Neoadjuvant SEMA4D blockade with nivolumab alters suppressive myeloid cells while elevating B cell and CD26^{hi} T cell infiltration in the tumors of patients with resectable stage III melanoma

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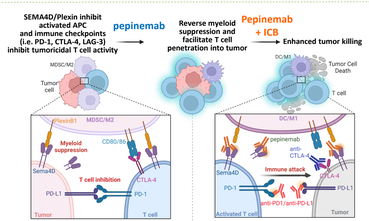
Objectives / Background

Semaphorin 4D (SEMA4D) modulates the balance of antigen presenting and suppressive myeloid cells to promote infiltration of effector immune populations into the tumor microenvironment (TME) and promote tumor killing. When combined with immune checkpoint blockade (ICB), SEMA4D antibody blockade enhances activity (1,2,3), but importantly does not increase toxicities associated with ICB (1).

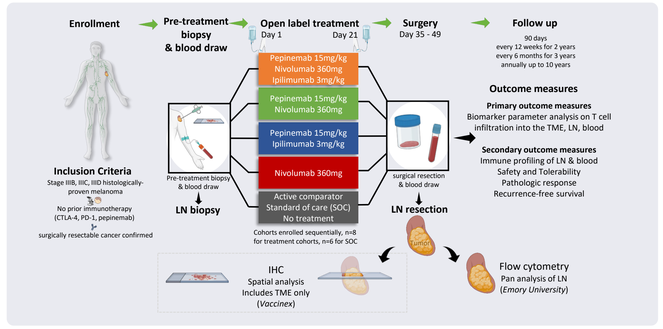
We hypothesized pepinemab (SEM4D-blocking Ab) can be safely combined with ICB to enhance immune infiltration in TME and may benefit patients with resectable stage III melanoma.



Methods

Trial Design NCT03769155

Patients with resectable stage IIIB/C/D melanoma were sequentially enrolled on one of four treatment cohorts (n = 8 each): pepi/nivolumab (nivo), pepi/ipilimumab (ipi), pepi/nivo/ipi or nivo alone. Two doses were given on days 1 and 21; surgery occurred on day 42. Patients received adjuvant nivo q4w to complete one year of treatment.



Tissue and Blood Assessments

Blood as well as surgical resection tissue was collected, and tumor tissue was dissected to obtain sections that contain both tumor and normal tissue. One piece of this section was collected into 10% formalin and sent for multiple immunohistochemical analysis, an adjacent piece was collected into hypothermosol (StemCell) and used for flow cytometry analysis.

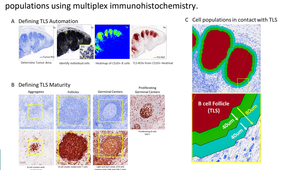
Biomarker Analysis Advantages

Generally, trends were consistent using independent assessments of TIL at two laboratories.

Flow Cytometry	• Entire LN is analyzed • Unique marker sets and high resolution gating • Increased sensitivity and power, due to high cell #s • Longitudinal analysis of blood samples
IHC	• Ability to define tumor area and exclude normal tissue • Spatial and cellular neighborhood comparisons and quantification • Access to archival pre-treatment tissues

Immunohistochemistry

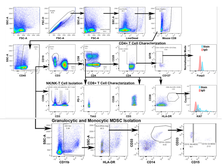
Pretreatment archival tissue was used in conjunction with on study surgical resection tissue to evaluate spatial distribution of immune populations using multiplex immunohistochemistry.



A. Tertiary lymphoid structures (TLS) were identified using algorithms trained for CD20 B cell stain using VISIOPHARM software. ROIs were created for individual TLS. B. TLS were categorized by maturation states as aggregates, follicles and follicles with germinal centers. C. B cell follicle ROIs were expanded by 40-80um to identify cell phenotypes in direct communication with B cells.

Flow Cytometry

Surgical tissue and blood underwent high dimensional immune analysis using 32-color flow cytometry.



For flow cytometry, tissue was digested using MACS Human Tumor Dissociation kit with a gentleMACS dissociator (Miltenyi Biotec Inc.). After filtration, single cell suspensions were stained flow cytometry panels to evaluate lymphocyte, regulatory T cells, myeloid cells, or stem-like T cells. Cells were fixed and then frozen for batched analysis, at which point samples were thawed, permeabilized and stained for intracellular markers. Samples were then analyzed on an LSRII or BDSymphony flow cytometer.

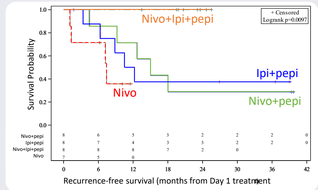
Results

Biomarkers of Response

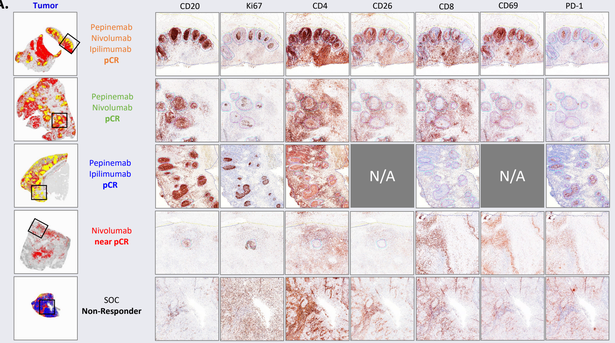
Increased infiltration and organization of multiple immune cells corresponds with improved clinical responses and recurrence free survival

Recurrence Free Survival

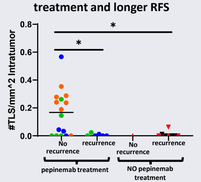
Triple combo (pepi+Nivo+Ipi) showed 100% recurrence free survival. Presented at ESMO 2022 (7).



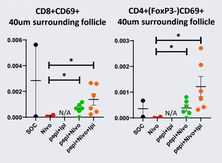
Increase in organized tertiary lymphoid structures (TLS) with response to Pepinemab + ICB Treatment



B. Higher density of TLS is associated with pepinemab treatment and longer RFS

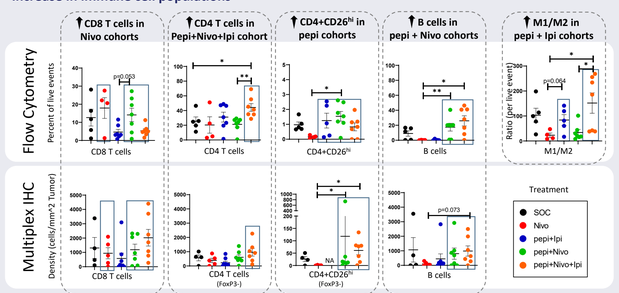


C. Increased activated T cells in proximity with B cell follicles with pepinemab + ICB Tx over Nivo alone



Patients who responded to pepinemab + ICB treatment showed increased density of mature TLS within the tumor bed. A. Representative images of responders in pepi+Nivo+Ipi, pepi+Nivo and pepi+Ipi cohorts show increased density of TLS structures. Insets show representative individual stains for immune markers demonstrate the complex organization of B cells and T cells within the pepi+Nivo treated cohorts in comparison with pepi+Ipi, Nivo alone and SOC which show unorganized immune cell infiltration. Germinal centers with proliferating B cells (Ki67) are predominantly seen in all pepinemab-containing treatment cohorts. CD4$^+$CD26^{hi} T cells are increased in pepi+Nivo cohorts. **B.** Density of TLS within the tumor bed (intratumor) were analyzed as per IHC methods section. Cohorts were combined to analyze patients with and without pepinemab treatment (pepiTx= pepi+Nivo, pepi+Ipi, pepi+Nivo+Ipi. No pepiTx= Nivo, SOC). Treatment groups were then split by recurrence free survival (RFS) at 12 months. Pepinemab treated patients with longer RFS showed increased density of TLS within the tumor bed. **C.** Spatial organization of TLS were analyzed as per IHC method section (C). Significant increase in density of activated (CD69+) CD8 and CD4 T cells within direct contact with B cell follicles (40um) was seen in pepi+Nivo and pepi+Nivo+Ipi cohorts in comparison with Nivo alone. Pepi+Ipi TLS analysis is in progress.

Increase in immune cell populations

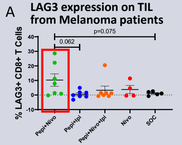


T cells expressing high surface CD26 (a costimulatory molecule with enzymatic activity) infiltrated tumors of patients treated in the neoadjuvant setting with pepinemab/immune checkpoint blockade (ICB) combination therapy versus patients receiving standard of care nivolumab or surgery alone and is associated with increased recurrence-free survival. This is a significant finding, as our team previously published that CD26 marks T cells with potent antitumor activity in the context of adoptive cellular therapy (6). Additional investigation reveals that CD26hiICD4+ T cells from patients with melanoma can co-secrete many cytotoxic molecules at once and possess long-lived durable memory responses in vivo (5) (see below). (NA= data pending, but not available at this time)

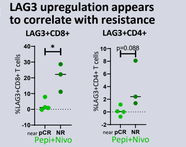
Biomarkers of Resistance

LAG3 appears to be a biomarker of resistance in patients who did *not* respond to Pepi + Nivo

A LAG3 expression on TIL from Melanoma patients



B LAG3 upregulation appears to correlate with resistance



Statistical analysis
A two-sample non-parametric Mann-Whitney test was performed on all biomarker results. * =p<0.05

Future Directions

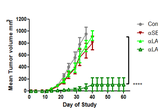
CD26^{high} T cells, B cells and TLS as biomarkers of response and **LAG3 as a biomarker of resistance** will be evaluated in ongoing studies, including studies evaluating combinations of pepinemab with pembrolizumab in first line recurrent and metastatic HNSCC (KEYNOTE-B84), and the combination of pepinemab with adoptive DC and CD4+T cell therapy in metastatic breast cancer (in collaboration with Moffitt Cancer Center).

These findings suggest several potential new applications for pepinemab-containing combos.

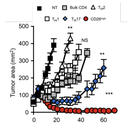
- CD26^{high} T cells, with enhanced multi-functionality (IL-17A, IFNγ, IL-2, TNFα, and IL-22), stemness properties (elevated β-catenin and Lef1), memory (long-term persistence and Bcl2 expression), and a rich profile of chemokine receptors (including CCR2 and CCR5) (4) induced by pepinemab may be expanded to improve adoptive T cell transfer therapies (including naturally arising TIL or T cell genetically engineered to express TCRs or CARs) (5, 6) to augment or rescue anti-tumor immune responses in patients with solid tumors.
- Upregulation LAG-3 appears to be a compensatory immune escape mechanism following treatment with nivo + pepi and, together with preclinical data and favorable safety profiles, suggest that a triple combination may be effective and well-tolerated.

Preclinical data to support Future Directions

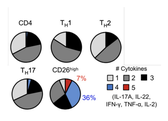
A. Anti-LAG3 + anti-SEMA4D



B. ACT with CD26^{hi}CD4$^+$ T cells



C. CD26^{hi}CD4$^+$ T cell cytokines



A. Colon26 mouse tumor model. B. Superior activity of ACT with human CD26^{hi}CD4$^+$ T cells: M108 in NSG mice (4) C. CD26^{hi}CD4$^+$ T cells are more cytotoxic and polyfunctional than bulk CD4 in blood of melanoma patients (n=10) (4).

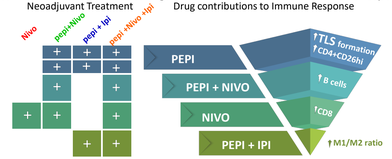
Conclusions

Summary of treatment effects

Triple combination of ipilimumab, nivolumab and pepinemab

1. Prolonged RFS compared to doubtlet therapies
2. Pepinemab did not enhance toxicities associated with ICB
3. Increases infiltration of immune cells into the tumor microenvironment, which are enhanced in clinical responders
4. Pepinemab Increases formation of tertiary lymphoid structures surrounded by effector T cells

Drug combinations contribute to organized and multi-functional immune response



Pending analysis of melanoma cohorts: IHC assessment of myeloid cells in TME, baseline and longitudinal analysis of blood.

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Other Clinical Trials

KEYNOTE-B84 (NCT04815720)
Emory HNSCC (NCT03690986)
Moffitt MBC (NCT05378464)

Illustrations made in BioRender

We would like to acknowledge Liz Tuttle for technical assistance in IHC

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