Reprogramming myeloid cells in TME with first-in-class Semaphorin 4D Mab enhances combination immunotherapy.

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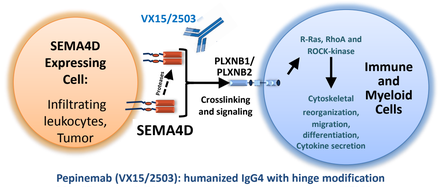
Abstract

Purpose: Tumor growth inhibition by anti-semaphorin 4D (SEMA4D, CD100) blocking antibody is enhanced when combined with various immunotherapies in preclinical animal models. Immune checkpoint combinations with pepinemab (VX15/2503), a humanized anti-SEMA4D antibody, are currently being evaluated in four clinical trials: (i) a Phase 1b/2a combination trial of pepinemab with avelumab in NSCLC (CLASSICAL-Lung) (NCT03268057); (ii) a phase 1 combination trial of pepinemab with nivolumab or ipilimumab in melanoma patients who have progressed on any anti-PD-1/PD-L1 (NCT03373188); (iii) a neoadjuvant integrated biomarker trial in patients with metastatic colorectal and pancreatic cancers treated with pepinemab in combination with nivolumab or ipilimumab (NCT03373188); and (iv) a Phase 1/2 trial of pepinemab in children with solid tumors and children and young adults with osteosarcoma (NCT03320330).

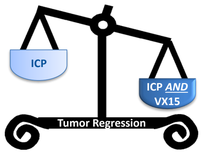
Results: SEMA4D exerts multi-faceted effects within the tumor microenvironment by creating a barrier at the tumor-stroma margin to restrict immune cell infiltration and promoting immunosuppressive activity of myeloid-derived cells. Blocking antibody to SEMA4D directly enhanced M1/M2 ratio, and both reduced expression of chemokines that recruit MDSC and the ability of MDSC to suppress T cell proliferation. Antibody blockade restores the ability of dendritic cells and cytotoxic T cells to migrate into the tumor while simultaneously reducing the function of immunosuppressive myeloid and regulatory T cells in the TME. Importantly, anti-SEMA4D Mab enhanced the activity of co-administered immunotherapies in preclinical models. For example, anti-SEMA4D plus anti-CTLA-4 resulted in 100% survival and 90% complete tumor rejection (CR) (p<0.0001) in an HNSCC model representative of a T cell inflamed tumor with high MDSC suppression. Entinostat has broad immunomodulatory effects, including reduction of MDSC suppressive function, and combination treatment of established Colon26 tumors with anti-SEMA4D and entinostat resulted in maximal tumor growth delay and 90% CR (p<0.0001).

Conclusions: SEMA4D blockade represents a novel approach to promote functional immune infiltration into the tumor, reduce mesenchymal suppression, and enhance immunotherapy effects. Pepinemab treatment was well tolerated in a Phase I trial in patients with advanced refractory solid tumors (NCT01313065, Patnaik A et al. Clin Cancer Res. 2016;22(4):827-36). Several clinical trials are in progress to evaluate safety, tolerability, efficacy, and biological endpoints, including immunophenotyping tumors and blood of patients treated with pepinemab in combination with immunomodulatory agents.



Pepinemab (VX15/2503): humanized IgG4 with hinge modification
MAb67: mouse IgG1, cross reacts with mouse and human SEMA

MOA: Antibody blockade of SEMA4D "opens the gates" to the tumor, facilitating penetration of activated immune cells, reverses myeloid suppression, and enhances activity of immunotherapy.

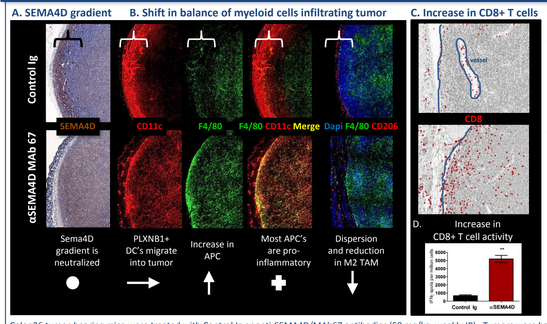


Immune Checkpoint (ICP):
- ↑ $T_{effectors}$
- ↑ Th1 cytokines: IFNg, TNFa
- ↑ CXCL9, CXCL10

Combination with anti-SEMA4D enhances effects of ICP and adds benefits of reversing mesenchymal suppression:
- ↓ M2 TAM
- ↓ MDSC
- ↓ $T_{regulatory}$
- ↓ Chemokines that recruit M2 and polarize Treg: CCL2, CXCL1, CXCL5, IL-10

1. Evans, EE et al 2015. Cancer Immunol Res. 3(6):689-701. http://cancerimmunolres.aacrjournals.org/content/early/2015/01/22/2326-6066.CIR-14-0171.full.pdf
2. Evans EE, Paris M, Smith ES & Zauderer M. 2015): OncoImmunology, DOI: 10.1080/2162402X.2015.1054599
3. Patnaik A et al. Clin Cancer Res. 2016 Feb 15;22(4):827-36., http://clincancerres.aacrjournals.org/content/22/4/827.full.pdf+html
4. Fisher et al, 2016. MAbs. 8(1): 150-162. http://www.tandfonline.com/doi/abs/10.1080/19420862.2015.1102813

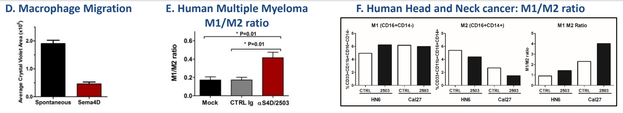
Anti-SEMA4D reverses immune suppression and regulates recruitment of myeloid cells to enhance T cell infiltration and activity within TME



- SEMA4D expression at tumor invasive margin restricts infiltration of PLXNB1+ DC into TME.
- Anti-SEMA4D promotes infiltration of pro-inflammatory CD11c+/F4-80+ APC, while reducing CD206+ M2 macrophage.
- Pro-inflammatory APC recruit and activate CD8+ T cells within TME.

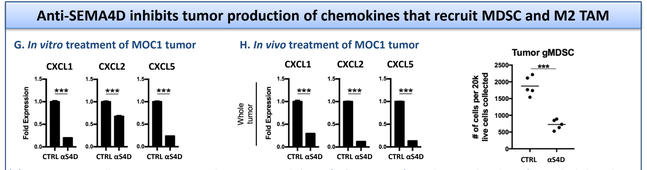
Colon26 tumor-bearing mice were treated with Control Ig or anti-SEMA4D/MAb67 antibodies (50 mg/kg, weekly IP). Tumors were harvested on day 27 and stained by IHC (A-C) or (D) tumors were dissociated, leukocytes enriched from whole tumor digests using lympholyte-M and cultured for 2-days, and T cell activity was assessed by ELISPOT against MHC-I restricted immunodominant peptide (AH-1)-pulsed targets.

SEMA4D regulates migration and polarization of tumor associated macrophage



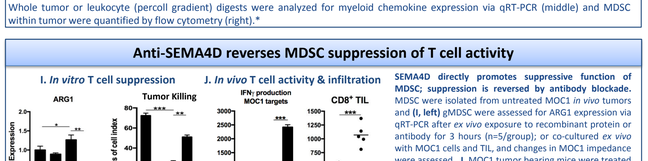
rSEMA4D inhibits spontaneous migration of mouse macrophage cell line, RAW264.7; rSEMA4D (1 μg/ml) added to the lower chamber of a transwell **(D). SEMA4D blockade increases ratio of M1/M2 when exposed to SEMA4D+ tumors.** Human PBMC were cultured with **(E)** conditioned media from co-culture of multiple myeloma RPMI 8226 with human bone marrow stroma (mock) or with **(F)** HNSCC lines HN6 and Cal27, and in presence of Control Ig or anti-SEMA4D/2503 (αS4D). M1 = $CD14^-CD16^+$ and M2 = $CD14^+CD16^+$.

Anti-SEMA4D inhibits tumor production of chemokines that recruit MDSC and M2 TAM



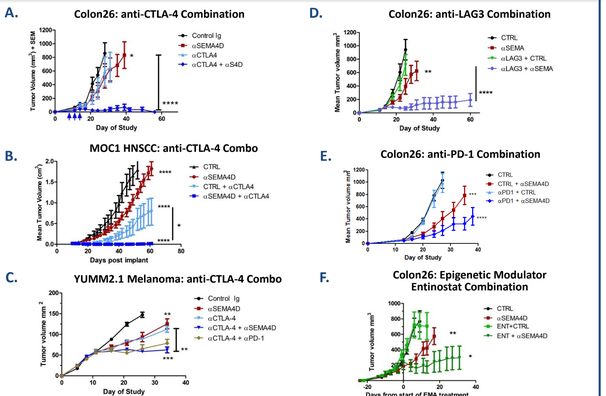
(G) HNSCC MOC1 cells *in vitro* were exposed to Sema4D mAb (10 μg/mL) or isotype for 24 hours and analyzed for myeloid chemokine expression by qRT-PCR. **(H)** Mice bearing MOC1 tumors were treated *in vivo* with isotype control or anti-Sema4D Ab (αS4D) (n=5/group). Whole tumor or leukocyte (percoll gradient) digests were analyzed for myeloid chemokine expression via qRT-PCR (middle) and MDSC within tumor were quantified by flow cytometry (right).*

Anti-SEMA4D reverses MDSC suppression of T cell activity



SEMA4D directly promotes suppressive function of MDSC; suppression is reversed by antibody blockade. MDSC were isolated from untreated MOC1 *in vivo* tumors and **(I, left)** gMDSC were assessed for ARG1 expression via qRT-PCR after *ex vivo* exposure to recombinant protein or antibody for 3 hours (n=5/group); or co-cultured *ex vivo* with MOC1 cells and TIL, and changes in MOC1 impedance were assessed. **J.** MOC1 tumor bearing mice were treated as in H. Tumor digests (n=5/group) were subjected to a percoll gradient, and leukocytes were analyzed for T cell markers by flow cytometry. T-lymphocytes were isolated from tumor draining LNs by negative magnetic selection, combined with irradiated MOC1 cells, and supernatant IFNγ (pg/ml) was measured by ELISA.

Combination Immunotherapy – Preclinical Models



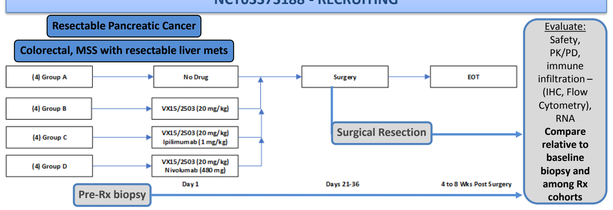
Immunomodulatory effects of SEMA4D blockade can enhance other immunotherapies. A, D, E. Colon26 (500,000 cells) were subcutaneously implanted into Balb/c mice, that were then treated with αSEMA4D / MAb67 (10 mg/kg, weekly IP X4), αLAG3/C9B7W (10 mg/kg 2x/week X4; n=20)*; αCTLA-4 / MAb UC10-4F10 (100/50/50 μg, q3 days; n=20), αPD-1 / MAb RMP1-14 (10 mg/kg, twice/week, n=20), **B. MOC1 HNSCC** ($5x10^6$ cells) were subcutaneously implanted into C57Bl/6 mice, that were then treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP), αCTLA-4 / MAb 9H-10 (5 mg/kg, q5D); n=10**. **C. YUMM2.1 melanoma*** ** were implanted into C57Bl/6 mice and treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP), αCTLA-4 / MAb UC10-4F10 (5 mg/kg 2x/wk X3 doses), αPD-1 / MAb RMP1-14 (10 mg/kg 3x/week); n=8. **F. Treatment of established tumors with Entinostat** (ENT, 20 mg/kg 3x/wk, at TV ~$250mm^3$, n=20).

Neoadjuvant "Window of Opportunity" IO Clinical Trial

Pepinemab (VX15/2503) Combo with Anti-PD-1 or with Anti-CTLA-4 Colorectal Cancer with metastasis to liver & Resectable Pancreatic Cancer

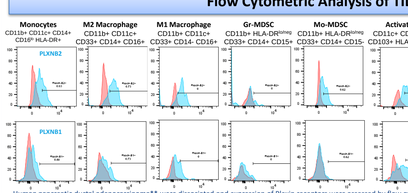
Integrated biomarker trial, Winship Cancer Institute of Emory University (Wu & Lesinski)

NCT03373188 - RECRUITING



Evaluate: Safety, PK/PD, immune infiltration – (IHC, Flow Cytometry), RNA **Compare relative to baseline biopsy and among Rx cohorts**

Flow Cytometric Analysis of TIL



Human pancreatic ductal adenocarcinoma** were dissociated and expression of Plexin receptors were assessed by flow cytometry.

Additional TIL evaluation includes:
- target (SEMA4D) saturation
- CD3+ CD4+/CD8+ T cells
- CD19+ B cells
- CD3+/- CD56+ NK-T and NK
- CD3+ CD4/8+ HLA-DR+ CD38+ Ki67+ stem-like PD-1 responsive T cell population
  - PD1/Tim3, CD26 expression
- Treg: CD3+ CD4+ CD25+ GITR+ CD127- T cells;
  - Foxp3 & SEMA4D
- SEMA4D, Plexin B1/B2, and PD-L1 expression on all cells

Pepinemab (VX15/2503) Combination and Immunotherapy Clinical Trials

CLASSICAL-Lung: pepinemab (VX15/2503) with avelumab (anti-PD-L1)

Vaccinex IND in collaboration with EMD Serono/Merck KGaA , Phase 1b/2 Combination Trial

NCT03268057 - RECRUITING

Pepinemab (VX15/2503) in combination with avelumab (anti-PD-L1)

	DOSE ESCALATION PHASE	EXPANSION PHASE
NSCLC immunotherapy naïve	To determine the recommended Phase 2 dose of pepinemab, up to 20 mg/kg, Q2W with avelumab, 10 mg/kg, Q2W (n=3-6/cohort)	Patients will be stratified but unselected for PD-L1; pre- and post-treatment biopsies mandatory
NSCLC Progressed following immunotherapy	*28-days for each escalation phase* ***COMPLETE***	*Up to 28 patients at recommended dose*

Co-funded by: MERCK

- Study to enroll up to ~62 subjects with advanced NSCLC
- Treatment of up to 6 subjects (3 + 3 design) in each of three VX15/2503 (pepinemab) dose levels (5, 10 and 20 mg/kg)
- A fixed standard dose of avelumab will be employed

Evaluate: Safety, PK/PD, clinical activity (ORR, DoR, PFS) and biomarkers including immune infiltration in tumor biopsies

VINO: pepinemab (VX15/2503) Combination with nivolumab or ipilimumab in anti-PD-1/PD-L1-refractory melanoma

UCLA (Ribas, Hu-Lieskovan) IND, Phase 1 Combination Trial

NCT03425461 - RECRUITING

Pepinemab (VX15/2503) in combination with nivolumab (anti-PD-1) or ipilimumab (anti-CTLA-4)

	DOSE ESCALATION PHASE	EXPANSION PHASE
pepinemab (VX15/2503) + nivolumab	Dose escalation of VX15 (pepinemab) from 10 to 20 mg/kg with nivolumab 480 mg Q4W or with ipilimumab 3 mg/kg Q3W x4 (n=3-6/cohort)	Repeat up to 12 months pre- and post-treatment biopsies mandatory 18 patients/cohort
pepinemab (VX15/2503) + ipilimumab		

- Randomized Phase 1 study to enroll up to 60 patients with advanced (stage III or IV) melanoma who have progressed on anti-PD-1/L1 based checkpoint inhibitors

Evaluate: Safety, PK/PD, clinical activity (ORR, DoR, PFS) and biomarkers including immune infiltration in tumor biopsies

Phase 1 Evaluation of Safety, Tolerability, PK & PD of Intravenous VX15/2503 in Patients With Advanced Solid Tumors

NCT013130: COMPLETE. Patnaik et al.

- **Humanized IgG4 anti-SEMA4D MAb (pepinemab, 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: 0.3-20 mg/kg; weekly infusions.
- **Safety**: **Well tolerated** up to 20 mg/kg (highest dose tested). No MTD was determined. Most common TEAE's were low grade (1 and 2): nausea, fatigue, arthralgia, decreased appetite (each 3 - 5%)
- **Disease Control**: Nineteen patients (45.2%) exhibited stable disease for ≥8 weeks. Of these, 8 patients (19%) had stable disease for ≥16 weeks, and **3 patients had stable disease for 48-55 weeks, 1 PR**.

A Phase 1/2 Trial of VX15/2503 in Children, Adolescents, or Young Adults With Recurrent or Relapsed Solid Tumors****

NCT03320330: RECRUITING

PRIMARY OBJECTIVES: I. To estimate the MTD and/or RP2D of VX15/2503 to children with recurrent or refractory solid tumors. (Part A) II. To define toxicities and III. PK. (Parts A-B) IV. To preliminarily define the antitumor activity of VX15/2503 for the treatment of **relapsed or refractory osteosarcoma**. (Part B)
SECONDARY OBJECTIVES: PD & immunogenicity of VX15/2503 in pediatric patients