# Inhibition of Semaphorin 4D in combination with immune checkpoint therapy induces organized lymphoid structures within the tumor microenvironment that correlate with clinical outcome

#3012

Crystal Mallow[1], Elizabeth E. Evans[1], Terrence L. Fisher[1], Elizabeth Tuttle[1], Vikas Mishra[1], Ellen Giampoli[2], Conor Steuer[3,6], Nabil Saba[3,6], Marya F. Chaney[4], Tarek Mekhail[5,6], Chrystal Paulos[3], Gregory Lesinski[3], Michael Lowe[3], Maurice Zauderer[1]

[1]Vaccinex, Inc.; [2]University of Rochester Medical Center; [3]Winship Cancer Institute of Emory University; [4]Merck & Co., Inc.; [5]Advent Health, Orlando FL; [6]On behalf of KEYNOTE-B84 investigators

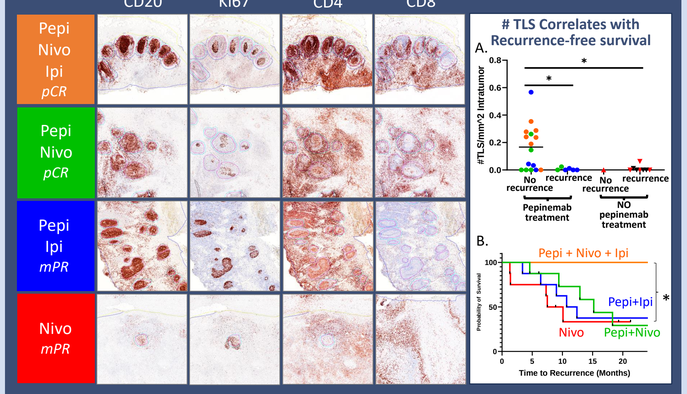
## Introduction

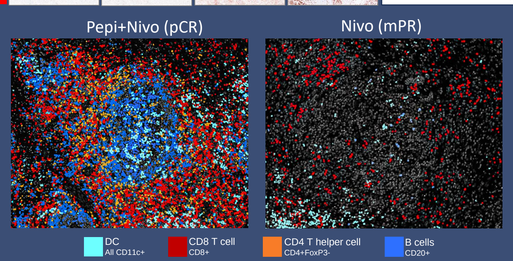
Myeloid cells play a critical role in suppression of adaptive immunity within the TME. Semaphorin 4D (SEMA4D) signaling through its receptors (PlexinB1/B2, CD72) excludes activated antigen presenting cells and promotes recruitment and suppressive function of myeloid suppressor cells (MDSC)(1). In preclinical and clinical studies, SEMA4D antibody blockade increased penetration of B cells, antigen presenting dendritic cells (DC) and T cells into the tumor and attenuated MDSC in the TME, leading to enhanced efficacy of immune checkpoint inhibitors (ICI) (2).

**Data presented here support the HYPOTHESIS that SEMA4D blockade regulates crosstalk of immune cells in TME to promote organized functional immune interactions as a novel mechanism of immune enhancement.**

## Background

Pepinemab combined with immune checkpoint inhibitors (ICI) appeared to induce mature TLS that correspond with recurrence free survival. In contrast, patients treated with nivolumab alone demonstrated few B cells and disorganized T cells (4) following neoadjuvant treatment in patients with Stage III metastatic melanoma (NCT03690986).




Melanoma patients received 2 doses of immunotherapy (Day1&21) before surgery (day 35-49), n=8/group. Data from resected tumors is shown. *Investigator sponsored trial NCT03690986, in collaboration with Emory University* **A. Pepinemab treatment was associated with higher density TLS and no recurrence.** ▼= no treatment. Statistical analysis: Two tailed unpaired t test, P<0.05 **B. Pepi combinations result in durable recurrence-free survival compared to Nivo alone.** Statistical analysis: Log-rank (Mantel-Cox) test P=0.0268. pCR=pathologic complete response; mPR=major pathologic response

## METHODS

*Sponsor: Vaccinex*
*Pembrolizumab provided by: Merck Sharp & Dohme Corp.*

### KEYNOTE-B84 R/M HNSCC Clinical Trial Design

- Recurrent & Metastatic HNSCC
- Immunotherapy naïve
- Enroll both PD-L1 high (CPS≥20) & PD-L1 low/neg (CPS<20)
  22C3 pharmDx kit (Dako)
- Treatment: 20mg/kg pepinemab + 200mg pembrolizumab, Q3W

**Phase 1b** Safety Well tolerated → **Phase 2** Pre-specified Interim Analysis 36 patients → **Outcome Measures** Safety Objective Response Biomarker Outcomes

NCT04815720

**IHC Biomarker Analysis**

Biopsies collected pre-treatment screening and at week 5 cycle 3

1. Stain → Image → Strip → Stain ~14 staining cycles per slide

| Lymphocyte Panel | APC Panel | Myeloid Panel |
|---|---|---|
| Hematoxylin | Hematoxylin | Hematoxylin |
| Sema4D | Sema4D | CD33 |
| PD-1 | CD163 | CD15 |
| CD69 | CD11c | CD14 |
| CD8 | HLA-DR | Arg1 |
| CD4 | CD68 | HLA-DR |
| FoxP3 | CD141 | Sema4D |
| CD26 | CD206 | S100A9 |
| CD20 | Arg1 | CD16 |
| CD39 | PanCK | PanCK |
| CD45 | PD-L1 | PD-L1 |
| TCF1 | | |
| PanCK | | |
| Ki67 | | |

2. Virtually align stains by panel using Visiopharm® Tissuealign

3. ROI drawn around **pathologist identified tumor area, includes tumor and tumor-associated stroma**

4. Image analysis software, Visiopharm® algorithms were written and **automated** to identify cell phenotypes, quantify density within entire tumor area, neighborhoods.

5. **Unbiased Software algorithm identifies B cell aggregates** using heatmaps. Heatmaps look at B cells clustering within 50um of each other.

6. **B cell aggregates classified by:**
   Low Density B cells (<20 cells)
   High Density (Mature) B cell aggregate(5,6) (≥20 cells)

7. Expand B cell hubs by 150um to **identify cells interacting with B cells.**

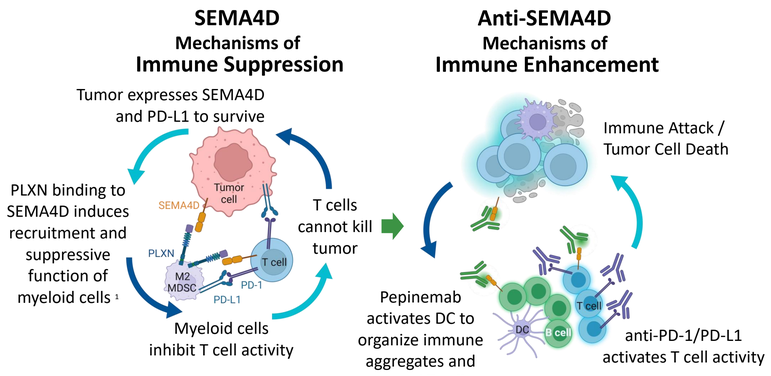
8. **Classification of cells within Immunity Aggregates**

## RESULTS: R/M HNSCC

### KEYNOTE-B84 interim analysis

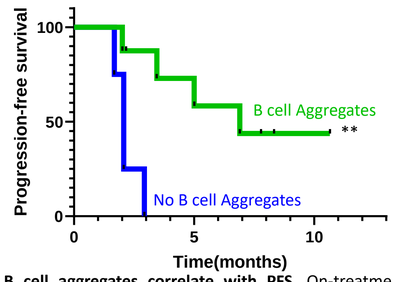
Spatial analysis of immune cells in TME revealed that combination therapy induced highly organized immune aggregates localized within HNSCC tumor bed with high density of activated B cells, DC's, CD4+ and CD8+ T cells, including stem-like CD8+TCF1+PD1+ T cells.

*Illustrations created using BioRender*



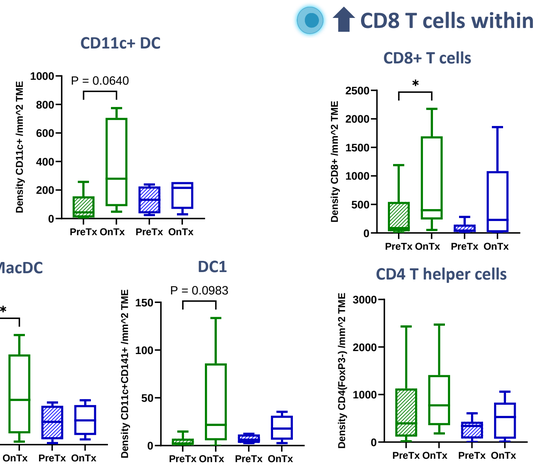

### Immune Aggregates correlate with PFS

Longer progression free survival (PFS) with presence of B cell aggregates




**B cell aggregates correlate with PFS.** On-treatment Patient biopsies with one or more B cell aggregates positively correlates with longer progression-free survival. N=12 on-treatment biopsies at interim analysis. Log Rank survival statistical analysis resulted in a ** p value of 0.0056.

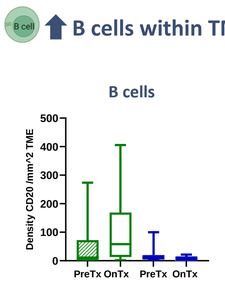
### Dendritic cells within TME




### CD8 T cells within TME

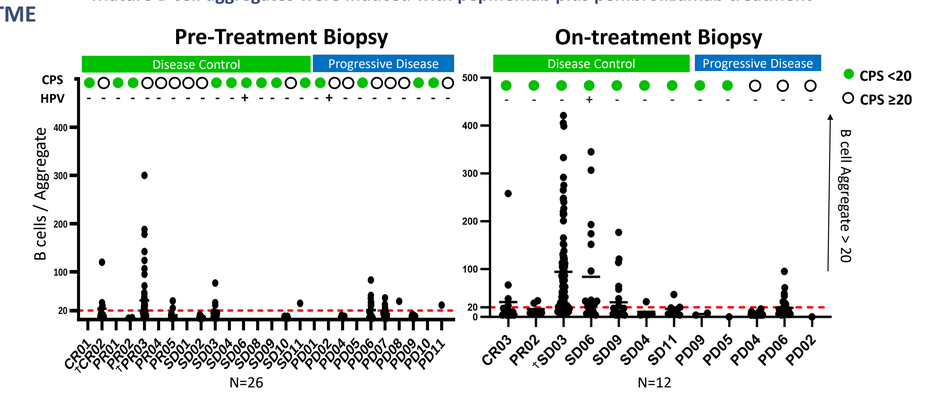



### B cells within TME




### Induction of B cell Aggregates within TME

Mature B cell aggregates were induced with pepinemab plus pembrolizumab treatment

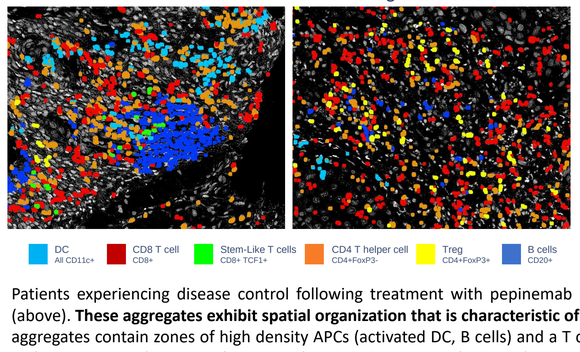



Disease Control
Disease Progression

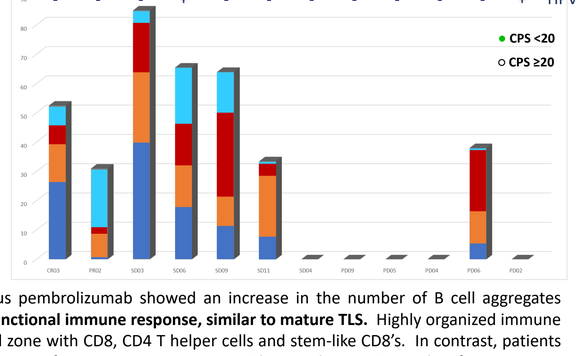
**Increase in CD11c+ dendritic cell populations and CD8 T cells** in pathologist-defined tumor area (TME) following treatment is associated with disease control (CR, PR, SD). **Presence of mature B cell aggregates is induced with treatment, associated with durable disease control, and is unexpected in HPV-negative HNSCC**. Mature B cell aggregates contain >20 B cells (5). B cell aggregates appear to be induced by treatment, as only 31% (5/16) of Pre-Tx biopsies among Disease Control tumors contained mature B cell aggregates, while 100% (7/7) contained mature aggregates following treatment. ϯ designates biopsies from distal metastasis to lung, all other samples are local to head and neck regions. *Disease Control (includes CR complete response, PR partial response, SD stable disease) determined by Response Evaluation Criteria (RECIST1.1) Statistical analysis: Two tailed unpaired t test, P<0.05.*

## Composition of Immune Aggregates

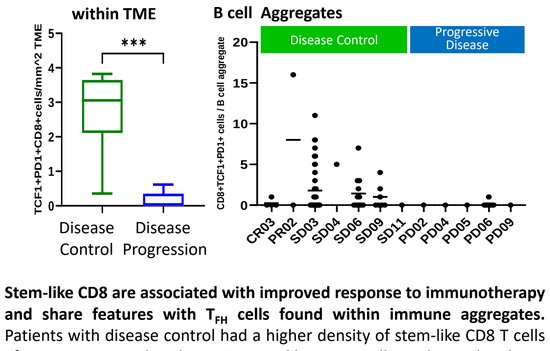
### B cell aggregates are highly organized with key immune cells for antigen presentation and expansion of T cells




### Durable disease control is associated with a higher percentage of DC's within TLS following treatment




### TCF1+PD1+CD8+ Stem-like progenitor cells are associated with Disease Control and located within B cell aggregates

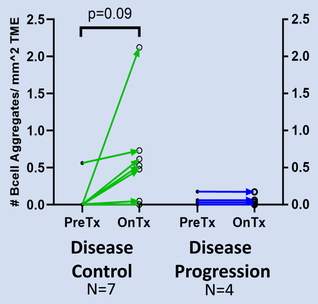



Patients experiencing disease control following treatment with pepinemab plus pembrolizumab showed an increase in the number of B cell aggregates (above). **These aggregates exhibit spatial organization that is characteristic of functional immune response, similar to mature TLS.** Highly organized immune aggregates contain zones of high density APCs (activated DC, B cells) and a T cell zone with CD8, CD4 T helper cells and stem-like CD8's. In contrast, patients with progressive disease and untreated patient tumors predominantly contain no or few immune aggregates with spatial interactions that favor immune suppression, including abundance of Treg.

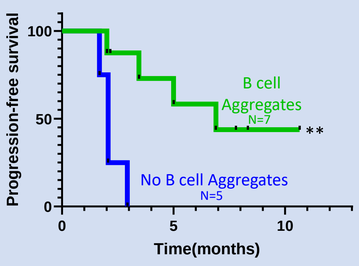
**Stem-like CD8 are associated with improved response to immunotherapy and share features with $T_{FH}$ cells found within immune aggregates.** Patients with disease control had a higher density of stem-like CD8 T cells after treatment within the TME. Stem-like CD8 T cells are located within B cell aggregates. Statistical analysis: Two tailed unpaired t test, P<0.05

## CONCLUSION

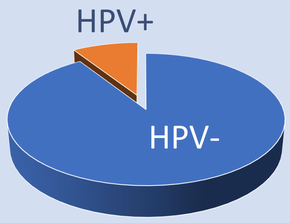
**1** **Pepinemab plus pembrolizumab induce B cell aggregates in patients experiencing disease control**




**2** **Immune Aggregates correlate with Progression Free Survival (PFS)**




**3** **B cell aggregates are induced in hard-to-treat HPV neg patients with disease control**
10/11 matched pre to on treatment biopsies were HPV-




**4** **Pepinemab plus Pembrolizumab showed ~2x increase in ORR and PFS in hard-to-treat PD-L1 low tumors** compared to historical response rate for checkpoint monotherapy in this population

| | PD-L1 Low (CPS < 20) | | |
|---|---|---|---|
| | KEYNOTE-B84 pepi + pembro | | KEYNOTE-048 (7) pembro |
| Total | (19) | | (168) |
| CR | 2 | 10.50% | 2.40% |
| PR | 2 | 10.50% | 9.50% |
| SD | 10 | 52.60% | 25.00% |
| ORR* | 4 | 21.10% | 11.90% |
| DCR | 14 | 73.70% | 36.90% |
| PFS, months (95% CI) | | 5.79 (2.2 - NR) | 2.2 (2.1 - 2.9) |

Results suggest that pepinemab combined with ICI induced formation of highly organized lymphoid aggregates in the tumor with a high density of activated B cells, DC and T cells including TCF1+PD1+CD8+ stem-like progenitors. Together with similar observations indicating that combination immunotherapy with pepinemab induces mature lymphoid structures in tumors of patients with metastatic melanoma, provides evidence of treatment-induced biologic activity corresponding with disease control and suggests a novel and independent mechanism of pepinemab to enhance immune interactions and activity of ICI in resistant settings.

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NOTE: CPS <20 was calculated post-hoc from analysis of CPS<1 and 1-19 assessments; these do not represent alpha controlled analyses.

SCAN ME