Development of an anti-SEMA4D monoclonal antibody for the treatment of Multiple Sclerosis

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Abstract

Semaphorin 4D (SEMA4D/CD100) is expressed on most immune cells, and its high affinity receptor, Plexin B1 (PLXNB1), is expressed on dendritic, endothelial, and neuronal cells. SEMA4D signaling through PLXNB1 induces growth cone collapse of neurons, inhibits differentiation of oligodendrocyte precursor cells (OPCs), induces oligodendrocyte (OD) process collapse and apoptosis, and disrupts CNS endothelial tight junctions. SEMA4D also plays an important role in glial cell activation and may influence induction of B and T cell responses. Antibody neutralization of SEMA4D could therefore reduce the severity of multiple sclerosis through several means. First, blocking SEMA4D could reduce the rate of disease relapse by reducing inflammation and secondary immune responses to CNS antigens. Second, to the extent that SEMA4D mediates apoptosis and inhibits maturation of OPCs, blocking SEMA4D could reduce the loss of ODs and promote remyelination. Third, SEMA4D may play a role in breakdown of the blood brain barrier (BBB), and blocking SEMA4D may reduce immune cell infiltration into the CNS.

We have demonstrated in several preclinical models the effects of SEMA4D in the central nervous system. Inhibitory effects on OD differentiation and myelination have been shown using recombinant SEMA4D, and anti-SEMA4D antibody has been shown to protect the integrity of the BBB both in vitro and in vivo, and to promote neural regeneration. Treatment with anti-SEMA4D MAbs also attenuates the disease severity in several rodent EAE models, including chronic EAE characterized by a combination of axonal and myelin loss and continuing inflammation.

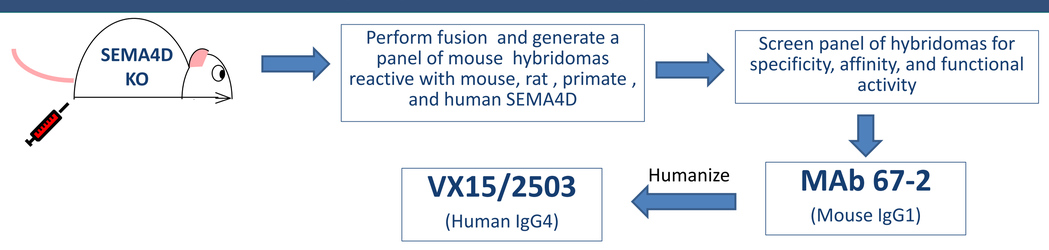
Antibody neutralization of SEMA4D represents a new therapeutic strategy for multiple sclerosis. We selected a humanized IgG4 antibody that blocks both cellular and soluble SEMA4D interactions with PLXNB1 in mouse, rat, monkey, and humans. SEMA4D signaling through PLXNB1 has also been implicated in tumor growth and angiogenesis. Using various tumor models we demonstrated that anti-SEMA4D antibody inhibits these processes. The dose escalation phase of a Phase I clinical trial in patients with advanced solid tumors has been completed; Infusions of VX15/2503 were well tolerated, with a maximum administered dose of 20 mg/kg. A randomized, placebo-controlled, single ascending dose Phase 1 study in MS patients began in 2012 and subjects are now being enrolled in the third dose cohort.

Introduction

- SEMA4D has a high affinity (1 nM) PLXNB1 Receptor and a low affinity (300 nM) CD72 Receptor
- SEMA4D is expressed abundantly on the surface of resting T cells and less strongly on B cells and APCs, and is upregulated and secreted as a biologically active soluble form upon cell activation
- SEMA4D signaling through CD72
  - B cell aggregation and differentiation
- SEMA4D signaling through Plexin-B1
  - activates endothelial cells
  - activates neuroinflammatory microglial cells
  - induces apoptosis of oligodendrocyte precursor cells
  - induces oligodendrocyte process collapse
  - inhibits neurite extension and axonal regeneration
- SEMA4D$^{-/-}$ mice do not develop EAE in response to immunization with myelin peptide

- Therapeutic Rationale: Neutralization of SEMA4D using a monoclonal antibody could inhibit Multiple Sclerosis progression by several mechanisms
  - reduce relapse rate by reducing neuroinflammation and secondary immune responses to CNS antigens
  - reduce disease severity by blocking inhibition of remyelination and axonal regeneration
  - maintain the integrity of the BBB, thereby reducing T cell infiltration into the CNS

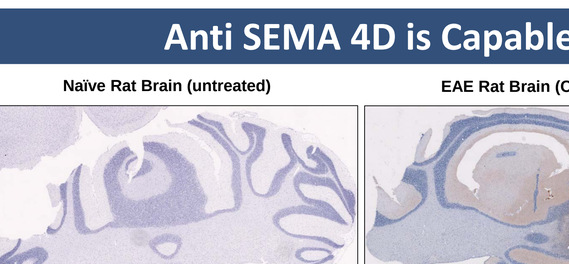
Generation of Anti-mouse SEMA4D MAbs



- Epitope mapping shows that the MAb 67-2 and VX15/2503 share the same epitope.
- Affinity of MAb 67-2 and VX15/2503 is approximately 5 nM as measured by Biacore on recombinant SEMA4D and 0.5 nM on native cell-bound SEMA4D by flow cytometry. It is possible that conformational changes in the recombinant protein account for the difference.

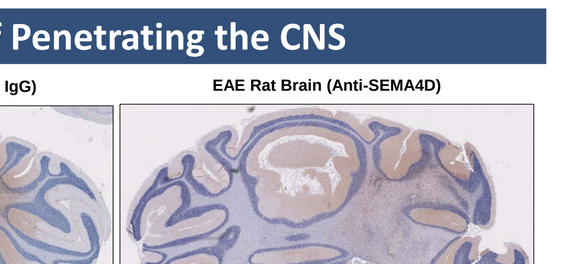
Anti-Sema4D Reduces EAE Clinical Scores

SJL Relapsing-Remitting EAE



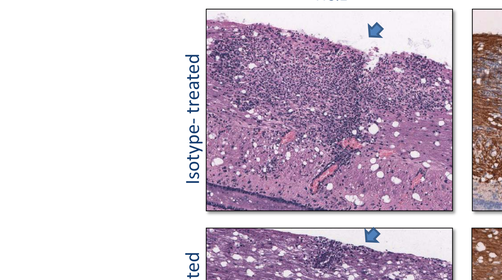
Objective
To determine if MAb 67-2 is effective in a relapsing - remitting SJL EAE model
Experimental Design
EAE was induced in SJL mice with $PLP_{139-151}$ in CFA. Starting on day 7, mice were injected 1x per week with 600 ug (~30 mg/kg) antibody, n=18/group
Results
A 50% reduction in clinical score was observed for the MAb 67-2 treated group.
Similar results observed in two additional studies with MAb 67-2 as well as with another independent anti-SEMA4D antibody and in two other labs.

SJL Adoptive Transfer EAE



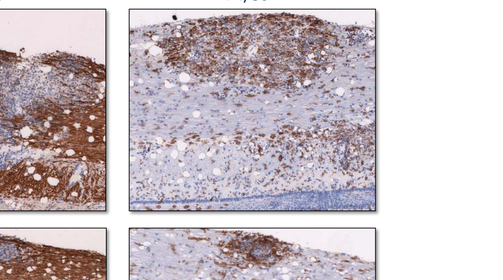
Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in the Th1 adoptive transfer EAE model in SJL mice.
Experimental Design
Starting at day 0 post transfer, mice were injected i.p. weekly with either 600 ug control IgG or 600 ug anti-SEMA4D MAb 67 (~30 mg/kg), or 3X/week with 1,000 Units rmuIFN-β. N=12/group
Results
Significant reduction in EAE score ($p<0.05$ using one way ANOVA) was observed for both MAb 67-2 and IFN-beta groups, but not control IgG.

B6 MOG EAE



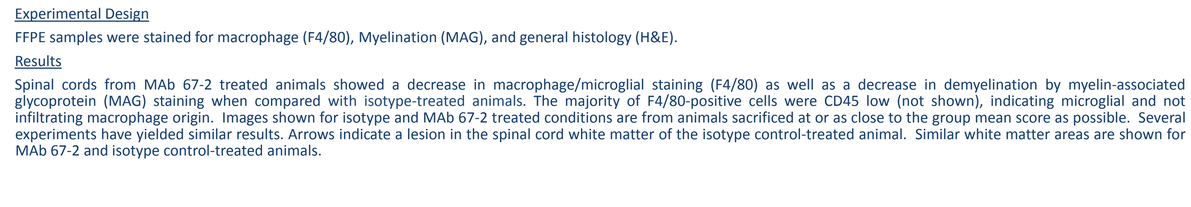
Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in the B6 MOG EAE model.
Experimental Design
EAE was induced in male B6 mice with MOG_{35-55} peptide in CFA with Pertussis Toxin; Mice were dosed beginning on Day 7 2X/week with 600ug/antibody (~30 mg/kg), n=14/group
Results
We observed approximately a 25% reduction in clinical score in the MAb 67-2 treated group (**p<0.01 by one way ANOVA).
Similar data observed with MAb 67-2 dosing 1X/week and every other week

Chronic Rat EAE



Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in Dark Agouti rat chronic EAE model.
Experimental Design
EAE is induced in Dark Agouti rats with MOG_{1-125} in IFA. Starting at day 3, rats were injected i.p. with 15 mg/kg of MAb 67-2 or an irrelevant murine IgG twice weekly up to peak disease, then weekly thereafter.
Results
A significant drop in clinical score was observed for the MAb 67-2 treated group (**p<0.01 by two way ANOVA)

Anti SEMA 4D is Capable of Penetrating the CNS

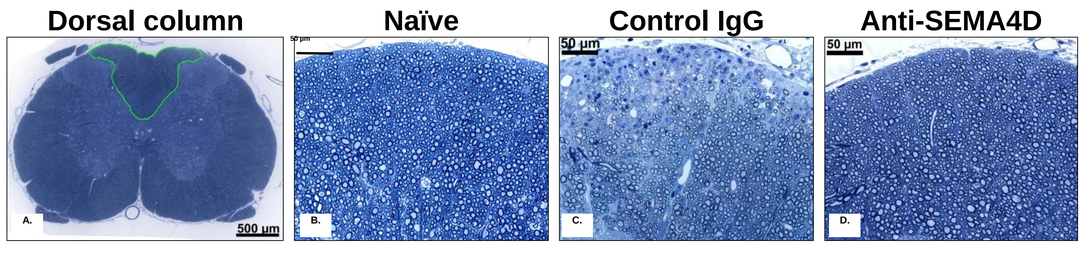


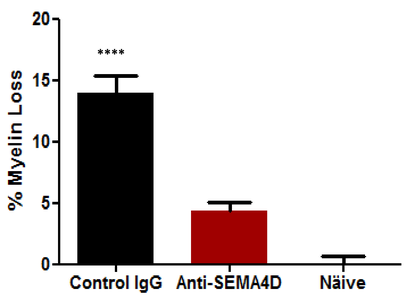
Objective To determine by IHC if MAb 67-2 can be detected in the CNS of EAE rats.
Experimental Design
The presence of drug was detected in FFPE sections using a rat-adsorbed goat anti-mouse antibody. This antibody does not detect rat IgG but readily identifies the presence of mouse IgG.
Results
Large areas of antibody penetration in the CNS were seen for MAb 67-2 and isotype control-treated EAE animals, but not untreated naïve animals.

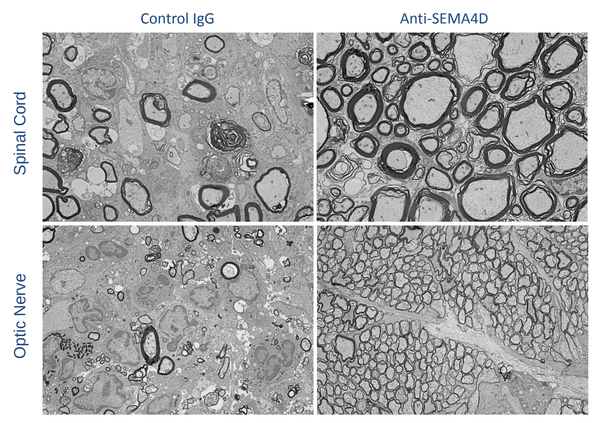
Anti-SEMA4D Protects Against Microglial Activation in EAE

Objective
To observe histochemical markers for myelin and macrophage/microglia in spinal cords of Th1 adoptively transferred SJL EAE mice
Experimental Design
FFPE samples were stained for macrophage (F4/80), Myelination (MAG), and general histology (H&E).
Results
Spinal cords from MAb 67-2 treated animals showed a decrease in macrophage/microglial staining (F4/80) as well as a decrease in demyelination by myelin-associated glycoprotein (MAG) staining when compared with isotype-treated animals. The majority of F4/80-positive cells were CD45 low (not shown), indicating microglial and not infiltrating macrophage origin. Images shown for isotype and MAb 67-2 treated conditions are from animals sacrificed at or as close to the group mean score as possible. Several experiments have yielded similar results. Arrows indicate a lesion in the spinal cord white matter of the isotype control-treated animal. Similar white matter areas are shown for MAb 67-2 and isotype control-treated animals.

Anti-SEMA4D Protects Against Demyelination in EAE

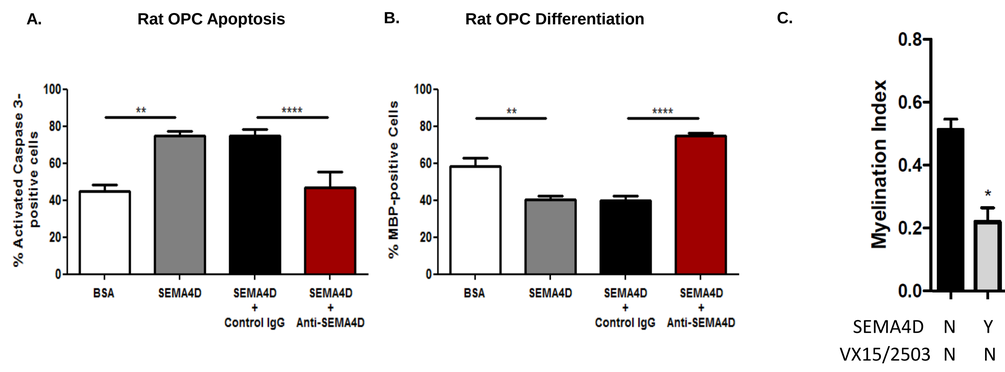






Objective
To evaluate and quantitate myelination in dorsal funicular region of spinal cords of anti-SEMA4D treated EAE rats.
Experimental Design
Glutaradehyde-fixed spinal cords from EAE rats were processed for EM and Toluidine Blue staining. DFR in 100 1µm Toluidine Blue sections were imaged at 60x for each group and were derived from 5 serial sections of 5 descending levels separated by 2mM within the lumbar spinal cord (total of 25 images/rat). Myelination index was determined by quantitating area of Myelin in DFR using algorithms generated in Image Pro software.
Results
Distinct regions of demyelination are apparent in spinal cords of rats treated with control IgG. Myelin is preserved in anti-SEMA4D treated spinal cords. Quantitation of all images reveals that relative to naïve animals loss of myelin is approximately 3-fold higher in IgG-treated animals compared to anti-SEMA4D treated animals. "****" indicates $p<0.0001$.

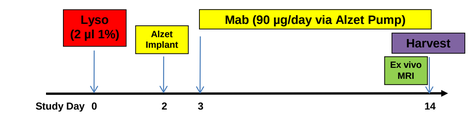
VX15/2503 Blocks SEMA4D-induced OPC Toxicity

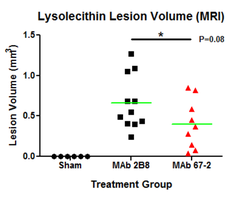


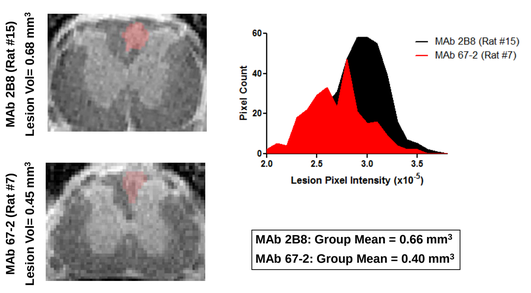
Objective
To determine if VX15/2503 protects rat OPCs from SEMA4D-induced apoptosis (A) and reverses inhibition of differentiation (B)
Experimental Design
Activated caspase 3 staining was performed to detect apoptotic cells and MBP staining was used as a marker of OPC differentiation.
Results
VX15/2503 significantly reduced both Sema4D- induced apoptosis and inhibition of differentiation in rat OPC (**$p<0.05$, Bonferroni's Multiple Comparison Test)

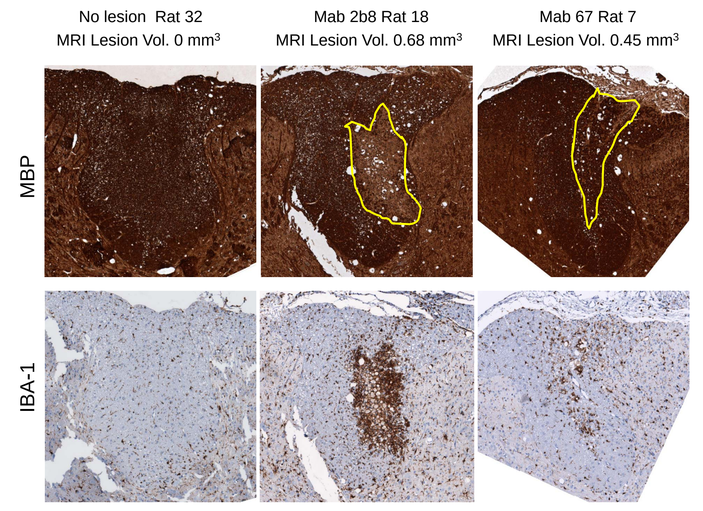
SEMA4D blocks remyelination following lysolecithin-induced injury in an *in vitro* Brain Slice Model (in collaboration with Dr Anna Williams at Edinburgh University)

Anti-SEMA4D Enhances Remyelination in a Lysolecithin-induced Lesion









Objective
To evaluate the effect of intrathecal MAb67-2 or control antibody, MAb2B8, in a spinal lysophosphatidylcholine (LPC) lesion model of focal demyelination in rats.

Experimental Design
Focal demyelination was induced by stereotactic infusion of 2 µL of 1% lysophosphatidylcholine (LPC; lysolecithin) directly into the dorsal column (T9/T10 level) of rat spinal cords. Two days later, an intrathecal catheter filled with either MAb 67-2 (n=9) or MAb 2B8 (n=11) and attached to Alzet minipump was implanted to deliver 90 µg MAb/day for 14 days. Sham control rats (n=6) did not receive LPC, but did receive intrathecal MAb 2B8 for 14 days. Lesion volumes were subsequently determined by MRI using a 3D TrueFISP sequence of 1-h duration on a 7T Bruker BioSpin instrument. The region of the spinal cord containing the lesion was dissected out and processed for further histological examination. Immunohistochemistry was performed on representative animal spinal cord mid-lesion sections for MBP to identify demyelinated areas and Iba1 for microglial activation.

Results
Anti-SEMA4D MAb treatment enhances spinal cord repair in lysolecithin-lesioned rats.

Development of an anti-SEMA4D Humanized MAb

- VX15/2503 is a high-affinity humanized antibody that was derived from the mouse MAb 67-2 antibody. It has been shown to block the functional activity of both the cellular and soluble forms of SEMA4D in a variety of *in vitro* assays.
- A stable CHO-S cell line expressing VX15/2503 was constructed and characterized; a master cell bank was similarly produced and characterized prior to manufacture of antibody for use in non-clinical and clinical studies.

Non-clinical Toxicology Studies

- Subchronic Toxicology
  - Rat and cynomolgus macaques were dosed during 1 month repeat dose GLP toxicology studies up to 100 mg/kg/dose
- Chronic Dose Toxicology
  - A GLP 6-month toxicology study employed 26 weekly iv doses of VX15/2503 at 0; 30; 100; or 200 mg/kg/dose in rats.
  - A similar six month toxicology study in cynomolgus macaques was completed

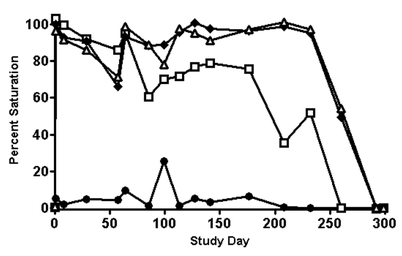
Non-clinical Toxicology Summary

- The no observed adverse effect level (NOAEL) was determined to be the highest dose administered in each completed study
- All animals displayed dose dependent PK in all studies and were exposed to significant drug levels
- Saturation results appear to be dose dependent and remarkably similar between species

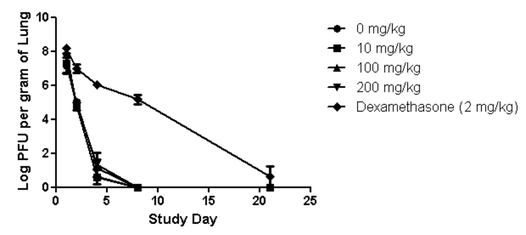
Immunotoxicology

- An influenza host resistance model was performed in rats
- VX15/2503 was dosed at 0; 10; 100; or 200 mg/kg/dose prior to influenza delivery, dexamethasone was used as a positive control
- VX15/2503 did not alter the ability of rats to clear influenza virus at any dose level, dexamethasone significantly delayed viral clearance.

Group Mean SEMA4D Saturation (Rat 6 month Toxicology)



Influenza Clearance in Rats After Vx15/2503 Dose



Clinical

- The dose escalation phase of a Phase I, non randomized, open label, dose-escalation study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of weekly intravenous infusions of VX15/2503 in adult patients with advanced solid tumors has been completed.
- Phase I, multicenter, randomized, double-blind, placebo-controlled, ascending single-dose study of the safety, tolerability, and pharmacokinetics of intravenous VX15/2503 in patients with Multiple Sclerosis began in late 2012. Enrollment is continuing for this trial.