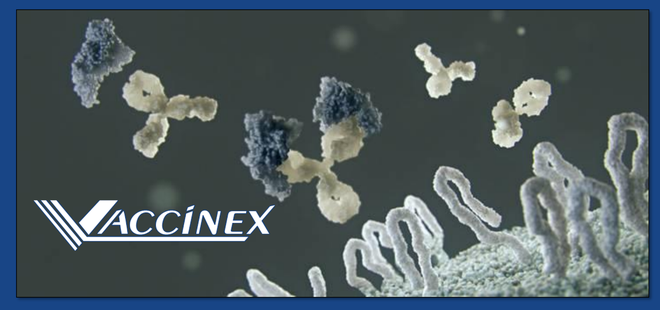
Pepinemab, an anti-semaphorin 4D blocking antibody as a potential treatment for neurodegenerative disease: Treatment rationale and SIGNAL HD and AD trial updates.



CHDI 2020

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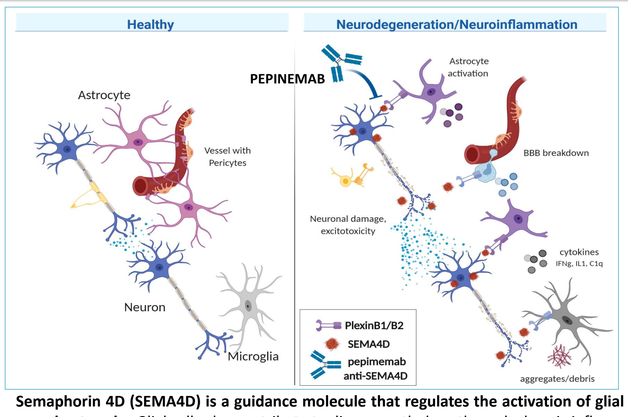
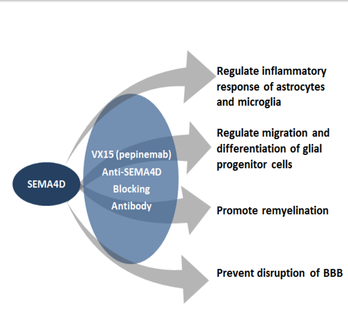
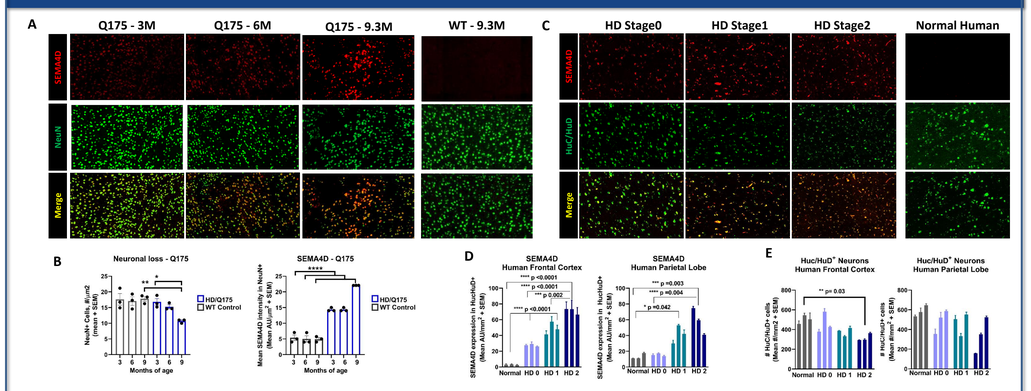
Biology of SEMA4D: Glial Cell Regulation





- **Semaphorin 4D (SEMA4D) is a guidance molecule that regulates the activation of glial cells that support neuronal function and shapes neural networks.** Glial cells also contribute to disease pathology through chronic inflammation and demyelination.
  - SEMA4D signals through Plexin-B1 and/or Plexin-B2 receptors connected to molecular switches, RhoA and R-Ras, that regulate cytoskeletal organization and cell adhesion.
- **How do glial cells recognize and respond to damage?**
  - CNS damage triggers upregulation of SEMA4D and dramatic change in glial cell morphology and function
  - Astrocytes and other glial cells express plexin receptors. SEMA4D signals through plexin receptors to trigger glial transformation from normal to activated "inflammatory" state at sites of injury. Reactive glial cells secrete cytokines that activate other inflammatory cells. (*Smith, et.al. Neurobiology of Disease, 73:254–268. 2015*).
- **Pepinemab (VX15/2503) is a humanized IgG4 antibody that blocks binding of SEMA4D to its receptors.**
  - Antibody blockade of SEMA4D inhibits changes associated with glial cell activation and promotes migration and differentiation of glial progenitor cells that can replenish glia and repair myelin.

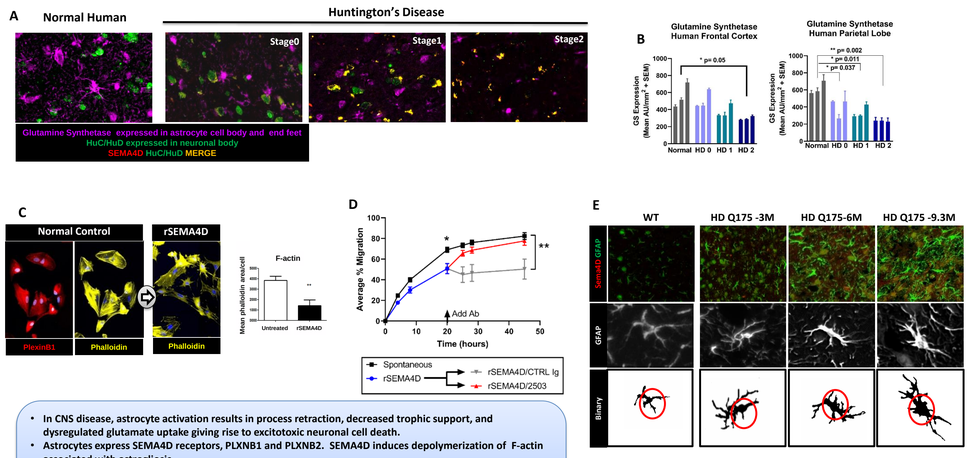
SEMA4D is upregulated in HD



A. Q175 knock-in mouse model of HD exhibit age-dependent upregulation and colocalization of SEMA4D in cortical neurons. **B.** Quantification of SEMA4D expression and number of neurons in Q175 model. Mean of entire coronal section +SEM (bar) with 3 mice/age group (closed circles). **C.** SEMA4D is increasingly upregulated in neurons of human frontal lobe with increasing HD stage and pathology, in parallel with neuronal loss. **D.** SEMA4D expression and **E.** number of neurons were quantified across entire human autopsy sections of frontal cortex (Inferior frontal gyrus, Brodmann area -BA 44-45) and parietal lobe regions (Somatosensory cortex- Brodmann area- BA 1,2,3 and part of frontal cortex BA4). Mean of 3-4 consecutive sections +SEM is shown for 3 subjects/condition. Group differences and statistical significance was determined using one-way ANOVA with Tukey post hoc analysis and is indicated by * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

- **SEMA4D expression is upregulated in HD mice as disease progresses**, compared to low expression in age matched wild type (WT) control mice.
  - SEMA4D is upregulated early in disease in Q175 HD mice, prior to onset of symptoms, which occurs approximately 5 months of age
- **SEMA4D in upregulated in brains of HD and AD subjects**
- **SEMA4D co-localizes with NeuN+ and HuC/HuD+ neurons**

Astrocytes lose normal form and function upon activation with SEMA4D

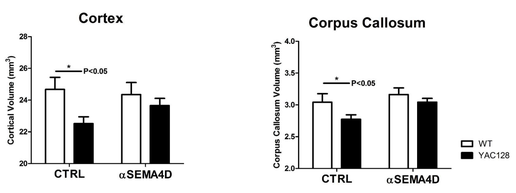


- In CNS disease, astrocyte activation results in process retraction, decreased trophic support, and dysregulated glutamate uptake giving rise to excitotoxic neuronal cell death.
- Astrocytes express SEMA4D receptors, PLXNB1 and PLXNB2. SEMA4D induces depolymerization of F-actin associated with astrogliosis.
- SEMA4D inhibits astrocyte process extension and migration. Antibody blockade reverses this effect
- Astrocytes transform to activated inflammatory state with increased soma size during disease progression
- Astrocytes are in close proximity to SEMA4D+ cells in diseased brain.

➢ **Blocking SEMA4D / Plexin interactions can help restore overall astrocyte health**

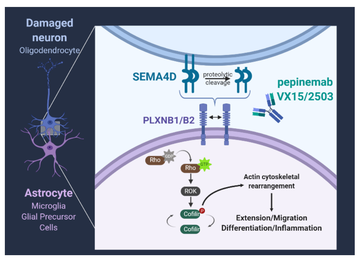
A. Human frontal cortex (Inferior frontal gyrus, Brodmann area -BA 44-45) sections were stained for glutamine synthetase (GS, astrocyte cell body and processes), SEMA4D, and Huc/HuD (neuronal cell body). **B.** Number of GS+ cells were quantified as described in Fig. 2d. **C.** Primary rat astrocyte cultures were stained for expression of receptor PLXNB1 (left), as well as F-actin filaments (phalloidin) and nuclei (Dapi) before (center) and after (right) one-hour treatment with recombinant SEMA4D. Representative images are shown. Mean phalloidin-positive area/cell in a field of ~ 300 cells was quantified using ImagePro software in each of five separate culture wells. **D.** SEMA4D inhibits process extension and migratory function of astrocytes; pepinemab reverses effects. Cell-free area in Radius 24-well Cell Migration Assay (Cell Biolabs) was determined following culture of purified astrocytes for the indicated time in the presence or absence of recombinant SEMA4D (15 ug/ml), added at time 0. Pepinemab "2503" or isotype control antibody "CTRL Ig" (50 ug/ml) was added at time = 20 hours to determine whether the effect is reversible. Results in replicate wells (n=6) at each time point are normalized to cell-free area at time 0. **E.** GFAP/Sema staining of caudoputamen region of Balb/c control and Q175 knock-in HD mice. (Representative images (20X) are shown from analysis of 3 mice/time-point, M = months of age.) Representative images of astrocyte fractal analysis, shown in lower panels demonstrate increased soma size in activated astrocytes present in diseased tissues Statistical significance was determined using two-way ANOVA and is indicated by * p<0·05 or ** p<0·01.

Antibody blockade preserves brain volume in YAC128 transgenic model of HD



Anti-SEMA4D preserves brain grey and white matter in YAC128 Huntington's Disease mice. Free-floating brain tissue sections from 12 month-old MAb-treated YAC128 and wild type (WT) mice (n=13-21/group) were stained with anti-NeuN antibody. Cortical and corpus callosum volumes were determined by tracing the perimeter of the desired structure in serial sections using StereoInvestigator software (Microbrightfield) and volumes determined using the Cavalieri principle. Statistical significance was determined by ANOVA with Bonferroni's Multiple Comparison Test where *=p<0.05 and **=p<0.01.

Southwell et.al., Anti-semaphorin 4D immunotherapy ameliorates neuropathology and some cognitive impairment in the YAC128 mouse model of Huntington disease. Neurobiology of Disease, 76:46–56 (2015).



Hypothesis:
Blocking F-actin depolymerization with anti-SEMA4D antibody pepinemab may reduce inflammatory transformation and increase glucose uptake

SIGNAL Clinical Trial Cohort A

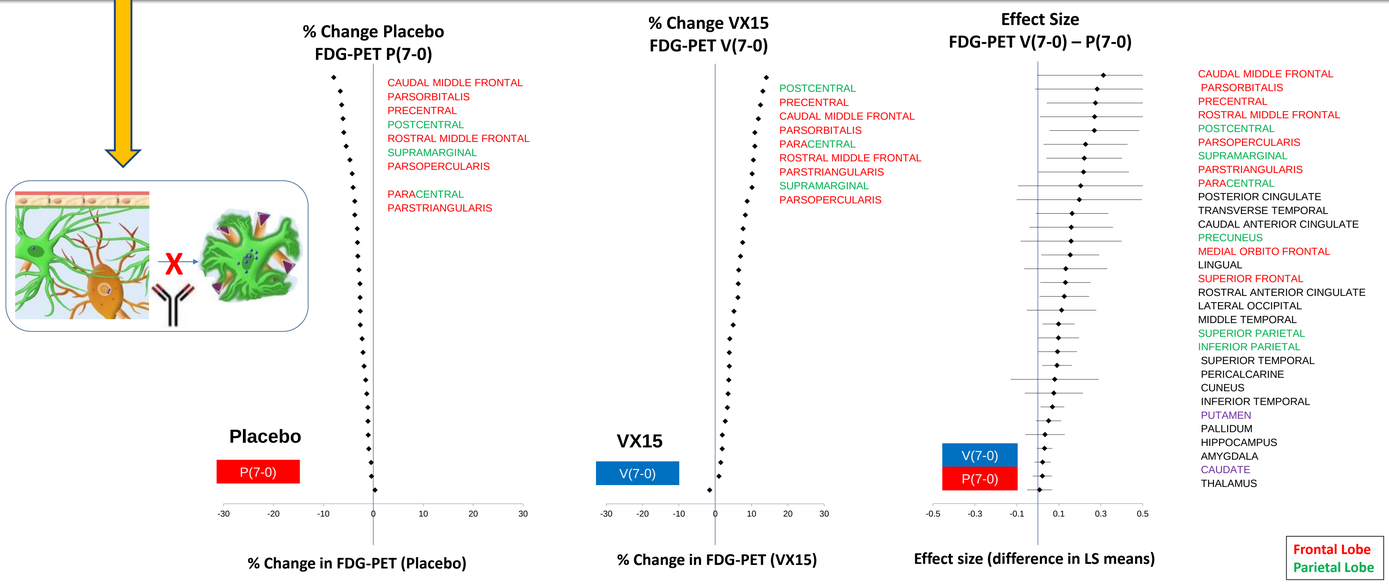
Enroll into SIGNAL **Cohort A** → 36 subjects randomized 1:1 Drug:Placebo, 6-months treatment → **Data analysis**

Cohort A identified a potential biomarker of treatment effect and provided data to estimate required sample size and treatment duration for potentially pivotal Cohort B

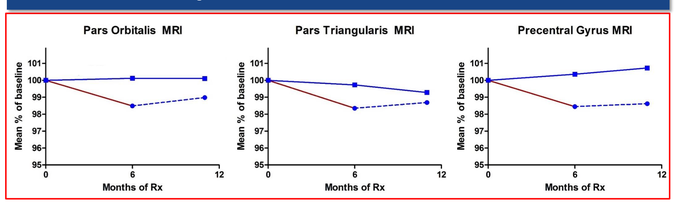
A Phase 2, multi-center, randomized, double-blinded, placebo-controlled clinical trial in subjects with early manifest (EM) and late prodromal (LP) Huntington disease (HD) to assess safety, tolerability, pharmacokinetics, and efficacy of VX15/2503 (*ClinicalTrials.gov Identifier: NCT02481674*).

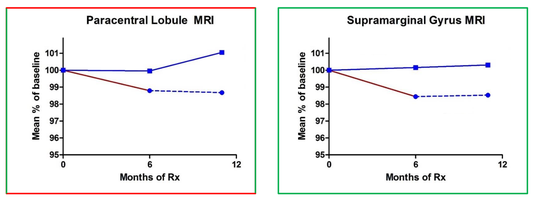
Program granted Orphan Drug and Fast Track Designation by the FDA Division of Neurology Products

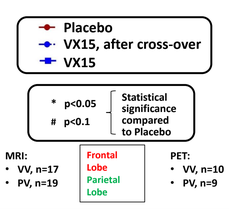
SIGNAL Cohort A: FDG-PET measures glucose metabolism



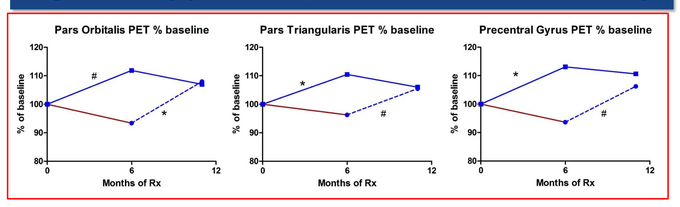
Cohort A SIGNAL MRI: Anti-SEMA4D trend to preservation of brain volume

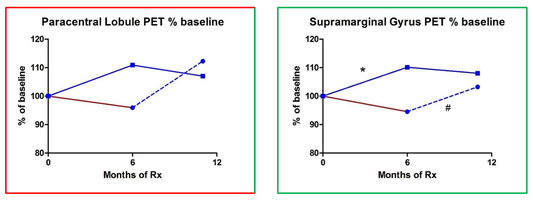






Cohort A SIGNAL FDG-PET: Anti-SEMA4D significantly preserves/restores metabolic activity





SIGNAL Clinical Trial Cohort B: Ongoing

N = 265

Cohort B Group 1 → 179 early manifest, randomized 1:1 Drug:Placebo → Double-blind treatment with pepinemab (VX15) or placebo, **18 months** → 1 month safety follow-up → Database lock and analysis for Cohort B, **Q4 2020**

Cohort B Group 2 → 86 late prodromal, randomized 1:1 Drug:Placebo → Double-blind treatment with pepinemab (VX15) or placebo, **18 or 36 months** → 1 month safety follow-up → Database lock and analysis for Cohort B, **Q4 2020**

Enrollment in Cohort B was completed by December 31, 2018

Last patient last visit anticipated June, 2020

Endpoints:
- Safety and Tolerability
- Imaging
  - FDG-PET reflecting glucose metabolism
  - Volumetric MRI as a measure of brain atrophy
- Cognitive assessments and Quantitative motor: HD-CAB, Q-motor, UHDRS-Motor, Total Functional Capacity, Patient Reported Outcomes
- PK, PD, sSEMA4D, NfL and mHTT levels in CSF, serum cytokine and biomarker levels

Conclusions

- While it is widely believed that neuronal loss is irreversible, other important elements that govern neurological activity, in particular glial cells and synapses, may be replenished or repaired with potentially significant impact on disease progression. We hypothesize that the imaging results from Cohort A suggest a partial restoration of glial function and/or restoration of disrupted neural networks.
- Pepinemab has been well-tolerated in in subjects with neurodegenerative disease, including those enrolled in SIGNAL-HD, and previously in a Phase 1 MS trial.
- Pepinemab treatment resulted in an increase in FDG-PET signal relative to the decrease observed in placebo group in SIGNAL-HD Cohort A.
- FDG-PET analysis favored pepinemab in all 31 ROI, achieving significance (p<0.05) in a majority of frontal and parietal brain ROI.

SIGNAL-AD

- Previous studies in Alzheimer's Disease concluded that glucose metabolism is a sensitive measure of change in cognition and functional ability and has value in predicting future cognitive decline or as an outcome measurement to monitor clinically-relevant change over time (Landau et. al., Neurobiol Aging. 2011; 32(7): 1207–1218).
- Clinical investigation of pepinemab in AD is being planned based on preclinical MOA data, clinical safety data, as well as SIGNAL FDG-PET data that suggests treatment-induced increase in metabolic activity and glial health.
  - Randomized monthly treatment: placebo (n=20), pepinemab 20 mg/kg (n=20), or pepinemab 40 mg/kg (n=20) for treatment duration of 32 weeks
  - Safety follow-up: weeks 32-40
  - FDG-PET imaging at baseline and 36 weeks
  - FPI planned in June
  - *Program funding supported by Alzheimer's Association and Alzheimer's Drug Discovery Foundation*

Acknowledgments

Vaccinex is very appreciative of the subjects who agreed to participate in this study in order to help investigate pepinemab as a novel potential treatment for HD. In addition, we wish to thank the Huntington Study Group and Elise Kayson and Jody Goldstein and their staff at the University of Rochester Clinical Trials Coordination Center for their excellent operational support and Dr. David Oakes and his colleagues at the University of Rochester for Biostatistical and Computational analysis. Finally, we wish to particularly thank the clinical staff at the thirty clinical sites that are participating in this study.