Development of Anti-SEMA4D Human Antibody for the Treatment of Multiple Sclerosis

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Disclosure

- Dr. Foster is a paid consultant of Vaccinex, Inc.
- All other authors of this abstract are employees of Vaccinex, Inc.

SEMA4D: Introduction

- SEMA4D (CD100) is a member of the semaphorin family of proteins first identified as mediators of axonal growth cone guidance in the developing nervous system
- SEMA4D has also been implicated in additional functions including:
  - Inhibition of neurite extension and axon regeneration in adults
  - Regulation of survival and differentiation of oligodendrocytes
  - Enhancement of CD40-induced activation of immune and inflammatory cells
  - Vascular development and angiogenesis
  - Tumor cell migration and proliferation

SEMA4D: Introduction

- In normal tissues, SEMA4D is expressed abundantly on the surface of resting T cells and less strongly on B cells, monocytes, dendritic cells, oligodendrocytes and platelets; expression is up-regulated upon cellular activation.
  - SEMA4D exists primarily as a cell surface homodimer, which can be cleaved to create a soluble form
  - Both membrane and soluble Sema4D have biological activity

- SEMA4D has two reported receptors
  - High affinity: Plexin-B1 (1nM) expressed on numerous tissues including microglia, neurons, oligodendrocytes, endothelial cells and tumor cells
  - Low affinity: CD72 (300nM), expressed primarily on B cells, APCs and platelets

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MAb VX15/2503

- As SEMA4D has been reported as an important mediator of both inflammatory responses and direct demyelination, it is an attractive target for the treatment of diseases like MS
- The clinical candidate MAb, VX15/2503, is a humanized IgG4 anti-SEMA4D MAb
  - Approximately 5 nM affinity by surface plasmon resonance (Biacore)
  - No demonstrable effector cell function (ADCC, CDC) in-vitro
- VX15/2503 is derived from the murine parent; MAb 67
- MAb 67 was generated in SEMA4D -/- mice and neutralizes rodent, monkey and human SEMA4D
- MAb 67 has been used to evaluate to role of blocking SEMA4D in murine models of EAE

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MAb VX15/2503: Binding Specificity

- VX15/2503 binds to SEMA4D with high specificity as demonstrated by ELISA, flow cytometry and immunohistochemistry
- ELISA
  - VX15/2503 binds to recombinant SEMA4D derived from several species including murine, rat, cynomolgus macaque, marmoset, rhesus macaque and human with no binding to a panel of 10 non-specific purified proteins
- Flow Cytometry
  - VX15/2503 binds to SEMA4D naturally expressing human cell lines; T cell (Jurkat), B cell (Daudi) and monocyte (U937) with no binding to non-SEMA4D expressing cell lines such as CHO and human epithelial cells (A431)
- Immunohistochemistry
  - Binding only demonstrated in resident or itinerant lymphoid cells in a fresh frozen human tissue panel

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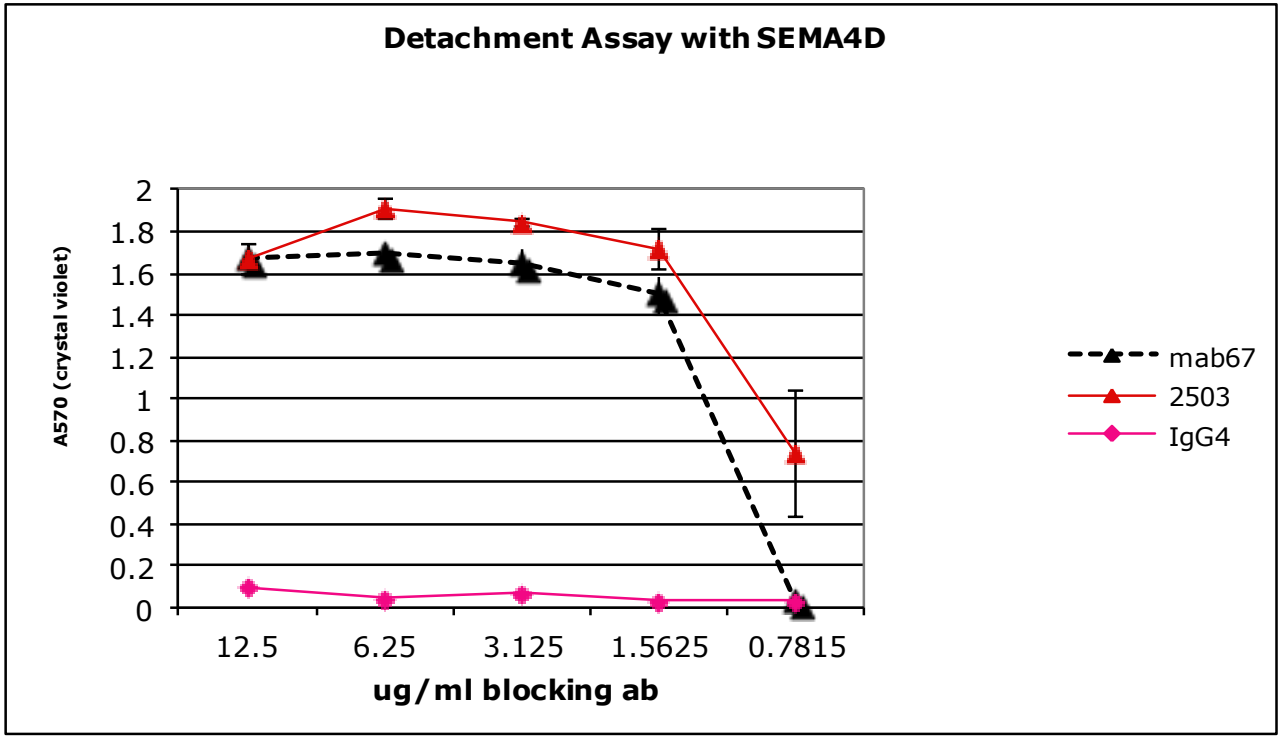
MAb VX15/2503: Functional Activity

- Ligation of Plexin-B1 by soluble SEMA4D regulates integrin function and cell adhesion via R-Ras and RhoA pathways
- A cell adhesion assay has been developed to measure the effect of VX15/2503 on this downstream signaling effect of the SEMA4D/Plexin-B1 axis
  - 293/Plexin-B1 expressing cells are plated on fibronectin coated microtiter wells and allowed to attach over night
  - The cells are then incubated with SEMA4D in presence or absence of anti-SEMA4D MAb
  - After washing and staining with crystal violet, the remaining attached cells are quantitated

SEMA4D/Plexin-B1 Detachment Assay



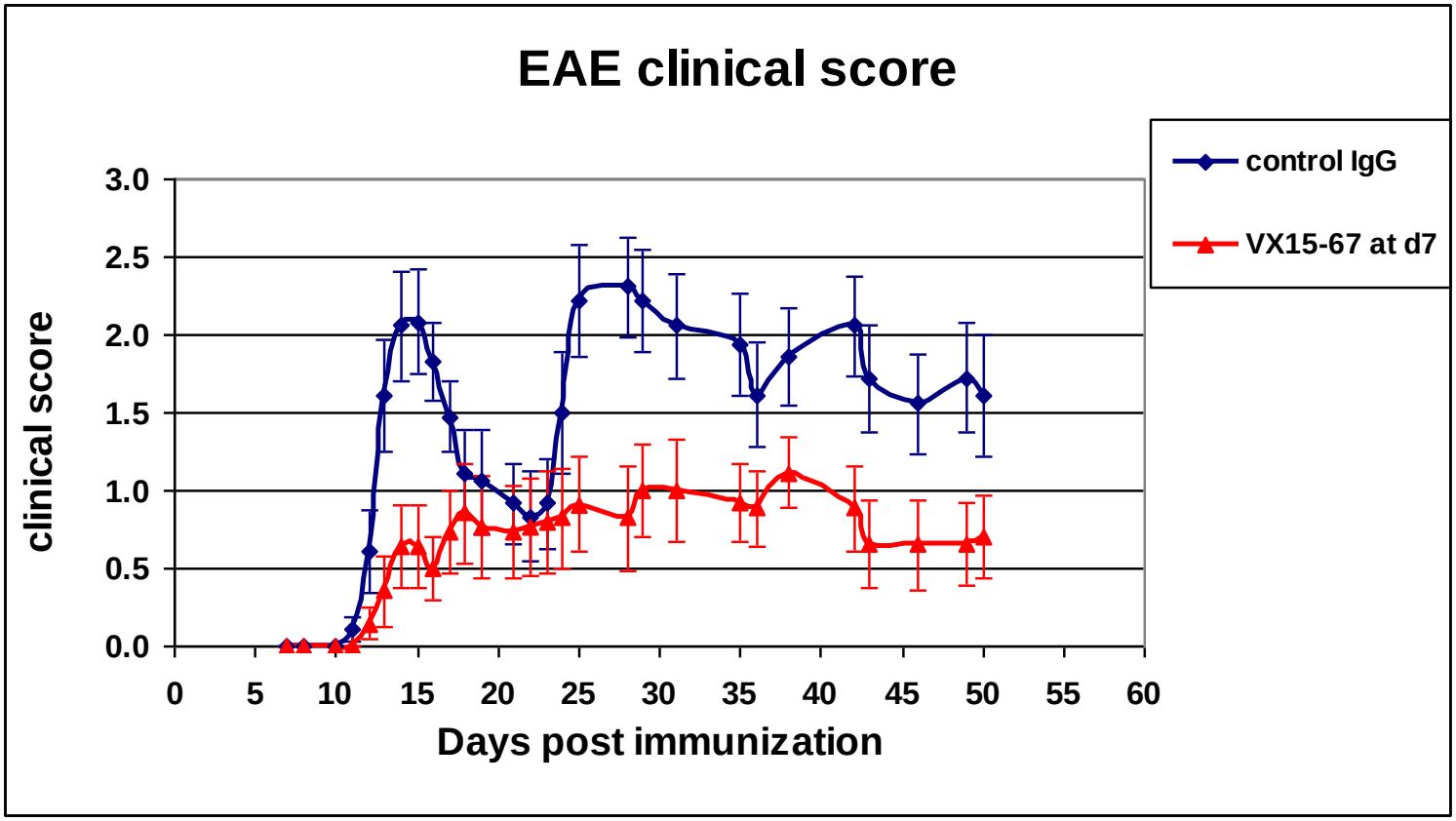
SJL RR-EAE Model

- MAb 67 was evaluated in a SJL mouse model of RR EAE

- EAE was induced in naive SJL female mice using an optimized commercial preparation of proteolipid protein $PLP_{139-151}$ in CFA (Hooke, Inc)

Group #	# Animals	Treatment	Dose	Treatment Days
1	18	Mouse IgG Isotype control	600µg ip	1X/week starting on Day 7
2	18	MAb 67	600µg ip	1X/week starting on Day 7

Anti-SEMA4D Antibody Ameliorates EAE in SJL Mice



50% reduction in mean disease score

Similar results observed in two additional SJL $PLP_{139-151}$ EAE studies

Similar but less pronounced effects were seen in a MOG model of EAE

Adoptive Transfer EAE Model – SJL Mice

- Day 0
  - SJL donor mice were immunized with $PLP_{139-151}$/CFA emulsion
- Day 10
  - CD4+ T cells were isolated from spleens and lymph nodes of the donor mice
  - Cells depleted of T cells were irradiated (3400 rad) and used as feeders
  - T cells were re-stimulated with $PLP_{139-151}$ peptide, IL12 and anti-IL4 MAb
- Day 17
  - The cells were re-stimulated with anti-CD3 and agonistic anti-CD28 MAbs
- Day 20
  - 2 million T cells were transferred into each of 20 recipient SJL mice
    - 10 animals were treated with 600ug control IgG
    - 10 animals were treated with 600ug anti-SEMA4D MAb 67
  - Treatments were repeated every 7 days

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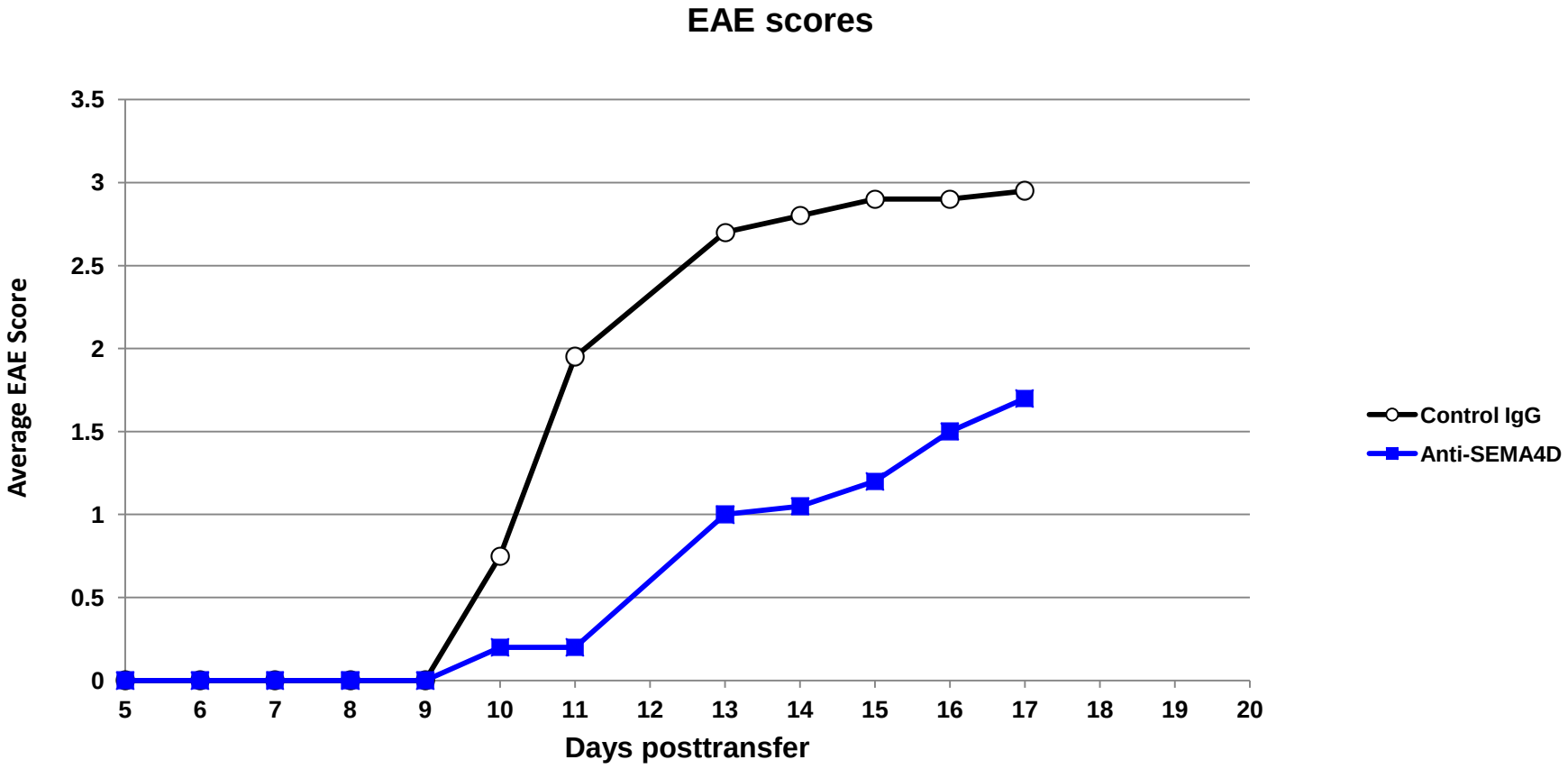
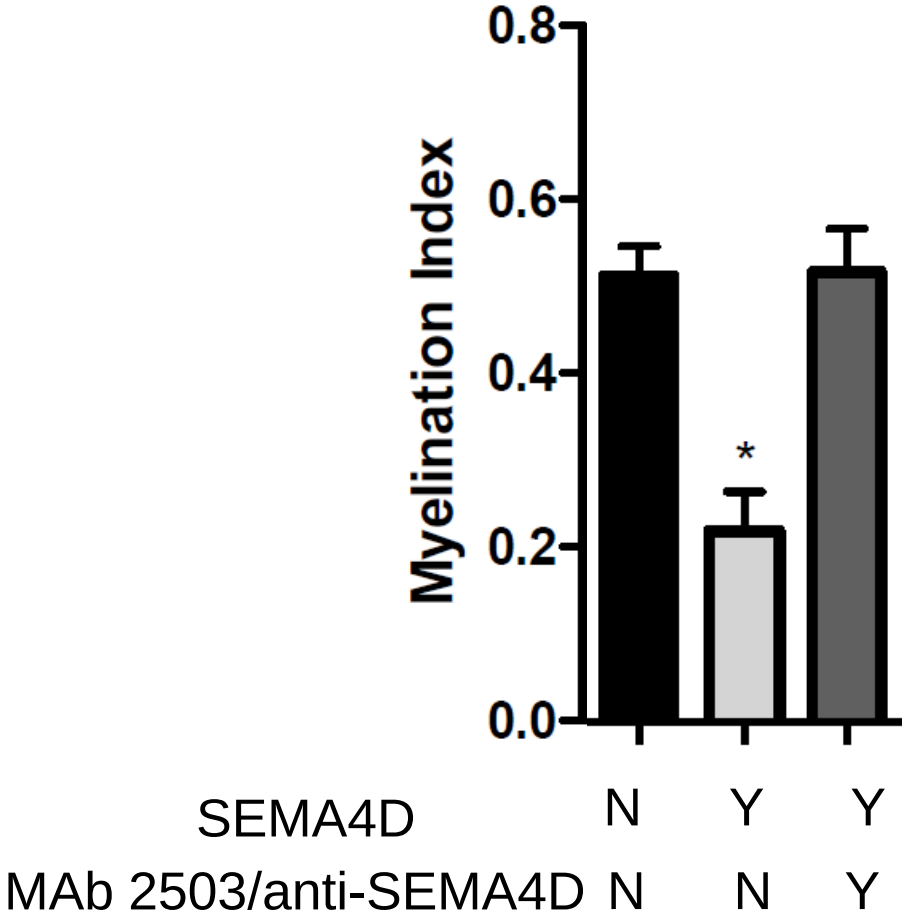
Anti-SEMA4D Ameliorates SJL-RR EAE: Adoptive Transfer Model



Neuroprotective and Anti-inflammatory Activity of Anti-SEMA4D Antibody

- SEMA4D signals through Plexin-B1 receptor on neurons to activate RhoA and inhibit axonal outgrowth and regeneration
- Morphologic differentiation of oligodendrocytes is also controlled by Rho family GTPases. SEMA4D signaling through Plexin-B1 on pre-myelinating oligodendrocytes activates RhoA and inactivates Rac to inhibit oligodendrocyte differentiation and myelination
- Culturing chronically activated T cells expressing SEMA4D with multipotent neural precursor cells or primary oligodendrocytes from rat brain induces neural precursor and oligodendrocyte apoptosis
- SEMA4D signaling also promotes inflammation by activation of microglia

SEMA4D Blocks Remyelination Following Lysolecithin Induced Injury in an *in vitro* Brain Slice Model



In collaboration with Dr. Anna Williams, Edinburgh University

Ongoing Investigations

- Treatment with anti-SEMA4D MAb is being tested following in vivo treatment of Balb/c mice with lysolecithin
  - Endpoint: Enhanced/accelerated remyelination

- SEMA4D deficient mice have increased numbers of oligodendrocytes following ischemic injury (Taniguchi, Y. et al. J Neuroscience Research 2009; 87:2833-41
  - Experiments are in progress to investigate the treatment of wild type mice with anti-SEMA4D MAb in this model

Clinical Development of VX15/2503

- Antibody production of VX15/2503 in CHO cell cultures at the 200L cGMP scale
- VX15/2503 GLP toxicology and pharmacokinetic studies have been performed in cynomolgus macaques and rats in both single and repeat dose settings
- NOAEL of 100mg/kg was demonstrated in both species
- Half-life increases with dose and ranges from approximately 1 day (1 mg/kg) to 10 days (100 mg/kg)
- Phase 1, open-label, dose-escalation, first-in-human study is being conducted in patients with relapsed or refractory solid tumors
- Phase 1 study in MS patients is planned for initiation Q1 2012