VX15 Anti-Semaphorin 4D Antibody (pepinemab) Increases FDG-PET Signal and is a Potential Treatment for Alzheimer's Disease

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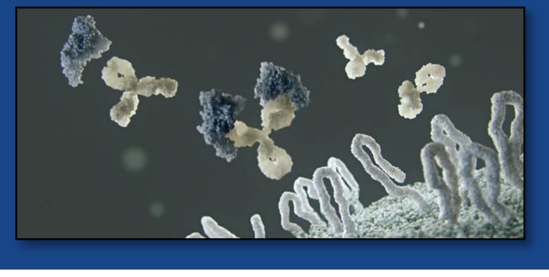
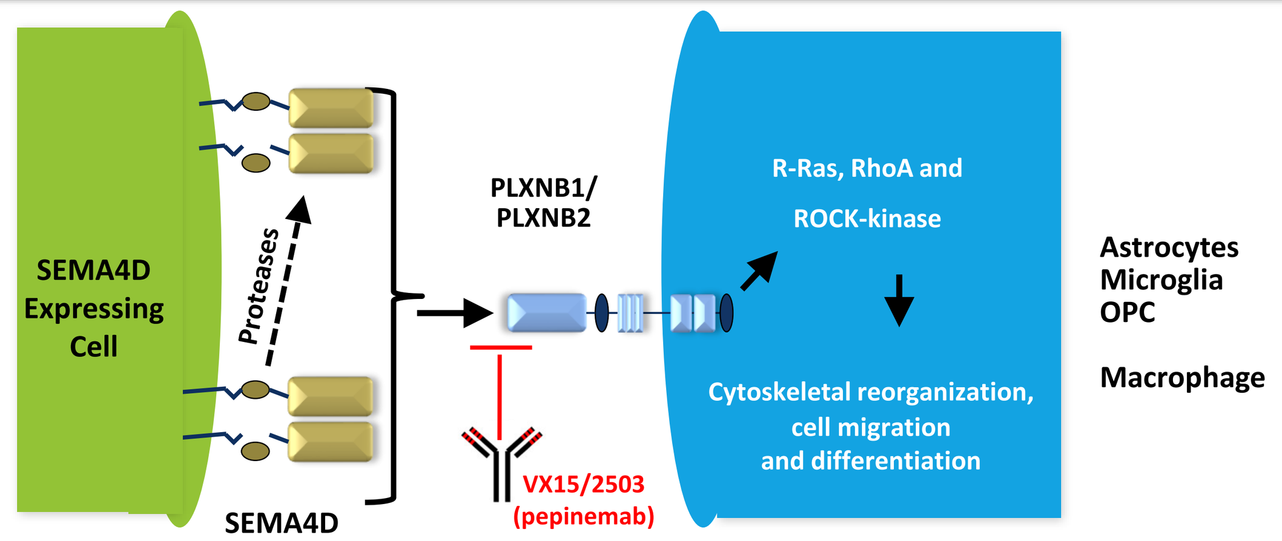
Poster # 30032

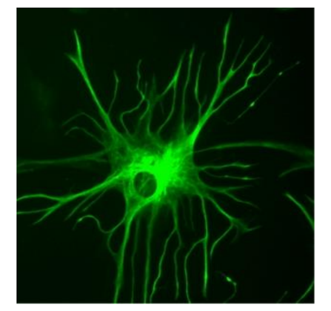


Biology of SEMA4D: Glial Cell Regulation



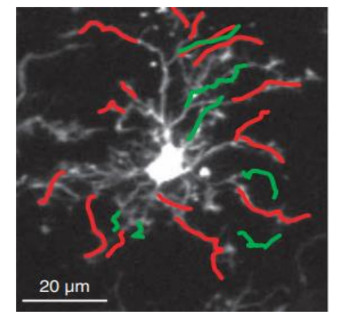
- Semaphorin 4D (SEMA4D) is a guidance molecule that regulates the activation of glial cells that support neuronal function and shape neural networks. Glial cells also contribute to disease pathology through chronic inflammation and demyelination.
- SEMA4D signals through Plexin-B1 and/or Plexin-B2 receptors connected to molecular switches, RhoA and R-Ras, that regulate cytoskeletal organization and cell adhesion.
- 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 replenish glia and repair myelin.

Astrocytes



A single astrocyte can make >200K contacts with capillaries, neurons and other astrocytes.

Microglia



Extensions (green) and retractions (red) of microglial processes over the time course of 20 min have an average velocity of 1.5 µm/minute. Nimmerjahn *Science* 308:1314 (2005)

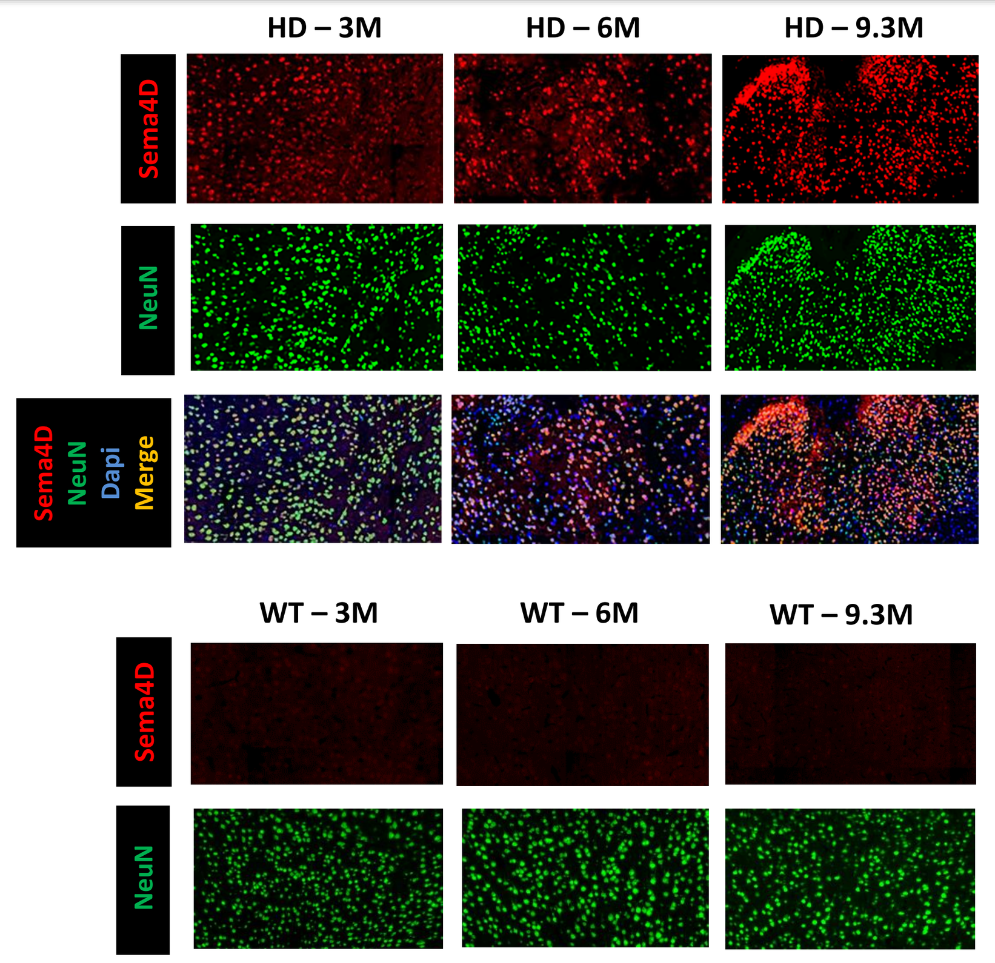
Glial cells are the most abundant cells in the brain cortex

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 and AD
3. **How do glial cells recognize and respond to damage?** 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.

→ **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*)

SEMA4D is upregulated in Neu+ neurons in disease



- **SEMA4D expression is upregulated in HD mice as disease progresses, compared to low expression in WT control.**
- **SEMA4D is upregulated early in disease, prior to onset of symptoms, which occurs app. 5 months of age in Q175 mice**
- **SEMA4D co-localizes with NeuN+ neurons**

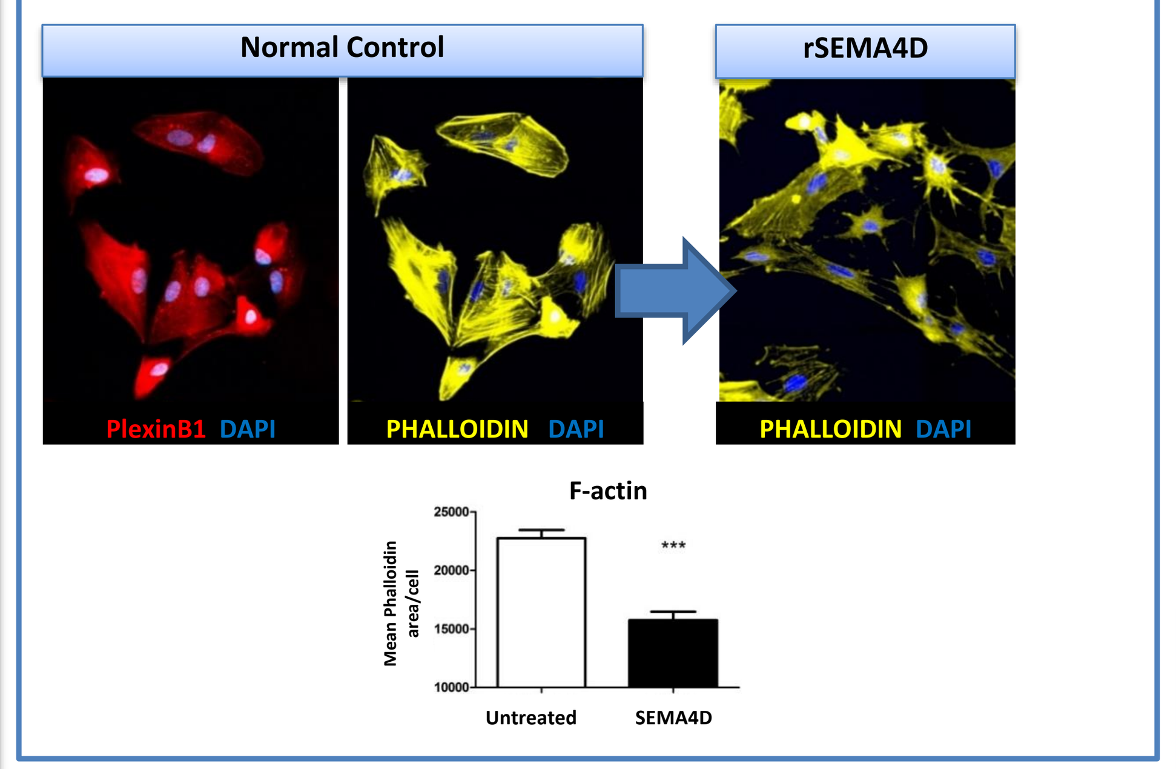
NeuN/Sema staining of cortical neurons in Q175 knock-in mouse model of HD and age-matched wild type (WT) littermate controls. Representative images, obtained from mouse brain coronal sections (~5µM) at 20X magnification, are shown from analysis of 3 mice/time-point. M = months of age.

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

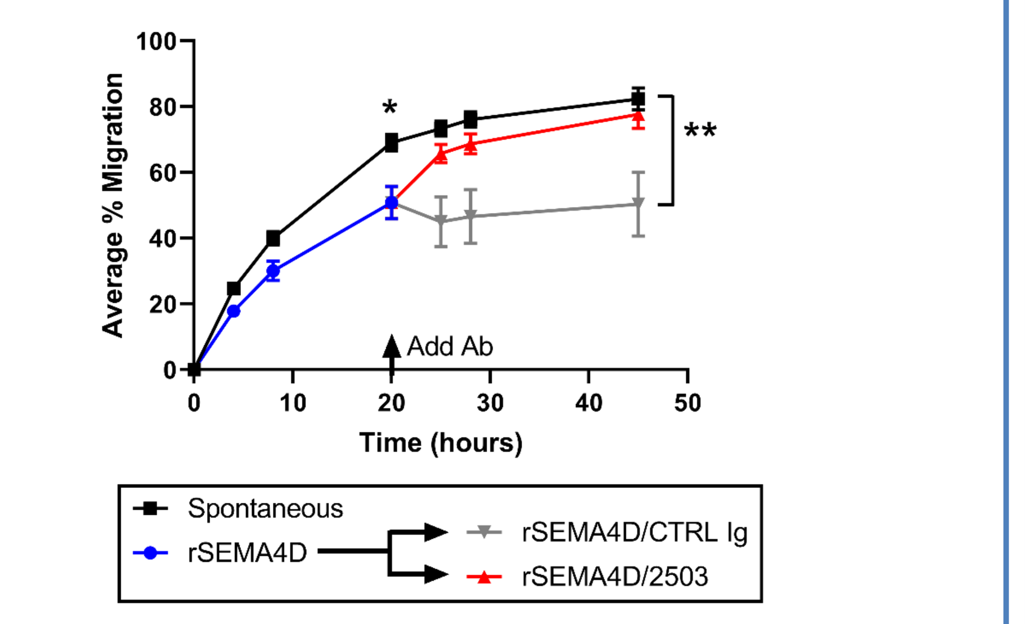
In CNS disease, astrocyte activation results in process retraction, decreased trophic support, and dysregulated glutamate uptake giving rise to excitotoxic neuronal cell death.

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

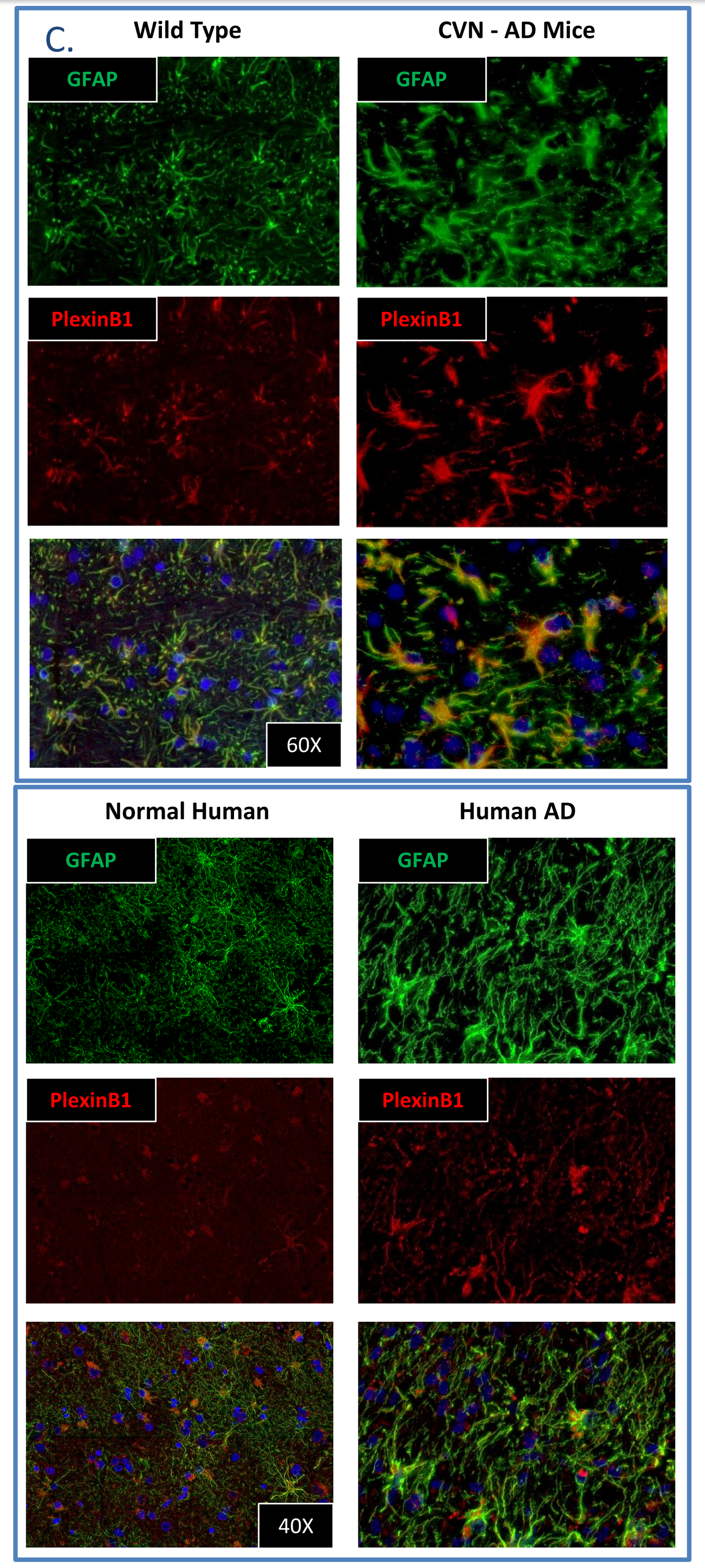
A. SEMA4D INDUCES DEPOLYMERIZATION OF CYTOSKELETON



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

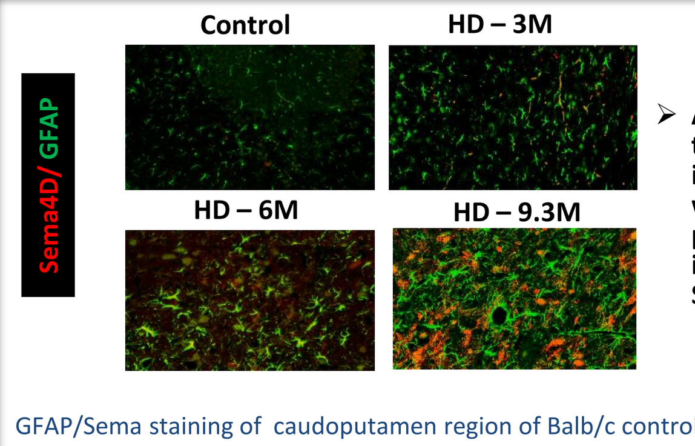


C.



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 addition of 10^5 purified astrocytes / well and culture for the indicated time in the presence or absence of recombinant SEMA4D (15 ug/ml), added at time 0. Anti-SEMA4D antibody ("2503", 50 ug/ml) or isotype control 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, ** p<0·01,*** p<0·005. **C.** Striatum of 12 month old YAC128 and WT mice, dentate gyrus of CVN and wild-type (WT) control mice (41 weeks of age), as well as occipital lobe of normal healthy and AD patient were stained for GFAP and PlexinB1.

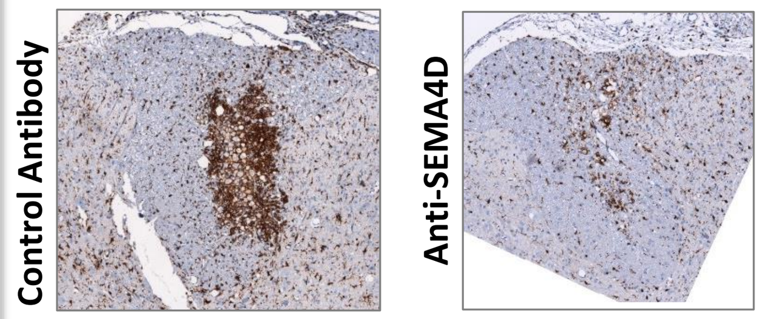
SEMA4D+ cells are in close proximity to PLXNB1+ astrocytes



- **Astrocytes transform to activated inflammatory state with disease progression and are in close proximity to SEMA4D+ cells**

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.

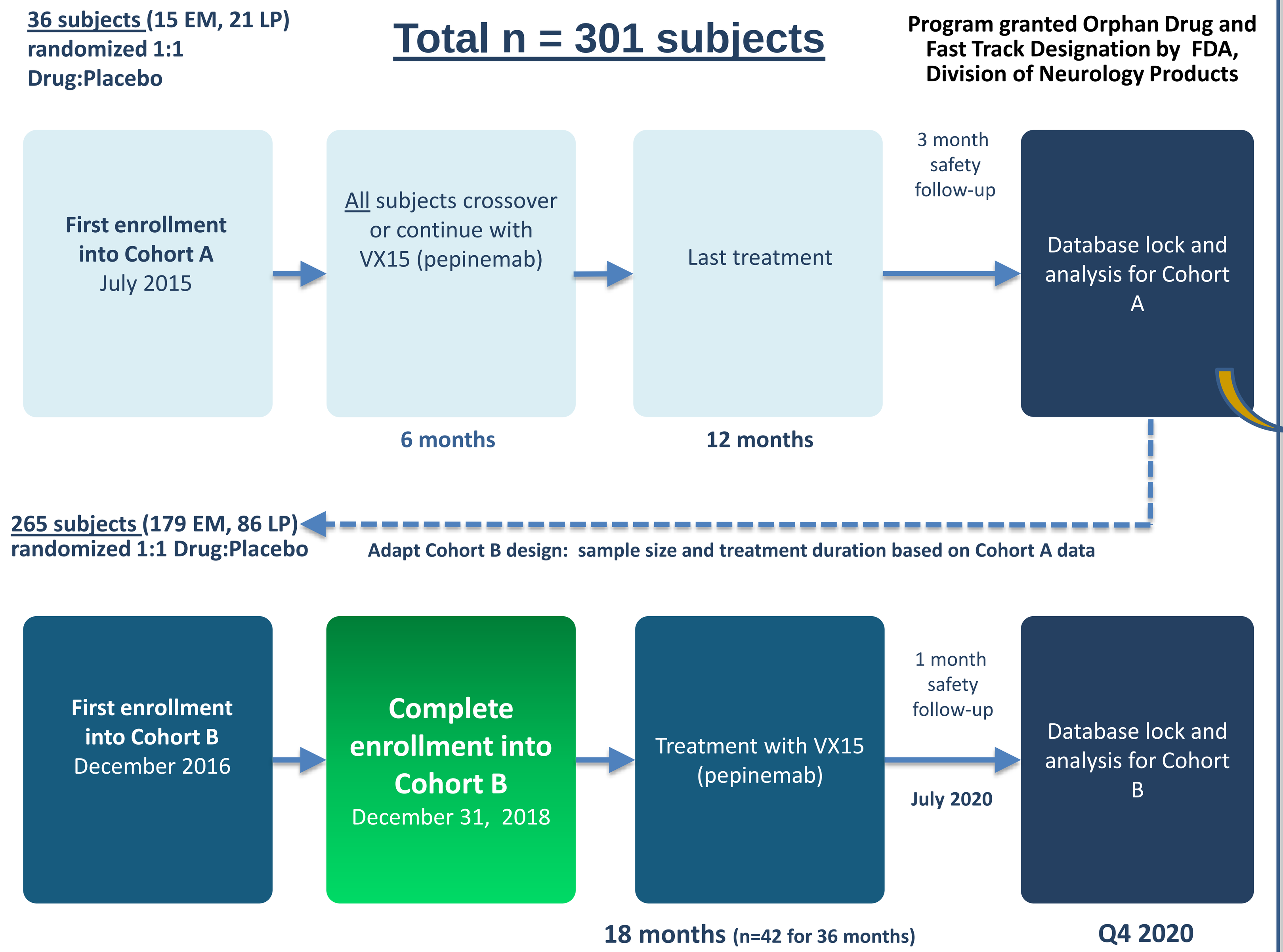
MICROGLIA: Antibody blockade inhibits SEMA4D-induced activation of microglia



Anti-SEMA4D antibody treatment inhibits activation of microglia in area of lysolecithin-induced demyelinated lesion in rat spinal cord (staining for Iba1 marker of activation)

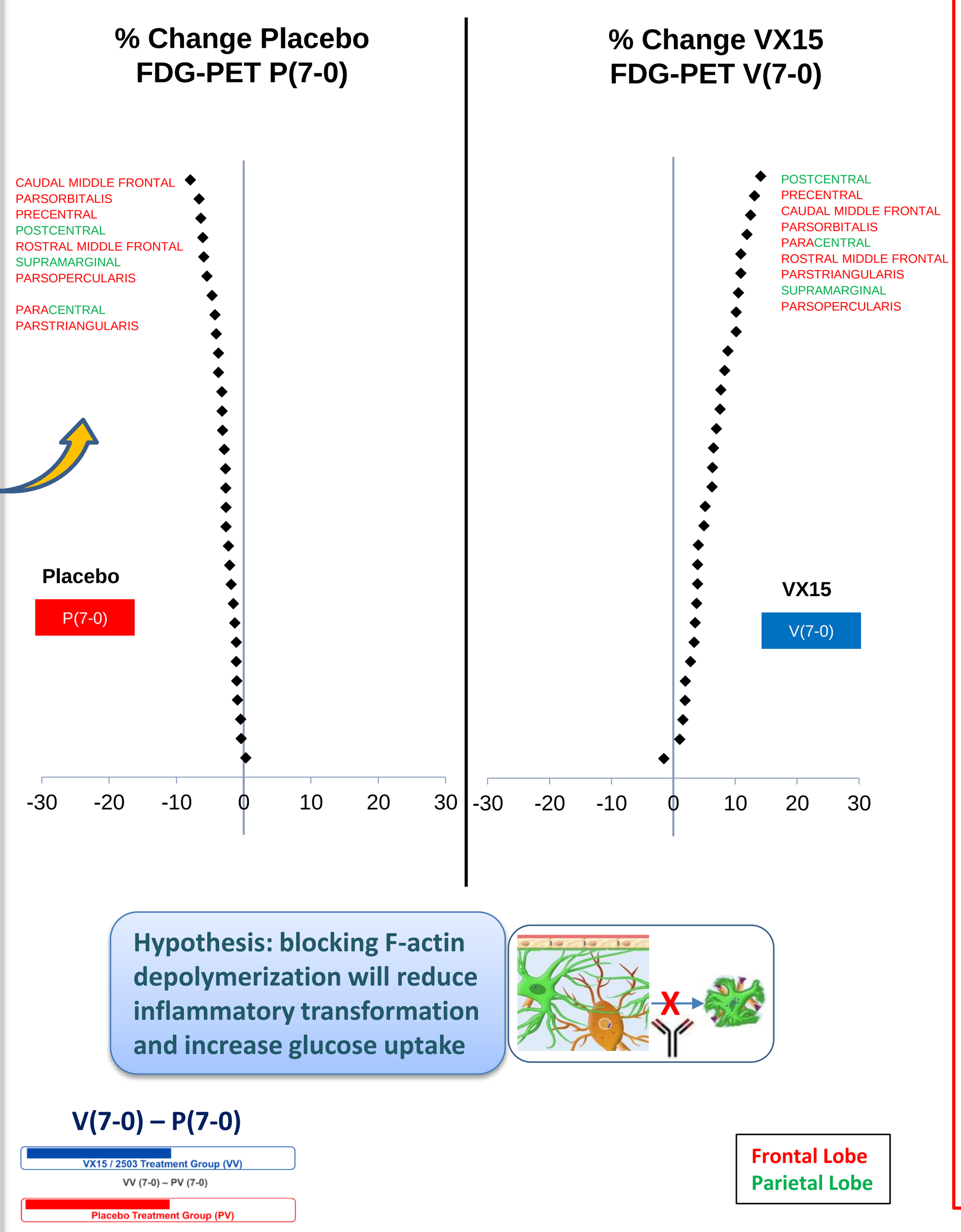
Smith et. al., Neurobiology of Disease, 73:254-268, 2015.

SIGNAL Clinical Trial Design

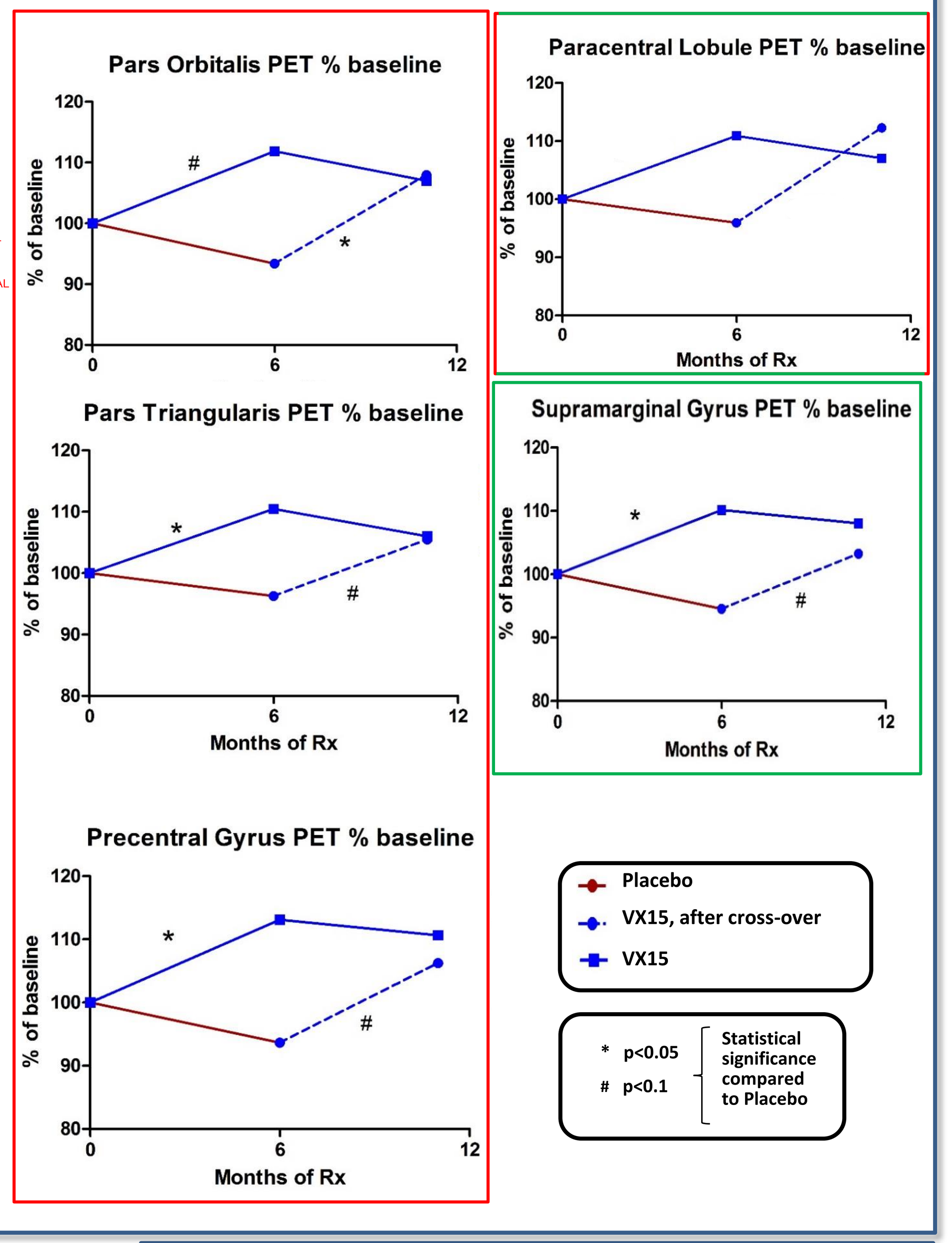

36 subjects (15 EM, 21 LP) randomized 1:1 Drug:Placebo
Total n = 301 subjects
Program granted Orphan Drug and Fast Track Designation by FDA, Division of Neurology Products
First enrollment into Cohort A July 2015
All subjects crossover or continue with VX15 (pepinemab)
Last treatment
3 month safety follow-up
Database lock and analysis for Cohort A
6 months
12 months
265 subjects (179 EM, 86 LP) randomized 1:1 Drug:Placebo
Adapt Cohort B design: sample size and treatment duration based on Cohort A data
First enrollment into Cohort B December 2016
Complete enrollment into Cohort B December 31, 2018
Treatment with VX15 (pepinemab)
1 month safety follow-up
July 2020
Database lock and analysis for Cohort B
18 months (n=42 for 36 months)
Q4 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*).

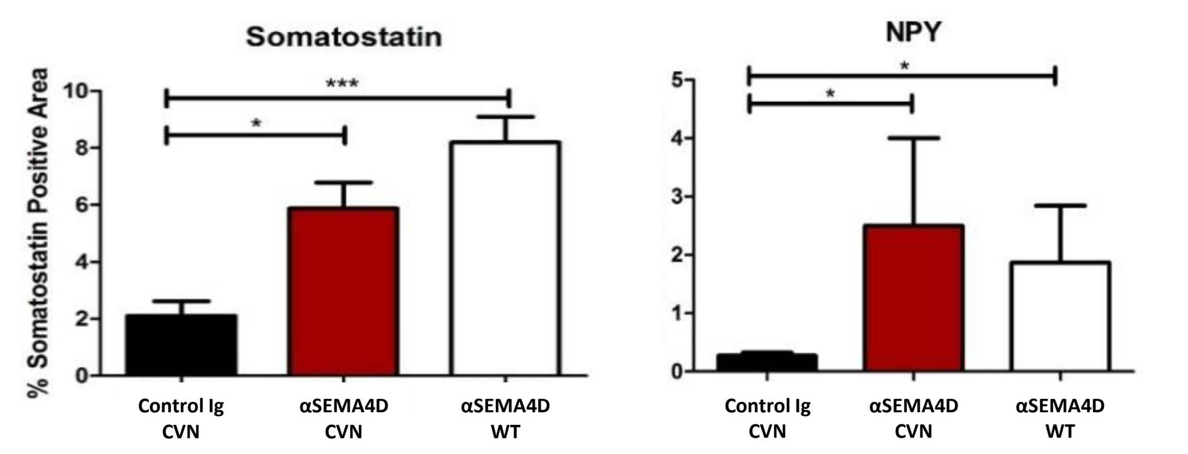
Cohort A SIGNAL FDG-PET: Anti-SEMA4D Significantly Preserves/Restores Metabolic Activity


% Change Placebo FDG-PET P(7-0)
% Change VX15 FDG-PET V(7-0)
CAUDAL MIDDLE FRONTAL
PARSORBITALIS
PRECENTRAL
POSTCENTRAL
ROSTRAL MIDDLE FRONTAL
SUPRAMARGINAL
PARSOPERCULARIS
PARACENTRAL
PARSTRIANGULARIS
Placebo
P(7-0)
VX15
V(7-0)
-30 -20 -10 0 10 20 30
V(7-0) – P(7-0)
VX15 / 2503 Treatment Group (VV)
VV (7-0) – PV (7-0)
Placebo Treatment Group (PV)
Frontal Lobe
Parietal Lobe

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

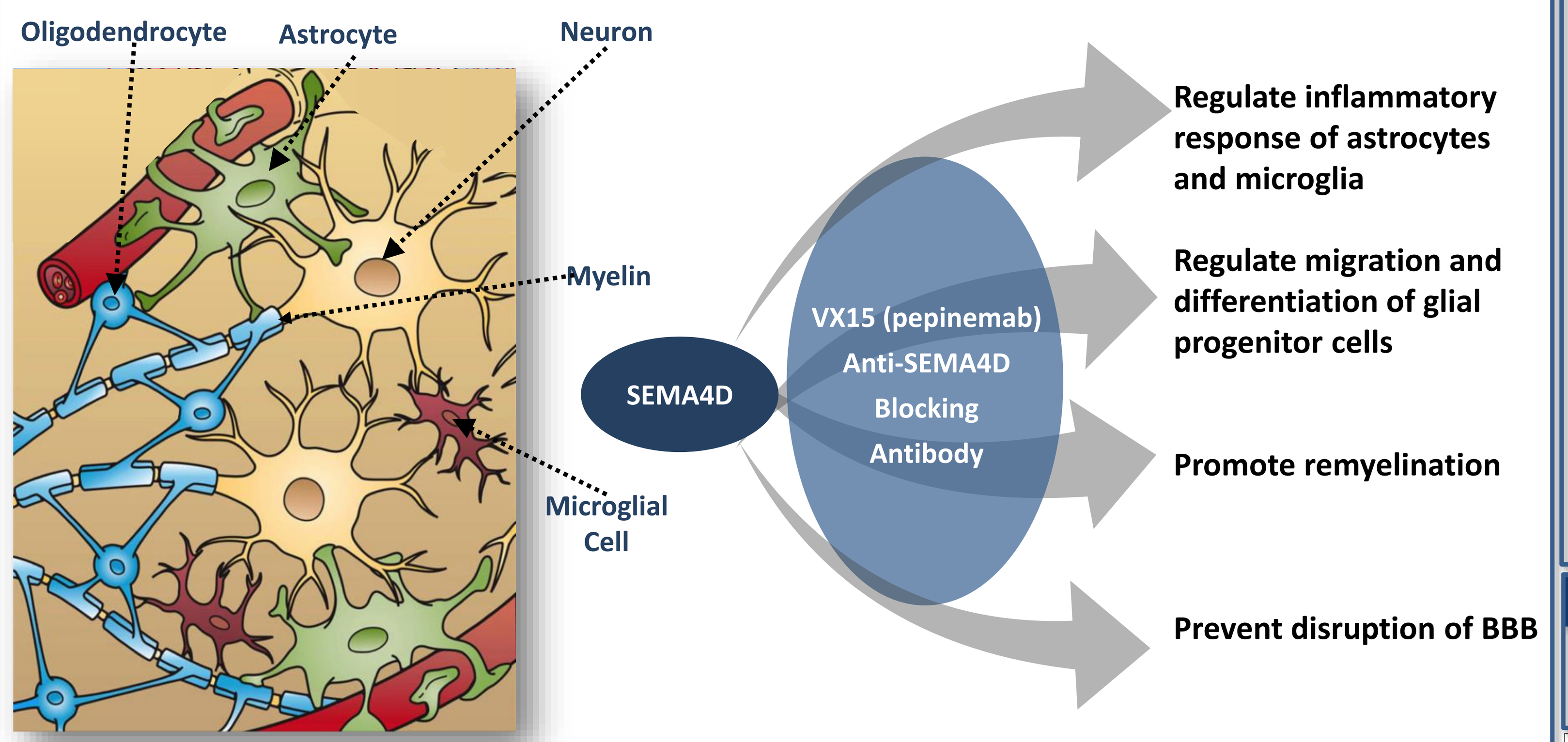

Pars Orbitalis PET % baseline
Pars Triangularis PET % baseline
Precentral Gyrus PET % baseline
Paracentral Lobule PET % baseline
Supramarginal Gyrus PET % baseline
% of baseline
Months of Rx
Placebo
VX15, after cross-over
VX15
* p<0.05
# p<0.1
Statistical significance compared to Placebo

Anti-SEMA4D protects against loss of inhibitory neurons



In CVN Murine Model of AD, blocking SEMA4D protects against loss of inhibitory neurons. (left). FFPE brain tissue sections from CVN and WT mice (n=9-13/group; treated 13 weekly injections at 30 mg/kg with Control Ig or anti-SEMA4D/MAb 67-2) were stained with anti-somatostatin antibody or anti-Neuropeptide-Y (NPY) to identify specific subsets of neurons that begin to degenerate during early AD pathogenesis. No effects on excitatory synapses were observed in diseased mice (as determined by Synaptophysin and VGLUT-1 staining, not shown). Percentages were quantified for all animals and normalized to total area scanned. Error bars indicate standard error. "*"=p<0.05 and "***"=p<0.005 by 1-way ANOVA with Bonferroni's Multiple Comparison Test.

Proposed MOA


Oligodendrocyte
Astrocyte
Neuron
Myelin
Microglial Cell
SEMA4D
VX15 (pepinemab) Anti-SEMA4D Blocking Antibody
Regulate inflammatory response of astrocytes and microglia
Regulate migration and differentiation of glial progenitor cells
Promote remyelination
Prevent disruption of BBB

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

Conclusions

- Based on data from SIGNAL Cohort A, pepinemab treatment resulted in an increase in FDG-PET signal relative to the decrease observed in placebo group.
  - FDG-PET analysis favored pepinemab in all 31 ROI, achieving significance (p<0.05) in a majority of frontal and parietal brain 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.
- Pepinemab has been well-tolerated in SIGNAL, and previously in a Phase 1 MS trial, suggesting there are no safety concerns in subjects with neurodegenerative disease.
- Clinical investigation of pepinemab in AD is warranted based on preclinical MOA data, clinical safety data, as well as SIGNAL FDG-PET data that suggests increased metabolic activity and glial health after treatment.

Acknowledgments

Vaccinex is very appreciative of the subjects who agreed to participate in SIGNAL in order to help investigate VX15/2503 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 SIGNAL.