Antibody blockade of semaphorin 4D promotes infiltration of activated tumor infiltrating leukocytes and reverses tumor growth. #278

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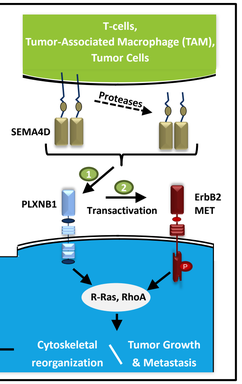
Introduction

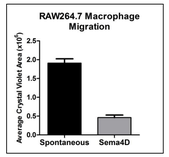
Semaphorin 4D (SEMA4D, CD100) is broadly expressed in cancer, including breast, pancreatic, colon, ovarian, urogenital and sarcoma. Expression correlates with invasive human disease. SEMA4D normally functions to regulate the motility and differentiation of multiple cell types, including those of the immune and vascular systems. In the tumor microenvironment, SEMA4D is expressed by activated inflammatory cells and regulates the infiltration, spatial distribution, and activity of antigen presenting cells and lymphocytes.

SEMA4D is a guidance molecule:

- SEMA4D/Plexin-B1 interaction inhibits movement and regulates the balance and localization of APC in tumor stroma and infiltration of tumor-specific cytotoxic T cells **(1)**.
- Crosslinking of Plexin-B1 receptor on tumor cells by SEMA4D transactivates oncogenic ErbB2 and MET membrane receptor kinases **(2).**



SEMA4D inhibits migration of APC



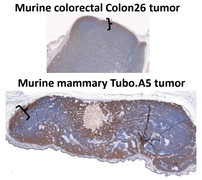
Presence of SEMA4D inhibits migration of macrophage cell line

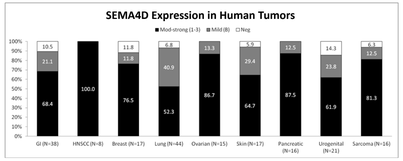
SEMA4D is a guidance molecule that inhibits movement of cells through receptor binding. rSEMA4D (1 µg/ml) added to the lower chamber inhibits spontaneous migration of macrophage cell line, RAW264.7, in a transwell assay.

SEMA4D antibodies

- A panel of mouse hybridomas were generated in Sema4D$^{-/-}$ mice to generate monoclonal antibodies that cross reacted with human, monkey, and mouse SEMA4D. A lead mouse IgG1 antibody was selected (MAb 67) and used in preclinical tumor models.
- 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 receptors: PLXNB1 (high affinity); PLXNB2 (intermediate affinity); CD72 (low affinity)
- Affinity for native human T cell SEMA4D is roughly 0.4 nM (Scatchard)
- Antibody is not generally immunosuppressive, as demonstrated in a GLP rat host resistance study

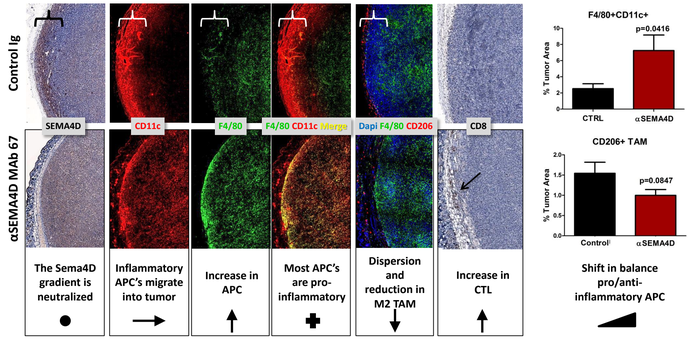
Strong SEMA4D expression at invasive tumor margin creates a barrier for immune cell penetration





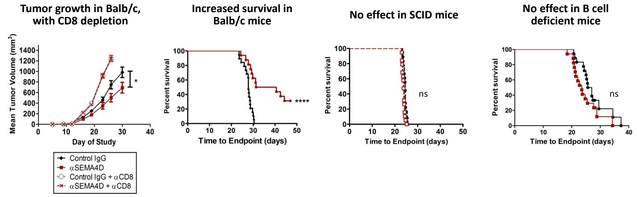
SEMA4D is expressed in many human and murine tumors. Expression of SEMA4D is especially strong at the invasive margin, as shown on low magnification images of murine tumors implanted subcutaneously in immunocompetent mice.

Hypothesis: SEMA4D regulates immune cell migration into tumor



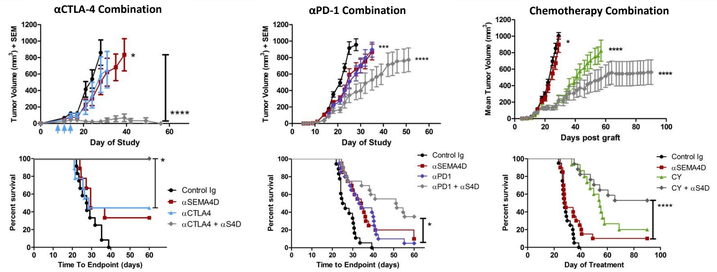
SEMA4D gradient at the leading edge of Colon26 tumor is neutralized with antibody blockade and facilitates migration of pro-inflammatory CD11c+/F4-80+ antigen presenting cells and cytotoxic T lymphocytes into the tumor, while reducing CD206+ M2 macrophage. A. Colon26 tumor-bearing mice were treated with Control Ig or anti-SEMA4D antibodies (50 mg/kg, IP weekly); tumors were harvested on day 27; FFPE sections were stained by IHC. **B.** The entire tumor section from each animal was scanned and quantitated. Overall changes in cellular densities are represented as group averages + SEM (n=10 animals).

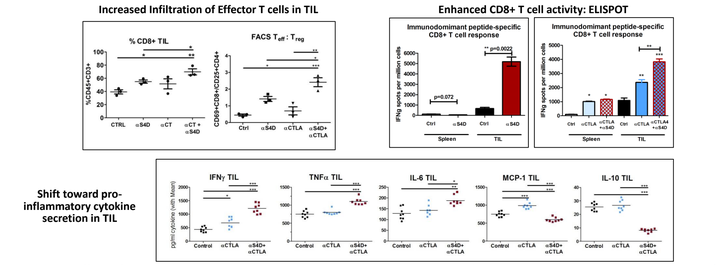
Hypothesis: α-SEMA4D antibody is immunomodulatory and affects tumor growth



Anti-SEMA4D delays tumor growth and significantly increases survival; this effect is dependent on adaptive immune response. Colon26 (500,000 cells, SC) cells were implanted into Balb/c mice and treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP), anti-CD8 / MAb 2.43 (0.15 mg IP). Mean tumor volume and Kaplan Meier survival curves are shown. * indicates level of statistical significance, ns (p >0.05).

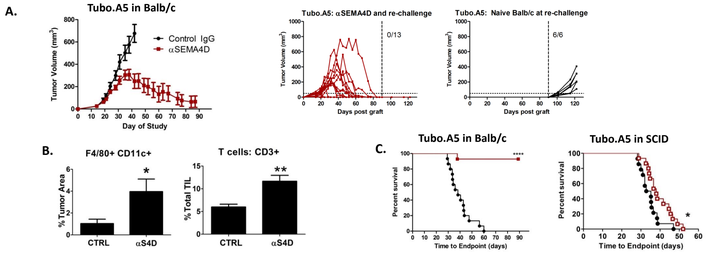
Hypothesis: increased immune infiltration facilitated by α-SEMA4D antibody enhances other immunotherapies





Immunomodulatory effects of SEMA4D blockade can enhance other immunotherapies. A. Colon26 (500,000 cells, SC) cells were implanted into Balb/c mice and treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP), αCTLA-4 / MAb UC10-4F10 (5/2.5/2.5 mg/kg), αPD-1 / MAb RMP1-14 (5 mg/kg twice/weekly), or cyclophosphamide (50 mg/kg, days 12 and 20). **B. Anti-tumor activity of αSEMA4D Ab enhances and focuses CTL activity within the TME.** Tissues from Colon26 tumor-bearing mice were harvested on day 15 for *ex vivo* analysis: ELISPOT, FACS, and CBA cytokine assay.

Hypothesis: SEMA4D blockade will be effective in PLXNB1+ ERBB2+ cancer
Hypothesis: α-SEMA4D antibody promotes immunological memory



α-SEMA4D inhibits growth of ERBB2+ PLXNB1+ mammary carcinoma as a result of both immune-dependent and immune-independent activity. Mice implanted with Tubo.A5 (30,000 cells, SC in Balb/c or 15,000 SC in SCID) were treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP). **A.** Mean tumor volume is shown (left). Mice with no measurable tumor on day 90 were re-challenged on opposite flank with viable tumor; individual tumor growth is shown (right panels). **B.** Tumors from Balb/c mice were harvested on day 39 for *ex vivo* IHC and FACS analysis . **C.** Treatment in Balb/c mice results in complete tumor regressions, while treatment in SCID mice delays growth, suggesting both immune-dependent and immune-independent activity.

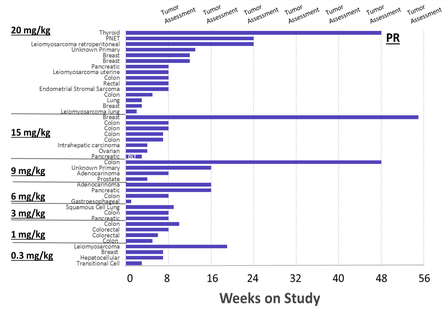
VX15/2503 Oncology Phase 1 Study

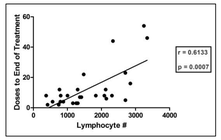
- Humanized IgG4 anti-SEMA4D MAb (VX15/2503)
- Phase I, two-center, non randomized, open-label, multiple-dose, dose-escalation in patients with advanced solid tumors
- Standard 3 + 3 Dose Escalation
- VX15/2503 Dose levels: 0.3, 1, 3, 6, 9, 15 and 20 mg/kg weekly infusion

Safety

- 460 doses administered to 42 patients
- Well tolerated up to 20 mg/kg (highest dose tested)
- No MTD was determined
- Most common TEAE's: nausea and fatigue (each < 10%)
- One DLT (15 mg/kg) - elevated GGT in setting of progressive pancreatic disease
- One neutralizing HAHA response (1 mg/kg)

VX15/2503 Progression Free Survival





- Patients with elevated immune cells in circulation at time of enrollment were progressing prior to initiation of VX15 therapy
- **Correlation of elevated lymphocyte counts with PFS following treatment with VX15/2503 is consistent with an immune-mediated mechanism of action for VX15/2503, as demonstrated in preclinical studies with MAb 67.**

Conclusions

- **SEMA4D modulates immune infiltration of tumor stroma. Antibody blockade of SEMA4D promotes infiltration and activation of an anti-tumor immune microenvironment; tumor growth inhibition and immunologic memory was observed in preclinical tumor models.**
- **Combination of anti-SEMA4D antibodies with immune checkpoint inhibitors and immunomodulatory chemotherapy improves anti-tumor immune responses and enhances tumor rejection.**
- **In Phase I trial of patients with advanced refractory solid tumors, VX15/2503 was well tolerated when administered as a weekly infusion at doses up to and including 20 mg/kg.**
- **Seven patients (Cohorts 4 – 7) with SD for ≥ 16 weeks had relatively elevated T or B cell levels at enrollment (T-cell count ≥ 1200/µl; B-cell count ≥ 250/µl)**
- **Future Phase 1a/2b clinical trials of VX15/2503 will be combination studies in selected tumor types**

1. Evans, EE et al 2015. Cancer Immunology Research, in press. http://cancerimmunolres.aacrjournals.org/content/early/2015/01/22/2326-6066.CIR-14-0171.full.pdf
2. Leonard et al. 2015. Molecular Cancer Therapies, http://mct.aacrjournals.org/content/14/4/964.full.pdf