Altered myeloid & lymphoid composition of tumor microenvironment following anti-SEMA4D and immunotherapies

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Summary

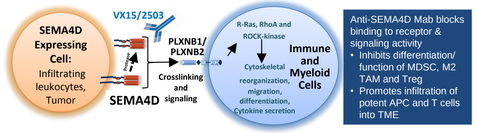
Anti-semaphorin 4D (SEMA4D, CD100) blocking antibody promotes immune infiltration, reduces immunosuppressive myeloid cells, and enhances T cell activity and tumor growth inhibition in combination with various immunotherapies in preclinical animal models. SEMA4D represents a novel target to regulate immune infiltration and mesenchymal suppression, sources of resistance to current immunotherapies.

PRECLINICAL MECHANISM OF ACTION STUDIES: Blocking antibody to SEMA4D directly enhanced M1/M2 ratio and reduced both expression of chemokines that recruit MDSC and the ability of MDSC to suppress T cell activity. Antibody blockade simultaneously restored the ability of dendritic cells and cytotoxic T cells to infiltrate the TME, increasing ratio of Teffector to Tregulatory cells, in syngeneic tumor models.

PRECLINICAL COMBINATION THERAPIES: anti-SEMA4D MAb enhanced the activity of co-administered immunotherapies, including antibodies to PD1, CTLA-4, and LAG3, and epigenetic modulators including entinostat.

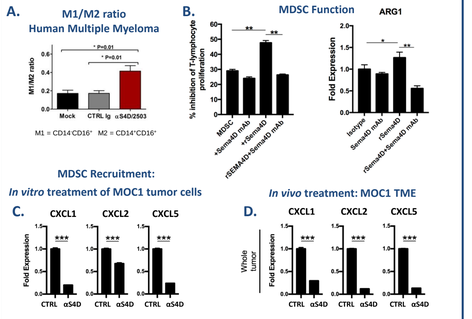
TRANSLATIONAL BIOMARKER ANALYSIS: Methods to assess immune phenotype and biomarkers in translational and clinical studies include flow cytometry and whole slide scans of multiplex IHC panels to examine MDSC, M1/M2 macrophage, monocytes, activated DC, B cells, exhausted, activated and stem-like populations of T cells in clinical samples.

CLINICAL TRANSLATION: Clinical trials of immune checkpoint inhibitors (ICI) in combination with pepinemab (VX15/2503), humanized anti-SEMA4D antibody, are currently underway in several cancer indications. Pepinemab treatment was well tolerated in a Phase I oncology trial (NCT01313065) and is currently being evaluated as single agent or in ICI combinations in: (i) a Phase 1b/2a combination trial of pepinemab with avelumab in ICI naive or ICI refractory 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 (NCT03425461); (iii) a neoadjuvant integrated biomarker trial in patients with metastatic colorectal, pancreatic (NCT03373188) and head and neck (NCT03690986) cancers treated with pepinemab in combination with nivolumab or ipilimumab; and (iv) a Phase 1/2 trial of pepinemab in children with solid tumors and children and young adults with osteosarcoma (NCT03320330). Clinical trials will evaluate safety, tolerability, efficacy, and biological endpoints, including immunophenotyping tumors and blood.



Anti-SEMA4D Mab blocks binding to receptor & signaling activity
- Inhibits differentiation/ function of MDSC, M2 TAM and Treg
- Promotes infiltration of potent APC and T cells into TME

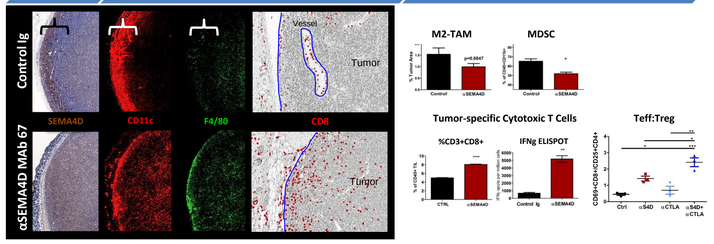
PRECLINICAL: SEMA4D blockade reverses myeloid suppression



SEMA4D blockade increases ratio of M1/M2 when exposed to SEMA4D+ tumors. (A) Human PBMC were cultured with conditioned media from co-culture of multiple myeloma RPMI 8226 with human bone marrow stroma (mock) or with Control Ig or anti-SEMA4D/2503 (α54D).
SEMA4D directly promotes function of myeloid derived suppressor cells (MDSC); suppression is reversed by antibody blockade. (B) MDSC were isolated from HNSCC MOC1 *in vivo* tumors and co-cultured *ex vivo* with rSEMA4D (10 μg/ml) or antibodies, in presence of naïve T cells labeled with CFSE in a T cell suppression assay (right panel). gMDSC were assessed for ARG1 expression via qRT-PCR after *ex vivo* exposure to recombinant protein or antibody for 3 hours (n=5/group). Arginase production in TME suppresses T cell function (right panel) **(C)** 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. **(D)** Mice bearing MOC1 tumors were treated *in vivo* with isotype control or anti-Sema4D Ab (α54D) (n=5/group). Whole tumor digests were analyzed for myeloid chemokine expression via qRT-PCR.

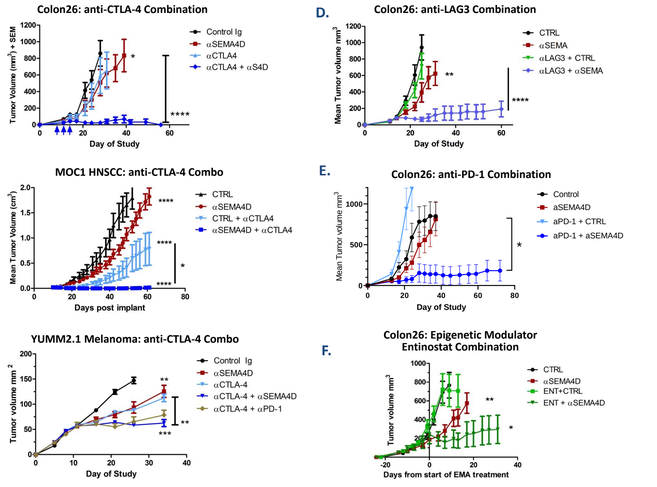
PRECLINICAL: Anti-SEMA4D Mab neutralizes SEMA4D barrier at tumor margin and shifts the balance of tumor immunity

A. Sema4D gradient → B. Increased infiltration of pro-inflammatory APC and CD8+ T cells → C. Shift in balance of tumor immunity in TME



SEMA4D is strongly expressed at the invasive margin of tumors. Antibody blockade of SEMA4D facilitates migration of APCs and T cells into the tumor. (A) SEMA4D expression at invasive margin of murine Colon26 tumors restricts infiltration of PLXNB1+ DC into TME. Brackets indicate area of SEMA4D gradient. (B) Anti-SEMA4D MAb promotes infiltration of pro-inflammatory CD11c+/F4-80+ antigen presenting cells, 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) and tumors were harvested on day 27 and FFPE sections were stained by IHC or tumors were dissociated and assessed for immune cell markers by flow cytometry. Leukocytes were enriched from whole tumor digests using lympholyte-M and cultured for 2-days and supernatants were assessed for T cell activity by ELISPOT (C), n=8-12 mice/group.

PRECLINICAL: Combination Immunotherapy



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, starting on day10, 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 ~250mm³, n=20).

CLASSICAL-Lung Phase 1/2b Trial: Combination with Avelumab

This phase 1b/2, open label, single arm, first-in-human combination study is designed to evaluate the safety, tolerability and efficacy of pepinemab in combination with avelumab in 62 subjects with advanced (IIIB/IV) NSCLC.

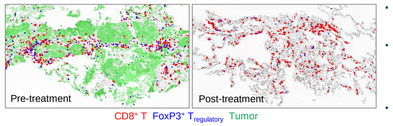
Phase 1b Dose Escalation (n=12): 5 mg/kg VX15 (n=3) → 10 mg/kg VX15 (n=6) → 20 mg/kg VX15 (n=3); + 10 mg/kg Avelumab Q2W — *Recruitment Closed*

Phase 2 Dose Expansion (n=50):
- IO Failure: 10 mg/kg VX15 (n=28); + 10 mg/kg Avelumab Q2W — *Recruitment Ongoing*
- IO Naïve: 10 mg/kg VX15 (n=22) — *Recruitment Ongoing*

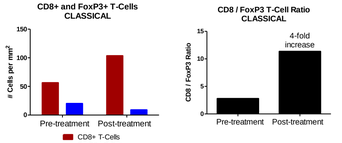
See POSTER #CT086

Study Objectives
- The primary objective of dose escalation is safety, tolerability, and identification of the RP2D for dose expansion.
- Secondary objectives include evaluation of efficacy, immunogenicity, and PK/PD, and an exploratory objective is to identify candidate biomarkers of activity
- Pre and post treatment biopsies are collected when possible

Exploratory Biomarker Analysis: shift in balance of CD8:Treg



- **Pre-treatment tissue shows infiltrating malignant cells (green), stained with pan-cytokeratin.**
- **Post-treatment biopsy demonstrates reactive alveolar parenchyma and inflammation. According to pathology report, "No evidence of tumor."**
- **CD8+ to FoxP3 T cell ratio increases in the tumor microenvironment post treatment**



Tumor IHC samples are from one subject and represent core biopsies that were isolated from the same lung lesion both at 2 days pre-treatment and 33 days post-treatment with pepinemab + avelumab. This patient was a PR according to RECIST, with a ~46% lesion decrease at first scan (day 50) and overall lesion shrinkage at every scan after that.
5 micron FFPE sections were stained sequentially with Hematoxylin, pan-cytokeratin, CD8 and FoxP3. Images were taken at 10x magnification with CD8 (red) and FoxP3 (blue) overlays of pre and post treatment biopsies. Scans were co-registered for each stain; total number of CD8+ and FoxP3+ cells were quantified from entire section and normalized by sample area using Visiopharm software.

Integrated Biomarker Window of Opportunity Study: MSS CRC with resectable liver metastases, PANC, HSNCC, Melanoma

See POSTER #CT016

Resectable melanoma (n=36)	Resectable HNSCC (n=36)	Resectable PDAC (n=16)
Total enrolled: 8	**Total enrolled: 3**	CRC resectable liver mets (n=16) **Total enrolled: 3**
No treatment (SOC)	VX15/2503 (20 mg/kg) Ipilimumab (1 mg/kg)	No treatment (SOC)
VX15/2503 (15 mg/kg)	VX15/2503 (20 mg/kg) Nivolumab (480 mg)	VX15/2503 (20 mg/kg)
VX15/2503 (15 mg/kg) Ipilimumab (3 mg/kg)	Nivolumab (480 mg)	VX15/2503 (20 mg/kg) Ipilimumab (1 mg/kg)
VX15/2503 (20 mg/kg) Nivolumab (480 mg)	Ipilimumab (1 mg/kg)	VX15/2503 (20 mg/kg) Nivolumab (480 mg)
VX15/2503 (15 mg/kg) Nivolumab (3 mg/kg) Ipilimumab (1 mg/kg)		
2 Doses	1 Dose	1 Dose

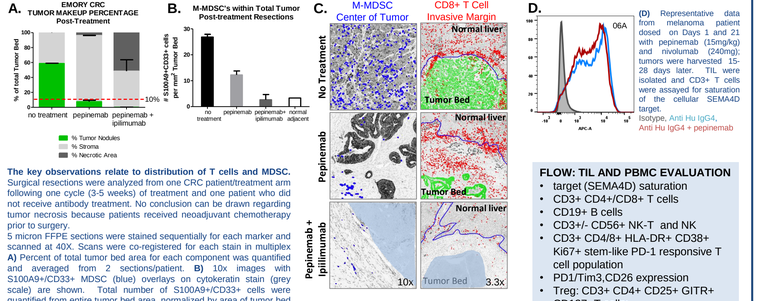
Pre-treatment Biopsy → Surgical Resection

EVALUATE — Safety: PK/PD — Biomarker Analysis:
- Compare relative to baseline biopsy and among Rx cohorts
- immune profile in tumor and peripheral blood

** Total enrolled, as of March 15, 2019.*

Exploratory Biomarker Analysis: Immune infiltration and drug penetration in TME

Shift in balance of infiltrating CD8+ T cells and MDSC | **T cells in TME are saturated with VX15**



The key observations relate to distribution of T cells and MDSC. Surgical resections were analyzed from one CRC patient/treatment arm following one cycle (3-5 weeks) of treatment and one patient who did not receive antibody treatment. No conclusion can be drawn regarding tumor necrosis because patients received neoadjuvant chemotherapy prior to surgery.
5 micron FFPE sections were stained sequentially for each marker and scanned at 40X. Scans were co-registered for each stain in multiplex **A)** Percent of total tumor bed area for each component was quantified and averaged from 2 sections/patient. **B)** 10x images with S100A9+/CD33+ MDSC (blue) overlays on cytokeratin stain (grey scale) are shown. Total number of S100A9+/CD33+ cells were quantified from entire tumor bed area, normalized by area of tumor bed using Visiopharm software, and 2 sections/patient were averaged in bar graphs. **C)** CD8+ T cells (red) overlays on cytokeratin stain (black/white) at tumor/normal liver margin are shown (3.3x).

(D) Representative data from melanoma patient dosed on Days 1 and 21 with pepinemab (15mg/kg) and nivolumab (240mg), tumors were harvested 15-28 days later. TIL were isolated and CD3+ T cells were assayed for saturation of the cellular SEMA4D target.
Isotype, Anti Hu IgG4, Anti Hu IgG4 + pepinemab

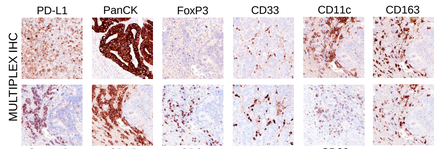
FLOW: TIL AND PBMC EVALUATION
- target (SEMA4D) saturation
- CD3+ CD4+/CD8+ T cells
- CD19+ B cells
- CD3+/- CD56+ NK-T and NK
- CD3+ CD4/8+ HLA-DR+ CD38+ Ki67+ stem-like PD-1 responsive T cell population
- PD1/Tim3,CD26 expression
- Treg: CD3+ CD4+ CD25+ GITR+ CD127- T cells;
- Foxp3 & SEMA4D
- SEMA4D,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

Melanoma – PD-1 or PD-L1 Refractory Ph 1/2b Trial: Combination with Ipilimumab or nivolumab

Phase 1b Dose Escalation (n=up to 30): 5 mg/kg VX15 Q2W (n=3) → 10 mg/kg VX15 Q2W (n=6); with nivolumab 240 mg, Q2W or ipilimumab 1 mg/kg, Q6W x4 — *Recruitment Ongoing*

Phase 2 Dose Expansion (n=36):
- VX15/2503, RP2D, Q2W + nivolumab, 240 mg, Q2W
- VX15/2503, RP2D, Q2W + ipilimumab, 1 mg/kg, Q6W x4

TRANSATIONAL Biomarker analysis: Serial Multiplex IHC



Evaluation of spatial and cell-specific expression of SEMA4D and its cognate receptors: Liver metastases from colorectal cancer patient (NCT03373188) was assessed for various cell types using sequential serial stains on the same section to allow multiplex IHC and colocalization of markers for various immune cell subsets.

Similar analysis will be applied to clinical biopsies from all trials.

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