  $\geq 26$  subgroup was not significant. In contrast, in participants with MoCA  $< 26$  at baseline there was a steady decline in HD-CAB scores in the placebo group during 18 months of treatment, while the HD-CAB index in the pepinemab group did not fall below baseline at any time point (Fig. 3d). The change from baseline at month 17 in the MoCA  $< 26$  subgroup resulted in a LS mean difference (s.e.) of  $0.24$  ( $0.08$ ) (nominal one-sided  $P=0.0025$ ). Similarly, for PTAP (Fig. 3e), the LS mean difference (s.e.) in the MoCA  $< 26$  subgroup was  $1.89$  ( $1.10$ ) (nominal one-sided  $P=0.044$ ), compared with no significant change in the MoCA  $\geq 26$  subgroup with LS mean difference (s.e.) of  $1.09$  ( $1.32$ ) (one-sided  $P=0.21$ ). Stratification by baseline MoCA score, however, does not have a similar impact on mean difference and  $P$  value for OTS (Fig. 3f). It appears that decline in OTS begins earlier in disease progression than for other HD-CAB domains: LS mean difference (s.e.) =  $-1.87$  ( $1.44$ ) (one-sided  $P=0.099$ ) for MoCA  $< 26$  and, for MoCA  $\geq 26$  subgroups =  $-1.73$  ( $1.34$ ) (one-sided  $P=0.101$ ). Indeed, because of the smaller group sizes ( $n=32$  and  $47$ ), this resulted in less significant one-sided nominal  $P$  values ( $P=0.099$  and  $0.101$ ) relative to the total cohort B1 population ( $n=79$ ,  $P=0.028$ ). These results suggest that HD-CAB is an effective instrument for capturing changes across different cognitive domains that may decline at different rates during disease progression.

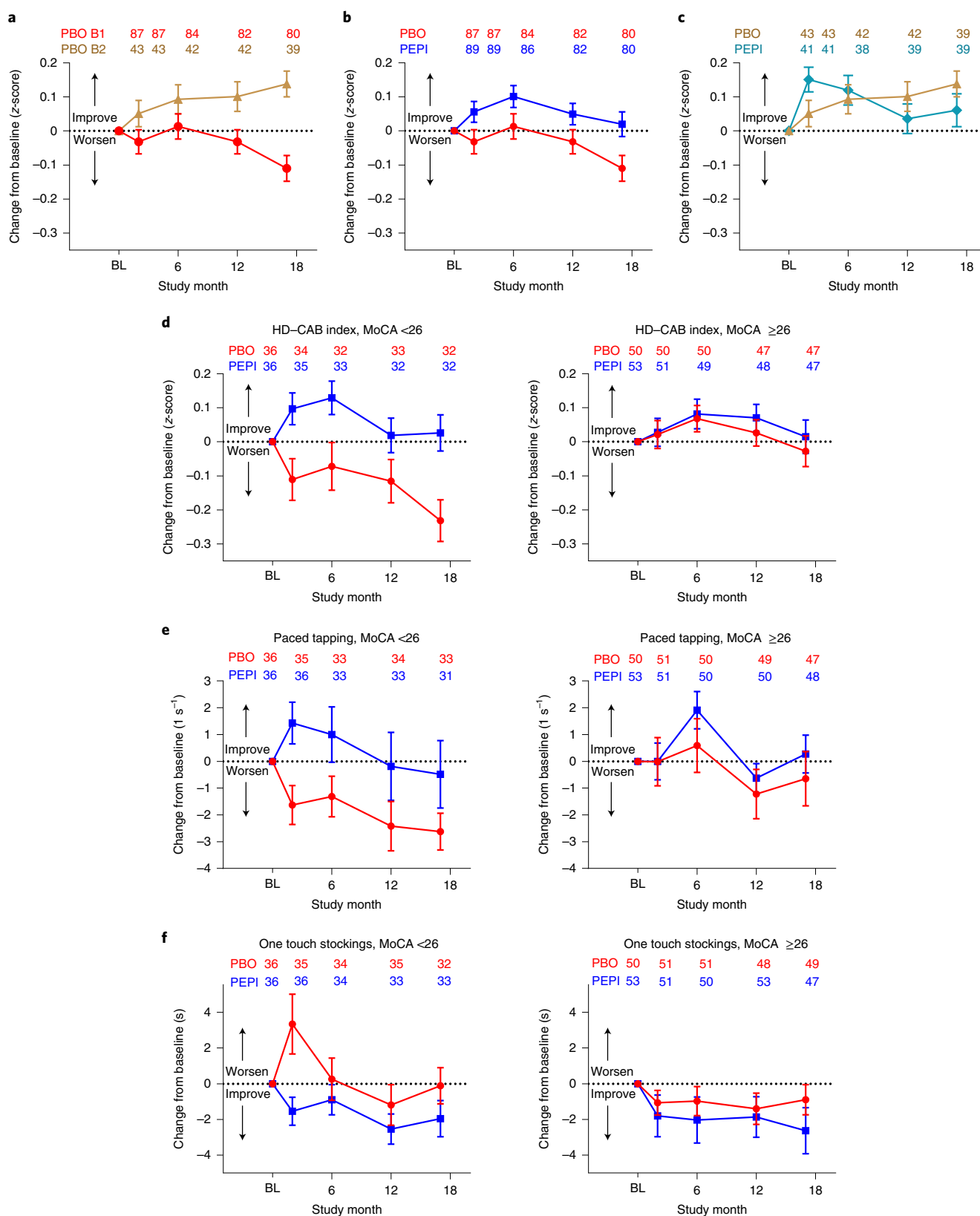
We next considered the possibility that a treating physician's ability to detect change, as reflected in CGIC, might be greater in subjects with somewhat more advanced disease. UHDRS-TFC is a 13-point, standardized disease-staging scale that rates a person's functional capacity and level of independence in five domains: occupation, ability to manage finances, ability to perform domestic chores, ability to perform personal activities of daily living and level of care<sup>47</sup>. Higher scores indicate higher functioning capacity, with a maximum score of 13. Subjects with TFC 11 would, therefore, be expected to have more advanced presentation of symptoms than those enrolling at the highest end of the scale, TFC 12–13. Using TFC as an independent

**Fig. 2 | Pepinemab delays brain atrophy and restores loss of metabolic activity in EM subjects. a–d**, Mean percentage changes from baseline by treatment group over time for the mITT sample of EM cohort B1 (PEPI,  $n=90$ ; PBO,  $n=88$ ) in vMRI measurement. **a**, Caudate BSI (atrophy); **b**, ventricular BSI (expansion); **c**, white matter (preservation); **d**, whole-brain BSI (atrophy). **e**, FDG-PET SUVR change from baseline to month 17 for each treatment group (mean and 1 s.d.) in each brain ROI for EM cohort B1. **f**, Treatment effect at month 17 calculated as difference between pepinemab ( $n=40$ ) and placebo groups ( $n=36$ ) as mean percentage change in SUVR. \* $P\leq 0.05$ ; exact two-sided  $P$  values for 15 brain regions (listed from top to bottom) are: extended frontal composite, 0.031; expanded cortical composite, 0.028; posterior cingulate, 0.008; lingual gyrus, 0.014; thalamus, 0.011; middle frontal gyrus, 0.033; occipital lobe, 0.029; precentral gyrus, 0.010; paracentral lobule, 0.014; post central gyrus, 0.028; precuneus cortex, 0.048; middle temporal gyrus, 0.044; inferior temporal gyrus, 0.033; superior parietal, 0.050; superior temporal gyrus, 0.037;  $P$  values for all regions are shown in Extended Data Table 5. Analysis results were determined from MMRM of scheduled measurements at months 2, 6 and 17, with estimation of the difference in means between groups at month 17.  $P$  values are indicated (two-sided); as described in Methods, stated  $P$  values for all statistical tests, besides the coprimary efficacy analyses, were not corrected for multiplicity and are thus presented as nominal and not under alpha control. Error bars show one standard error on either side of the mean, with sample sizes at each time point for each group listed above the profile lines.

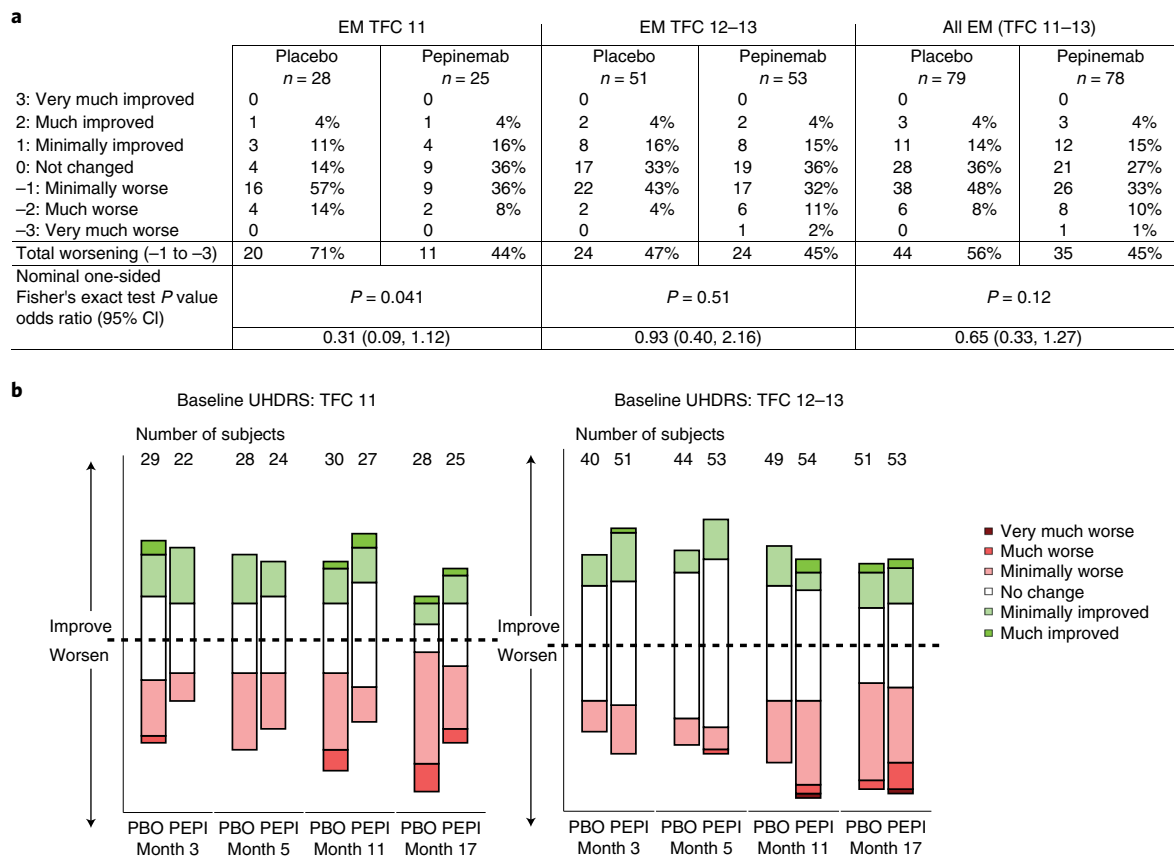
functional measure to stratify CGIC scores, among the subgroup of patients with baseline TFC 11, significantly fewer subjects had a worsened disease CGIC status in the pepinemab group: specifically, 44% PEPI versus 71% PBO, with an odds ratio (95% CI) of 0.31 (0.09, 1.12) (nominal one-sided  $P=0.041$ ), as compared with 45% PEPI versus

47% PBO for the TFC 12–13 subpopulation of EM cohort B1 (Fig. 4). Although the TFC 11 subgroup sample size is small, this is consistent with the hypothesis that potentially greater treatment benefit can be detected in subjects with somewhat more advanced disease and could be an important consideration in the design of subsequent studies.





**Fig. 3 | Exploratory cognitive measures and post hoc subgroup analysis of baseline MoCA as a biomarker for treatment response in early HD. a-c.** Observed mean changes from baseline by treatment group over time for the placebo groups alone of cohort B1 (red circles) and B2 (brown triangles) (a), for both placebo and pepinemab treatment groups of EM cohort B1 (b) and LP cohort B2 (c). Cognitive assessments stratified by baseline MoCA scores of <26 and 26–30 for assessments of HD-CAB index (d), PTAP (e) and OTS (f). Error bars in each panel show one standard error on either side of the mean, with sample sizes at each time point for each group listed above the profile lines. PEPI (blue squares) and PBO (red circles) in EM cohort B1, and PEPI (teal diamonds) and PBO (brown triangles) in LP cohort B2.



**Fig. 4 | Effects of pepinemab treatment on CGIC in EM cohort B1. a**, Observed categorical CGIC values at visit 17 for the entire EM cohort B1 CGIC analysis population, and in two subgroups stratified by baseline TFC value (that is, 11 and 12–13). **b**, Observed categorical CGIC in the subgroups stratified by TFC value over the study duration. The CGIC is a seven-point Likert scale, ranging from very much worse (–3) to very much improved (+3). Values were set to –3 following a patient death adjudged by a blinded data review committee to be related to Huntington's disease. *P* values determined by one-sided Fisher's exact test; odds ratio (95% CI) are also shown.

**Pharmacokinetics/pharmacodynamics.** The 20 mg kg<sup>-1</sup> dose of pepinemab administered i.v. every four weeks was selected to achieve a projected dose in cerebrospinal fluid (CSF) of 100–300 ng ml<sup>-1</sup>, estimated as the effective range from earlier dose-titration studies. Pepinemab drug levels and total soluble SEMA4D were measured in CSF in a subset of 54 subjects (*n* = 28 placebo and *n* = 26 pepinemab) predominantly during months 13–18 (mean, 15.6; median, 15). Pepinemab was detected in CSF at a mean level (95% CI) of 348 ng ml<sup>-1</sup> (220.7, 437.2) and the average CSF/serum concentration ratio of pepinemab was 0.38 (0.39%) (Extended Data Fig. 2a). No drug was detected in subjects treated with placebo, as expected. Evidence of target engagement in CSF based on increased concentration of soluble SEMA4D is reported in Extended Data Fig. 2b.

## Discussion

Predefined primary efficacy endpoints did not achieve statistical significance in the SIGNAL study. Nevertheless, a significant pepinemab treatment-related reduction in caudate brain atrophy and increase in FDG–PET SUVR in most brain ROIs, along with multiple prespecified exploratory assessments that suggest a cognitive benefit of treatment, encourage continued development of pepinemab as a potential therapy for EM HD.

In two broad surveys, patients with HD have identified cognitive decline as a major concern. Even subtle cognitive changes can affect functional abilities such as work performance, driving and financial management<sup>21,48,49</sup>. While predefined primary endpoints

of cognition, OTS and PTAP, failed to achieve statistical significance (one-sided *P* = 0.028 and 0.06, respectively) other indications of cognitive benefit of pepinemab treatment include a nominally significant effect on the HD–CAB index (one-sided *P* = 0.007), a prespecified exploratory endpoint, as well as post hoc analysis demonstrating a nominally significant treatment effect on apathy severity (*P* = 0.017), a component of the prespecified exploratory PBA-s that has been reported to correlate with cognitive decline. It was also of particular interest that learning effects appear to be compromised early in HD progression, as previously reported for early Alzheimer's disease<sup>44,45</sup>, and that pepinemab treatment appears to improve or rescue this cognitive feature. It would be desirable to add a direct assessment of ability to learn in future studies.

The SIGNAL clinical study has established the safety and tolerability of pepinemab immunotherapy in individuals with HD. No concerning clinically relevant safety issues were identified, concordant with other completed clinical studies of pepinemab in individuals with multiple sclerosis and advanced solid tumors<sup>18–20</sup>. One suicide death was observed in an individual with EM disease following treatment with pepinemab, but was deemed unrelated to treatment. Natural history studies report that the prevalence of suicidal ideation is a common symptom of HD and occurs in about 24% of individuals during the early stage of the disease<sup>50</sup>. Nevertheless, suicidal ideation and suicide attempts should be carefully monitored in future studies of pepinemab in HD.

A major goal of phase 2 studies is to identify the patient population likely to benefit from treatment. Post hoc subgroup analysis suggested



a potential treatment benefit to patients who were somewhat more advanced in disease progression, as indicated by improved CGIC score in patients with a baseline TFC score of 11 relative to less advanced TFC 12–13, and also supported by increased relative response to treatment for OTS, PTAP and the HD–CAB index in patients with MoCA <26 relative to MoCA ≥26 at baseline. This hypothesis, based on post hoc analysis of relatively reduced group sizes, will require empirical testing in a future phase 3 study of pepinemb that should include a broader TFC range—for example, enrollment of patients with baseline TFC 8–12. It may naturally occur that this population will also be substantially enriched for MoCA <26, but further stratification could also be considered if that were not the case.

There are considerations related to the mechanism of action of pepinemb that may explain a delay in the ability to detect treatment-related changes very early in disease. As reported elsewhere<sup>13</sup> (and in E.E.E. et al., in press Ibid), SEMA4D plays an important role in the promotion of astroglial and microglial reactivity. This entails complex interactions between astrocytes and microglia and among different subsets of each. Counterbalancing activity of different inflammatory cells may, in normal circumstances, maintain a homeostatic balance. However, under the influence of a disease-related disturbance, this process may fail to arrive at a healthy equilibrium. In a slowly progressive disease like HD it could, therefore, be a matter of time before the homeostatic process is exhausted and the benefits of treatment become evident. As discussed below, it is, of course, also possible that there are other early-stage pathogenic events unrelated to glial reactivity that are not impacted by SEMA4D blockade.

Consistent with the natural history of HD, brain atrophy progressed and metabolic activity decreased in most brain ROIs in the placebo group for EM HD. This was in contrast to a pepinemb-associated decrease in caudate brain atrophy (95% CI) of  $-1.54\%$  ( $-2.79, -0.29$ ),  $P=0.017$  and a trend of reduced ventricular expansion (95% CI) of  $-2.47\%$  ( $-5.04, 0.10$ ),  $P=0.060$ . Importantly, decline in metabolic activity detected by FDG–PET is a regular feature of other slowly progressive neurodegenerative diseases, and multiple studies in Alzheimer's disease have demonstrated that decline in FDG–PET SUVR correlates with cognitive decline and clinical progression<sup>28,51</sup>. The observation that treatment with pepinemb results in a significant increase in FDG–PET SUVR in most brain ROIs is a notable reversal of the previously reported decline in FDG–PET signal during HD progression<sup>25–27</sup>. It was unexpected that regions of the basal ganglia, including caudate and putamen, did not manifest the same degree of treatment-related increase in FDG–PET SUVR observed in most other brain ROIs of EM HD subjects. Striatal regions are known to degenerate at a rate three- to fourfold greater than other brain regions even before the time of motor diagnosis<sup>22,52</sup>. Loss of neurons is certain to impact energy metabolism and may be triggered by mechanisms that directly affect medium spiny neurons in striatum and are independent of SEMA4D activity and, therefore, relatively unaffected by pepinemb treatment. In keeping with the absence of a treatment effect on glucose utilization in striatum, we also did not observe an effect on early motor dysfunction—for example, chorea—in either EM or LP populations as detected by either UHDRS–TMS or Q-motor assessments. It is possible that later stages of motor progression could be affected by treatment but are less prominent in the TFC ≥11 population.

As might be expected, given their important role in glucose transport, recent reports demonstrate that astrocytes contribute substantially to the FDG–PET signal in the brain<sup>53</sup>. Normal astrocytes extend numerous cytoplasmic projections with endfeet that express glucose transporter and fully cover brain capillaries. These projections are reduced and glucose transport is downregulated in reactive astrocytes<sup>54,55</sup>. SEMA4D is known to trigger collapse of the cell cytoskeleton and to downregulate glucose transporters on astrocytes, while SEMA4D blockade restores glucose uptake (E.E.E. et al.

in press, Ibid) and may, therefore, counteract decline in FDG–PET signal associated with astrocyte reactivity. There may be other potential effects of SEMA4D blockade that could impact FDG–PET. We have previously reported that SEMA4D blockade protects the integrity of the BBB<sup>13</sup>. Compromise to the BBB could have a profound impact on transport and synaptic activity that may impact energy metabolism. We considered the possibility that changes in FDG–PET SUVR might reflect an undesirable increase in inflammation, but evidence of our own and from others<sup>13,56</sup> suggests that the effects of SEMA4D antibody blockade are anti-inflammatory. For example, we previously reported that SEMA4D antibody prevents inflammatory activation of murine Iba-1<sup>+</sup> microglia<sup>13</sup>, and others have recently demonstrated that the SEMA4D–plexin-B1/B2 signaling pathway governs crosstalk between astrocytes and microglia that triggers glial activation<sup>56</sup>. SEMA4D blockade should, therefore, be expected to reduce inflammation. These novel findings suggest that FDG–PET may hold promise as an early biomarker of potentially effective treatments in HD.

In parallel with metabolic effects in multiple cortical regions, there appeared to be a clear effect of pepinemb treatment in EM disease on OTS (one-sided  $P=0.028$ ), a measure of executive function that is a key cognitive domain related to learning, as well as on direct learning effects evident during sequential HD–CAB assessments. In contrast to Alzheimer's disease, for which the clinical dementia rating scale and Alzheimer's disease assessment scale-cognitive subscale are well established as meaningful composite and cognitive endpoints, there is no established measure of cognition in HD that is currently accepted as intrinsically meaningful by both US and European Union regulators. We suggest that direct measures of learning could constitute an intrinsically meaningful cognitive endpoint in manifest HD.

Important limitations of the SIGNAL study design include exclusion of patients with TFC <11. This was highlighted by post hoc subgroup analysis which suggested that pepinemb treatment effects appear to be more evident in patients with early cognitive or functional deficits. While MMRM analyses provided mathematical power for independent imaging and cognitive outcomes, power for correlation analysis with imaging endpoints was limited due to further reduced group sizes associated with scheduling issues, quality control and a subset of sites unable to perform FDG–PET. Despite these limitations, key observations suggesting a trend of benefit in a two-item cognitive family, supported by significant change in pre-specified exploratory analysis of the HD–CAB index, improved apathy score, a reduction in caudate atrophy and increase in metabolic activity as reflected by FDG–PET SUVR, collectively suggest clinically relevant changes in patients with early manifest Huntington's disease. Further studies are warranted to confirm and extend these observations, with adjustments to enroll patients with early signs of cognitive or functional deficits.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-022-01919-8>.

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## Methods

**SIGNAL study design and participants.** SIGNAL cohort B is a phase 2, multicenter, randomized (1:1), double-blind, placebo-controlled, parallel-group study of pepinemab in subjects with EM (cohort B1) and LP (cohort B2) HD (<https://clinicaltrials.gov/ct2/show/NCT02481674>) (no. VX15-2503-N-131B). A total of 265 subjects (179 EM and 86 LP) were enrolled at 30 outpatient clinical sites in the United States and Canada. Only a subset of sites ( $n=25$ ) were able to perform FDG-PET imaging. All subjects received at least 17 planned monthly infusions.

Eligible participants were aged 21 years and older, had genetically confirmed presence of  $\geq 36$  CAG repeats in one huntingtin gene and no features of juvenile HD (Westphal variant). EM participants were defined by UHDRS-TFC  $\geq 11$ ; they were determined by the site investigator to have a clinical diagnosis of HD as defined by a DCL of 4. LP was defined as DCL of 2 or 3 and a CAP score of  $>200$ . CAP score, a measure of HD mutation burden, was calculated as the product of age and (CAG-33.66)<sup>0.7</sup>. Stable dosages of concomitant medications (including tetrabenazine and deutetabenazine) were permitted if initiated at least 1 month before baseline (visit 0), as were newly prescribed anxiolytics for use as premedication before imaging at screening, which were permitted on a case-by-case basis. Exclusion criteria included participation in an investigational drug or device study within 30 days of baseline (or 180 days if the previous investigational drug was a monoclonal antibody therapeutic), suicide risk (as determined by the Columbia-Suicide Severity Rating Scale), MoCA score  $\leq 22$ , ECG abnormalities at screening, pregnancy and conditions that would exclude MRI participation.

**Randomization and blinding.** Subjects who satisfied all eligibility criteria were participants and were randomized to one of two treatment arms through an interactive web response system. The subjects, site investigators, site personnel and study statisticians—as well as representatives of these organizations and staff at Vaccinex—were blinded as to treatment assignments until database lock. The investigational agent and placebo were in vials of identical appearance. During the course of the study, the Safety Monitoring Committee maintained access to treatment code information. Omnicomm Inc. eClinical Solutions v.5.2 was utilized for data collection on SIGNAL.

**Investigational agent and treatment.** Pepinemab (VX15/2503) is a humanized IgG4 monoclonal antibody<sup>58</sup> with a hinge mutation that prevents in vivo Fab arm recombination<sup>59</sup>. Bulk pepinemab (Catalent Pharma Solutions) was produced using a proprietary CHO cell line. The bulk antibody was purified using standard techniques and formulated at approximately 20 mg ml<sup>-1</sup> in preservative-free 20 mM sodium acetate pH 5.4, containing 130 mM sodium chloride and 0.02% polysorbate 80. Pepinemab and matching placebo were supplied by the sponsor as single-use vials. Subjects enrolled in cohort B were randomized to receive monthly i.v. 60-min infusions of either placebo or 20 mg kg<sup>-1</sup> pepinemab. Selection of the 20 mg kg<sup>-1</sup> i.v. dose of pepinemab for the SIGNAL cohort A study was based on findings from nonclinical studies<sup>60</sup>, as well as on single- and repeat-dose Phase 1 and 2 studies of pepinemab up to 20 mg kg<sup>-1</sup> in subjects with multiple sclerosis<sup>49</sup> and in subjects with advanced solid tumors<sup>18</sup>, respectively. Placebo or drug was administered for up to 18 months of treatment. Subjects were treated at baseline through to visit 17. Efficacy endpoints were assessed on visit 17 after 18 months of study drug exposure, and a month 18 visit or phone call at the end of the safety analysis period, which spans a total of 18 months. Subjects were then followed for an additional ~3 months (minimum of 2 months and up to 6 months after last infusion) for safety and laboratory assessments. Additionally, under protocol amendments 1–3, the first 53 subjects enrolled in cohorts B1 or B2 were offered the option to receive 18 additional months of treatment for a total of up to 36 months safety evaluation. From protocol amendments 4–6, the treatment period was 18 months. Of the 53 subjects who were offered entry into the extended treatment period, 42 agreed.

**Outcome measures.** Primary outcome measures were defined as: (1) to evaluate the safety and tolerability of monthly i.v. administration of pepinemab relative to placebo over 18 months of double-blind treatment in the entire study population (cohorts B1 and B2); and (2) to evaluate the efficacy of monthly i.v. administration of pepinemab relative to placebo over 18 months of double-blind treatment in EM subjects (cohort B1), including change from baseline to month 17 in the two-item cognitive family (PTAP and OTS components of the HD-CAB) and CGIC at month 17 relative to baseline. Secondary and exploratory outcome measures reported here include UHDRS-TFC and TMS, Q-motor, pharmacokinetics (PK), pharmacodynamics (PD) and immunogenicity; additional cognitive assessments, in particular the HD-CAB index; and imaging assessments, including change from baseline to visit 17 in FDG-PET SUVR and in brain volume as measured by volumetric MRI.

The ITT population consisted of all randomized subjects categorized by their randomized treatment assignment. The mITT population consisted of all randomized subjects who had received at least one complete infusion of study drug and had at least one post-infusion efficacy evaluation for one of the coprimarily efficacy outcomes—that is, cognition or CGIC. The safety population consisted of subjects who had received at least one dose of study drug (including one patient who received a partial infusion); efficacy analyses was performed on the mITT population. A subset of subjects in the mITT population were assessed for CGIC, due to addition of this outcome measure in amendment 5, and a different subset were assessed by FDG-

PET due to the limited number of participating sites and consenting individuals, as described in the CONSORT diagram (Extended Data Fig. 1).

**Description of outcomes.** Tolerability, defined as the ability to complete the study on the assigned study arm, accounted for subjects' study disposition (for example, reason for study discontinuation of 'did not tolerate study drug'), treatment disposition and duration of exposure. Adverse events were monitored monthly for each subject during the study period, defined as from signing informed consent through to final study contact. A TEAE was defined as an adverse event with onset on or after the date of first dose of study drug. AEs were coded using the Medical Dictionary for Regulatory Activities v.16.1. The investigator assessed the causality of each AE to the study drug and the severity of each AE using his/her clinical expertise, and designated a grade to each AE according to the current CTCAE. The Columbia-Suicide Severity Rating Scale and Hospital Anxiety and Depression Scale were both assessed. The HD-CAB was measured at six visits during the primary analysis period: screening, baseline and months 2, 6, 12 and 17. The HD-CAB comprises six component tests<sup>39</sup>, among which are PTAP and OTS, and is described in the table below. PTAP measures the consistency of tapping rate, measured as the reciprocal of the standard deviation of ITI duration. OTS measures the mean time (in seconds) to a correct response. CGIC<sup>40</sup> is a single-item questionnaire that asks the investigator to assess a subject's HD symptoms compared with those immediately before starting the study drug, using a seven-point Likert scale ranging from very much worse (-3) to very much improved (+3), to assess overall response to the study drug relative to baseline. CGIC was evaluated at four visits during the primary analysis period: months 3, 5, 11 and 17. The TFC scale and TMS are components of the UHDRS, including UHDRS-TFC and UHDRS-TMS, and have been used in observational studies and randomized controlled trials in both premanifest and manifest HD populations<sup>47,61,62</sup>. The UHDRS-TFC score is the sum of five items ranging from 0 to 13, with a higher score representing better functioning. Q-motor and TMS allow objective monitoring of motor effects. The UHDRS-TMS score is the sum of scores of 31 items in four domains, the response to each being rated on a four-point scale for a maximum score of 124, with higher scores indicating more severe motor impairment<sup>37</sup>. The Q-motor battery is composed of precalibrated and temperature-controlled force transducers and three-dimensional position sensors that are used to assess (1) grasping forces, (2) involuntary choreatic movements, (3) regularity of index finger tapping and (4) regularity of alternating pronation/supination hand movements. PBA-s is a semistructured clinical interview that contains 11 items, each measuring a different behavioral problem rated for both severity and frequency on a five-point scale (0–4)<sup>63</sup>. HD-CAB component tests and variables of interest were as follows.

Test name	Cognitive function assessed	Variable of interest	Value range for variable of interest
HVLT-r	Verbal memory	Total correct recall trials over three learning and one delayed recall trials; higher score indicates better performance	0–48
SDMT	Visuospatial attention, processing speed, working memory	Number of correctly coded terms; higher score indicates better performance	0–110
TMT-B*	Flexibility, visuospatial attention, psychomotor speed	Time (seconds) to complete the task; higher value indicates poorer performance	0–240
EMO	Emotion recognition	Number of negative emotions correctly identified; higher score indicates better performance	0–24
<b>PTAP</b>	<b>Timing, psychomotor coordination</b>	<b>Reciprocal of the standard deviation of the ITI duration that occurred following cessation of pacing tones over all trials taken; higher value indicates better performance</b>	<b>Not defined</b>
<b>OTS*</b>	<b>Planning, working memory</b>	<b>Mean time to reach a correct response (seconds), averaged across ten trials; higher values indicate poorer performance</b>	<b>Not defined</b>

Bold font indicates membership in the HD-CAB two-item cognitive family for coprimarily endpoint in EM cohort B1. For tests with an asterisk (\*), a lower value of the variable of interest indicates better performance; for all others, a higher value indicates better performance.



**COVID considerations.** As a result of the COVID-19 pandemic declared by the World Health Organization in March 2020, and in accordance with the FDA Guidance ‘Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic’, released in March 2020 and updated in June 2020, changes were made to allow flexibility in data collection at clinical study sites while maintaining participant safety and clinical trial integrity. Visit windows and visit schedule were updated to allow less time between visits and to permit key data collection planned for the final visit to be collected at an earlier or later visit within an allowed time frame (plus or minus one visit) if a site was open; if a site had closed, the site could collect these data when local guidance permitted. Subjects able to attend visit 16 or 17 received an infusion at the investigator’s discretion. The date 11 March 2020 was selected as the date on or after which the COVID-19 pandemic was likely to have influenced data collection for this study.

**MRI imaging.** A subset of 178 EM subjects (90 pepinemab, 88 placebo) and 86 LP subjects (41 pepinemab, 45 placebo) participated in MRI imaging. MRI neuroimaging was scheduled at screening and months 6 and 17. IXICO, Ltd ([www.ixico.com](http://www.ixico.com)) reported volumes at screening and changes from baseline at postscreening visits according to the BSI analysis method<sup>42</sup>. White matter volume change was calculated with a Jacobian method. Other region segmentations were calculated with the ‘Learning Embeddings for Atlas Propagation’ (LEAP) method, both for volume measures and for use in the FDG–PET SUVR pipeline. All sites and their respective 3T MRI scanners were qualified before the start of the study. The protocol included the following scan sequences: (1), localizer; (2), T1w; (3), field map DTI; (4), DWI; (5), T2\_SPACE; (6), T2\_FLAIR; (7) field map functional MRI; (8), resting state functional MRI; and (9) PD, but analysis involved only analysis of volumetric changes (T1w). IXICO reports the pseudo-total intracranial volume (pTIV) factor, which is a measure of how a subject’s head size compares with the standard template. Screening results were normalized to the pTIV to account for differences in head size. The normalized screening result for each brain region was calculated as screening volume/pTIV factor. At each post-baseline time point, percentage change from baseline was calculated as (reported change value/ reported screening value) × 100. MRI preprocessing software included MRICron DICOM to Nifti conversion (v.6/2013), DCMTK extraction of orientation information (v.3.5.4), image registration toolkit (IRTK, v.1.95) for multiatlas brain extraction and registrations, N4BiasFieldCorrection (v.1.9 from the ANTs package), MIDAS for semiautomated delineation (v.5.11.1), KN-BSI for boundary shift integral calculation of volume change (v.1.2), NiftyReg/NiftiSeg (v.0.9.4) and NiftK for BSI computation (v.12.11).

**FDG–PET.** A subset of 76 EM subjects (40 pepinemab, 36 placebo) and 51 LP subjects (22 pepinemab, 29 placebo) participated in FDG–PET imaging.<sup>18</sup> Fluoro-2-deoxy-D-glucose was employed as ligand in FDG–PET. FDG–PET imaging was performed at screening and again post baseline, approximately at months 2, 6 and 17. Primary FDG–PET scans were analyzed without knowledge of treatment assignments by IXICO. For imaging data used for this investigation in regard to PET images specifically preprocessed with the IXICO PET Regional SUVR pipeline, PET dynamic average was registered to native 3D–T1w space and WB–LEAP segmentation<sup>64–66</sup> of the same subject to allow for regional uptake sampling. FDG–PET images underwent image quality control. All FDG–PET images that passed image quality control were entered into the quantitative SUVR workflow for computation of SUVR for a number of brain regions, using the pons as the reference region. This involved computation of regional PET tissue activity values, correcting for radioactive decay and patient weight and standardizing according to a tracer-specific reference region, to allow comparison between patients. Brain regions for which SUVR was calculated were found using LEAP with the subject 3D–T1w MR image, which is registered to the FDG–PET image. SUVR quantification was performed on standard FDG–PET images acquired within 30-min intervals, 40 min after injection. FDG–PET data for the left and right hemispheres were averaged because within-subject changes in both hemispheres were strongly correlated ( $r = 0.98$ ,  $P < 0.001$ ). Prespecified brain ROIs ( $n = 26$ ) were analyzed; additionally, composite SUVRs were computed using the mean SUVR in the masked region consisting of the following regions that comprise (1) the extended frontal composite: anterior orbital gyrus, central operculum, frontal operculum, postcentral gyrus medial segment, post central gyrus, opercular part of inferior frontal gyrus, triangular part of inferior frontal gyrus (pars triangularis), frontal pole, gyrus rectus, lateral orbital gyrus, precentral gyrus medial segment, precentral gyrus, superior frontal gyrus, supramarginal gyrus, medial frontal cortex, middle frontal gyrus, middle frontal gyrus, superior frontal gyrus medial segment, supplementary motor area; and (2) expanded cortical composite: extended frontal composite plus posterior cingulate cortex, inferior parietal lobule, superior temporal gyrus, anterior cingulate cortex, superior parietal lobule, middle temporal gyrus, precuneus cortex, paracentral lobule.

**Statistics.** According to the protocol, the planned cohort B sample size of 240 allows a minimal detectable effect size of 0.38 (Cohen’s  $D$ ) with two-sided alpha of 0.05, 80% power and 10% dropout. Power calculations and endpoint selection were based on a pilot study (cohort A) of 36 patients with HD (15 EM and 21 LP) treated with pepinemab or placebo in a double-blinded comparison for 6 months.

The 2CARE study provided additional information on estimates of variance at month 18 (ref. 67).

The statistical analysis protocol, finalized before locking the database and unblinding, specified a plan to control the overall type I error rate in multiple testing of two coprimaries plus a series of secondary analyses to be tested in hierarchy. The coprimaries were the two-item HD–CAB family (OTS and PTAP) and CGIC. Both coprimaries were required to meet a critical one-sided  $P$  value of 0.025 (equivalent to two-sided alpha of 0.05) for a successful trial overall. The success of the two-item HD–CAB family was assessed according to the Hochberg procedure for multiple testing between two items. Because the coprimaries did not collectively reach the threshold needed to declare a successful finding, the prespecified hierarchy of secondary endpoints was not tested formally. Thus, stated  $P$  values for all statistical tests besides the coprimaries efficacy analyses were not corrected for multiplicity and are thus presented as nominal and not under alpha control.

The efficacy analyses for this study were performed in a mITT population. Missing values were not imputed before fitting the models for statistical testing, and estimation and inference were based on estimates of the difference or ratio between means (that is, PEPI versus PBO) at month 17. No data beyond month 17 were included in the efficacy analyses.

For continuous outcomes, the dependent variable is generally change from baseline over time, and these values were analyzed using MMRM with categorical time, treatment group, screening value (if applicable) and time by treatment as explanatory variables and with an unstructured covariance structure. By contrast, CGIC is inherently a change score and does not have a baseline value for inclusion in the model. The relevant summary statistics presented for continuous outcomes include LS means, standard errors, 95% CI and the  $P$  value from an MMRM. For binary outcomes, the results presented include proportions by arm, an odds ratio, exact CIs and  $P$  values from Fisher’s exact test. The  $P$  values presented are generally one-sided to reflect the known direction of benefit; two-sided  $P$  values are presented only for exploratory outcomes where the direction of benefit was not necessarily established. Statistical analyses of clinical data were performed using SAS software v.9.4.

**PK/PD.** Blood samples for the analysis of pepinemab drug levels were obtained on a monthly basis through to the last visit. Blood was collected on all dosing days before the first and after the last infusion. Blood draw for SEMA4D saturation in whole blood, T cell SEMA4D levels, total soluble SEMA4D levels in serum and exploratory serum cytokine and biomarker analysis was collected at baseline, visits 1 and 3 and every 3 months thereafter. In a subset of subjects who volunteered ( $n = 54$ ), CSF was collected by lumbar puncture during one visit among visits 13–18 (mean, 15.6; median, 15.0), within 24 h post infusion, to be evaluated for pepinemab concentration, total SEMA4D and other biomarkers. Validated assays were utilized. Briefly, pepinemab drug levels were measured using a sandwich ELISA format with an anti-idiotypic antibody, and total soluble SEMA4D was measured using a sandwich ELISA format with two SEMA4D-specific monoclonal antibodies that do not react with the same epitope of pepinemab, thus allowing measurement of both free and bound soluble SEMA4D<sup>18,19</sup>.

**Ethics.** Institutional review board (IRB) approvals for the study protocol, amendments and informed consent documents were obtained before use in the study; written informed consent was obtained from study participants before the initiation of study procedures. This study utilized both a central IRB—Western Institutional Review Board—as well as local IRBs at sites that did not utilize the central IRB. As per the International Classification of Functioning, Disability and Health, participants received US\$450 for each routine visit, an additional US\$250 for LP/CSF and an additional US\$250 for radioligands targeting translocator protein (TSPO)–PET; an additional reimbursement for transportation or lodging costs was considered on a case-by-case basis. This study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization guidelines and applicable portions of the United States Code of Federal Regulations. The ClinicalTrials.gov identifier (no. NCT02481674) was obtained before study initiation. All experiments including human specimens were performed in compliance with the relevant ethical regulations.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

Requests for access to the patient-level data from this study can be submitted via email to [medinfo@vaccinex.com](mailto:medinfo@vaccinex.com) with detailed proposals for use of information. A signed data access agreement with the sponsor is required before accessing shared data. All requests for study protocol and data will be reviewed by the study sponsor, Vaccinex, to verify whether the request is subject to any intellectual property or confidentiality obligations. Patient-related data were generated as part of a clinical trial and may be subject to patient confidentiality. The SIGNAL study is registered with ClinicalTrials.gov (no. NCT02481674).

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## Author contributions

M.Z., E.E.E., T.L.F., J.E.L., K.D.K. and A.F. were involved in design of the study. D.O., K.A.W., L.K., M.Z., T.L.F., E.E.E. and V.M. planned and performed statistical analysis. M.Z., E.E.E., T.L.F., K.D.K., E.S. and A.F. interpreted the data. A.R. compiled clinical data. R.B., K. Marder, P.D., H.D.R., S.K., J.K., B.R., J. Bang, D.C., K. McDonnell, S.F., F.W., C.G., J.W., L.A.R., J.C.-B., V.S., M.D., M.G., A.N., S. Frank, K.L., A.D., L.E., A.S., Y.-H.L., S.C., L.S., B.S., J. Boyd, N.M., E.S., O.S., C.T., K.A., E.K., J.G. and R.M. acquired key clinical data. M.Z., E.E.E. and T.L.F. wrote the first draft of the manuscript. A.F., V.M., E.S.S., J.E.L., R.M., K.W., K.D.K., D.O., S.K. and H.D.R. revised the manuscript for content and clarity.

## Competing interests

Vaccinex employment and stock: E.E.E., T.L.F., J.E.L., E.S., V.M. and M.Z. Vaccinex patents and applications related to SEMA4D (USPTO nos. 8,816,058, 9,090,709, 10,800,853 and 7,919,594): E.E.E., T.L.F., E.S. and M.Z. IXICO employment and stock: R.M. Research grant support from HSG, Neurocrine, Uniqure and Vaccinex: P.D. Payments to Clintrex Research Corporation for provision of research services during the conduct of the trial: K.D.K. Research funding from Vaccinex, CHDI, HDSD, Roche/Genentech, UniQure, HSG/NBI and Cures within Reach, consulting role for Teva and on Speaker's Bureau of Sunovion Pharmaceuticals: E.F.S. Consultation fees from Amylyz, Novartis, Sage, Teva and Uniqure, grant funding from uniQure, Roche/Genentech, Triplet Therapeutics and CHDI and salary support from HSG for serving as chair of DSMB of a study funded by Neurocrine and the virtual UHDRS study: S. Frank. The remaining authors declare no competing interests.

## Additional information

**Extended data** is available for this paper at <https://doi.org/10.1038/s41591-022-01919-8>.

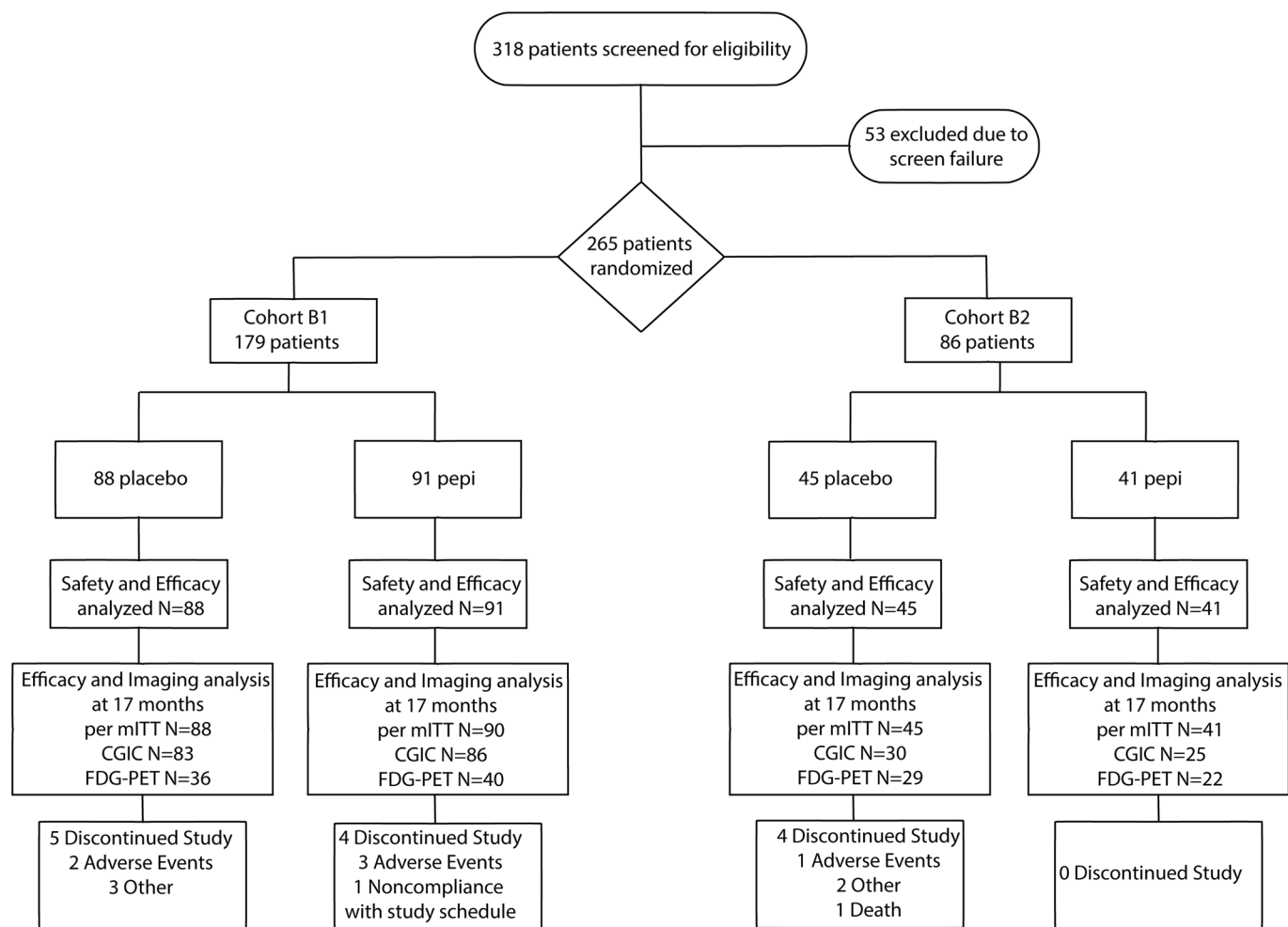
**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41591-022-01919-8>.

**Correspondence and requests for materials** should be addressed to Maurice Zauderer.

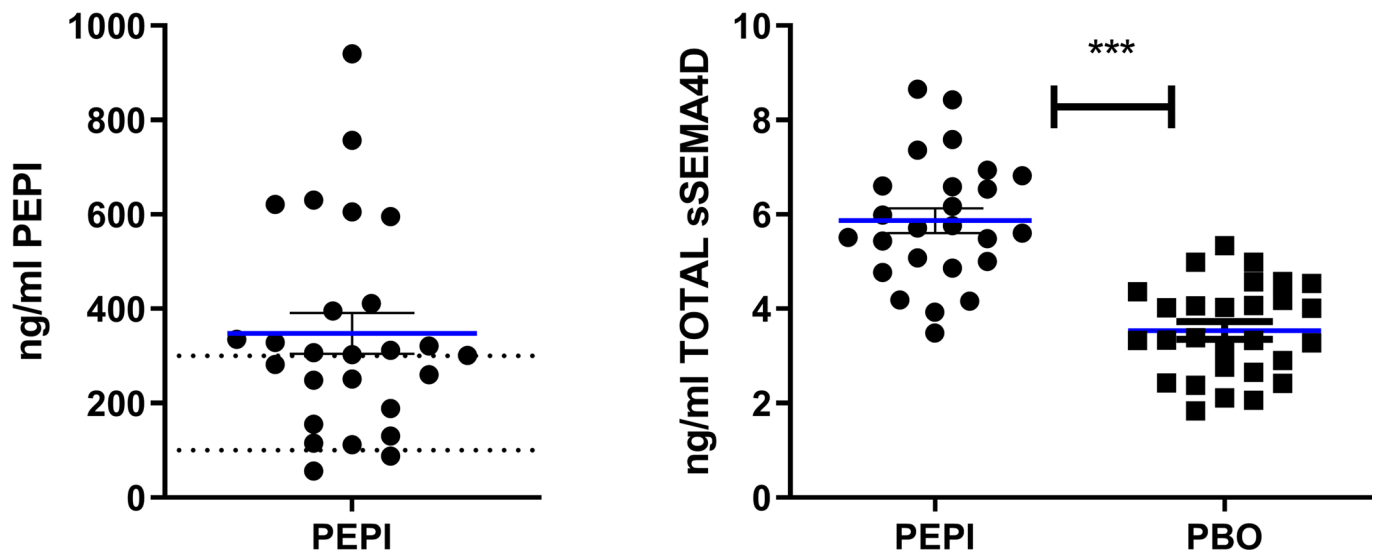
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**Extended Data Fig. 1** | CONSORT diagram indicating participant numbers and disposition.



**Extended Data Fig. 2 | Pepinemb is detected in CSF at expected level for target engagement.** **a.** Drug concentration in CSF of subjects treated with pepinemb. Mean+SEM is shown; dotted lines indicate target concentration (~100-300 ng/ml). **b.** Concentration of soluble SEMA4D (sSEMA4D) in CSF, mean+SEM are shown for each treatment group (n = 26 PEPI, n = 28 PBO). \*\*\* indicates statistical significance,  $p = 0.000000001855$ . As seen in previous trials<sup>18,19</sup>, levels of total soluble SEMA4D (including complex of drug bound to target) increased 1.7-fold upon dosing due to the increased half-life of the pepinemb/SEMA4D complex in subjects treated with pepinemb compared to those treated with placebo (average of 5.9 vs 3.5 ng/ml respectively,  $p < 0.001$ ), demonstrating evidence of target engagement in CSF. The mean (SD) observed maximum serum concentration ( $C_{max}$ ) after all infusions post visit 12 in Cohort B overall was 218 (115)  $\mu\text{g}/\text{mL}$  and the  $AUC_{tau}$  over the dosing interval was 68,900 (14,447)  $\mu\text{g}\cdot\text{hr}/\text{mL}$ . Based on the empirical Bayesian estimates of the pharmacokinetic parameters, the terminal elimination half-lives for Cohorts B1 and B2 were calculated to be approximately 25 and 23 days, respectively. In general, the clearance of pepinemb was low and the volume of distribution small, which is common to other therapeutic monoclonal antibodies and similar to observations in previously completed studies with pepinemb<sup>18,19</sup>.



Extended Data Table 1 | Demographics and baseline characteristics for all cohorts (ITT population)

Category	Cohort B1		Cohort B2		Cohort B Overall	
	PBO N=88	PEPI N=91	PBO N=45	PEPI N=41	PBO N=133	PEPI N=132
Age (years)	47.5 (10.6)	50.0 (11.4)	42.7 (10.8)	47.3 (10.8)	45.9 (10.8)	49.2 (11.3)
Sex, n (%)						
Female	48 (55)	44 (48)	27 (60)	23 (56)	75 (56)	67 (51)
Male	40 (45)	47 (52)	18 (40)	18 (44)	58 (44)	65 (49)
Race, n (%)						
White	82 (93)	88 (97)	45 (100)	40 (98)	127 (95)	128 (97)
Black or African American	2 (2)	0	0	1 (2)	2 (2)	1 (1)
Asian	1 (1)	1 (1)	0	0	1 (1)	1 (1)
Multiple	0	1 (1)	0	0	0	1 (1)
Other	3 (3)	1 (1)	0	0	3 (2)	1 (1)
Ethnicity, n (%)						
Not Hispanic or Latino	84 (95)	87 (96)	45 (100)	40 (98)	129 (97)	127 (96)
Hispanic or Latino	4 (5)	4 (4)	0	1 (2)	4 (3)	5 (4)
Education (years)	15.5 (2.3)	14.7 (2.2)	15.7 (2.7)	15.4 (2.6)	15.6 (2.4)	14.9 (2.3)
Weight (kg)	75.5 (16.2)	79.9 (22.4)	79.6 (15.3)	83.9 (20.3)	76.9 (15.9)	81.1 (21.8)
Height (cm)	170.5 (9.5)	171.8 (10.3)	170.8 (9.9)	169.7 (9.6)	170.6 (9.6)	171.1 (10.1)
BMI (kg/m <sup>2</sup> )	26.0 (5.6)	26.8 (6.0)	27.3 (5.0)	29.2 (7.1)	26.4 (5.4)	27.6 (6.4)
Time since HD symptom onset (years)	4.7 (3.7)	5.8 (4.9)	3.3 (4.3)	3.8 (4.2)	4.3 (4.0)	5.2 (4.8)
Time since clinical HD diagnosis (years) [a,b]	2.9 (3.2)	2.5 (2.6)	NA	NA		
CAG repeat length	44.1 (3.8)	43.5 (3.1)	42.8 (2.3)	42.4 (2.7)	43.7 (3.4)	43.2 (3.0)
CAP score [c]	469.9 (95.9)	466.3 (84.6)	374.4 (72.4)	403.6 (98.1)	437.6 (99.3)	447.2 (93.2)
CAP score median (1 <sup>st</sup> , 3 <sup>rd</sup> Quartiles)	467 (419, 513)	476 (411, 527)	367 (336, 418)	402 (367, 444)	442 (374, 490)	448 (386, 509)
CAP score minimum, maximum	224, 988	226, 680	228, 612	209, 735	224, 988	209, 735
UHDRS-DCL at baseline, n (%)						
0, 1—Normal or non-specific signs	0	0	0	0	0	0
2—May be HD signs (50%-89% confident)	0	1 (1)	28 (62)	25 (61)	28 (21)	26 (20)
3—Likely HD signs (90%-98% confident)	1 (1)	1 (1)	15 (33)	13 (32)	16 (12)	14 (11)
4—Unequivocal (>99% confident)	87 (99)	89 (98)	2 (4)	3 (7)	89 (67)	92 (70)
UHDRS-TFC at screening [d]	12.0 (0.9)	12.0 (0.8)	12.7 (0.6)	12.5 (0.8)	12.3 (0.9)	12.1 (0.8)
TFC 11 stratified subgroup	11.0 (0)	11.0 (0)	11.0 (0)	11.0 (0)	11.0 (0)	11.0 (0)
TFC 12-13 stratified subgroup	12.67 (0.47)	12.41 (0.50)	12.83 (0.38)	12.85 (0.36)	12.74 (0.44)	12.57 (0.48)
UHDRS-TFC at screening, n (%) [d]						
11	33 (38)	29 (32)	4 (9)	7 (17)	37 (28)	36 (27)
12	18 (20)	37 (41)	7 (16)	5 (12)	25 (19)	42 (32)
13	37 (42)	25 (27)	34 (76)	29 (71)	71 (53)	54 (41)
MoCA score	26.02 (2.04)	26.14 (2.30)	26.84 (2.17)	27.56 (1.80)	26.30 (2.11)	26.59 (2.25)
MoCA <26 stratified subgroup	23.97 (0.94)	23.78 (1.07)	24.08 (1.0)	23.67 (0.58)	24.00 (0.95)	23.77 (1.04)
MoCA ≥26 stratified subgroup	27.44 (1.21)	27.72 (1.34)	27.85 (1.5)	27.87 (1.5)	27.60 (1.34)	27.78 (1.40)
MoCA at screening, n (%)						
MoCA <26 stratified subgroup	36 (40.9)	36 (40.0)	12 (27)	3 (7.3)	48 (36.1)	39 (29.8)
MoCA ≥26 stratified subgroup	52 (59.1)	54 (60.0)	33 (73)	38 (92.7)	85 (63.9)	92 (70.3)
HD-CAB OTS (sec) [e]	17.13 (13.42)	17.88 (10.57)	12.19 (5.52)	11.61 (4.80)	15.48 (11.62)	15.93 (9.60)
HD-CAB PTAP (1/sec) [f]	16.96 (7.82)	15.74 (9.99)	25.59 (9.74)	25.51 (9.57)	19.84 (9.40)	18.77 (10.82)
HD-CAB index	-0.14 (0.65)	-0.30 (0.68)	0.43 (0.58)	0.52 (0.50)	0.05 (0.68)	-0.04 (0.74)
PBA-s apathy severity n (%)						
Absent	64 (73%)	67 (75%)	35 (81%)	29 (74%)	99 (76%)	96 (75%)
Slight, questionable, or mild	20 (23%)	20 (22%)	7 (16%)	8 (21%)	27 (21%)	28 (22%)
Moderate	4 (5%)	2 (2%)	1 (2%)	2 (5%)	5 (4%)	4 (3%)
Caudate Brain Volume BSI (mL)	5.13 (1.19)	4.83 (1.19)	5.93 (1.56)	5.92 (1.31)	5.40 (1.38)	5.17 (1.32)
Ventricular Volume BSI (mL)	32.7 (18.2)	35.2 (21.8)	19.5 (12.1)	21.4 (8.5)	28.2 (17.5)	30.9 (19.7)

Abbreviations: CAG, cytosine, adenine, and guanine; CAP, CAG/age product; DCL, Diagnostic Confidence Level; HD, Huntington disease; HD-CAB, Huntington's Disease Cognitive Assessment Battery; ITI, inter-tap interval; ITT, intent-to-treat; NA, Not applicable; OTS, One Touch Stockings of Cambridge; PBO, placebo; PEPI, pepinemab; PTAP, Paced Tapping; SD, standard deviation; Tap-Speed, speeded index finger tapping; TFC, Total Functional Capacity; UHDRS, Unified Huntington's Disease Rating Scale.

Note(s): Column header counts and denominators are the number of subjects in the ITT population. The sample sizes are the number of subjects in the ITT population with non-missing data. Mean (SD) are listed, unless otherwise specified.

[a] Cohort B2 clinical HD diagnosis data has been excluded from this table.

[b] Only the year of clinical HD diagnosis is reported on the case report form; to calculate time since HD diagnosis, the month and day of HD diagnosis is imputed to January 1.

[c] CAP score=age×(CAG repeat length-33.66). The relevant inclusion criterion requires a CAP score>200.

[d] Score ranging from 0 to 13; higher scores indicate better functioning.

[e] Time to a correct response (averaged over all trials per visit); lower values indicate better performance.

[f] Tapping consistency measured as the reciprocal of the average standard deviation of the ITI durations (over all trials per visit); higher scores indicate better performance.

**Extended Data Table 2 | Common TEAEs ( $\geq 10\%$  of Subjects) Cohort B overall for the safety analysis period\***

MedDRA Preferred Term	Cohort B Overall N=265	
	PBO N=132	PEPI N=133
Subjects with $\geq 1$ TEAE, n (%)	123 (93%)	125 (94%)
Headache	25 (19%)	50 (38%)
Nasopharyngitis	34 (26%)	40 (30%)
Fall	23 (17%)	33 (25%)
Nausea	21 (16%)	25 (19%)
Vomiting	11 (8%)	20 (15%)
Fatigue	17 (13%)	20 (15%)
Anxiety	20 (15%)	17 (13%)
Contusion	13 (10%)	16 (12%)
Dizziness	15 (11%)	14 (11%)
Pain	9 (7%)	15 (11%)
Back pain	8 (6%)	15 (11%)
Upper RTI	19 (14%)	13 (10%)
Sinusitis	12 (9%)	13 (10%)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; PBO, placebo; PEPI, pepinemab; TEAE, treatment-emergent adverse event.

Notes: Subjects are counted at most once in each cell regardless of the number of events they may have had. TEAEs are those that occur during the safety analysis period with onset on or after the date of the first exposure to study drug (PEPI or PBO).

\*Analysis of the safety analysis period included subjects treated up to 18 months. A subset of patients was offered an additional 18 months of treatment for a total of 36 months of treatment and safety follow up. A total of 42 subjects (9 in EM Cohort B1 and 33 in LP Cohort B2) enrolled in this safety extension cohort. Among the 36 month safety extension cohort, no significant group differences were observed among frequency or severity of treatment emergent adverse events (TEAEs) (n=18 PBO and n=19 PEPI) or SAEs (n=1 PBO and n=0 PEPI), the majority of subjects had TEAEs considered by the Investigator to be mild-to-moderate in severity (100% versus 95%), and no subjects had a TEAE leading to permanent study drug discontinuation.

**Extended Data Table 3 | Exploratory analysis of motor assessments in EM Cohort B1, mean change from Baseline to Month 17**

Population / Parameter <sup>a</sup>	PBO		PEPI		LS mean Difference, PEPI - PBO	
	N <sup>b</sup>	Mean (SE)	N <sup>b</sup>	Mean (SE)	Estimate (95% CI)	One-sided p-value (+ favors PEPI) <sup>c</sup>
<b>Cohort B1 mITT (N=178)</b>	88		90			
UHDRS - Total Motor Score	88	2.81 (0.78)	89	1.61 (0.77)	-1.20 (-3.37, 0.96)	0.14 (+)
<b>Q-Motor Assessments</b>						
Q-Motor Lift Grip Force CV Hand (%)	86	-0.36 (0.84)	89	-0.30 (0.83)	0.06 (-2.27, 2.40)	0.53 (-)
Q-Motor Lift Orientation Index Mean Hand (rad/sec)	87	0.07 (0.01)	82	0.05 (0.01)	-0.02 (-0.06, 0.02)	0.13 (+)
Q-Motor Lift Position Index Mean Hand (m/sec)	87	0.010 (0.002)	82	0.007 (0.002)	-0.003 (-0.008, 0.003)	0.17 (+)
Q-Motor Pro-Sup Frequency Mean Hand (Hz)	87	0.07 (0.04)	89	0.09 (0.04)	0.02 (-0.08, 0.12)	0.33 (+)
Q-Motor Pro-Sup IOI SD Hand (msec)	87	-5.72 (9.62)	89	-2.20 (9.57)	3.52 (-23.27, 30.30)	0.61 (-)
Q-Motor Pro-Sup Inter-Tap-interval Mean Hand (msec)	87	1.44 (9.31)	89	-1.46 (9.25)	-2.90 (-28.82, 23.01)	0.42 (+)
Q-Motor Tap-Speed Frequency Mean Foot (Hz)	85	0.16 (0.06)	89	0.17 (0.06)	0.01 (-0.15, 0.17)	0.45 (+)
Q-Motor Tap-Speed Frequency Mean Hand (Hz)	87	-0.17 (0.06)	89	-0.14 (0.06)	0.03 (-0.15, 0.21)	0.36 (+)
Q-Motor Tap-Speed IOI Mean Hand (msec)	87	19.03 (8.21)	89	17.58 (8.14)	-1.44 (-24.26, 21.38)	0.46 (+)
Q-Motor Tap-Speed IOI SD Foot (msec)	85	-33.98 (22.25)	89	-52.16 (21.76)	-18.17 (-79.61, 43.27)	0.29 (+)
Q-Motor Tap-Speed IOI SD Hand (msec)	87	9.37 (5.92)	89	16.45 (5.88)	7.07 (-9.40, 23.55)	0.81 (-)
Q-Motor Tap-Speed Inter-Tap-interval Mean Foot (msec)	85	-13.53 (13.85)	89	-4.02 (13.58)	9.51 (-28.79, 47.81)	0.69 (-)
Q-Motor Tap-Speed Inter-Tap-interval Mean Hand (msec)	87	13.54 (6.65)	89	13.10 (6.58)	-0.44 (-18.91, 18.03)	0.49 (+)

Abbreviations: CI=confidence interval; CV=coefficient of variation; IOI=inter-onset-interval; mITT=modified intent-to-treat; MMRM=mixed model repeated measures; PBO=placebo; PEPI=pepinemab; Q-Motor=Quantitative Motor; SD=standard deviation; SE=standard error.

Note(s): Analysis results are from an MMRM of measurements from scheduled postbaseline assessments up to and including Month 17, with estimation of the difference in means between groups at Month 17. The dependent variable is the change from baseline value and the independent variables are normalized outcome screening value, treatment group assignment, visit, and a treatment group by visit interaction term. The pooled analyses also include HD status at screening (Cohort B1, Cohort B2) as an independent variable.

[a.] In the most-affected limb at baseline and averaged over all trials per visit. A higher value is beneficial for Pro-Sup Frequency Mean Hand, Tap-Speed Frequency Mean Foot, Tap-Speed Frequency Mean Hand. A lower value is beneficial for all other parameters. The most affected limb at baseline is defined for each parameter as the side with the worse score at baseline. If a subject has a baseline score for only one limb for a parameter, that limb will be the most affected limb for the parameter.

[b.] The number of subjects included in an analysis may differ from the full analysis population size if any subjects do not have a baseline and postbaseline observation for a parameter.

[c.] The sign next to the p-value indicates whether the direction of the estimated difference indicates better (+) or worse (-) performance for PEPI relative to PBO.

**Extended Data Table 4 | Exploratory analyses of volumetric MRI in cortical brain regions of interest in EM Cohort B1 and LP Cohort B2, percent change from Baseline to Month 17**

Cohort B1 or B2 [a] Region of interest	N [b]	PBO	N [b]	PEPI	LS mean difference, PEPI - PBO	
		LS Mean (SE)		LS Mean (SE)	Estimate (95% CI)	Two-sided p-value (+ favors PEPI) [c]
<b>Cohort B1 (N=178)</b>	88		90			
Caudate brain volume BSI (atrophy)	80	5.93 (0.45)	81	4.39 (0.44)	-1.54 (-2.79, -0.29)	0.017 (+)
Ventricular volume BSI (expansion)	76	12.64 (0.94)	79	10.17 (0.90)	-2.47 (-5.04, 0.10)	0.060 (+)
Whole brain volume BSI (atrophy)	69	1.52 (0.15)	67	1.37 (0.15)	-0.15 (-0.57, 0.27)	0.48 (+)
White brain matter volume (increase)	81	-0.85 (0.07)	79	-0.73 (0.07)	0.12 (-0.06, 0.31)	0.20 (+)
<b>Cohort B2 (N=86)</b>	45		41			
Caudate brain volume BSI (atrophy)	40	3.13 (0.47)	38	3.70 (0.45)	0.57 (-0.74, 1.88)	0.39 (-)
Ventricular volume BSI (expansion)	36	9.52 (1.81)	39	8.73 (1.62)	-0.79 (-5.67, 4.09)	0.75 (+)
Whole brain volume BSI (atrophy)	34	0.89 (0.19)	36	1.09 (0.17)	0.20 (-0.32, 0.72)	0.45 (-)
White brain matter volume (increase)	39	-0.41 (0.10)	41	-0.61 (0.09)	-0.20 (-0.47, 0.07)	0.15 (-)

Abbreviations: BSI, boundary shift integral; CI, confidence interval; mITT, modified intent-to-treat; MRI, magnetic resonance imaging; MMRM, mixed-model repeated measures; PBO, placebo; PEPI, pepinemab; SE, standard error.

Note(s): Analysis results are from an MMRM of measurements from scheduled post-baseline assessments at Month 6 and Month 17, with estimation of the difference in means between groups at Month 17. The dependent variable is the percent change from baseline value and the independent variables are normalized outcome screening value, treatment group assignment, visit, and a treatment group by visit interaction term.

[a] A positive difference in BSI is theoretically detrimental (atrophy for caudate and whole brain and expansion for ventricles), while a positive difference in white brain matter volume is theoretically beneficial.

[b] The number of subjects included in an analysis may differ from the full analysis population size if any subjects do not have a baseline and post-baseline observation for a parameter that passed quality control processes.

[c] The sign next to the p-value indicates whether the direction of the estimated difference indicates better (+) or worse (-) performance for PEPI relative to PBO.



**Extended Data Table 5 | Exploratory analysis of FDG-PET SUVR in brain regions of interest in EM Cohort B1 and LP Cohort B2, percent change from Baseline to Month 17 a. EM Cohort B1. b. LP Cohort B2**

FDG-PET subgroup [a] region of interest	N [b]	PBO		PEPI	LS mean difference, PEPI - PBO	
		LS mean (SE)	N [b]		LS mean (SE)	Estimate (95% CI)
<b>a. Cohort B1 (N=76)</b>	36		40			
Amygdala	31	-1.16 (0.77)	28	0.61 (0.81)	1.77 (-0.48, 4.02)	0.13 (+)
Anterior cingulate cortex	31	-1.45 (0.88)	28	0.29 (0.92)	1.74 (-0.81, 4.29)	0.18 (+)
Caudate nucleus	31	-5.28 (1.06)	28	-4.47 (1.10)	0.81 (-2.26, 3.89)	0.60 (+)
Globus pallidus	31	-1.43 (0.81)	28	-0.85 (0.85)	0.58 (-1.78, 2.93)	0.63 (+)
Hippocampus	31	-0.94 (0.70)	28	0.48 (0.74)	1.42 (-0.63, 3.48)	0.17 (+)
Inferior parietal lobule	31	-1.73 (1.01)	28	0.84 (1.06)	2.58 (-0.38, 5.53)	0.086 (+)
Inferior temporal gyrus	31	-1.17 (0.78)	28	1.34 (0.82)	2.51 (0.22, 4.79)	0.033 (+)
Lingual gyrus	31	-1.18 (1.08)	28	2.84 (1.13)	4.02 (0.87, 7.16)	0.014 (+)
Medial frontal gyrus	31	-2.56 (1.12)	28	1.05 (1.18)	3.61 (0.32, 6.89)	0.033 (+)
Medial orbitofrontal cortex	31	-1.68 (0.96)	28	0.70 (1.00)	2.38 (-0.40, 5.16)	0.092 (+)
Medial temporal gyrus	31	-1.40 (0.84)	28	1.14 (0.88)	2.54 (0.08, 5.00)	0.044 (+)
Occipital lobe	31	-0.68 (1.08)	28	2.83 (1.13)	3.52 (0.38, 6.66)	0.029 (+)
Paracentral lobule	31	-1.39 (0.81)	28	1.61 (0.85)	3.00 (0.64, 5.35)	0.014 (+)
Posterior cingulate cortex	31	-2.87 (1.01)	28	1.24 (1.07)	4.11 (1.14, 7.08)	0.008 (+)
Postcentral gyrus	31	-1.24 (0.85)	28	1.55 (0.89)	2.79 (-0.43, 5.26)	0.028 (+)
Precuneus	31	-1.94 (0.93)	28	0.83 (0.98)	2.76 (0.03, 5.49)	0.048 (+)
Precentral gyrus	31	-1.47 (0.80)	28	1.68 (0.84)	3.15 (0.81, 5.49)	0.010 (+)
Putamen	31	-2.46 (0.63)	28	-2.41 (0.65)	0.05 (-1.77, 1.88)	0.96 (+)
Superior frontal gyrus	31	-2.21 (1.04)	28	0.77 (1.09)	2.97 (-0.06, 6.01)	0.055 (+)
Superior parietal lobule	31	-1.58 (0.85)	28	0.91 (0.89)	2.50 (0.01, 4.98)	0.050 (+)
Superior supramarginal gyrus	31	-1.78 (0.96)	28	0.58 (1.01)	2.37 (-0.43, 5.16)	0.096 (+)
Superior temporal gyrus	31	-2.06 (0.80)	28	0.44 (0.84)	2.50 (0.17, 4.83)	0.037 (+)
Thalamus	31	-2.56 (0.95)	28	1.14 (0.99)	3.69 (0.93, 6.46)	0.011 (+)
White matter brain	31	-0.32 (0.63)	28	1.33 (0.66)	1.64 (-0.20, 3.48)	0.079 (+)
Expanded cortical composite region	31	-1.87 (0.85)	28	0.93 (0.89)	2.80 (0.33, 5.28)	0.028 (+)
Extended frontal composite region	31	-2.06 (0.91)	28	0.89 (0.96)	2.96 (0.29, 5.62)	0.031 (+)
<b>b. Cohort B2 (N=51)</b>	29		22			
Amygdala	22	0.43 (0.78)	18	-0.47 (0.93)	-0.90 (-3.38, 1.58)	0.47 (-)
Anterior cingulate cortex	22	0.05 (0.85)	18	-0.42 (0.99)	-0.47 (-3.14, 2.21)	0.73 (-)
Caudate nucleus	22	-3.56 (1.08)	18	-3.13 (1.23)	0.43 (-2.94, 3.80)	0.80 (+)
Globus pallidus	22	0.32 (1.03)	18	0.56 (1.21)	0.24 (-2.99, 3.47)	0.89 (+)
Hippocampus	22	0.04 (0.73)	18	-0.57 (0.87)	-0.60 (-2.92, 1.71)	0.61 (-)
Inferior parietal lobule	22	0.15 (1.13)	18	-0.89 (1.33)	-1.04 (-4.59, 2.51)	0.56 (-)
Inferior temporal gyrus	22	-1.19 (0.91)	18	-0.28 (1.06)	0.91 (-1.93, 3.75)	0.52 (+)
Lingual gyrus	22	-0.53 (1.08)	18	-0.16 (1.29)	0.36 (-3.05, 3.78)	0.84 (+)
Medial frontal gyrus	22	-0.43 (1.10)	18	-0.46 (1.29)	-0.03 (-3.49, 3.43)	0.99 (-)
Medial orbitofrontal cortex	22	-1.70 (0.86)	18	-0.56 (1.00)	1.14 (-1.59, 3.87)	0.41 (+)
Medial temporal gyrus	22	-0.51 (0.97)	18	-0.42 (1.16)	0.09 (-2.98, 3.17)	0.96 (+)
Occipital lobe	22	0.81 (1.23)	18	-0.54 (1.47)	-1.35 (-5.25, 2.55)	0.49 (-)
Paracentral lobule	22	0.76 (1.01)	18	0.72 (1.20)	-0.04 (-3.23, 3.14)	0.98 (-)
Posterior cingulate cortex	22	-1.56 (1.18)	18	-1.41 (1.37)	0.15 (-3.53, 3.83)	0.94 (+)
Postcentral gyrus	22	0.69 (1.02)	18	0.98 (1.21)	0.28 (-2.93, 3.49)	0.86 (+)
Precuneus	22	-0.04 (1.10)	18	-0.79 (1.30)	-0.75 (-4.22, 2.71)	0.67 (-)
Precentral gyrus	22	0.84 (1.03)	18	0.54 (1.21)	-0.29 (-3.53, 2.94)	0.86 (-)
Putamen	22	-1.70 (0.93)	18	-1.78 (1.08)	-0.08 (-3.00, 2.84)	0.96 (-)
Superior frontal gyrus	22	-0.33 (1.02)	18	-1.06 (1.20)	-0.73 (-3.95, 2.49)	0.65 (-)
Superior parietal lobule	22	0.31 (1.10)	18	-0.60 (1.30)	-0.91 (-4.36, 2.54)	0.60 (-)
Superior supramarginal gyrus	22	-0.35 (1.09)	18	-0.47 (1.28)	-0.13 (-3.55, 3.29)	0.95 (-)
Superior temporal gyrus	22	0.48 (1.17)	18	0.41 (1.40)	-0.07 (-3.79, 3.64)	0.97 (-)
Thalamus	22	0.84 (1.03)	18	-1.75 (1.21)	-2.59 (-5.83, 0.65)	0.12 (-)
White matter brain	22	0.88 (0.72)	18	0.15 (0.85)	-0.73 (-2.98, 1.51)	0.52 (-)
Expanded cortical composite region	22	-0.04 (0.98)	18	-0.37 (1.15)	-0.33 (-3.41, 2.75)	0.84 (-)
Extended frontal composite region	22	-0.22 (0.98)	18	-0.46 (1.15)	-0.24 (-3.33, 2.85)	0.88 (-)

Abbreviations: CI, confidence interval; FDG-PET, [18F]-Fluoro-2-Deoxy-D-Glucose positron emission tomography; mITT, modified intent-to-treat; MMRM, mixed-model repeated measures; PBO, placebo; PEPI, pepinemab; SE, standard error; SUVR, standardized uptake value ratio.

Note(s): FDG-PET assessments were performed at a subset of sites and were voluntary for subjects at those sites. Each analysis includes subjects who had both a baseline (screening) and post-baseline FDG-PET result from a scan that was judged as suitable for analysis.

[a] Analysis results are from an MMRM of measurements from scheduled post-baseline assessments at Month 2 (Month 1 - Month 3), Month 6, and Month 17, with estimation of the difference in means between groups at Month 17. The dependent variable is the percent change from baseline value and the independent variables are outcome screening value, treatment group assignment, visit, and a treatment group by visit interaction term. Within-subject correlations were modeled using an unstructured covariance structure. A positive difference in SUVR is theoretically beneficial.

[b] The number of subjects included in an analysis may differ from the full analysis population size if any subjects do not have a baseline and post-baseline observation for a parameter.

[c] Indicates whether the direction of the estimated difference indicates better (+) or worse (-) performance for PEPI relative to PBO.

**Extended Data Table 6 | Exploratory analyses of HD-CAB Index and six components in EM Cohort B1 and LP Cohort B2 (mITT Population), mean change from Baseline to Month 17**

Population / Parameter	PBO		PEPI		LS mean difference (PEPI-PBO)	
	N [a]	LS Mean (SE)	N [a]	LS Mean (SE)	Estimate (95% CI)	One-sided p-value (+ favors PEPI) [b]
<b>Cohort B1 mITT (N=178)</b>	88		90			
OTS (sec) [c]	88	-0.33 (0.72)	89	-2.30 (0.73)	-1.98 (-4.00, 0.05)	0.028 (+)
PTAP (1/sec) [d]	87	-1.67 (0.65)	89	-0.24 (0.64)	1.43 (-0.37, 3.23)	0.060 (+)
SDMT [e]	88	-3.59 (0.70)	89	-2.97 (0.71)	0.62 (-1.35, 2.59)	0.27 (+)
EMO [f]	88	-0.09 (0.33)	89	0.28 (0.33)	0.37 (-0.55, 1.30)	0.22 (+)
HVLT-R [g]	88	0.21 (0.73)	89	0.65 (0.73)	0.44 (-1.59, 2.47)	0.34 (+)
TMT-B (sec) [h]	88	8.27 (4.24)	89	1.06 (4.26)	-7.21 (-19.09, 4.66)	0.12 (+)
HD-CAB Index [i]	87	-0.12 (0.04)	89	0.01 (0.04)	0.13 (0.03, 0.23)	0.007 (+)
<b>Cohort B2 mITT (N=86)</b>	45		41			
OTS (sec) [c]	44	-0.89 (0.74)	41	-0.94 (0.74)	-0.05 (-2.14, 2.05)	0.49 (+)
PTAP (1/sec) [d]	44	0.08 (1.02)	41	-1.00 (1.04)	-1.08 (-3.98, 1.82)	0.77 (-)
SDMT [e]	45	1.07 (1.05)	41	-0.29 (1.09)	-1.36 (-4.38, 1.65)	0.82 (-)
HVLT-R [g]	45	2.86 (0.83)	41	3.25 (0.86)	0.39 (-1.99, 2.77)	0.38 (+)
TMT-B (sec) [h]	45	-9.58 (3.63)	41	0.72 (3.76)	10.30 (-0.21, 20.80)	0.98 (-)
HD-CAB Index [i]	44	0.18 (0.06)	41	0.08 (0.06)	-0.11 (-0.27, 0.06)	0.90 (-)

Abbreviations: CI, confidence interval; EMO, Emotion Recognition; HD-CAB, Huntington's Disease Cognitive Assessment Battery; HVLT-R, Hopkins Verbal Learning Test – Revised; ITI, inter-tap interval; ITT, intent-to-treat; MMRM, mixed-model repeated measures; OTS, One Touch Stockings of Cambridge; PBO, placebo; PEPI, pepinemab; PTAP, Paced Tapping; SD, standard deviation; SE, standard error; TMT-B, Trail-Making Test – Part B.

Note(s): Analysis results are from an MMRM of measurements from scheduled post-baseline assessments up to and including Month 17, with estimation of the difference in means between groups at Month 17. The dependent variable is the change from baseline value and the independent variables are outcome screening value, treatment group assignment, visit, and a treatment group by visit interaction term.

[a] The number of subjects included in an analysis may differ from the full analysis population size if any subjects do not have a baseline (if applicable) and post-baseline observation for a parameter. As described in Methods, stated p-values for all statistical tests besides the co-primary efficacy analyses are not corrected for multiplicity and are thus presented as nominal and not under alpha control.

[b] Indicates better (+) or worse (-) performance for PEPI relative to PBO.

[c] Time to a correct response (averaged over all trials per visit); lower values indicate better performance.

[d] Tapping consistency measured as the reciprocal of the average SD of the ITI durations (over all trials per visit); higher scores indicate better performance.

[e] Number of correctly coded terms; higher scores indicate better performance.

[f] Total number of negative emotion stimuli (face images) for which the subject's response was correct; higher scores indicate better performance.

[g] Total correct responses across all four learning trials; higher scores indicate better performance.

[h] Time taken to complete test; lower values indicate better performance.

[i] HD-CAB Index is the average of z-scores for HVLT-R, SDMT, TMT-B, EMO, PTAP, and OTS. The sample baseline mean and SD used to compute the z-scores are limited to Cohort B1 subjects for Cohort B1 analysis and Cohort B2 subjects for Cohort B2 analysis.

**Extended Data Table 7 | Post-hoc analyses of selected PBA-s severity subscores at Month 17 in EM Cohort B1**

Presence of Behavior	PBO n/N (%)	PEPI n/N (%)	Odds ratio (95% CI) PEPI vs. PBO	Nominal one-sided p-value (+ favors PEPI)[a]
Apathy	33/83 (39.76)	19/82 (23.17)	0.46 (0.22, 0.94)	0.017 (+)
Depression	28/83 (33.73)	16/82 (19.51)	0.48 (0.22, 1.02)	0.030 (+)
Irritability	35/83 (42.17)	32/82 (39.02)	0.88 (0.45, 1.71)	0.41 (+)
Anxiety	40/83 (48.19)	40/82 (48.78)	1.02 (0.53, 1.97)	0.60 (-)

Abbreviations: CI, confidence interval; PBA-s, Problem Behaviors Assessment-short form; PBO, placebo; PEPI, pepinemab.

Note(s): Column header counts are the number of subjects in the mITT population. The analysis includes observed data only and dichotomizes original responses into absence or presence of the problem behavior. The confidence limits around the odds ratio are exact and the p-value is from a Fisher's exact test of association between treatment group assignment and the problem behavior.

[a] The sign next to the p-value indicates whether the conditional probability of worsening indicates better (+) or worse (-) performance for PEPI relative to PBO.

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- For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
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## Life sciences study design

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Sample size	According to the protocol, the planned Cohort B sample size of 240 allows a minimal detectable effect size of 0.38 (Cohen's D) with two-sided alpha of 0.05, 80% power, and 10% dropout. Power calculations and endpoint selection were based on an earlier pilot study (Cohort A) of 36 HD patients (15 Early Manifest and 21 Late Prodromal) treated with pepinemab or placebo in a double-blinded comparison for six months. The 2CARE study provided additional information on estimates of variance at Month 18.
Data exclusions	No individual data was excluded from reported endpoints.
Replication	Group sizes were sufficiently large to afford multiple subject replicates for each assessment and time point. In addition, one previous pilot study was conducted and trends of safety, tolerability, PK, cognitive and FDG-PET imaging results were reproduced in current report.
Randomization	Subjects who satisfied all eligibility criteria were participants and were randomized to 1 of 2 treatment arms through an Interactive Web Response System (IWRS). Subjects in Cohorts B1 and B2 were independently randomized. This system also prescribed for each patient specific numbered vials of pepinemab or matching placebo. The placebo used in the study matched the vial of pepinemab in that the vial, vial stopper and overwrap, formulation, vial fill volume and label appearance were identical to that of the vial of pepinemab.
Blinding	The subjects, site investigators, site personnel, study statisticians, as well as representatives of these organizations and staff at Vaccinex were blinded as to treatment assignments until database lock. The investigational agent and placebo were in vials identical in appearance. During the course of the study, the Safety Monitoring Committee (SMC) maintained access to treatment code information.

## Reporting for specific materials, systems and methods

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### Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
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### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input type="checkbox"/>	<input checked="" type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used	Pepinemab (VX15/2503) is a humanized IgG4 monoclonal antibody with a hinge mutation to prevent in vivo Fab arm recombination. Bulk pepinemab (Catalent Pharma Solutions, Madison, WI) was produced using a proprietary CHO cell line.
Validation	The bulk antibody was purified using standard techniques and formulated at approximately 20 mg/mL in preservative-free 20 mM sodium acetate, pH 5.4, containing 130 mM sodium chloride and 0.02 % polysorbate 80. Pepinemab and matching placebo were supplied by the Sponsor as single-use vials.

## Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	See Extended Data Table 2.
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## Recruitment

Eligible participants were aged 21 years and older, had genetically confirmed presence of  $\geq 36$  cytosine-adenine-guanine (CAG) repeats in one huntingtin gene and no features of juvenile HD (Westphal variant). EM participants were defined by UHDRS-TFC  $\geq 11$ ; they were determined by the site investigator to have a clinical diagnosis of HD as defined by a DCL of 4. LP was defined as DCL of 2 or 3 and a CAP score of  $>200$ . CAP score, a measure of HD mutation burden, was calculated as the product of age x (CAG-33.66) <sup>55</sup>. Stable dosages of concomitant medications (including tetrabenazine and deutetabenazine) were permitted if initiated at least one month prior to baseline (Visit 0), with the exception of newly prescribed anxiolytics for the use of premedication prior to imaging at screening, which were permitted on a case-by-case basis. Exclusion criteria include participation in an investigational drug or device study within 30 days of baseline, or 180 days if previous investigational drug was a monoclonal antibody, therapeutic, suicide risk, MoCA score  $\leq 22$ , ECG abnormalities at screening, pregnancy, conditions which would exclude MRI participation. Per the ICF, participants received \$450 for each routine visit, an additional \$250 for LP/CSF, and additional \$250 for TSPO-PET, and additional reimbursement for transportation or lodging costs was considered on a case-by-case basis.

## Ethics oversight

Institutional review board approvals for the study protocol, amendments, and informed consent documents were obtained before use in the study; written informed consent was obtained from study participants before the initiation of study procedures. This study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization (ICH) guidelines, and applicable portions of the United States Code of Federal Regulations. The ClinicalTrials.gov identifier (NCT02481674) was obtained before study initiation. All experiments including human specimens were performed in compliance with the relevant ethical regulations. This study utilized both a central IRB; Western Institutional Review Board (WIRB) as well as local IRBs at sites that did not utilize the central IRB.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Clinical data

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## Clinical trial registration

NCT02481674

## Study protocol

Clinicaltrials.gov

## Data collection

SIGNAL Cohort B is a Phase 2, multi-center, randomized (1:1), double-blind, placebo controlled, parallel-group study of pepinemab in subjects with EM (Cohort B1) and LP (Cohort B2) HD (<https://clinicaltrials.gov/ct2/show/NCT02481674>) (VX15-2503-N-131B). A total of 265 subjects (179 EM, and 86 LP) were enrolled at 30 outpatient clinical sites in USA and Canada. Subjects were enrolled from 28 December 2015 through 31 December 2018. Data was collected at each clinical site and entered into the EDC (eClinical Solutions), which was reviewed regularly by the CRAs. The EDC was centrally managed and reviewed by the clinical CRO.

## Outcomes

Tolerability, defined as the ability to complete the study on the assigned study arm, accounted for subject's study disposition (e.g. reason for study discontinuation of "Did not tolerate study drug"), treatment disposition, and duration of exposure. Adverse events (AEs) were monitored monthly on each subject during the study period, defined as from signing informed consent through the final study contact. A treatment-emergent AE (TEAE) is defined as an adverse event with onset on or after the date of first dose of study drug. AEs are coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 16.1. The Investigator assessed the causality of each AE to study drug and the severity of each AE using his/her clinical expertise and designated a grade to each AE per the current Common Terminology Criteria for Adverse Events (CTCAE). Columbia-Suicide Severity Rating Scale and Hospital Anxiety and Depression Scale were assessed. The Huntington's Disease Cognitive Assessment Battery (HD-CAB) was measured at six visits during the Primary Analysis Period: screening, baseline, and Months 2, 6, 12, and 17. The HD-CAB is comprised of six component tests two of which are PTAP and OTS, and are described in METHODS. CGIC is a single item questionnaire that asks the investigator to assess a subject's HD symptoms compared to immediately before starting study drug, using a 7-point Likert Scale, ranging from very much worse (-3) to very much improved (+3), to assess overall response to study drug relative to baseline. CGIC was evaluated at four visits during the Primary Analysis Period: Months 3, 5, 11, and 17. The Total Functional Capacity (TFC) scale is a component of the Unified Huntington's Disease Rating Scale (UHDRS; together referred to as "UHDRS-TFC") and has been used in premanifest and manifest HD populations in many observational studies and randomized controlled trials. The UHDRS-TFC score, is the sum of five items, ranges from 0 to 13, with a higher score representing better functioning. Q-motor and TMS allow objective monitoring of unintentional motor side-effects. The UHDRS-TMS assesses motor features of HD with standardized ratings in the following five domains: eye movement, chorea (jerky movement), dystonia (muscle spasm and twisting), bradykinesia (slowness in movement), and rigidity (stiffness). Items in each of the five domains are individually rated on a five-point scale ranging from 0 (normal) to 4 (most severe impairment). The sum of the scores of all 31 items is referred to as the UHDRS-TMS. The range of the UHDRS-TMS is 0 to 124, with higher scores indicating more severe motor impairment. (REF ) The Q-Motor battery is composed of pre-calibrated and temperature-controlled force transducers and 3D position sensors that are used to assess (1) grasping forces, (2) involuntary choreatic movements, (3) regularity of index finger tapping, and (4) regularity of alternating pronation/supination hand movements. PBA-s is a semi structured clinical interview that contains 11 items, each measuring a different behavioral problem which is rated for both severity and frequency on a five-point scale (0-4).

## Magnetic resonance imaging

### Experimental design

## Design type

Volumetric MRI only

## Design specifications

NA; The design type question above is about block or event-based design, which refers to functional MRI, but we are only doing volumetric MRI here, so there is no design spec.

Behavioral performance measures NA; no behavioral measures taken as acquisition was resting state volumetric scans only.

## Acquisition

Imaging type(s) Structural, T1W sequence

Field strength 3T

Sequence & imaging parameters 3DT1W Sequence image parameters: Gradient echo T1W sequence, Cartesian readout, based on ADNI 1 guidelines.  
Voxel size: 1x1x1mm, FOV: 256mm  
Sequence type: FFE (Philips), Bravo (GE), TFL (Siemens)  
Orientation: Sagittal  
TE/TR/flip angle: vary between scanners/sequences, but typical example:  
Siemens: TE 3.3ms, TR 2530ms, FA 7 deg, TI 1100ms

Area of acquisition Whole brain

Diffusion MRI  Used  Not used

## Preprocessing

Preprocessing software MRICron DICOM to Nifti conversion (version 6/2013), DCMTK extraction of orientation information (version of 3.5.4), Image registration toolkit (IRTK, version 1.95) for mult-atlas brain extraction, registrations, N4BiasFieldCorrection (version 1.9 from ANTs package), MIDAS for semi-automated delineation (version 5.11.1), KN-BSI for boundary shift integral calculation of volume change (version 1.2), NiftyReg/NiftiSeg (version 0.9.4), NiftTK for BSI computation (version 12.11), LEAP (Learning Embeddings for Atlas Propagation) and LLEAP (Longitudinal LEAP) used for regional brain segmentations.

Normalization Individual brain volume measurements can be adjusted for intracranial volume to correct for subject-variability in head size. Following the work of (Buckner 2004), an affine scaling factor is used, which relates the subject's brain size to that of an atlas to calculate intracranial volume (ICV). This measurement of ICV is referred to as pseudo total intracranial volume (pTIV), and entails affinely registering the patient T1-weighted image to an atlas and calculating the overall scaling factor as the magnitude of the scaling vector in the transformation matrix calculated from the registration. Rigid and affine registrations are used with the MNI atlas.

Normalization template Subject 3DT1w image affine transformation to whole head MNI atlas in MNI space, with MNI305 template

Noise and artifact removal N4 bias field correction

Volume censoring NA; Each MRI acquisition was a 3D single image, rather than a 4D time series, so no volume censoring is required.

## Statistical modeling & inference

Model type and settings All sites and their respective 3T MRI scanners were qualified prior to the start of the study. The protocol included the following scan sequences; 1. Localizer, 2. T1w, 3. Field Map DTI, 4. DWI, 5. T2\_SPACE, 6. T2\_FLAIR, 7. Field Map fMRI, 8. Resting State fMRI, 9. PD, but analysis only involved analysis of volumetric changes (T1w). IXICO reports the pseudo total intracranial volume (pTIV) factor, which is a measure of how a subject's head size compares to the standard template. Screening results were normalized to the pTIV to account for differences in head size. The normalized screening result for each brain region was calculated as screening volume / pTIV factor. At each post-baseline timepoint, percentage change from baseline was calculated as (reported change value / reported screening value) × 100.

Effect(s) tested Volumetric change from baseline

Specify type of analysis:  Whole brain  ROI-based  Both

Anatomical location(s) automated labeling algorithms

Statistic type for inference (See [Eklund et al. 2016](#)) voxel-wise

Correction NA; Imaging vendor reported absolute and and % volume change, with no correction needed.

## Models & analysis

n/a | Involved in the study

Functional and/or effective connectivity

Graph analysis

Multivariate modeling or predictive analysis

Multivariate modeling and predictive analysis The statistical analysis plan, finalized prior to locking the database and unblinding, specified a plan to control the overall Type I error rate in multiple testing of two co-primary analyses plus a series of secondary analyses to be tested in hierarchy. The co-primary endpoints: were the two-item HD-CAB family (OTS and PTAP) and

the CGIC. Both co-primary endpoints were required to meet a critical one-sided p-value of 0.025 (equivalent to a two-sided alpha of 0.05) for a successful trial overall. The success of the HD-CAB family was assessed according to the Hochberg procedure for multiple testing among the two items. Since the co-primary endpoints did not collectively reach the threshold needed to declare a successful finding, the prespecified hierarchy of secondary endpoints were not tested formally. Thus, stated p-values for all statistical tests besides the co-primary efficacy analyses are not corrected for multiplicity and are thus presented as nominal and not under alpha control.

For the continuous outcomes, the dependent variable is generally change from baseline over time, and these values were analyzed using mixed models for repeated measures (MMRM) with categorical time, treatment group, screening value (if applicable), and time by treatment as explanatory variables and with an unstructured covariance structure. Alternately, CGIC is inherently a change score and does not have a baseline value to include in the model. The relevant summary statistics presented for continuous outcomes include the LS means, standard errors (SE), 95% confidence intervals (CI), and the p-value from an MMRM. For the binary outcomes, the results presented include proportions by arm, an odds ratio, exact confidence intervals, and the p-value from a Fisher's exact test. The p-values presented are generally one-sided to reflect the known direction of benefit; two-sided p-values are presented only for exploratory outcomes where the direction of benefit is not necessarily established.