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 the induction of B and T cell responses and glial cell activation. 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.

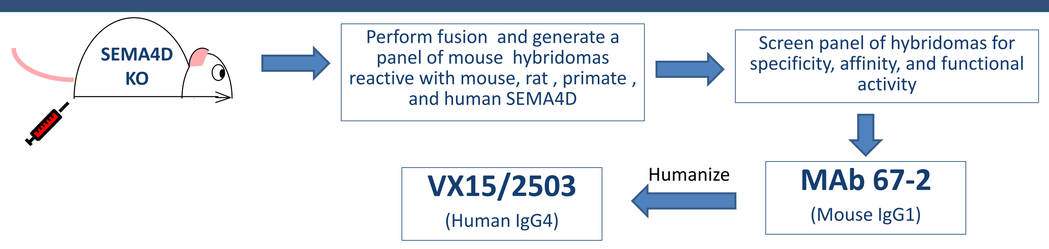
We have demonstrated in several preclinical models the effects of SEMA4D in the central nervous system. Inhibitory effects on OD differentiation and myelination have been shown using recombinant SEMA4D, and anti-SEMA4D antibody has been shown to protect the integrity of the BBB both *in vitro and in vivo,* and to promote neural regeneration. Treatment with anti-SEMA4D MAbs attenuates the severity of EAE in several rodent EAE models.

Antibody neutralization of SEMA4D represents a new therapeutic strategy for multiple sclerosis. We selected a humanized IgG4 antibody that recognizes mouse, rat, monkey and human SEMA4D with high affinity. We utilized several *in vitro* functional assays to demonstrate that this antibody blocks SEMA4D – PLXNB1 interactions. This antibody was characterized in single dose, one month, as well as six month non-clinical IND-enabling toxicology studies. 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. A Phase I clinical trial in patients with advanced solid tumors is ongoing; no dose limiting toxicities have been observed following weekly infusions at dose levels up to 9 mg/kg. A randomized, placebo-controlled, single ascending dose Phase 1 study in MS patients is planned for late 2012.

Introduction

- SEMA4D has a high affinity (1nM) 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
    - activates B lymphocytes and induces dendritic cell maturation for antigen presentation to T lymphocytes
  - 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 by SEMA4D
  - maintain the integrity of the BBB, thereby reducing T cell entry into the CNS

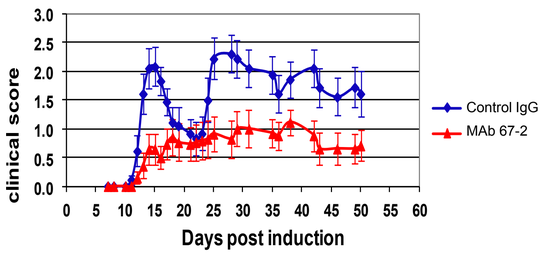
Generation of anti-mouse SEMA4D MAbs



- 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 via 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.

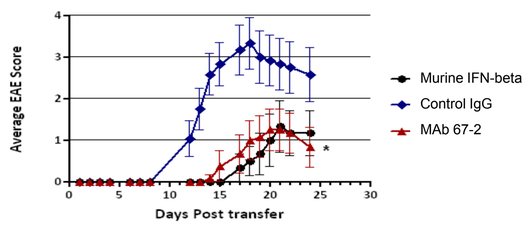
MAb 67-2 Reduces EAE clinical scores

SJL Relapsing-Remitting EAE



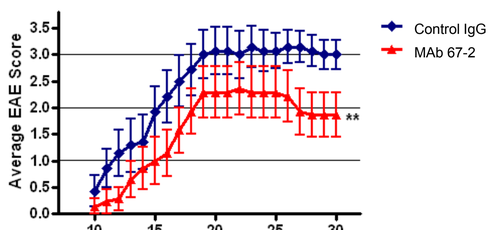
Objective
To determine if MAb 67-2 is effective in a relapsing - remitting SJL EAE model
Experimental Design
EAE was induced in SJL mice with $PLP_{139-151}$ in CFA. Starting on day 7, mice were injected 1x per week with 600 ug (~30 mg/kg) antibody, n=18/group
Results
A 50% reduction in clinical score 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



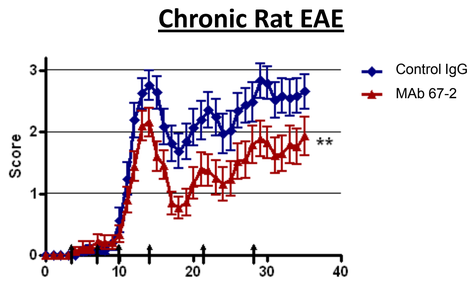
Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in the Th1 adoptive transfer EAE model in SJL mice.
Experimental Design
Starting at day 0 post transfer, mice were injected i.p. weekly with either 600 ug control IgG or 600 ug anti-SEMA4D MAb 67 (~30 mg/kg), or 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



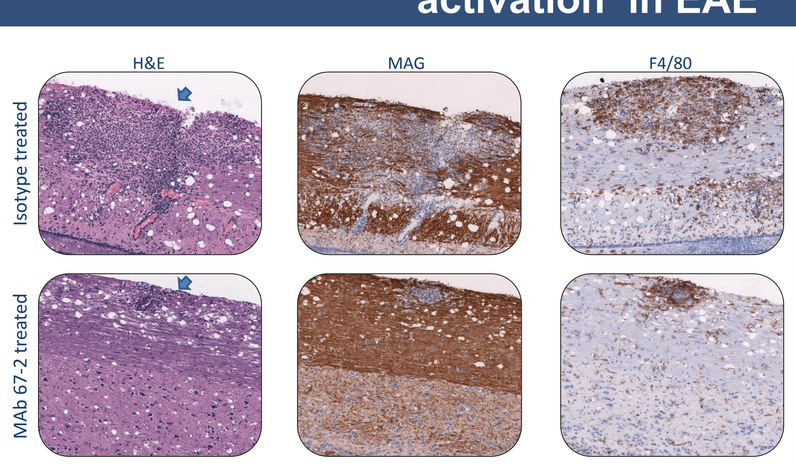
Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in the B6 MOG EAE model.
Experimental Design
EAE was induced in B6 mice with MOG_{35-55} peptide in CFA with Pertussis Toxin; Mice were dosed beginning on Day 7 2X/week with 600ug/antibody (~30 mg/kg), n=14/group
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



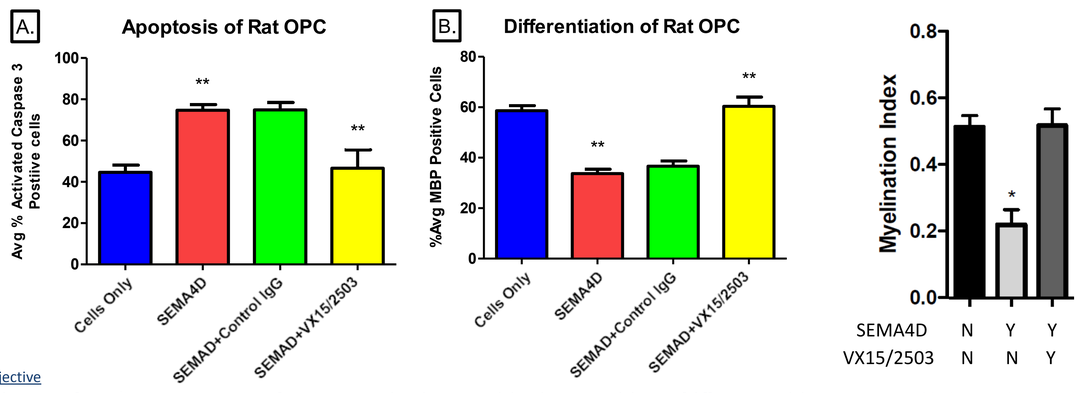
Objective
To test the anti-SEMA4D antibody MAb 67-2 for its ability to reduce EAE scores in Dark Agouti rat chronic EAE model.
Experimental Design
EAE is induced in Dark Agouti rats with MOG_{1-125} in IFA. Starting at day 3, rats were injected i.p. with 15 mg/kg of MAb 67-2 or an irrelevant murine IgG 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)

MAb 67-2 protects against demyelination and microglial activation in EAE



Objective
To observe histochemical markers in Th1 adoptive transfer model in SJL 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 treated animal. Similar white matter areas are shown for MAb 67-2 and isotype treated animals.

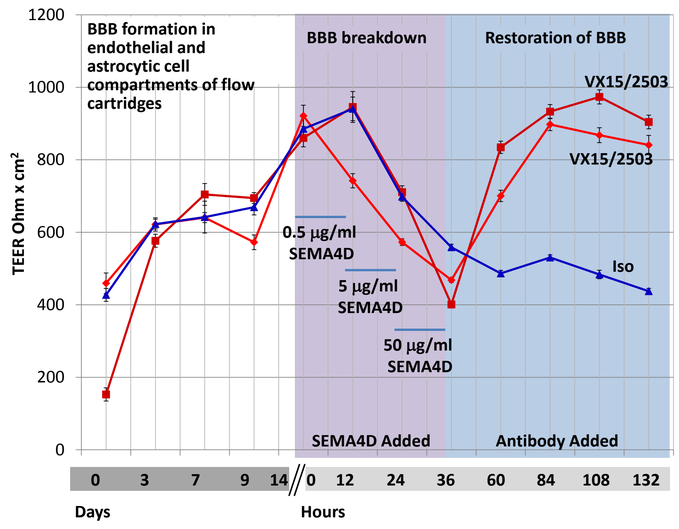
VX15/2503 blocks SEMA4D-induced OPC Toxicity



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 is performed to detect apoptotic cells and MBP is stained 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 Mutiple Comparison Test)

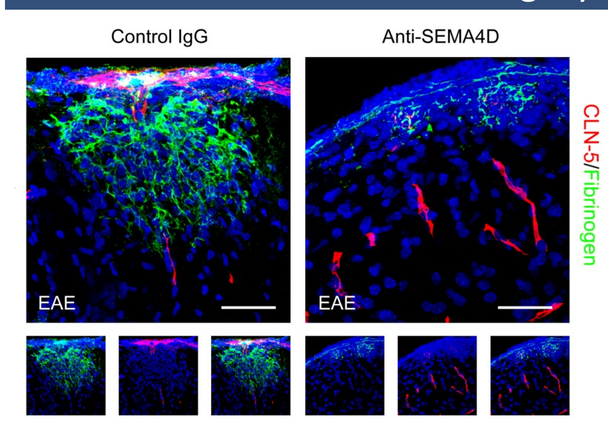
- SEMA4D blocks remyelination following lysolecithin induced injury in an *in vitro* Brain Slice Model (in collaboration with Dr Anna Williams at Edinburgh University)

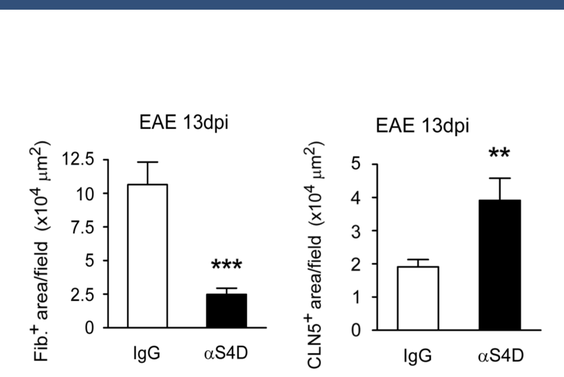
VX15/2503 Restores SEMA4D-induced breakdown of the BBB



Objective
To determine if SEMA4D is capable of disrupting a synthetic blood- brain barrier, and to determine if VX15/2503 is capable of restoring the integrity of the barrier.
Experimental Design
A DIV-BBB (dynamic *in vitro* blood-brain barrier) was allowed to form over two weeks culture of endothelial cells and astrocytes in hollow fiber cartridge. Once stable, increasing concentrations of SEMA4D were added at 12-hour intervals. Transendothelial electrical resistance (TEER) was measured to monitor the integrity of the BBB at each time point. VX15/2503 or isotype control antibody were then added to neutralize SEMA4D and determine effect on BBB.
Results
The addition of SEMA4D resulted in the breakdown of BBB integrity as measured by TEER. The addition of VX15/2503 resulted in the restoration of the BBB in presence of SEMA4D. Isotype control was not able to restore BBB integrity.
(In collaboration with Dr. Damir Janigro, Cleveland Clinic)

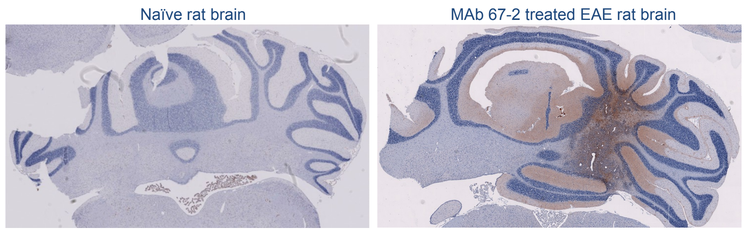
MAb 67-2 Protects Integrity of Blood-Brain Barrier in EAE



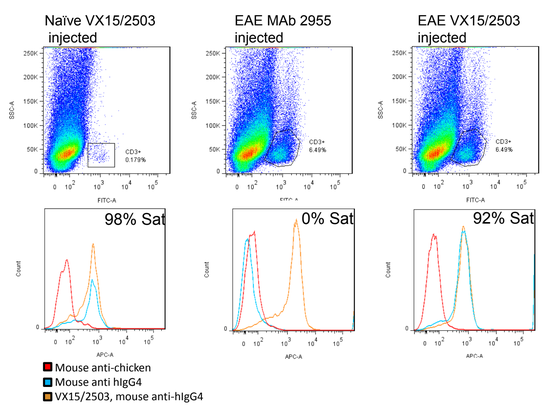


Objective
To determine if MAb 67-2 can protect BBB integrity in EAE
Experimental Design
Mice (12wk SJL/J) were sensitized with PLP 139-151 in CFA, then treated once per week from 7d post induction with 600 µg i.p. anti-SEMA4D antibody (MAb 67-2) or control IgG. Neurological Signs were first observed at 11d postinduction (dpi). At 13 dpi, during the acute phase of disease, 4 representative mice per group were sacrificed and lumbar spinal cord samples prepared for histopathologic analysis. Staining was performed for Fibrinogen and Claudin-5 and imaged using confocal microscopy.
Results
Fibrinogen does not normally penetrate the blood-brain brain barrier (BBB). In EAE, with the BBB compromised, the green fibrinogen stain is detected in brain matter (left panel). In addition, expression of claudin-5 (CLN-5, red stain), a component of the tight junctions that make up the BBB, is reduced. Anti-SEMA4D antibody prevents BBB breakdown and both prevents extravascular leakage of fibrinogen and preserves claudin-5 as detected by red stain (right panel). Scale 50 µm.
(In collaboration with Dr. Gareth John, Mount Sinai, New York)

MAb 67-2 and VX15/2503 are 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 treated EAE animals, but not untreated naïve animals.



Objective To analyze T cells isolated from brains of SJL EAE mice for VX15/2503 saturation by flow cytometry.

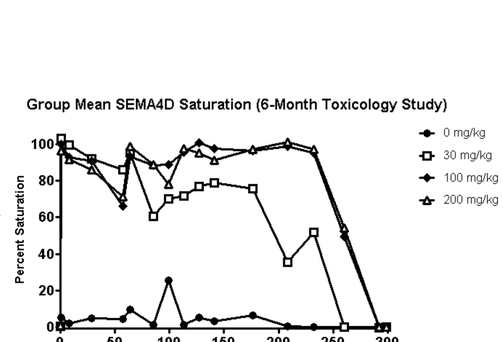
Experimental Design
PBMC were isolated from PBS perfused brains treated as follows:
- Naïve mice injected with VX15/2503
- EAE induced mice injected with MAb 2955 (IgG4 isotype control)
- EAE induced mice injected with VX15/2503
- Mice from all groups were sacrificed 2 days after antibody injection
- Mononuclear cells were prepared by mechanical separation followed by a two layer Percoll gradient

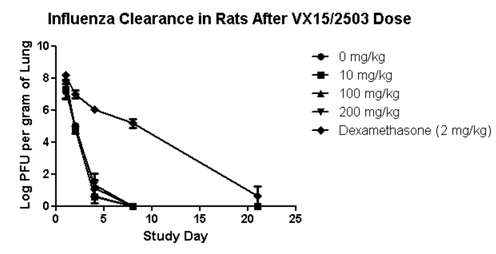
Results
- Mice without EAE that were injected with VX15/2503 did not have many CD3+ cells in the brain, as expected, though they were fully saturated with VX15/2503.
- Mice with EAE that received just isotype control antibody had no saturation of SEMA4D on their CD3+ cells from the brain.
- Mice with EAE that were injected with VX15/2503 showed complete saturation of SEMA4D by VX15/2503 antibody on their CD3+ cells from the brain.

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 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 clinical studies.

- **Non-clinical Toxicology Studies**
  - Subchronic Toxicology
    - Rat and cynomolgus macaques were dosed during one month repeat dose GLP toxicology studies up to 100 mg/kg/dose
  - Chronic Dose Toxicology
    - A GLP six 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 is ongoing
- **Non-clinical Toxicology Summary**
  - No observed adverse effect level (NOAEL) has been designated as the high dose 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**





- **Clinical**
  - Phase I, non randomized, open label, dose-escalation study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of weekly intravenous infusion of VX15/2503 in adult patients with advanced solid tumors. Enrollment is continuing for this trial.
  - 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 will begin in late 2012.

Summary

We have generated a high affinity mouse antibody, MAb 67-2, that blocks both SEMA4D-PLXNB1 and SEMA4D-CD72 interactions and significantly reduces the severity of disease in murine and rat EAE disease models.

VX15/2503, a humanized antibody derived from MAb 67-2, exhibits specificity and functional characteristics similar to the murine progenitor antibody.

Single and repeat dose intravenous infusion toxicity, PK, and PD studies with VX15/2503 in cynomolgus monkeys and in rats with a recovery phase have been completed, with no toxicity observed.

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 will begin in late 2012.