SEMA4D antibody blockade overcomes mechanisms of immune suppression and combination immunotherapy including TGFβ blockade promotes efficient tumor regression

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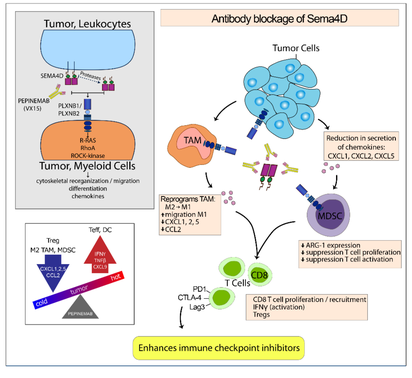
Summary

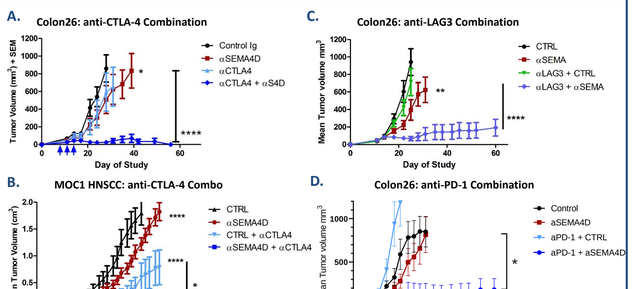
Background: Despite progress of immune checkpoint blockade therapies, resistance mechanisms including myeloid suppression and upregulation of TGFβ signaling prevent durable clinical benefit in many cancer patients. Anti-semaphorin 4D (SEMA4D, CD100) blocking antibody promotes immune infiltration, reduces immunosuppression, and enhances T cell activity in the tumor microenvironment (TME), resulting in increased tumor control when combined with various immunotherapies in preclinical models (1-3). Clinical trials of immune checkpoint inhibitors (ICI) in combination with pepinemab (VX15/2503), a humanized anti-SEMA4D antibody (4,5), are currently underway in several cancer indications.
Methods: Activity of anti-SEMA4D antibody in combination with immune checkpoint inhibitors and TGFβ blockade was evaluated in preclinical mouse tumor models. Ongoing clinical trials of immune checkpoint inhibitors (ICI) in combination with pepinemab include: (i) a Phase 1b/2a combination trial of pepinemab with avelumab in ICI naïve or ICI refractory or relapsed NSCLC (CLASSICAL-Lung) (NCT03268057, N=65); (ii) neoadjuvant integrated biomarker trials in patients with metastatic melanoma (NCT03769155, n=36), metastatic colorectal, pancreatic (NCT03373188, n=32) and head and neck (NCT03690986, n=36) cancers treated with pepinemab in combination with nivolumab or ipilimumab.
Results: Anti-SEMA4D antibody enhanced tumor regression when combined with antibodies targeting CTLA-4, PD-1, PD-L1, LAG3, and TGFβ in several preclinical models. For example, anti-SEMA4D plus anti-TGFβ treatment resulted in maximal tumor growth delay (TGD) of 239% ($p<0.01$) and 10/15 complete tumor regressions (CR) ($p<0.05$), compared to 10% TGD and 0/13 CR with single agent anti-TGFβ or 29% TGD and 1/10 CR with anti-SEMA4D alone in MC38 colon carcinoma model. Additionally, the combination of anti-SEMA4D, folfirinox, and ICI improved survival in KP2-tumor bearing mice, a KPC-derived pancreatic adenocarcinoma model of immune exclusion, myeloid suppression and active TGFb signaling. In clinical trials, pepinemab was well-tolerated and analysis of pre and on-treatment biopsies revealed increased CD8 density and reduced presence of myeloid derived suppressor cells within TME.
Conclusions: SEMA4D antibody blockade modulates the TME to enhance anti-tumor immunity and combination therapies further enhance anti-tumor activity and overcome important resistance mechanisms. Preliminary data suggest the combination of pepinemab plus immune checkpoint therapy is well tolerated and shows initial signals of antitumor activity in patients. Ongoing analysis of various therapeutic combinations and immunophenotyping of tissue biopsies will shed light on mechanism of action of SEMA4D antibody blockade in several combination therapies.

Anti-SEMA4D Mab blocks binding to its cognate receptors & receptor-mediated signaling activity

- Promotes infiltration of potent APC and T cells into TME
- Inhibits function of MDSC, M2 TAM and Treg



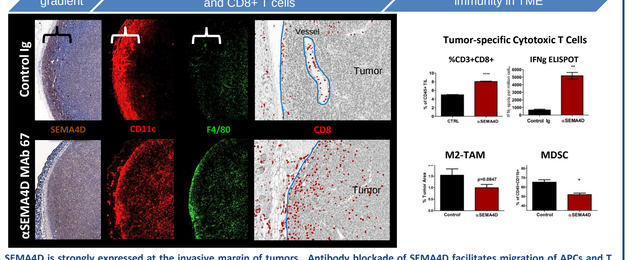
Immune Checkpoint Combinations



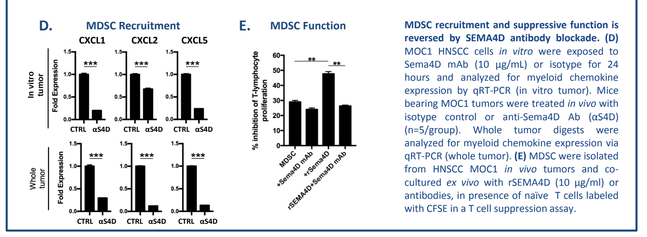
Immunomodulatory effects of SEMA4D blockade can enhance immune checkpoint therapies. A,C,D. Colon26 (500,000 cells) were subcutaneously implanted into Balb/c mice, that were then treated with αSEMA4D / MAb67 (10 mg/kg, weekly IP X4), αLAG3/C9B7W (10 mg/kg 2x/week X4; n=20); αCTLA-4 / MAb UC10-4F10 (100/50/50 µg, q3 days; n=20), αPD-1 / MAb RMP1-14 (10 mg/kg, twice/week, starting on day10, n=20), **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.

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

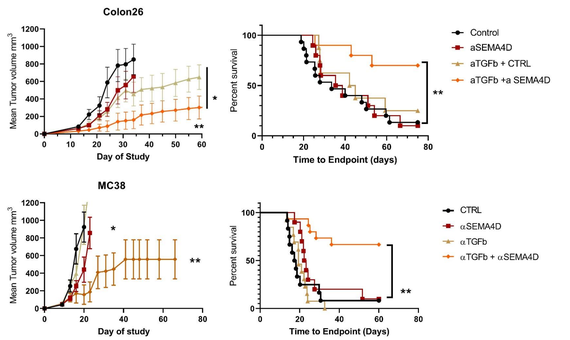


SEMA4D is strongly expressed at the invasive margin of tumors. Antibody blockade of SEMA4D facilitates migration of APCs and T cells into the TME. (A) SEMA4D expression at invasive tumor margin restricts infiltration of PLXNB1+ DC into TME. Brackets indicate area of SEMA4D gradient. **(B)** Anti-SEMA4D MAb treatment promotes infiltration of pro-inflammatory CD11c+/F4-80+ antigen presenting cells, while reducing CD206+ M2 macrophage and MDSC. 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 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 T cell activity by ELISPOT, n=8-12 mice/group.



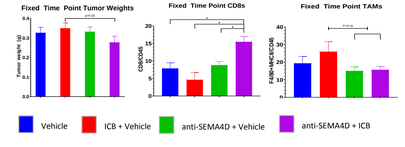
MDSC recruitment and suppressive function is reversed by SEMA4D antibody blockade. (D) MOC1 HNSCC cells *in vitro* were exposed to Sema4D mAb (10 µg/mL) or isotype for 24 hours and analyzed for myeloid chemokine expression by qRT-PCR (in vitro tumor). Mice bearing MOC1 tumors were treated *in vivo* with isotype control or anti-Sema4D Ab (αS4D) (n=5/group). Whole tumor digests were analyzed for myeloid chemokine expression via qRT-PCR (whole tumor). **(E)** 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.

TGFβ Combinations

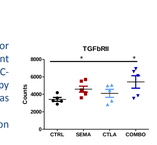


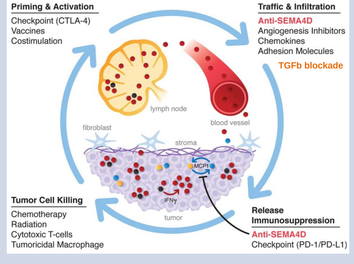
Immunomodulatory effects of SEMA4D blockade can enhance TGFβ blockade. A) Colon26 (500,000 cells) were subcutaneously implanted into Balb/c mice, that were then treated with αSEMA4D / MAb67 (10 mg/kg, weekly IP X4) and αTGFβ/Mab1D11.16.8 (5 mg/kg, 2x/week IP); n=15. **B) MC38** (80,000 cells) were subcutaneously implanted into C57Bl/6 mice, that were then treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP) and αTGFb/Mab1D11.16.8 (10 mg/kg, 3x/week IP); n=15.

Anti-SEMA4D enhances T cell infiltration and reduces immunosuppressive M2 TAM in TGFβ driven, highly desmoplastic and immune excluded pancreatic cancer model (6). A) C57b/6 mice were injected orthotopically with KP2 cells* (derived from the spontaneous KRASG12D; TP53Flox/Wt; P48-Cre autochthonous tumors from David Denardo, Washington University) and treated with αSEMA4D / MAb67, αPD1/MAb RMP1-14, αCTLA-4 / MAbUC10-4F10 (10 mg/kg, twice weekly IP). Tumors were harvested after 2 weeks of treatment, weighed and dissociated for flow cytometric analysis of CD8+ T cells and M2 TAM.



Anti-SEMA4D enhances expression of TGFbRII. Upregulation of TGF receptor may result from down-regulation of its ligand, or upregulation of an independent immune resistance mechanism. Upregulation of TGFbRII is associated with APC-independent and fibroblast-dependent resistance to immune checkpoint therapy (7). Potential interaction of SEMA4D/PLXN and TGFβ pathways and EMT has been reported (8,9); further investigation in ongoing.
Colon26 tumor bearing mice were treated as above. Tumors were harvested on day15 and RNA expression was analyzed using Nanostring.





Adapted from:
Evans EE, Paris M, Smith ES & Zauderer M. 2015, OncoImmunology

Overcoming Immune Exclusion in TME can enhance immune checkpoint therapy by

- Reduction and polarization of suppressive myeloid cells
- Reduction of fibroblast-specific TGF-β signaling (9)

➢ Anti-SEMA4D facilitates infiltration of T cells and pro-inflammatory APC into TME and reverses recruitment and activity of MDSC and M2 TAM.
➢ TGFβ represents an independent fibroblast-driven mechanism of immune exclusion and resistance to immune checkpoint therapy.
➢ **Anti-SEMA4D can enhance activity of both immune checkpoint and TGFβ blockade.**
➢ Targeting multiple pathways may overcome immune exclusion and promote anti-tumor immunity.

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

This ongoing completely enrolled phase 1b/2, open label, single arm, first-in-human combination study is designed to evaluate the combination of pepinemab with avelumab in 62 subjects (pts) with advanced (stage IIIB/IV) NSCLC.

Study Design
- The trial is split into dose escalation (n=12) and dose expansion (n=50) phases.
- The dose escalation portion includes subjects who are immunotherapy naïve and have either progressed or declined standard first or second-line systemic anticancer therapy.
- Subjects in the three dose escalation cohorts received ascending doses of pepinemab (5, 10, 20 mg/kg, Q2W) in combination with avelumab (10mg/kg, Q2W).
- The expansion phase includes an IO naïve (ION) cohort as well as a second cohort of subjects whose tumors progressed during or following immunotherapy (IO failure, IOF).

Study Objectives
- The primary objective 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

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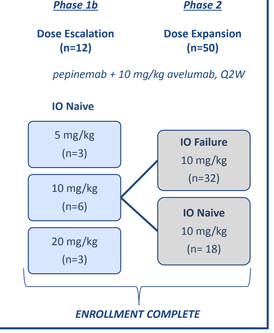
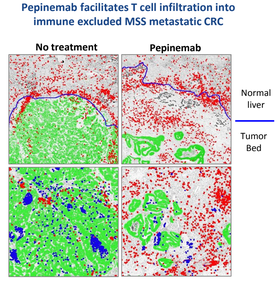
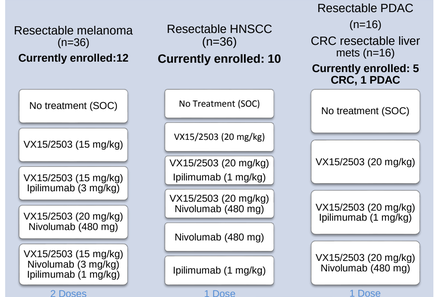
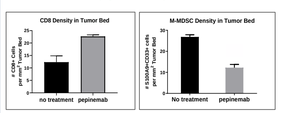


Integrated Biomarker Window of Opportunity Clinical Study

In collaboration with Winship Cancer Institute, Emory University







CRC patients received neoadjuvant chemotherapy before immunotherapy and surgery

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 NOV 1, 2019.*

The key observations relate to distribution of T cells and MDSC in MSS metastatic CRC. Surgical resections were analyzed from one CRC patient following 3-5 weeks of treatment with pepinemab and one patient who did not receive antibody treatment. 5 micron FFPE sections were stained sequentially for each marker and scanned at 40X. Scans were co-registered for each stain in multiplex. **A)**) CD8+ T cells (red) overlays on cytokeratin stain (green) at tumor/normal liver margin are shown (3.3x). 10x images from center of tumor are shown below with S100A9+/CD33+ MDSC (blue) overlays on cytokeratin stain (green) are shown. **B)** Total number of CD8 or MDSC 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.
Analysis of additional patient samples is ongoing.

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