Integrated Biomarker Study of Pepinemab in Combination With Ipilimumab or Nivolumab to Evaluate Immune Cell Composition of TME in Patients With Head And Neck Squamous Cell Carcinoma and Other Solid Tumors

Conor E Steuer[2], Gregory B. Lesinski[2], Elizabeth E. Evans[1], Terrence L. Fisher[1], Crystal Mallow[1], Elaine Gersz[1], Brian Olson[2], Desa Rae Pastore[1], John E. Leonard[1], Ernest Smith[1], Maurice Zauderer[1], Michael Lowe[2], Ragini Kudchadkar[2], Christina Wu[2], Mihir Patel[2], Nabil F Saba[2]

1Vaccinex, Inc., 2 Winship Cancer Institute of Emory University

MULTIDISCIPLINARY HEAD and NECK CANCERS SYMPOSIUM
EXPANDING TREATMENT HORIZONS
The Westin Kierland Resort and Spa | Scottsdale, Arizona | February 27-29, 2020

PURPOSE / OBJECTIVE

Immunosuppressive myeloid cells activated in the tumor microenvironment (TME) are 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, while simultaneously restoring the ability of dendritic cells and cytotoxic T cells to infiltrate the TME. Importantly, this coordinated shift from immunosuppression to tumoricidal activity complemented effects of other immunotherapies in syngeneic tumor models, whereby combinations of anti-SEMA4D with ICIs enhanced T cell activity and tumor regression.

Objectives: Evaluation of immunomodulatory effects of pepinemab, a humanized monoclonal antibody targeting SEMA4D, and combinations with ICI within periphery and TME. Additional objectives include extension of the previously reported safety profile of pepinemab to ICI combination therapies and overall survival in patients with HNSCC.

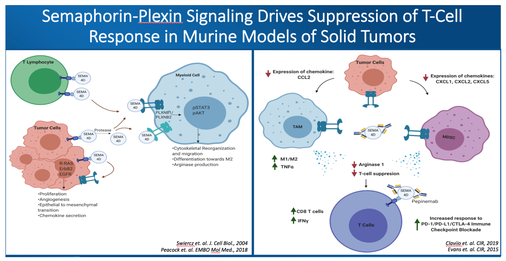
MATERIAL & METHODS

Biomarker-driven window of opportunity studies are recruiting patients to investigate novel combinations of pepinemab with ICIs in HNSCC (NCT03690986, n=36); as well as colorectal cancer with resectable liver metastases and pancreatic ductal adenocarcinoma (NCT03373188, n=32); and metastatic melanoma (NCT03769155, n=36). HNSCC patients will be stratified by HPV status and randomly assigned into cohorts receiving one dose of a combination of pepinemab (20 mg/kg) with nivolumab (480 mg) or ipilimumab (1 mg/kg), single agents, or no treatment. Three to five weeks later, surgically resected tumors are collected under the guidance of a pathologist. Blood is collected for PK, PD, and correlative biomarker assessments. Multiplex flow cytometric (FC) and immunohistochemistry (IHC) panels have been established to phenotype cells in the TME and periphery, including cytotoxic T cells, Tregs, DCs, monocytes, macrophages, and myeloid-derived suppressor cells. Target engagement and expression of SEMA4D and its receptors will be evaluated.

REFERENCES / ACKNOWLEDGEMENTS

1. Clavijo PE et al. Cancer Immunol Res. 2019 Feb;7(2):282-291
2. Evans, EE et al 2015. Cancer Immunol Res. 3(6):689-701.
3. Evans EE, Paris M, Smith ES & Zauderer M. 2015): OncoImmunology
4. Patnaik A et al. Clin Cancer Res. 2016 Feb 15;22(4):827-36.
5. Fisher et al, 2016. MAbs. 8(1): 150-162.
6. Shafique et al. 2020. ASCO-SITC Clinical Immuno-Oncology Symposium.

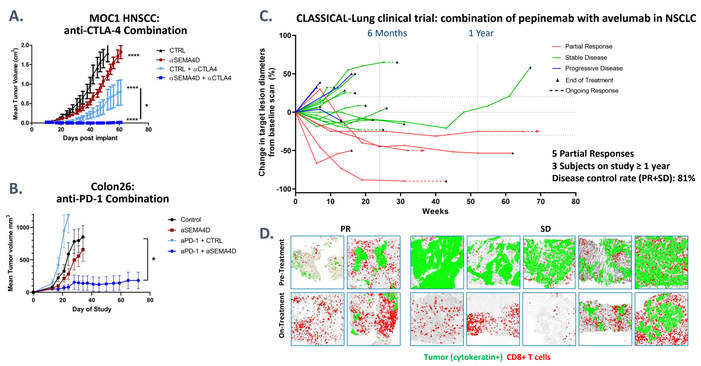
BACKGROUND



Immune checkpoint combinations

Preclinical Proof of Concept

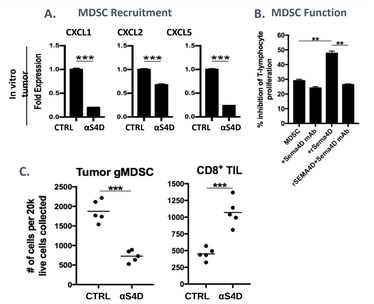
CLASSICAL-Lung (NCT03268057)



Immunomodulatory effects of SEMA4D blockade can enhance immune checkpoint therapies and promote T cell infiltration. Two preclinical models: A) MOC1 HNSCC ($5x10^6$ cells) were subcutaneously implanted into C57Bl/6 mice, then treated with αSEMA4D/MAb67 (10 mg/kg, weekly IP), αCTLA-4 / MAb 9H-10 (5 mg/kg, q5D); n=10. **B) Colon26** (500,000 cells) were subcutaneously implanted into Balb/c mice, and treated with αSEMA4D / MAb67 (10 mg/kg, weekly IP X4), αPD-1 / MAb RMP1-14 (10 mg/kg, twice/week, starting on day10, n=20). **C)** (CLASSICAL-Lung) (NCT03268057): a phase 1b/2, 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. Patients were treated Q2W (10 mg/kg). Clinical response (RECIST1.1) of IO-naïve patients shown. **D)** Pre and on-treatment (~5 weeks of treatment) biopsies were assessed for tumor content (cytokeratin) = green and CD8 T cells = red. Examples of matched lung biopsies from patients experiencing partial response (PR) and stable disease (SD) demonstrate increased T cell infiltration into tumors following treatment; this trend was not observed with progressive disease (not shown).

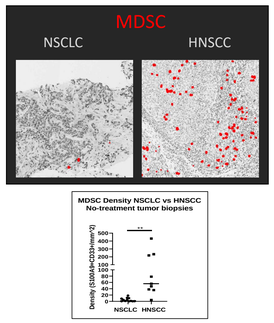
Myeloid derived suppressor cells in HNSCC

Preclinical: SEMA4D blockade reverses MDSC recruitment and function



SEMA4D antibody blockade shifts the balance of MDSC and T cells within TME of HNSCC model. A) 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). **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. C) 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, gMDSC and CD8+ T cells were analyzed by flow cytometry.

HNSCC have high MDSC content, compared to other cancers such as NSCLC



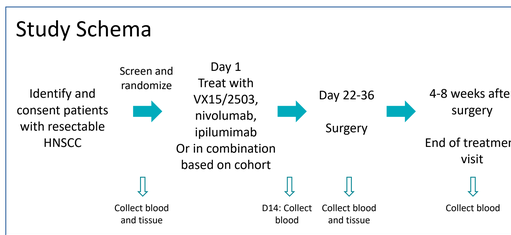
MDSC levels were assessed in patient biopsies: NSCLC: pre-treatment biopsies from CLASSICAL-Lung HNSCC: pre-treatment and no treatment biopsies from integrated biomarker trial (NCT03690986).

STUDY DESIGN

Trial Design/Methodology: A neoadjuvant, window of opportunity trial of VX15/2503, ipilimumab, or nivolumab alone or in combination in patients with HNSCC. Total number will be a maximum of 36 patients with HNSCC, stratified by HPV status. Patients receive no or one treatment prior to surgery.

Disease	*Group*	*Treatment*
HNSCC	A	VX15/2503 (20 mg/kg)
HNSCC	B	VX15/2503 (20 mg/kg) + Ipilimumab (1 mg/kg)
HNSCC	C	VX15/2503 (20 mg/kg) + Nivolumab (480mg)
HNSCC	D	Nivolumab (480mg)
HNSCC	E	Ipilimumab (1 mg/kg)
HNSCC	F	No Treatment

Study Schema



Key Inclusion Criteria:

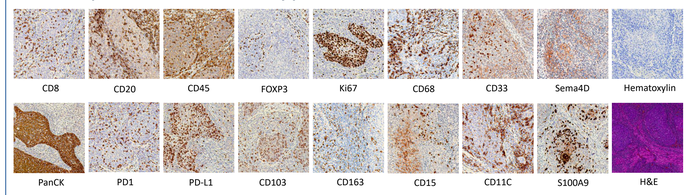
- **Must be at least 18 years of age**
- **Stage I-IVA cytologically or histologically-proven HNSCC, p16 positive and negative allowed.**
- **Oropharyngeal tumors must have p16 testing done**
- **Cancer confirmed to be surgically resectable, with surgery evaluation with planned resection.**
- **Archival tissue prior to treatmentavailable from at most 6 months prior to study enrollment. Otherwise new pre-treatment biopsy mandatory.**
- **No prior lines of therapy**
- **Eastern Cooperative Oncology Group (ECOG) Performance Status 0 or 1 (Appendix I)**
- **Adequate Labatory Values**

Key Exclusion Criteria:

- **Nasopharynx cancer, cancer of unknown primary, sinonasal cancer**
- **Determined not to be a surgical candidate due to medical co-morbidities**
- **Treatment with chronic immunosuppressants (e.g., cyclosporine following transplantation)**
- **Prior organ allograft or allogeneic bone marrow transplantation.**
- **Subjects with any active autoimmune disease or history of known or suspected autoimmune disease except for subjects with vitiligo, resolved childhood asthma/atopy, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.**
- **Women who are pregnant or lactating**
- **Clinical evidence of bleeding diathesis or coagulopathy**
- **Patients with prior malignancies, including pelvic cancer, are eligible if they have been disease free for > 5 years. Patients with prior in situ carcinomas are eligible provided there was complete removal.**
- **No archival tissue available pre-study treatment, and repeat biopsy not feasible.**

Exploratory biomarker analysis: immune infiltration

A. Multiplex immunohistochemistry panel



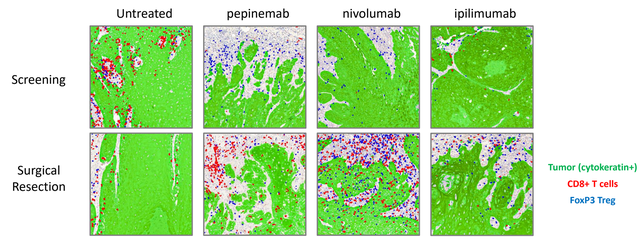
B. Multiparameter flow cytometric analysis

Surgical samples and peripheral blood processed and stained using four different flow panels

Lymphocyte	• Live/Dead, CD45, mCD8, CD3, CD4, CD8, CD19, CD26, CD38, CD56, HLA-DR, PD1, Tim3, LAG3, Ki67, hIgG/SEMA-4D
Treg	• Live/Dead, CD45, mCD8, CD3, CD4, CD25, CD127, GITR, Foxp3, hIgG/SEMA-4D
Myeloid	• Live/Dead, CD45, mCD8, CD11b, CD11c, CD14, CD15, CD16, CD33, CD40, CD103, HLA-DR, Plexin-B1, Plexin-B2, PD-L1
Stem-like T cells	• Live/Dead, CD45, mCD8, CD3, CD4, CD8, PD1, TCF1/7

Cell Type	Markers
target (SEMA4D) saturation	Sema4D
T Cells	CD3+ CD4+/CD8+
B Cells	CD19+
NK-T and NK	CD3+/- CD56+
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
Targets and receptors	SEMA4D, Plexin B1/B2, and PD-L1

C. Representative staining: T cell infiltration



Immunophenotype analysis in HNSCC cohorts. A) 5 micron FFPE sections were stained sequentially for each marker and scanned at 40X. Scans were co-registered for each stain in multiplex. This will also allow evaluation of spatial and cell-specific expression of SEMA4D and its cognate receptors. B) Single cell suspensions of PBMC and dissociated tumor were assessed by flow cytometry. C) examples of T cell staining in biopsy samples: CD8+ T cells (red) overlays on cytokeratin stain (green) at tumor invasive margin are shown (3.3x). 10x images from center of tumor are shown below with FoxP3+ (blue) overlays on cytokeratin stain (green) are shown. Tumor areas were confirmed by pathologist review.

CONCLUSION

Correlative FC and IHC panels utilizing a sequential probe and strip procedure that allows co-localization and quantification of multiple immune markers have been established. Analysis of tumors from HNSCC patients suggest an increase in T cell infiltration following treatment with immune modulating antibodies. 11 HNSCC patients have been enrolled as of 02 Feb 2019.

Conclusions: These studies will provide the first integrated clinical assessment of the use of anti-SEMA4D antibody to reprogram the TME.

eevans@vaccinex.com
csteuer@emory.edu

#HNCS20