Integrated biomarker trials of VX15/2503 (pepinemab) in combination with checkpoint inhibitors in window of opportunity studies in solid tumors

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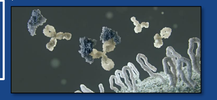
Abstract #CT016



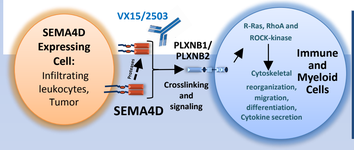
Abstract

Interrogation of the tumor microenvironment (TME) is crucial to provide insight into biological activity, resistance mechanisms and implementation of rational combination immunotherapies. Semaphorin 4D (SEMA4D, CD100) has broad immunomodulatory effects in the TME. In preclinical models, antibody blockade of SEMA4D promoted immune infiltration and reduced function and recruitment of immunosuppressive myeloid cells within the TME. Importantly, preclinical combinations of anti-SEMA4D with immune checkpoint inhibitors (ICIs) enhanced T cell activity and tumor regression. VX15/2503 (pepinemab), an IgG4 humanized monoclonal antibody targeting SEMA4D, is currently being evaluated in window of opportunity, integrated biomarker trials to characterize immunomodulatory effects in pancreatic (PDAC), colorectal (CRC), and head and neck squamous cell (HNSCC) carcinomas, and melanoma.

At present, three biomarker trials are recruiting patients with four resectable indications to investigate novel combinations of pepinemab with ICIs; 1) PDAC and CRC with resectable liver metastases (NCT03373188, n=32), 2) HNSCC (NCT03690986, n=36), and 3) metastatic melanoma (NCT03769155, n=36). Prior to surgery, patients enroll in treatment cohorts including combinations of pepinemab with nivolumab and /or with ipilimumab, single agents, or no treatment. Three to seven weeks later, patients will undergo surgery and a substantial surgical section will be collected under the guidance of a pathologist for comparison across treatment groups and with a pre-dose tissue biopsy. Blood will be collected for PK, PD, and additional correlative biomarker assessments. The primary objective is to evaluate the treatment-induced effects on the immune profile in the TME and in peripheral blood. Additional objectives include, extending the previously reported safety profile of single agent pepinemab to ICI combination therapies, as well as exploring pathologic and radiographic responses in the melanoma study.

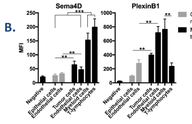
Correlative multiplex flow cytometric flow panels have been established to phenotype cells in the TME and periphery. A multiplex IHC assay utilizing a sequential probe and strip procedure has also been qualified that allows co-localization, spatial orientation, and quantitation of multiple immune markers. Analysis of immune subsets include but are not limited to activated T cells, neutrophils, Treg cells, DCs, monocytes, macrophages, and importantly myeloid-derived suppressor cells (MDSCs). Target engagement and expression of SEMA4D and its cognate receptors will also be evaluated.

Fourteen subjects have been enrolled in these studies as of 15 MAR 2019. These trials will provide the first integrated clinical assessment of anti-SEMA4D antibody activity to reprogram the TME.

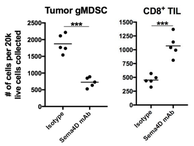


Anti-SEMA4D Mab blocks binding to receptor & signaling activity
- Inhibits differentiation/ function of MDSC, M2 TAM and Treg
- Promotes infiltration of potent APC and T cells into TME

SEMA4D/Plexin signaling regulates MDSC and T cells in TME



Murine MOC1 HNSCC tumors and normal oral mucosa were dissociated and expression of SEMA4D and Plexin receptors were assessed by flow cytometry. PLXN receptors are also expressed in human PBMC (see top right panel)

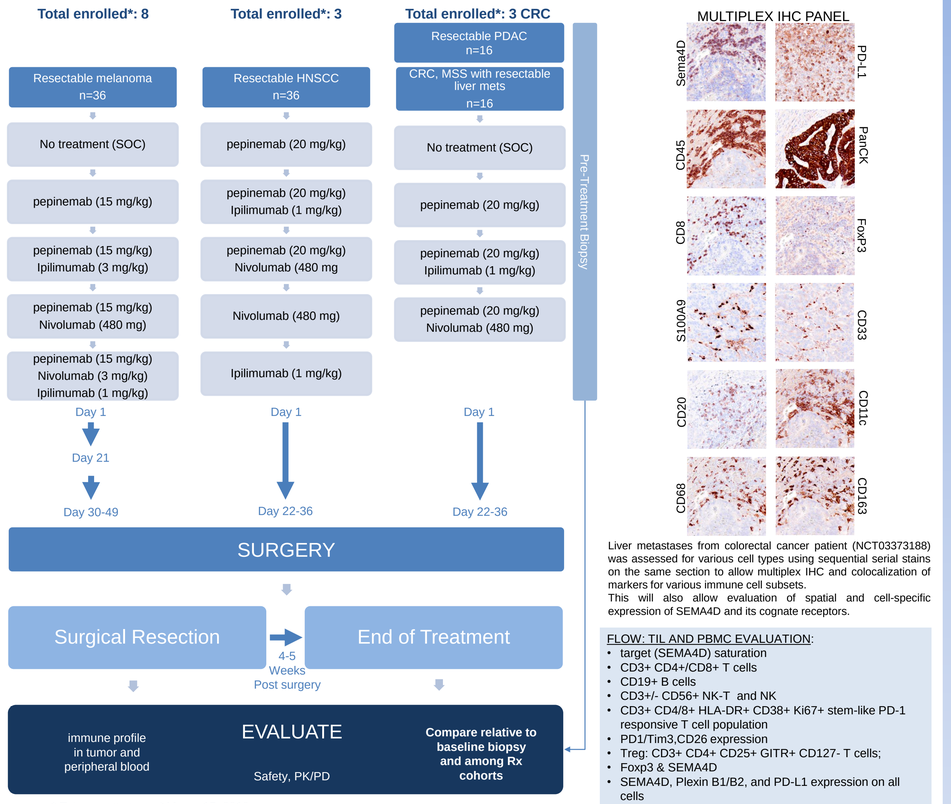


Mice bearing MOC1 tumors were treated *in vivo* with anti-SEMA4D antibody/Mab67 or isotype control (10 mg/kg weekly). Tumor digests (n=5/group) were subjected to a percoll gradient, and leukocytes were analyzed for MDSC and T cell markers by flow cytometry.

Clavijo PE et al. 2019 . Cancer Immunol Res. 7(2): 282-291

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
5. Clavijo PE et al. 2019 . Cancer Immunol Res. 7(2): 282-291

Trial Design: window of opportunity, integrated biomarker trials

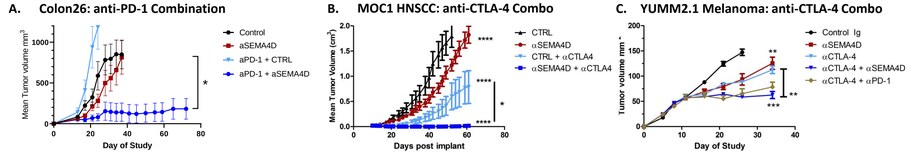


** Total enrolled, as of March 15, 2019.*

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.
This will also allow evaluation of spatial and cell-specific expression of SEMA4D and its cognate receptors.

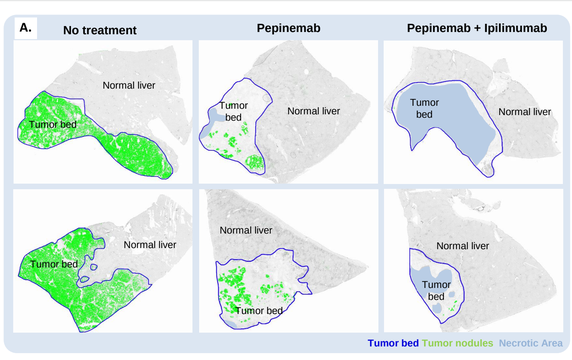
FLOW: TIL AND PBMC EVALUATION:
- target (SEMA4D) saturation
- CD3+ CD4+/CD8+ T cells
- CD19+ B cells
- CD3+/- CD56+ NK-T and NK
- CD3+ CD4/8+ HLA-DR+ CD38+ Ki67+ stem-like PD-1 responsive T cell population
- PD1/Tim3,CD26 expression
- Treg: CD3+ CD4+ CD25+ GITR+ CD127- T cells;
- Foxp3 & SEMA4D
- SEMA4D,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

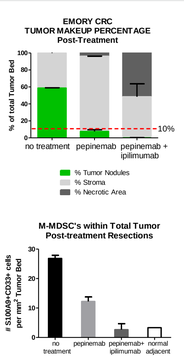
Preclinical Rationale: Immune Checkpoint Combinations

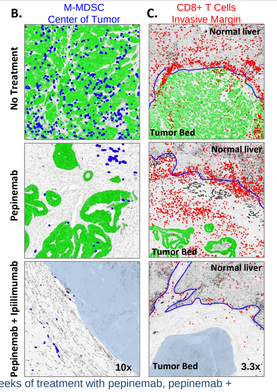


Immunomodulatory effects of SEMA4D blockade can enhance other immunotherapies. A) Colon26 (500,000 cells) were subcutaneously implanted into Balb/c mice, that were then treated with αSEMA4D / MAb67 (10 mg/kg, weekly IP X4), αPD-1 / MAb RMP1-14 (10 mg/kg, twice/week, n=20). **B) MOC1 HNSCC** ($5x10^6$ 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.

Biomarker Analysis of Clinical Samples: Distribution of T cells and MDSC

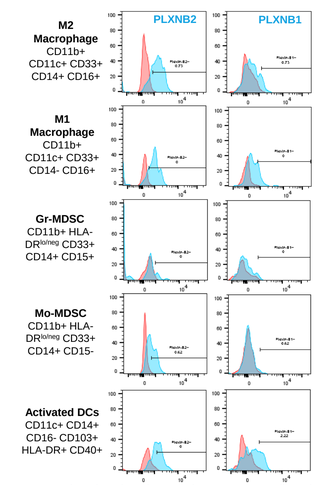






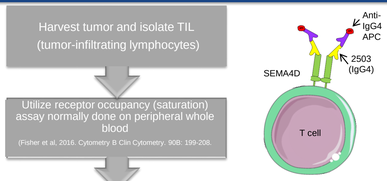
The key observations relate to distribution of T cells and MDSC. Surgical resections were analyzed from one CRC patient/treatment arm following 3-5 weeks of treatment with pepinemab, pepinemab + ipilimumab, and one patient who did not receive antibody treatment. No conclusion can be drawn regarding tumor necrosis because patients received neoadjuvant chemotherapy prior to surgery.
5 micron FFPE sections were stained sequentially for each marker and scanned at 40X. Scans were co-registered for each stain in multiplex. **A)** 0.5x images with cytokeratin stain are shown; tumor bed (tumor/normal boundary) is outlined in blue, cytokeratin+ tumor cells are pseudo colored green, necrotic areas were defined by morphology and outlined in grey. 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 (green) 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). **Analysis of additional patient samples is ongoing.**

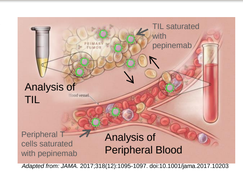
Plexin expression in the tumor

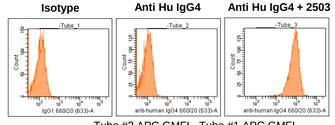


Human pancreatic ductal adenocarcinoma were dissociated and expression of Plexin receptors were assessed by flow cytometry.

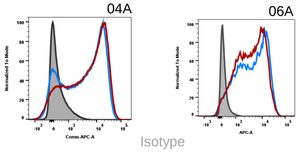
Pepinemab penetration into the tumor







$$\% \text{ Saturation} = \frac{\text{Tube \#2 APC GMFI - Tube \#1 APC GMFI}}{\text{Tube \#3 APC GMFI - Tube \#1 APC GMFI}} \times 100$$



ID	% Saturation
01A	83%
02A	79%
03A	74%
04A	79%
06A	108%

The first five subjects in the melanoma arm were dosed on Days 1 and 21 with pepinemab (15mg/kg) and nivolumab (240mg) and tumors were harvested 15-28 days later. TIL were isolated and CD3+ T cells were assayed for saturation of the cellular SEMA4D target. Example histograms from patient 04A and 06A are shown as well as % saturation for all 5 subjects.