Blocking Semaphorin 4D to regulate glial cell activation and neurodegeneration – a potential treatment for Huntington's Disease

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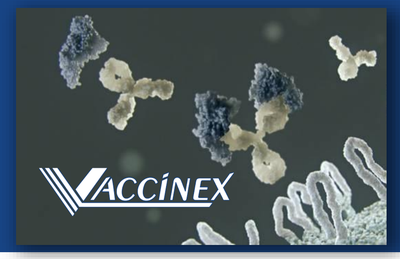
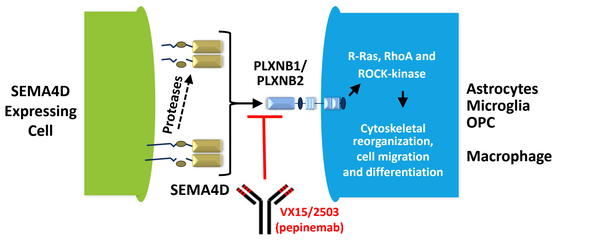


Biology of SEMA4D: Glial Cell Regulation



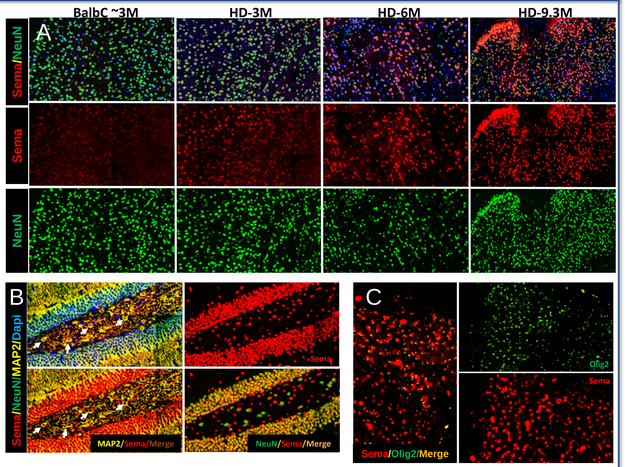
- Semaphorin 4D (SEMA4D) is a guidance molecule that regulates the activation of glial cells that support neuronal function and shape neural networks. Glial cells also contribute to disease pathology through chronic inflammation and demyelination.
- SEMA4D signals through Plexin-B1 and/or Plexin-B2 receptors connected to molecular switches, RhoA and R-Ras, that regulate cytoskeletal organization, cell adhesion, and cytokine synthesis and secretion.
- VX15/2503 (pepinemab) 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 repair and remyelinate brain lesions
  - *Smith, et.al., SEMA4D compromises blood–brain barrier, activates microglia, and inhibits remyelination in neurodegenerative disease. 2015 Neurobiology of Disease, 73:254–268*)
- Glial cells are the most abundant cells in the brain cortex

1. They provide essential functional support to neurons. **Glial cells couple glucose transport and metabolism to synaptic activity.**
2. CNS damage triggers dramatic change in glial cell morphology and function (a) Beneficial in the context of acute focal injury, but (b) maladaptive in broad chronic injury such as HD

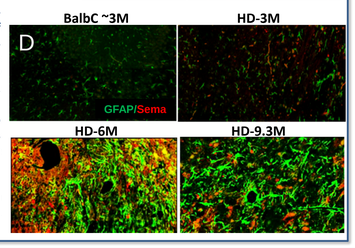
How do glial cells recognize and respond to damage?

1. SEMA4D is upregulated at site of CNS injury/disease
2. Astrocytes express high levels of receptors for SEMA4D
3. SEMA4D triggers depolymerization of F-actin associated with transformation of astrocytes from normal to inflammatory state

SEMA4D is upregulated in HD



Q175 knock-in mouse model of HD exhibit age-dependent upregulation and colocalization of SEMA4D in neurons (A,B), as well as oligodendrocytes (C). Expression of SEMA4D is observed in both NeuN+ mature and Map2 isoform+ immature neurons (B - White arrows indicate Map2+SEMA4D+ neurons)



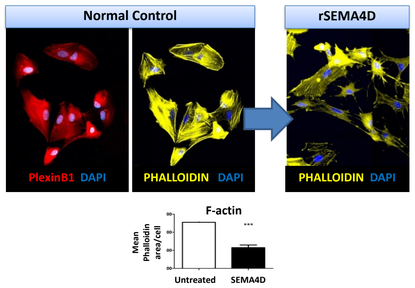
(D) SEMA4D+ cells are in close proximity to PLXNB1+ astrocytes; astrocyte activation is apparent with disease progression. Representative images from analysis of 3 mice/time-point
A) NeuN/Sema from Retrosplenial cortex.
B) NeuN/MAP2/Sema and C) Olig2/Sema are from dentate gyrus regions in HD mice-9.3 months of age.
D) GFAP/Sema from caudoputamen.

ASTROCYTES: Express cognate Plexin receptors. Antibody blockade inhibits SEMA4D-induced cytoskeletal changes and activation.

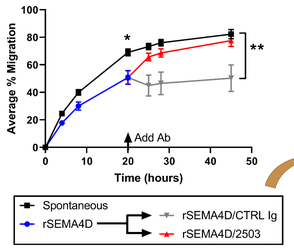
In CNS disease, astrocyte activation results in process retraction, decreased trophic support, and dysregulated glutamate uptake giving rise to excitotoxic neuronal cell death.

1. Astrocytes express SEMA4D receptors, PLXNB1 and PLXNB2
2. SEMA4D induces depolymerization of F-actin associated with astrogliosis.
3. SEMA4D inhibits astrocyte process extension and migration.

A. **SEMA4D INDUCES DEPOLYMERIZATION OF CYTOSKELETON**

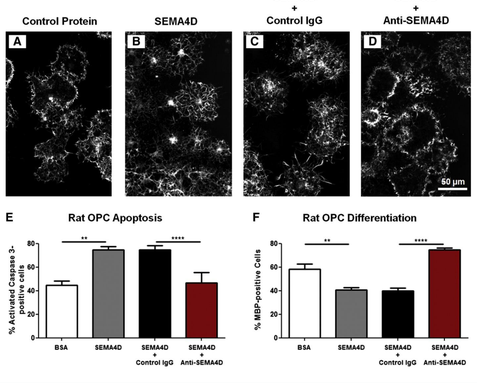


B. **SEMA4D INHIBITS ASTROCYTE MIGRATION, ANTI-SEMA4D / Mab2503 REVERSES EFFECTS**



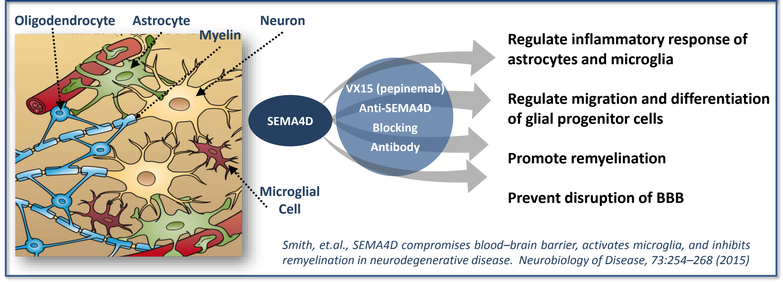
A. Primary rat astrocyte cultures were treated with recombinant SEMA4D for 1 hour, and were stained for expression of receptor PLXNB1, as well as F-actin filaments (phalloidin) and nuclei (Dapi). Representative images are shown. Mean phalloidin-positive area/cell in a field of ~ 300 cells was quantified using ImagePro software in each of 5 separate culture wells. **B.** 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 µg/ml), added at time 0. Anti-SEMA4D "2503" or isotype control antibody "CTRL Ig" (50 µg/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. Statistical significance was determined using two-way ANOVA and is indicated by * p<0.05 or ** p<0.01

OPC: Anti-SEMA4D promotes migration & differentiation of OPC that can repair and remyelinate brain lesions



Anti-SEMA4D rescues oligodendrocyte precursor cells (OPC) from SEMA4D-induced cytoskeletal collapse, apoptosis and inhibition of differentiation. Oligodendrocyte precursor cells (OPCs) were established from dissociated spinal cord or cortices of P2 rat pups. OPCs were seeded for a collapse assay and subsequently treated with A) control protein (C35 (Evans et al., 2006); B) 50 µg/ml recombinant SEMA4D alone, C) 50 µg/ml SEMA4D + 500 µg/ml control IgG (MAb 2955; or D) 50 µg/ml SEMA4D + 500 µg/ml anti-SEMA4D antibody (VX15/2503). Cells were fixed with 4% PFA and cytoskeletal changes were visualized by Phalloidin-A488 staining. For the differentiation assay, OPCs were plated onto wells coated with poly-L-lysine (PLL; 10 µg/ml) + BSA (50 µg/ml), PLL (10 µg/ml) + SEMA4D (50 µg/ml), PLL (10 µg/ml) + SEMA4D (50 µg/ml) + control IgG (MAb 2955; 250 µg/ml), or PLL (10 µg/ml)+ SEMA4D (50 mg/ml)+ anti-SEMA4D (VX15/2503; 250 µg/ml). E) Cells were cultured for 4 days and then fixed with 4% PFA and processed for immunohistochemistry to assess extent of apoptosis via enumeration of activated caspase 3-positive cells. F) Companion cultures were established under an identical set of treatment conditions for 8 days, at which time cells were fixed with 4% PFA and processed for immunohistochemistry to assess number of cells expressing the mature oligodendrocyte marker, myelin basic protein (MBP). Error bars represent standard deviation. Statistical significance was determined by ANOVA with Bonferroni's Multiple Comparison Test where "**"=p < 0.05 and "****"=p < 0.001.

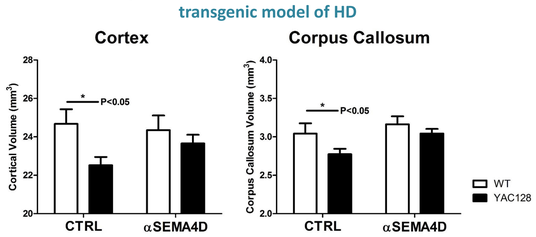
Proposed Mechanism of Action



Smith, et.al., SEMA4D compromises blood–brain barrier, activates microglia, and inhibits remyelination in neurodegenerative disease. Neurobiology of Disease, 73:254–268 (2015)

Huntington's Disease (HD)

Antibody blockade preserves brain volume in YAC128 transgenic model of HD

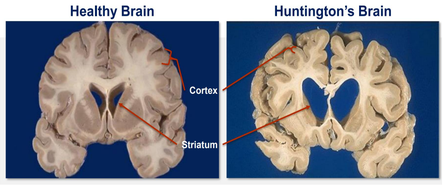


Anti-SEMA4D preserves brain grey and white matter in YAC128 Huntington's Disease mice. Free-floating brain tissue sections from 12 month-old MAb-treated YAC128 and wild type (WT) mice (n=13-21/group) were stained with anti-NeuN antibody. Cortical and corpus callosum volumes were determined by tracing 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).

HD is an autosomal dominant neurodegenerative disease caused by mutation in a single gene

- Neuronal degeneration and severe atrophy is observed in multiple brain regions
- Symptoms usually appear between the ages of 30 to 50



Estimated patient population ~30,000 manifest disease and 100,000 pre-manifest with inherited mutation (prodromal) in the U.S. Similar number in EU 5

There are currently no approved treatments to alter the course of HD

→ **Reconstitution of HD transgenic mice with normal human astrocytes ameliorates disease.** (*Benraiss et al. 2016 Nature Communications*)
→ **Glial precursor cells derived from HD patients exhibit deficiencies in oligodendrocyte and astrocyte functions** (*Osipovitch et al. 2019 Cell Stem Cell*)

SIGNAL Clinical Trial Design for HD

Total n = 301 subjects

36 subjects (15 EM, 21 LP) randomized 1:1 Drug:Placebo

Cohort A July 2015 → All subjects crossover or continue with VX15 (pepinemab) **6 months** → Last treatment **12 months** → 3 month safety follow-up → Database lock and analysis for Cohort A

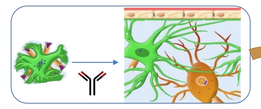
Adapt Cohort B design: sample size and treatment duration based on Cohort A data

265 subjects (179 EM, 86 LP) randomized 1:1 Drug:Placebo

Cohort B ENROLLMENT COMPLETE December 2018 → Treatment with VX15 (pepinemab) **18-36 months** → 1 month safety follow-up → Database lock and analysis for Cohort B **2020**

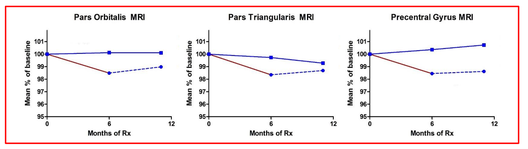
A Phase 2, multi-center, randomized, double-blinded, placebo-controlled clinical trial in subjects with early manifest (EM) and late prodromal (LP) Huntington disease (HD) to assess safety, tolerability, pharmacokinetics, and efficacy of VX15/2503 (*ClinicalTrials.gov Identifier: NCT02481674*).
Program granted Orphan Drug and Fast Track Designation by FDA, Division of Neurology Products

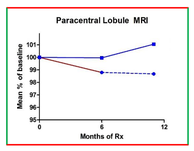
Hypothesis: blocking F-actin depolymerization may reduce inflammatory transformation and increase glucose uptake

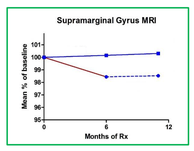


Cohort A SIGNAL MRI: Anti-SEMA4D

Trend to Preservation of Brain Volume







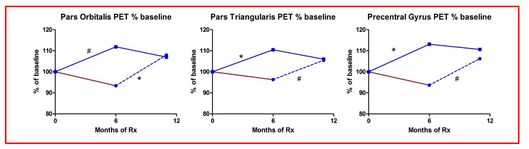
MRI:
- VV, n=17
- PV, n=19

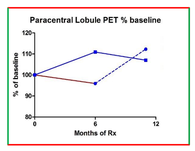
Frontal Lobe
Parietal Lobe

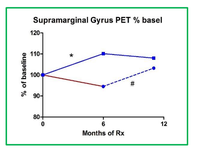
PET:
- VV, n=10
- PV, n=9

Cohort A SIGNAL FDG-PET: Anti-SEMA4D

Significantly Preserves/Restores Metabolic Activity



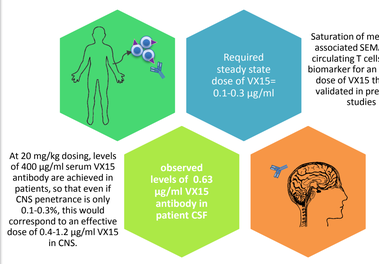




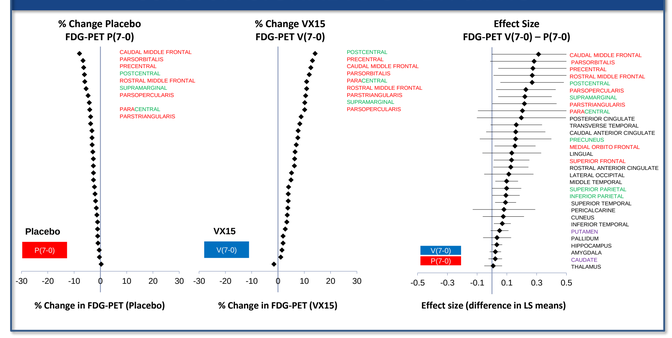
Placebo
VX15, after cross-over
VX15

* p<0.05
\# p<0.1
Statistical significance compared to Placebo

VX15 Antibody Penetration into the CNS



Cohort A SIGNAL FDG-PET: all ROI



Highlights of Analysis – Cohort A

- VX15 (pepinemab) treatment significantly increases metabolic activity as detected by FDG-PET:
  - FDG-PET analysis favored pepinemab in all 31 ROI, achieving significance (p<0.05) in a majority of frontal and parietal brain ROI.
  - 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 for monitoring clinically-relevant change over time (*Landau et. al., Neurobiol Aging. 2011; 32(7): 1207–1218*).
- Encouraging trends of treatment effects on preservation of brain matter (reduced atrophy) and improvement in multiple motor and cognitive assessments were also seen in Cohort A
  - Pepinemab treatment trended toward stabilization of disease-related reduction in MRI volume and was favored over placebo in 24/31 ROI.
- **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 could suggest a partial restoration of glial function and / or restoration of disrupted neural networks.**
- No concerning safety signals were identified
  - Monthly i.v infusions were well tolerated, no subjects discontinued, no concerning safety signals identified.
- Cohort A provide direction for power calculations of required group size in Cohort B.
  - Increased the number of patients in Cohort B:
    - ✓ Total of 179 Early Manifest patients to be randomized 1:1 VX15 to placebo for 18 months of treatment.
    - ✓ Total of 86 Late Prodromal patients to be randomized 1:1 VX15 to placebo for 18 months of treatment.
  - Increased the duration of treatment in Cohort B:
    - ✓ A subset of 42 ate Prodromal subjects currently enrolled will continue randomized treatment for an extended 36 months to obtain long-term safety data and greater insight into potential clinical changes over a longer treatment period.