Reprogramming suppressive myeloid cells in tumor microenvironment with first-in-class Semaphorin 4D Mab enhances combination immunotherapy

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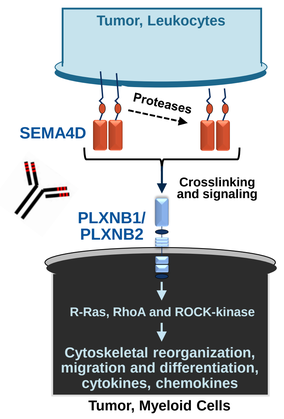
Semaphorin 4D/Plexin signaling to myeloid and tumor cells

Semaphorins and cognate receptors are overexpressed in many malignancies and reported to be associated with poor prognosis. Semaphorin 4D (SEMA4D, CD100) and its receptors are expressed on precursor cells, including those of immune, neural and vascular systems, as well as tumor cells. Myeloid precursor cells are immunosuppressive within the TME.

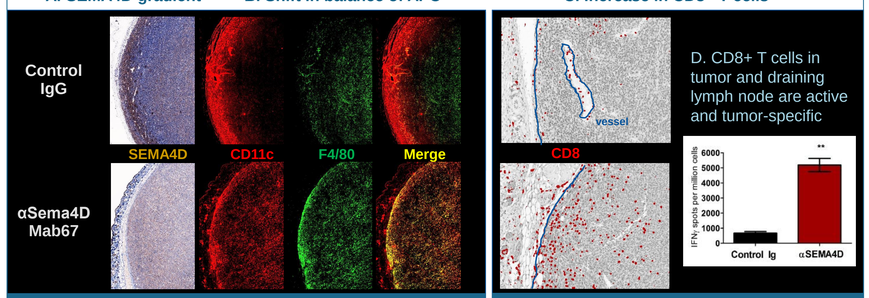
- SEMA4D signals through PLXNB1 and PLXNB2 receptors to regulate (1) cell cytoskeleton (2) cytokine synthesis and secretion
- **In TME, SEMA4D inhibits migration and promotes immunosuppressive functions of receptor-positive myeloid cells.**

- **Anti-SEMA4D antibody blocks binding to its receptor and signaling activity to**
- **Promote infiltration of potent APC and T cells**
- **Inhibit differentiation/function of MDSC, M2 TAM and Treg**

- Pepinemab (VX15/2503): humanized IgG4 with hinge modification
- MAb67: mouse IgG1, cross reacts with mouse and human SEMA4D
  - MAbs do NOT deplete immune cells *in vivo* and do NOT generally affect immune responses in the periphery.



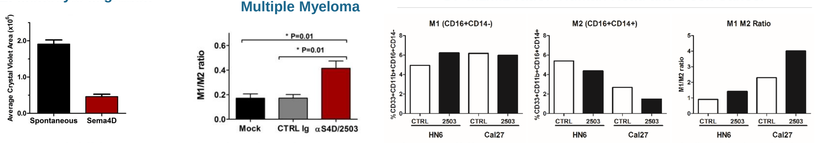
Anti-SEMA4D reverses immune suppression and regulates recruitment of myeloid cells to enhance T cell infiltration and activity within TME



- SEMA4D expression at tumor invasive margin restricts infiltration of PLXNB1+/PLXNB2+ DC into TME.
- Anti-SEMA4D promotes infiltration of pro-inflammatory CD11c+/F480+ APC, while reducing CD206+ M2 TAM, MDSC, CCL2, CXCL1/5
- Pro-inflammatory APC recruit and activate CD8+ T cells within tumor microenvironment.
- Coordinated increase in Th1 cytokines - IFNγ, TNFα
- Increase in T cell recruiting CXCL9, CXCL10

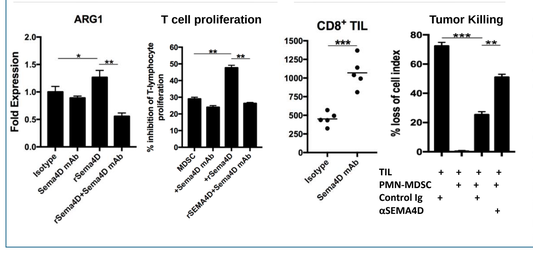
Colon26 tumor-bearing mice were treated with Control Ig or anti-SEMA4D/MAb67 antibodies (50 mg/kg, weekly IP). Tumors were harvested on day 27 and stained by IHC (A-C), or (D) tumors were dissociated, leukocytes enriched from whole tumor digests using lympholyte-M and cultured for 2-days, and T cell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

SEMA4D regulates migration and polarization of tumor associated macrophage



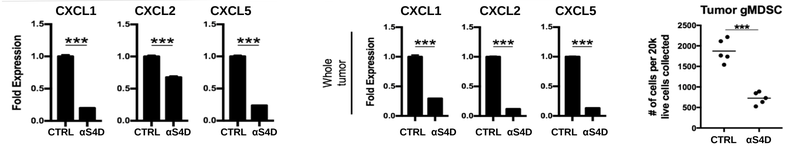
rSEMA4D inhibits spontaneous migration of mouse macrophage cell line, RAW264.7; rSEMA4D (1 µg/ml) added to the lower chamber of a transwell (E). **SEMA4D blockade increases ratio of M1/M2 when exposed to SEMA4D+ tumors.** Human PBMC were cultured with (F) conditioned media from co-culture of multiple myeloma RPMI 8226 with human bone marrow stroma (mock) or with (G) 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 reverses MDSC suppression of T cell activity



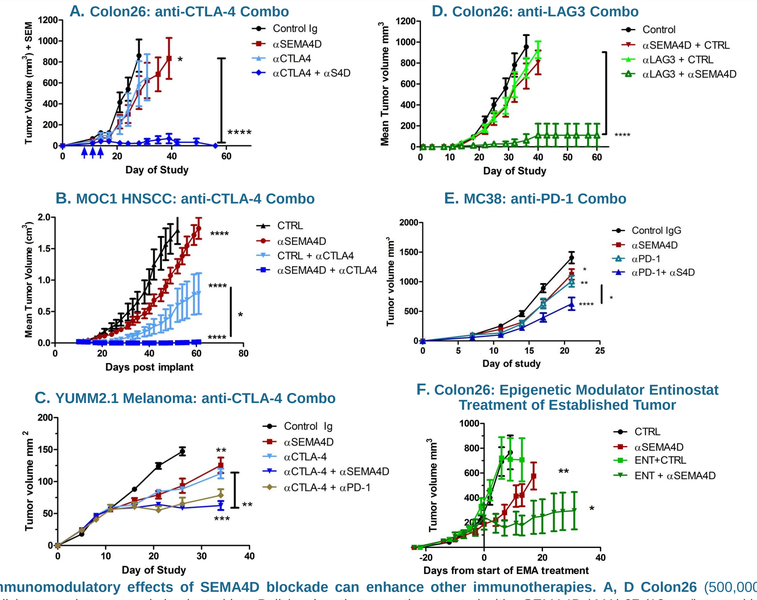
SEMA4D directly promotes suppressive function of MDSC; suppression is reversed by antibody blockade. H. MDSC were isolated from untreated HNSCC MOC1 *in vivo* tumors and co-cultured *ex vivo* with rSEMA4D or antibodies (10 ug/ml), in presence of naïve T cells labeled with CFSE in a T cell suppression assay (right); gMDSC were assessed for ARG1 expression via qRT-PCR after ex vivo exposure for 3 hours (left). **I.** Mice bearing MOC1 tumors were treated *in vivo* with Sema4D mAb or isotype control (0.2 mg weekly, n=5/group), tumors were subjected to a percoll gradient, and CD8+ T cells were analyzed by flow cytometry. TIL were isolated and co-cultured with MOC1 tumor cells in the presence or absence of Ly6G^{hi}Ly6C^{low} myeloid cells, and changes in MOC1 cells impedance were assessed in real-time.*

Anti-SEMA4D inhibits tumor production of chemokines that recruit MDSC



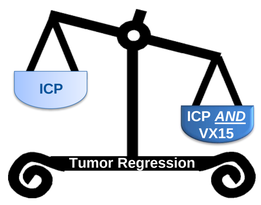
(J) HNSCC MOC1 cell cultures were exposed *in vitro* to Sema4D mAb (10 µg/mL) or isotype control for 24 hours and analyzed for expression of CXCR2-binding chemokines by qRT-PCR. **(K)** Mice bearing MOC1 tumors were treated *in vivo* with isotype control or anti-Sema4D Ab (αS4D) (n=5/group). Whole tumor or leukocyte (percoll gradient) digests were analyzed for chemokine expression via qRT-PCR (middle) and MDSC within tumor were quantified by flow cytometry (right).*

Rational Combinations:
Anti-SEMA4D increases immune infiltration & reduces suppression
Immune Checkpoint sustains T cell activity



Immunomodulatory effects of SEMA4D blockade can enhance other immunotherapies. A, D 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 (10 mg/kg 2x/week X4; n=10)*; αCTLA-4 / MAb UC10-4F10 (100/50/50 µg, q3 days; 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. **E. MC38***** (300,000 cells) were subcutaneously implanted into C57Bl/6 mice, treated with αSEMA4D/MAb67 and αPD-1 / MAb RMP1-14 (10 mg/kg, twice/week), n=8. **F. Treatment of established tumors with Entinostat** (ENT, 20 mg/kg 3x/wk, at TV ~250mm^3, n=20).

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 inhibitors (ICP) or anti-SEMA4D:
- ↑ $T_{effectors}$
- ↑ 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

Pepinemab (VX15/2503) Phase I Clinical Trials: pepinemab is well tolerated

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

NCT013130: COMPLETE. Patnaik et al.

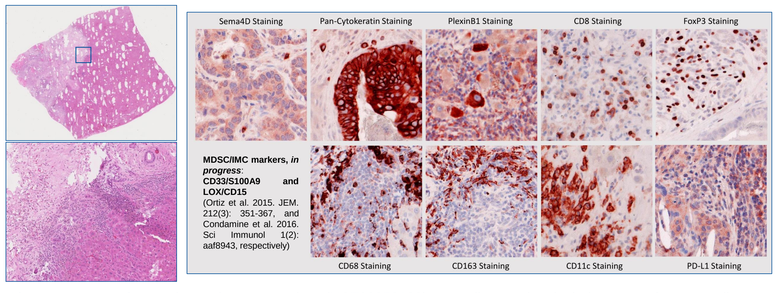
- **Humanized IgG4 anti-SEMA4D MAb (pepinemab, VX15/2503)**
- Phase I, two-center, non randomized, open-label, multiple-dose, dose-escalation in patients with advanced solid tumors. Standard 3+3 Dose escalation: 0.3-20 mg/kg; weekly infusions.
- **Safety**: **Well tolerated** up to 20 mg/kg (highest 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 partial response (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

Biomarker Analysis of Clinical Samples: Multiplex IHC



Liver metastases from colorectal cancer patient (NCT03373188) were dissected to include areas of tumor, adjacent normal, and tumor margin (H/E). Sections were assessed for various cell types using serial stains on the same section to allow multiplex IHC and colocalization of markers for various immune cell subsets.
This will also allow evaluation of spatial and cell-specific expression of SEMA4D and its cognate receptors.

Pepinemab (VX15/2503) Combination and Immunotherapy Clinical Trials in Progress

CLASSICAL-Lung: pepinemab (VX15/2503) with 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 **NSCLC** Progressed following immunotherapy	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) *28 days for each escalation phase* ***COMPLETE***	Patients will be stratified but unselected for PD-L1; pre- and post-treatment biopsies mandatory *Up to 28 patients with pepinemab + avelumab, 10 mg/kg Q2W*

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) with Anti-PD-1 or with Anti-CTLA-4 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 **pepinemab (VX15/2503) + ipilimumab**	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

- 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

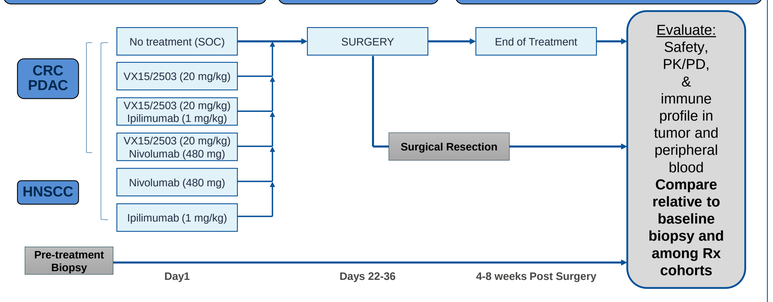
Neoadjuvant "Window of Opportunity" IO Clinical Trials

Pepinemab (VX15/2503) with Anti-PD-1 or with Anti-CTLA-4 Colorectal Cancer with metastasis to liver & Pancreatic Cancer, Head and Neck Cancer#

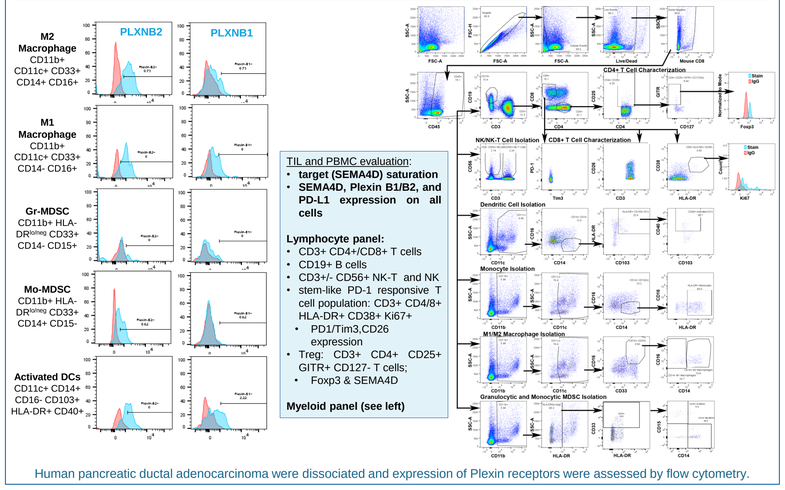
Integrated biomarker trial, Winship Cancer Institute of Emory University ** (Lesinski, Wu and Steuer#)

NCT03373188 – RECRUITING, NCT03690986# - OPEN

Colorectal Cancer	Pancreatic Cancer	Head and Neck squamous cell cancer*
MSS, stage IV with resectable liver mets, n=4	Resectable,Stage I-III, n=4	Resectable Stage I-IVA, n=6



Biomarker Analysis of Clinical Samples: Flow Cytometric Analysis of TIL



TIL and PBMC evaluation:
- **target (SEMA4D) saturation**
- **SEMA4D, Plexin B1/B2, and PD-L1 expression on all cells**

Lymphocyte panel:
- CD3+ CD4+/CD8+ T cells
- CD19+ B cells
- CD3+/- CD56+ NK-T and NK
- stem-like PD-1 responsive T cell population: CD3+ CD4/8+ HLA-DR+ CD38+ Ki67+
  - PD1/Tim3,CD26 expression
- Treg: CD3+ CD4+ CD25+ GITR+ CD127- T cells;
  - Foxp3 & SEMA4D

Myeloid panel (see left)

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