Reduction of tumor growth by an antibody to SEMA4D/MAb67, a murine version of humanized MAb VX15/2503

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Abstract

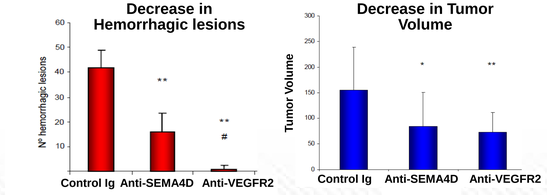
Semaphorin 4D (SEMA4D; CD100) has been implicated in several key mechanisms of tumor progression, including transactivation of several oncogene products, neovascularization, and metastasis. Expression of SEMA4D and its plexin-B receptors correlates with invasive disease in several human tumors. SEMA4D is over-expressed in a wide array of tumor types and is also produced by inflammatory cells recruited to the tumor microenvironment. SEMA4D binding to plexin-B1 (PLXNB1) on endothelial cells activates RhoA and AKT signaling pathways, which promotes formation of new blood vessels and tumor growth in vivo. In addition to its effects on endothelial cells, the interaction of PLXNB1 with MET and ERBB2 and crosslinking by SEMA4D can lead to transactivation of these membrane receptor kinases with a direct effect on tumor cell migration and invasive growth. It is well known that tumor growth and metastasis involve a complex process of cross talk amongst the tumor cells, stroma and immune infiltrate, as well as the endothelial cells and vasculature. Our understanding of the role of SEMA4D in this process is evolving.
We selected a humanized IgG4 antibody, VX15/2503, that blocks SEMA4D interaction with the broadly expressed PLXNB receptors and an alternate receptor, CD72, expressed on immune cells. The antibody binds with between 1-5 nM affinity to rat, mouse, primate, and human recombinant SEMA4D, and with 0.5 nM affinity for native, cell-associated SEMA4D on primary human T cells. We demonstrate that antibody-mediated SEMA4D neutralization delays tumor growth in several in vivo tumor models. In some tumor models this effect is, in part, mediated by inhibition of SEMA4D-induced angiogenesis. We demonstrate here that SEMA4D also regulates the balance and localization of classically activated and alternatively activated macrophage in the tumor and that modulation of the infiltrating immune microenvironment can result in anti-SEMA4D antibody-mediated tumor growth delay. In summary, blockade of SEMA4D reduces tumor growth through effects on tumor microenvironment, angiogenesis and vascular permeability, and, possibly, direct effects on tumor. Antibody neutralization of SEMA4D may, therefore, represent a new therapeutic strategy for cancer treatment. The humanized antibody, VX15/2503, has successfully completed IND-enabling toxicology testing and the dose escalation phase of a Phase I trial in adult patients with advanced solid tumors.

Introduction

- **SEMA4D**
  - **Binds PLXNB1 with 1 nM affinity and CD72 with 300 nM affinity**
  - **Exists in both cellular and soluble forms, both forms are biologically active**
  - **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**

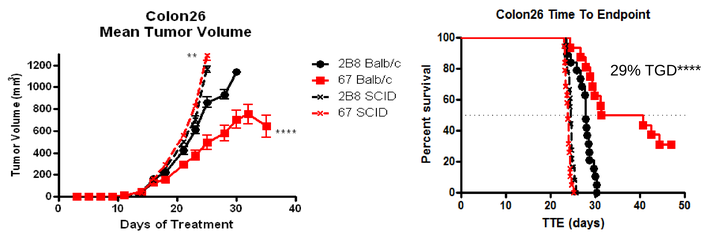
- **A panel of mouse hybridomas producing MAbs specific for human, monkey, and mouse SEMA4D were generated in Sema4D$^{-/-}$ mice. Several antibodies were selected for further analysis.**
  - **In *vitro* biochemical and functional characterization assays, including included affinity measurement and inhibition of SEMA4D-mediated activities, were conducted using antibody purified from several hybridomas.**
  - **Based on these data a lead mouse IgG1 antibody was selected (MAb 67-2).**
  - **A humanized IgG4 version of this MAb was created (VX15/2503).**
  - **Lead antibodies bind the SEMA4D dimerization domain of both soluble and membrane-bound SEMA4D and block binding to PLXNB and CD72 receptors.**

Anti-SEMA4D antibody reduces angiogenesis and growth of spontaneous pancreatic neuroendocrine tumors in Rip1 Tag2/Rag1-null Model



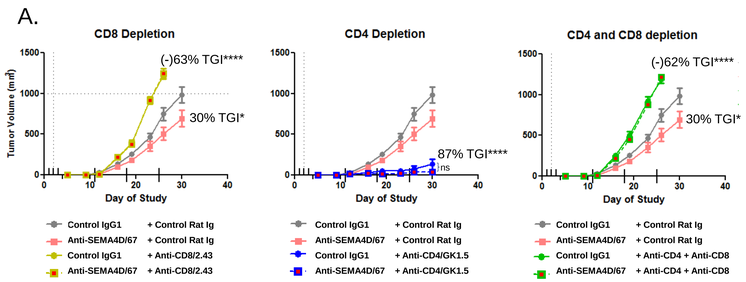
Anti-angiogenic antibodies reduced the frequency of hemorrhagic lesions and reduced tumor growth. Rip1Tag2/Rag1-null mice develop highly vascularized spontaneous pancreatic neuroendocrine tumors due to expression of the oncogene SV40 T antigen driven by the insulin promoter. The Rag-1 background confers deficiency in V(D)J recombination of immunoglobulins and T cell receptor genes during immune cell maturation. Groups of 10 tumor-bearing mice were treated with anti_VEGFR2/Ab DC101 (IP 60 mg/kg, 3x/week), or MAb 67-2 or an irrelevant murine isotype control antibody (IP 50 mg/kg, weekly). There is a statistical difference in all the treatment groups by the Mann-Whitney test (*: p<0.05; **p < 0.01) when compared to control group. Error bars indicate ± SD. This experiment was performed in collaboration with Oriol Casanovas (Barcelona).

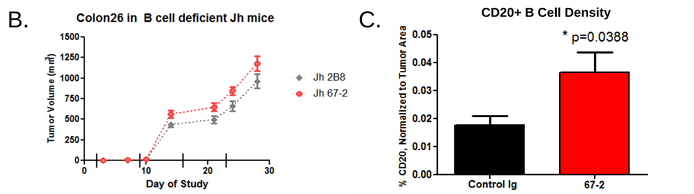
Treatment with anti-SEMA4D MAb 67-2 delays growth of Colon26 tumors in Balb/c Mice. Anti-tumor effect is dependent on competent immune system



Anti-SEMA4D/MAb 67-2 delays growth of subcutaneous Colon26 tumors in immune competent mice, while efficacy is abrogated in SCID mice, suggesting a role for immune cells, as either producers and/or effectors, in the SEMA4D regulated mechanism of action. Anti-SEMA4D antibody treatment resulted in 29% Tumor Growth Delay (TGD), which was determined to be highly statistically significant using Mantel Cox Log Rank Analysis. Groups of 20 Balb/c mice were implanted with 500,000 cells, SCID mice were implanted with 50,000 cells. Two days post implant, mice were treated with intraperitoneal injections of MAb 67-2 or an irrelevant murine isotype control antibody (50 mg/kg, weekly). Similar results were observed in 6 other experiments in this model, as well as other syngeneic tumor models.

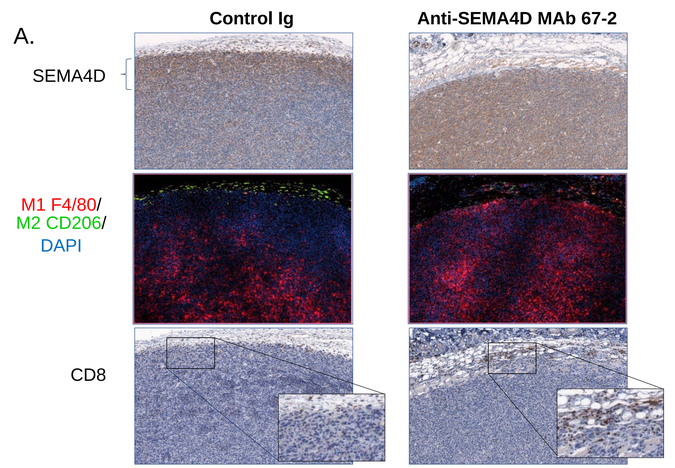
T and B cells mediate efficacy of Anti-SEMA4D antibody





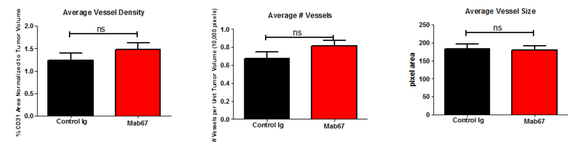
A. CD8+ T cells suppress growth of Colon26, while CD4+ T cells, likely Treg, promote growth of Colon26. CD8+ T cells are required for efficacy of MAb 67-2. Groups of 20 tumor-bearing Balb/c mice each were treated with intraperitoneal injections of MAb 67-2 or an irrelevant murine isotype control antibody (50 mg/kg, weekly, starting 2 days post tumor implant). Depleting antibodies or control Rat IgG were administered on days 1, 2, 3, and weekly thereafter (0.15 mg IP). Mean tumor volume for each group (n=20) are graphed above. **B. B cells contribute to anti-tumor effect of MAb 67-2.** Growth of Colon26 tumors is not delayed, and may be augmented, in B cell-deficient Jh mice. **C. Treatment with MAb 67-2 increases B cell density in Balb/c mice.** Formalin fixed paraffin embedded sections of tumors from treated mice were stained with anti-CD20 antibody and assessed for B cell infiltration by scanning an entire section (n=10/group).

Anti-SEMA4D antibody allows penetration of CD8+ T cells and M1 macrophage into areas of actively proliferating tumor



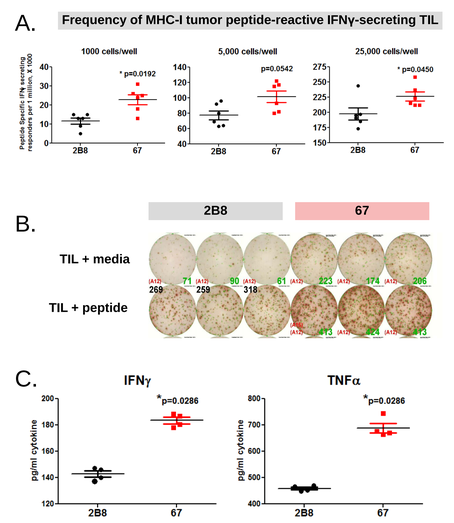
A. Neutralization of SEMA4D gradient at the leading edge of the tumor allows migration of anti-tumor macrophage and cytotoxic T lymphocytes into this area. A band of strongly SEMA4D+ tumor is bounded by M2 and M1 macrophage. Neutralization of SEMA4D with MAb 67-2 facilitates entry of anti-tumor M1 macrophage into the zone of highly proliferating tumor cells and CD8+ T cells throughout the zone and extending into the leading edge (inset). Furthermore, an overall increase in the CD8+ T cell density was observed within entire tumor sections isolated from MAb 67-treated mice. FFPE tumor sections (n=10/group) were stained with polyclonal anti-SEMA4D, M1 are F4/80^{hi} CD206^{-} and M2 macrophage are F4/80^{lo} and CD206^{hi}, cytotoxic T cell anti-CD8, and DAPI nuclear stain. Representative images are shown. **B.** The entire section from each tumor was scanned and quantitated.

Tumor growth delay is not dependent on angiogenesis in Colon26 tumors



Neither vessel density, vessel #, nor vessel size decreased with anti-SEMA4D MAb 67-2. This is consistent with previous reports that Colon26 is resistant to anti-angiogenic (anti-VEGFR) treatment (Fischer et al. 2007, Cell131(3): 463–475). FFPE tumor sections were stained for CD31 using Cy5 detection. Whole tumor sections were imaged and CD31-positive signal was thresholded for vessel detection. Vessel size was determined by filling in autofluorescence of erythrocytes in the vessels. The number and size of vessels were enumerated using ImagePro software. Vessel density was calculated by dividing the number of vessels in total tumor by tumor pixel area for each section.

Anti-SEMA4D antibody increases tumor infiltration of tumor-specific cytotoxic CD8+ T cells



MAb 67-2 treatment increases the frequency of tumor-specific TIL and secretion of pro-inflammatory cytokines. Following four weeks of in-vivo anti-SEMA4D treatment, tumors were dissociated and enriched for CD45+ cells by magnetic separation. CD45+ TIL, pooled from 5 mice, were incubated in the presence and absence of immunodominant tumor peptide, AH-1, at various cell densities. **A. IFNγ secreting cells were measured by ELISPOT**; peptide specific response was determined by subtracting average of wells without peptide. Each sample was tested in replicates of 6 and is graphed above. Statistical significance was determined with Mann-Whitney non-parametric t test. An increase in IFNγ secreting cells was observed in MAb 67-treated mice both in the presence and absence of peptide. CD45+ TIL, especially MHC-I-restricted peptide-specific CD8+ cytotoxic T cells, represent activated effector cells following treatment with MAb 67-2. **B. Representative ELISPOT images shown. C. CD45+ TIL were cultured ex vivo for 48 hr and assayed for cytokine secretion using CBA analysis.** Mab 67-2 promotes secretion of anti-tumor cytokines, such as IFNγand TNFα, in TIL. Statistical significance was determined with Mann-Whitney non-parametric t test.

Summary

- **We have generated a high affinity mouse MAb 67-2 that significantly reduces tumor growth in mice.**
- **The antibody has demonstrated anti-angiogenic activity in a highly vascularized neuroendocrine tumor model.**
- **The antibody also alters the number and localization of tumor infiltrating macrophage and lymphocytes, promoting an anti-tumor immune microenvironment and resulting in delayed tumor growth in an immunogenic tumor model.**
- **SEMA4D was first described as a repulsive axon guidance molecule during development. It is shown here to also inhibit immune infiltration of tumor stroma. Antibody blockade of SEMA4D reverses this effect.**

- **These studies, in conjunction with immunohistology, PK/PD, and toxicology studies in rat and cynomolgus monkeys supported a phase 1 clinical study to evaluate the safety and tolerability of VX15/2503 in adult patients with advanced solid tumors. This study is ongoing and results of the trial will be reported elsewhere.**