

Reduction of *In Vivo* Tumor Growth and Angiogenesis by a Humanized IgG4 Monoclonal Antibody to SEMA4D

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Abstract

Semaphorin 4D (SEMA4D; CD100) has been implicated in several key mechanisms of tumor progression, including neovascularization, tumor invasion, and metastasis. SEMA4D binding to its receptor plexin-B1 (PLXNB1) on endothelial cells transactivates MET and promotes formation of new blood vessels and tumor growth *in vivo*. SEMA4D is over-expressed in a wide array of tumor types, and is also produced by recruited inflammatory cells present in the tumor microenvironment. Several recent papers have shown that in an environment lacking SEMA4D, the ability of mouse cancer cells to originate tumor masses and metastases is severely impaired. Furthermore, SEMA4D produced by tumor-associated macrophages has been shown to support tumor angiogenesis and growth. In addition to its effects on endothelial cells, SEMA4D has a direct effect on tumor invasive growth and migration. A recent clinical study in soft tissue sarcomas correlates strong SEMA4D expression in tumors with a higher mitotic count and poor prognosis. SEMA4D binding to PLXNB1 on tumor cells results in MET transactivation and migration of tumor cells. It has been further reported that overexpression of PLXNB1 and MET in breast and ovarian cancers is a negative prognostic factor. Tumors co-expressing PLXNB1 and MET were characterized as having a higher grade and an increased frequency of metastases. Collectively, these results suggest that expression of SEMA4D, either by tumor cells or by tumor associated inflammatory cells, functions as a crucial factor in tumor neovascularization, and that expression of the SEMA4D and/or its high affinity receptor in tumors may further induce tumor growth rate and metastatic potential. Antibody neutralization of SEMA4D thus may represent a new therapeutic strategy for cancer treatment. We selected a humanized IgG4 antibody that binds with high affinity to rat, mouse, primate, and human SEMA4D, and utilized several *in vitro* functional assays to demonstrate that this antibody blocks SEMA4D – PLXNB1 interactions. Using syngeneic, xenograft and orthotopic tumor models we demonstrated that antibody mediated neutralization of SEMA4D *in vivo* inhibits tumor growth and tumor angiogenesis. This humanized antibody has successfully completed IND-enabling toxicology testing and is currently undergoing clinical trials.

Introduction

- SEMA4D
 - Binds PLXNB1 with 1 nM affinity and CD72 with 300 nM affinity
 - Exists in both cellular and soluble forms
 - Is expressed abundantly on the surface of resting T cells and less strongly on B cells and APCs; it is upregulated upon cellular activation
 - Activates B lymphocytes and induces dendritic cell maturation for antigen presentation to T lymphocytes
 - Binding to PLXNB1 transactivates MET promoting angiogenesis and stimulating invasive growth of tumors
 - Is overexpressed in a variety of human tumors including head and neck, prostate, colon, and lung
- Use of shRNA to knockdown the expression of SEMA4D reduced tumor growth and vascularization in mice
- Therapeutic Rationale for anti-SEMA4D Antibody: Neutralization of SEMA4D using a monoclonal antibody could inhibit tumor growth and invasion
- VX15/2503 binds with 5 nM affinity to cellular and soluble SEMA4D and was selected for clinical development to treat patients with advanced solid malignancies

Generation of anti- SEMA4D MAbs

- A panel of mouse hybridomas specific for human, monkey, and mouse SEMA4D or only human and monkey SEMA4D were generated in Sema4D^{-/-} mice. Several hybridomas were selected for further analysis.
- In vitro* biochemical and functional characterization was carried out with purified antibody from independent hybridomas:
 - Affinity measurement
 - Functional Activity
- Based on this data a lead mouse antibody was selected (67-2).
- A humanized version of this MAb was created (VX15/2503)

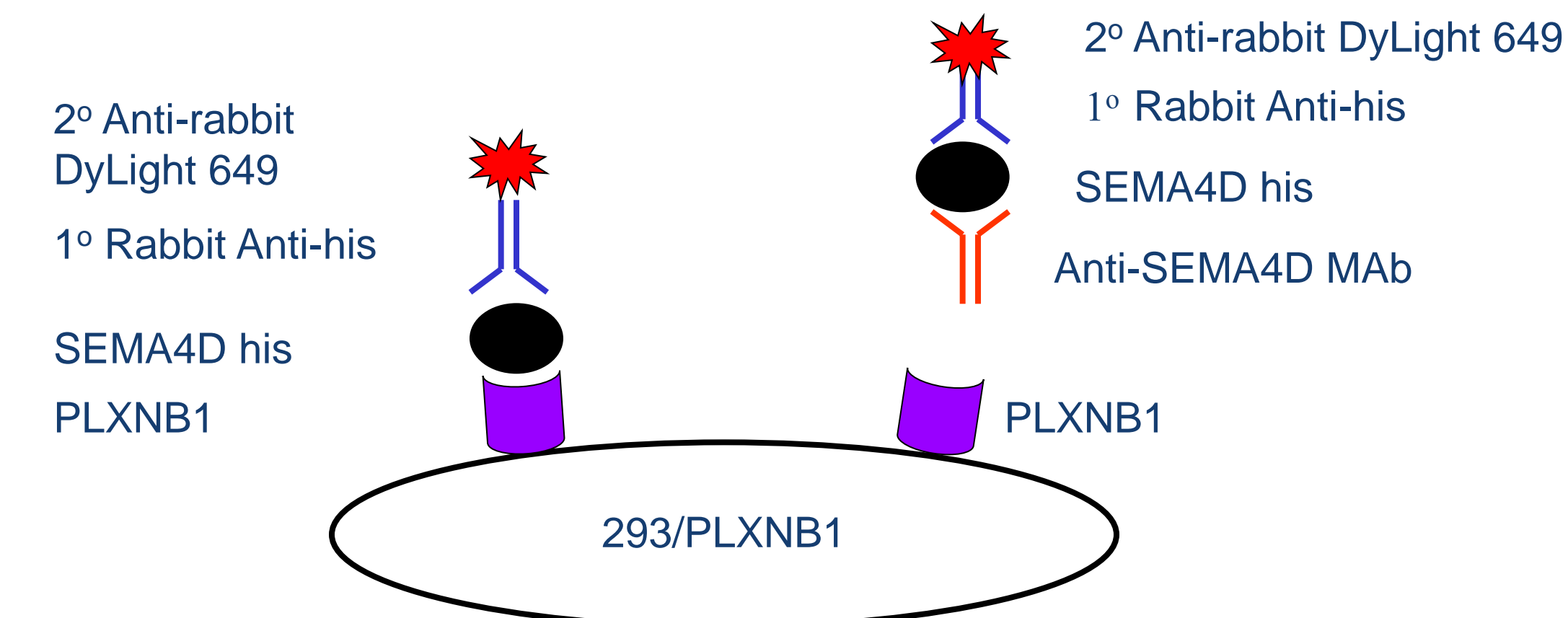
Mouse and Human MAbs With High Affinity for Mouse and Human SEMA4D Have Been Selected

Table shows Affinity (nM) Measured by Biacore

MAb	Isotype	Affinity for Mouse SEMA4D	Affinity for Marmoset SEMA4D	Affinity for Human SEMA4D
Murine 2503 parent (67-2)	mIgG1	1.3	2.7	5.1
Human (2503)	hIgG4	1.5	2.4	5.4

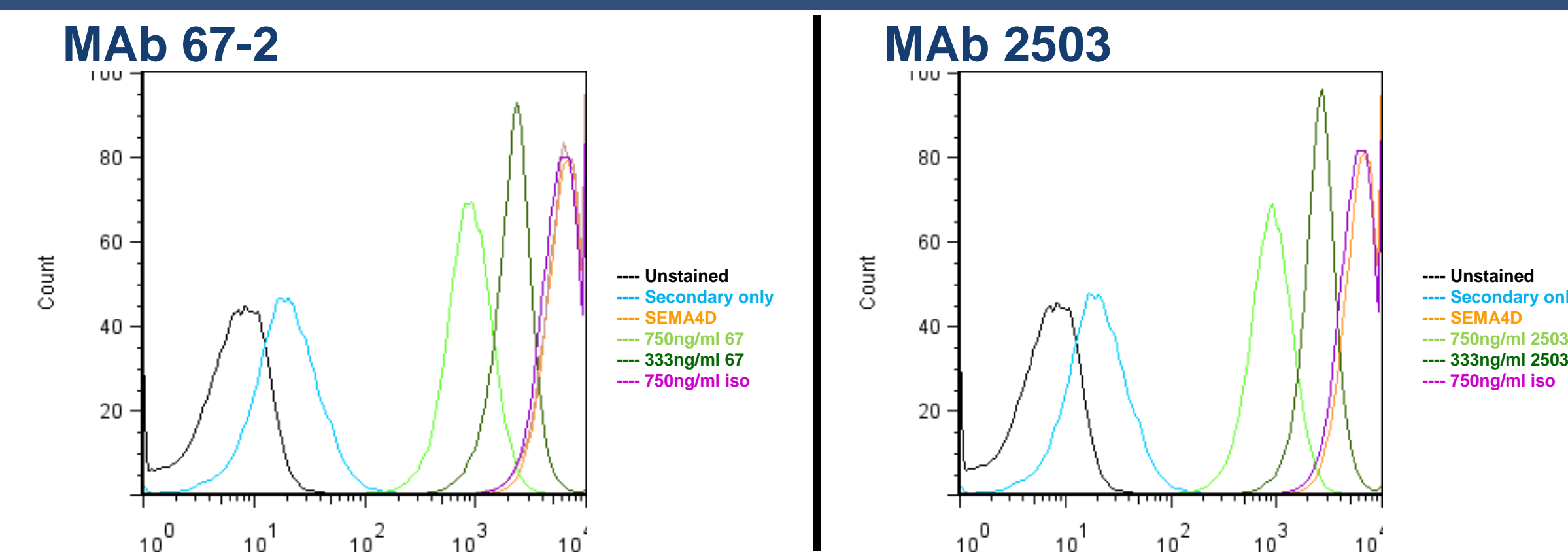
MAbs 67-2 and VX15/2503 have high affinity for mouse, monkey and human SEMA4D
 Note: VX15/2503 affinity for Rat and Cynomolgus SEMA4D is also less than 5 nM by Biacore

SEMA4D receptor blocking assay

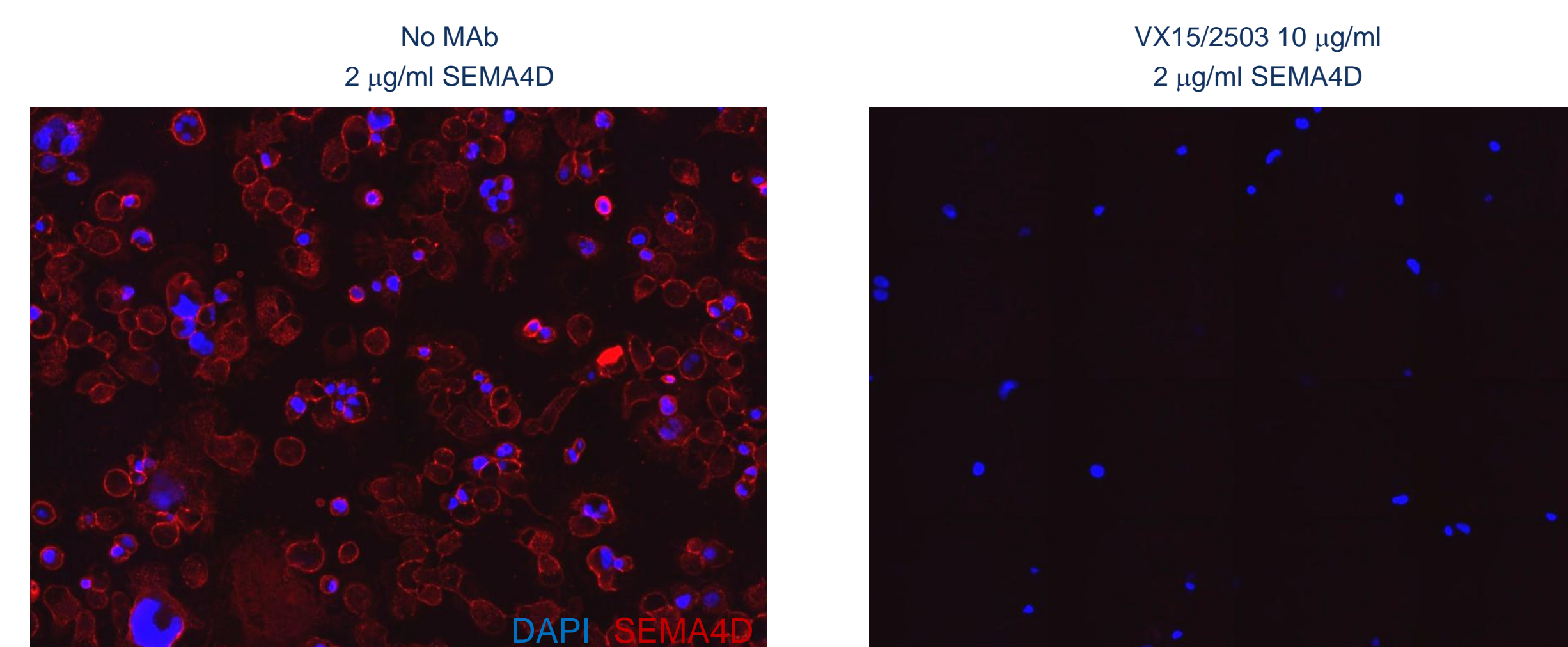


If MAb is able to block binding of SEMA4D to PLXNB1, then no SEMA4D will be detected on the cell, resulting in lower fluorescence

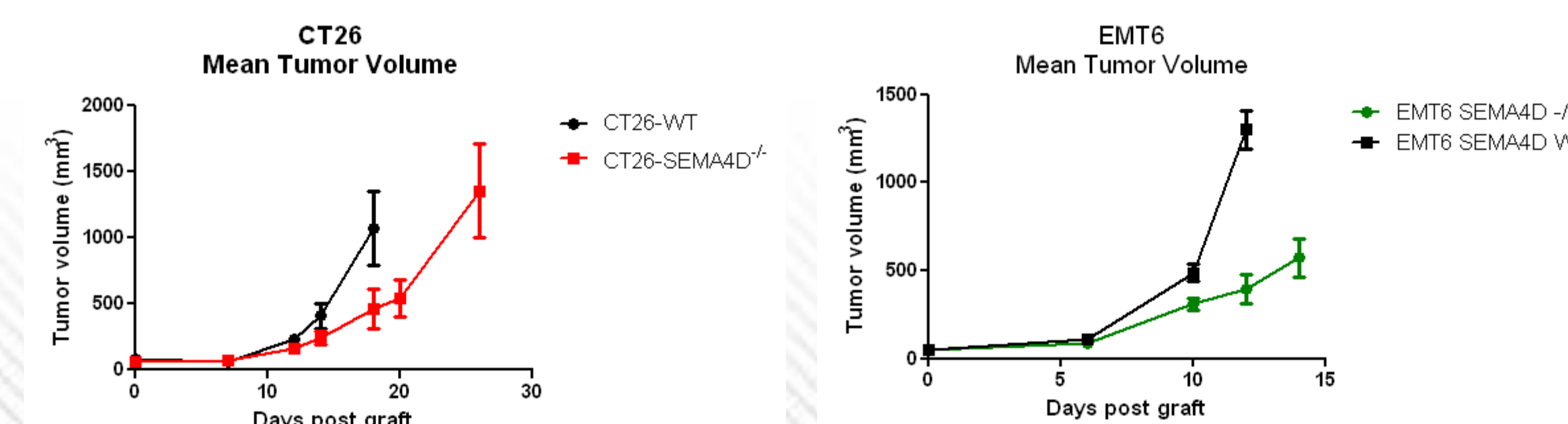
Both Mab 67-2 and VX15/2503 block SEMA4D binding to PLXNB1 on HEK 293-PLXNB1 cells by flow cytometry



VX15/2503 blocks SEMA4D binding to PLXNB1 on HEK 293-PLXNB1 cells by Immunofluorescence

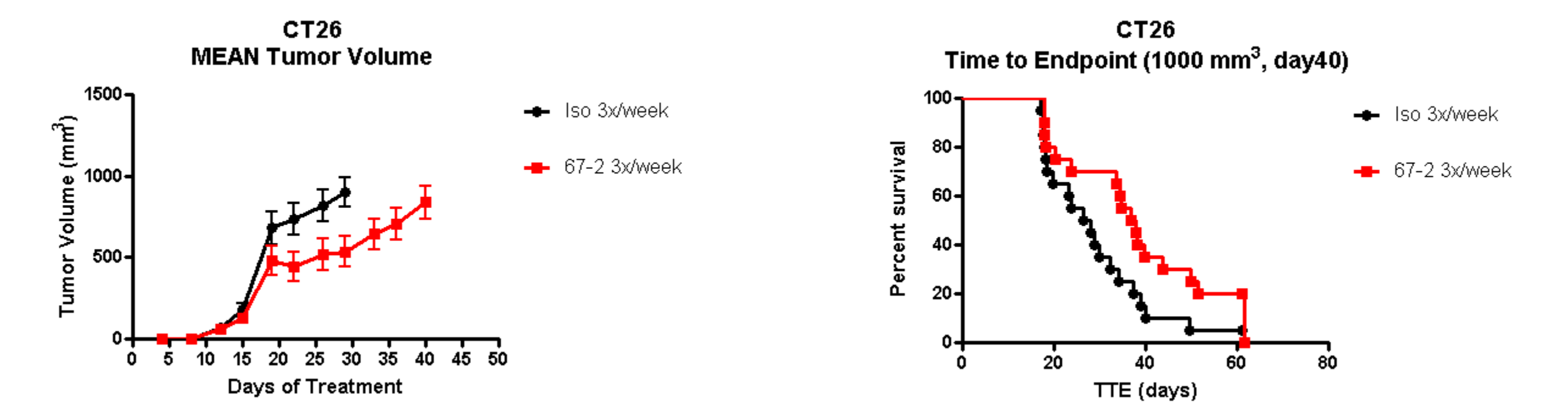


CT26 and EMT6 tumors exhibit slower growth rate in BALB/c SEMA4D^{-/-} versus WT mice



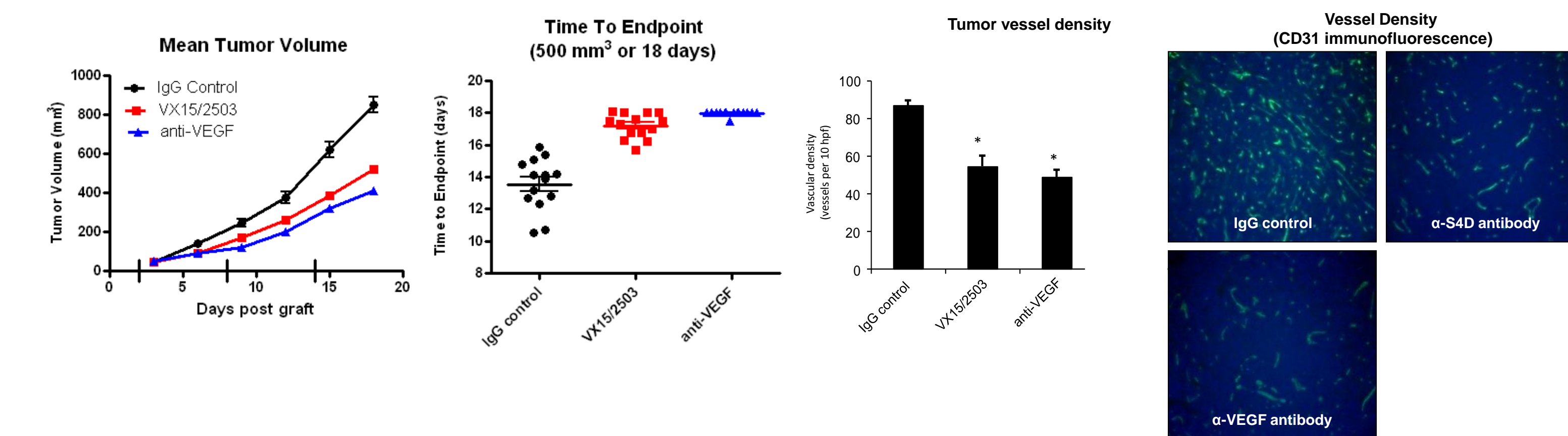
Two syngeneic mouse tumor lines, CT26 and EMT6 were tested for their growth rate in BALB/c SEMA4D Knockout versus WT Balb/c mice. CT26 or EMT6 cells were injected into WT and SEMA4D^{-/-} BALB/c mice and measured for their growth rate. The data shown demonstrate that both CT26 and EMT6 tumors grew slower in SEMA4D deficient mice. Similar results were observed using BCA34-pt4 cells.

Treatment with anti-SEMA4D MAb 67-2 slows growth of CT26 tumors in BALB/c Mice



The anti-SEMA4D antibody 67-2 was tested for its ability to reduce the growth of syngeneic CT26 tumors. Tumor bearing mice were divided into groups of 20 mice each, and treated 3x/week with 60 mg/kg intraperitoneal injections of MAb 67-2 or an irrelevant murine isotype control antibody. The data shown demonstrate that treatment with MAb 67-2 slowed the growth of tumors in these mice. The median Time To Endpoint (TTE) for 67-2 -treated animals was 37.3 days, or 38% Tumor Growth Delay (TGD). Logrank results were significant ($P < 0.05$). A similar result was observed for BCA34 tumors (median TTE for 67-2 -treated animals was 18.45 days, or 18% TGD. Logrank results were very significant ($P < 0.01$)). Similar results were observed in four other experiments.

Treatment with anti-SEMA4D MAb VX15/2503 inhibits vascularization and slows growth of HN6 tumors



The humanized anti-SEMA4D antibody VX15/2503 was tested for its ability to block SEMA4D and reduce tumor growth and vascularity in HNSCC-6-HIF1a mODD xenografts, which express high levels of HIF1a and SEMA4D. Treatment included VX15/2503, anti-VEGF (R&D Ab), and an isotype control. Mice were divided into 3 groups of 10 nude mice, each injected with 2 subcutaneous tumors and treated weekly with 1 mg (~ 50 mg/kg) of antibody by ip injection, starting day 2 post graft for a 3 week duration. All mice were sacrificed on day 18 as a tumor growth inhibition (TGI) study. Treatment with VX15/2503 or anti-VEGF significantly ($P < 0.001$) inhibited tumor growth and vascularization compared to IgG control.

Preclinical Evaluation

- Tissue cross reactivity analysis demonstrated similar binding of VX15/2503 to lymphoid tissues in human, cynomolgus, and rat tissues.
- Single and repeat dose intravenous infusion toxicity, PK, and PD studies with VX15/2503 in cynomolgus macaques and in rats with a recovery phase have been completed. Similar PK and PD profiles were observed for rats and cynomolgus monkeys.
- Anti- VX15/2503 antibodies were detected in both rat and cynomolgus macaques, typically within two weeks of injection. These exerted only marginal effects on total exposure in low dose animals only.
- No adverse events were identified in any nonclinical study; NOAEL = 100 mg/kg (highest dose tested).
- Additional preclinical data for SEMA4D and VX15/2503 are presented as part of abstract #4578

Summary

- We have generated a high affinity mouse MAb, 67-2, that blocks SEMA4D-PLXNB1 interactions and significantly slows the growth of both mouse and human tumors in mice.
- MAb VX15/2503, a humanized antibody derived from MAb 67-2, inhibits tumor growth and vascularization, similar to that observed for anti-VEGF.
- VX15/2503 binds with high affinity to rat, cynomolgus macaque and human SEMA4D
- VX15/2503 binds to and is internalized by T cells in a time dependent manner.
- These studies, in conjunction with PK/PD, safety, and immunolocalization studies in rat and cynomolgus monkeys, supported the initiation of a phase 1 clinical study to evaluate the safety and tolerability of VX15/2503 in patients with advanced solid tumors