Integrated biomarker trials to evaluate myeloid and lymphoid composition of HNSCC and solid tumors treated with pepinemab and combinations with checkpoint inhibitors

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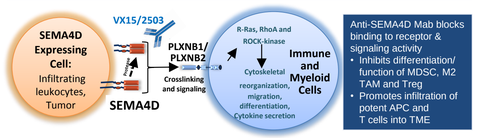
Summary

Anti-semaphorin 4D (SEMA4D, CD100) blocking antibody promotes immune infiltration, Recruitment of immunosuppressive myeloid cells into the tumor microenvironment (TME) is a critical limitation to the efficacy of immune checkpoint inhibitors (ICIs) in patients with head and neck squamous cell carcinoma (HNSCC). In preclinical models, antibody blockade of Semaphorin 4D (SEMA4D, CD100) reduced function and recruitment of immunosuppressive myeloid cells within the TME. Importantly, combinations of anti-SEMA4D with ICIs enhanced T cell activity and tumor regression. Pepinemab, an IgG4 humanized monoclonal antibody targeting SEMA4D, is currently being evaluated in window of opportunity, integrated biomarker trials to characterize immunomodulatory effects of treatment.

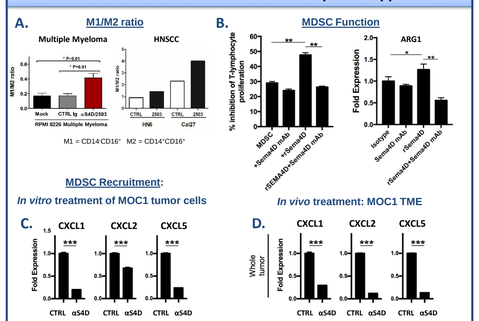
SEMA4D exerts multi-faceted effects within the tumor microenvironment by creating a barrier at the tumor-stroma margin restricting immune cell infiltration and promoting immunosuppressive activity of myeloid-derived cells. In preclinical in vitro and in vivo studies, blocking antibody to SEMA4D directly enhanced M1/M2 ratio and reduced both expression of chemokines that recruit MDSC and the ability of MDSC to suppress T cell activity. SEMA4D antibody treatment simultaneously restored the ability of dendritic cells and cytotoxic T cells to infiltrate the TME and increased ratio of Teffector to Tregulatory cells. This coordinated shift from immunosuppression to tumoricidal activity complemented effects of other immunotherapies in syngeneic tumor models.

At present, three biomarker-driven window of opportunity trials are recruiting patients with four resectable indications to investigate novel combinations of pepinemab with ICIs; 1) HNSCC (NCT03690986, n=36), 2) pancreatic ductal adenocarcinoma and colorectal cancer with resectable liver metastases (NCT03373188, n=32), and 3) metastatic melanoma (NCT03769155, n=36). Presurgical treatment cohorts include combinations of pepinemab with nivolumab and /or ipilimumab, single agents, or no treatment. Three to seven weeks later, surgically resected tumors are collected under the guidance of a pathologist for comparison of tumor infiltrates across treatment groups and with a pre-dose tissue biopsy. Blood is collected for PK, PD, and additional correlative biomarker assessments. Correlative multiplex flow cytometric and immunohistochemistry panels have been established to phenotype cells in the TME and periphery; preliminary biomarker analysis will be presented.



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



MDSC Recruitment:

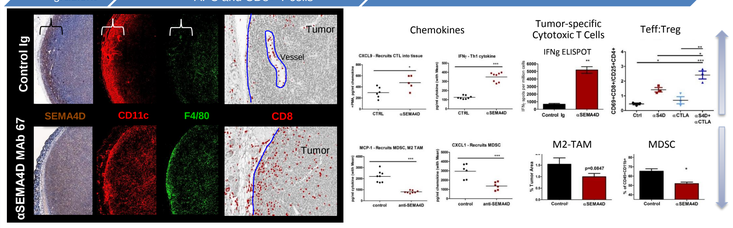
In vitro treatment of MOC1 tumor cells

In vivo treatment: MOC1 TME

SEMA4D blockade increases ratio of M1/M2 when exposed to SEMA4D+ tumors. (A) Human PBMC were cultured with conditioned tumor media from co-culture of multiple myeloma RPMI 8226 with human bone marrow stroma (mock), HNSCC tumors HN6 or Cal27, and incubated with Control Ig or anti-SEMA4D/2503 (αS4D) for 24 hours. M1 and M2 were determined by flow cytometry.
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)
SEMA4D Mab inhibits cytokines that recruit MDSC (C) MOC1 tumor cells cultured *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 (αS4D) (n=5/group). Whole tumor digests were analyzed for myeloid chemokine expression via qRT-PCR.

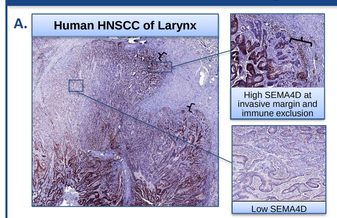
PRECLINICAL: Anti-SEMA4D Mab neutralizes SEMA4D barrier at tumor margin and shifts the balance of tumor immunity

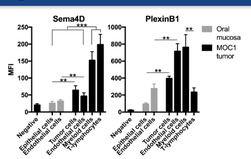
A. Sema4D gradient → B. Increased infiltration of pro-inflammatory APC and CD8+ T cells → C. Shift in balance of tumor immunity in TME



SEMA4D is strongly expressed at the invasive margin of tumors. Antibody blockade of SEMA4D facilitates migration of APCs and T cells into the tumor and a shift toward anti-tumor immune activity. 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, which 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 **C)** 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 chemokine/cytokine secretion using cytometric bead array, or for T cell activity by ELISPOT, n=8-12 mice/group.

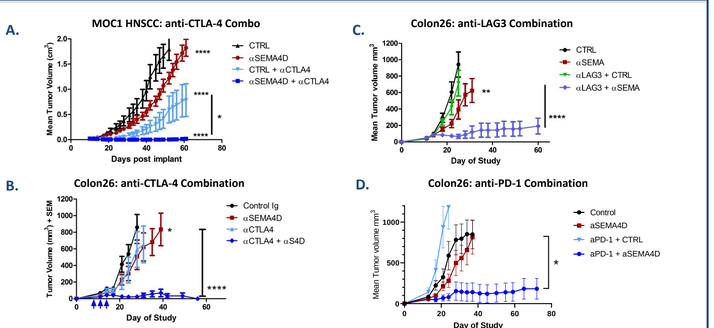
SEMA4D and PLXNB1 are over-expressed in HNSCC tumor and myeloid cells compared to non-malignant tissue





A) SEMA4D is strongly expressed at the invasive margin of human HNSCC. SEMA4D+ immune cells reside in the stroma but are excluded from the epithelial tumor. B) SEMA4D and Plexin receptors are over-expressed in tumor tissue. Murine MOC1 HNSCC tumors and normal oral mucosa were dissociated and expression of SEMA4D and Plexin receptors were assessed by flow cytometry.

PRECLINICAL: Combination Immunotherapy



Immunomodulatory effects of SEMA4D blockade can enhance other immunotherapies. A) MOC1 HNSCC (5x10⁶ 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. **B-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/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).

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

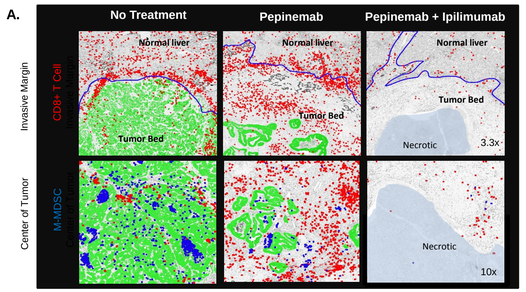
Resectable melanoma (n=36) **Total enrolled: 8**	Resectable HNSCC (n=36) **Total enrolled: 5**	CRC resectable liver mets (n=16) Resectable PDAC (n=16) **Total enrolled: 4 CRC**
No treatment (SOC)	VX15/2503 (20 mg/kg) Ipilimumab (1 mg/kg)	No treatment (SOC)
VX15/2503 (15 mg/kg)	VX15/2503 (20 mg/kg) Nivolumab (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

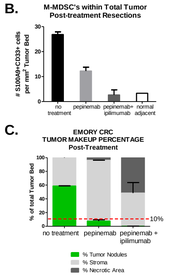
Pre-treatment Biopsy → Surgical Resection

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 April 19, 2019.*

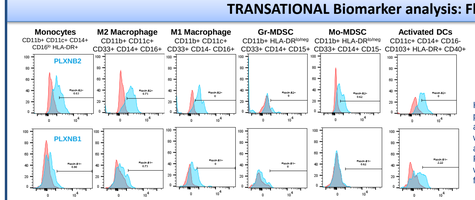
Shift in balance of Immune infiltration and drug penetration in TME





The key observations relate to distribution of T cells and MDSC. Surgical resections were analyzed from one CRC patient/treatment arm following 3-5 weeks of treatment with pepinemab, pepinemab + ipilimumab, 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.
A) 5 micron FFPE sections were stained sequentially for each marker and scanned at 40X. Scans were co-registered for each stain in multiplex. (Top) Invasive margin: CD8+ T cells (red) overlays on cytokeratin stain (green) at tumor/normal liver margin are shown (3.3x). (Bottom) Center of tumor: with S100A9+/CD33+ MDSC (blue) overlays (10x). **B)** 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)** Percent of total tumor bed area for each component was quantified and averaged from 2 sections/patient. **D) Target saturation in TME:** Representative data from melanoma patient 06A 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 **Similar data were observed in TIL isolated from HNSCC patients.**

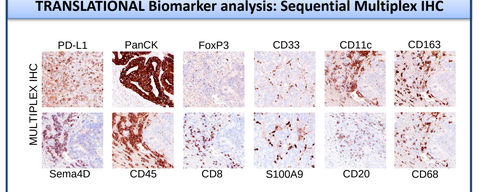
TRANSATIONAL Biomarker analysis: Flow Cytometry



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

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

TRANSLATIONAL Biomarker analysis: Sequential Multiplex IHC



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

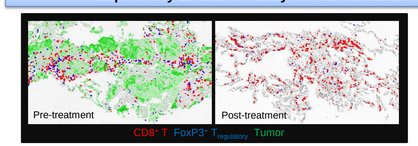
This phase 1b/2, open label, single arm, first-in-human combination study is designed to evaluate the safety, tolerability and efficacy of pepinemab in combination with avelumab in 62 subjects with advanced (IIIB/IV) NSCLC.

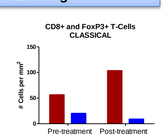
Phase 1b — Dose Escalation (n=12): 5 mg/kg VX15 (n=3) → 10 mg/kg VX15 (n=6) → 20 mg/kg VX15 (n=3) → *Recruitment Closed*
+ 10 mg/kg Avelumab Q2W

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

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

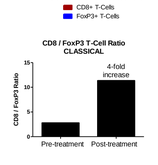
Exploratory Biomarker Analysis: shift in balance of CD8:Treg





- **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.



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