# Neoadjuvant pepinemab enhances DC function associated with mature tertiary lymphoid structures and activity of immune checkpoint blockade in patients with metastatic melanoma

VACCINEX 6007

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## ABSTRACT

Neoadjuvant treatment with immune checkpoint blockade (ICB) improves clinical benefit relative to adjuvant therapy in multiple types of cancer. Window of opportunity studies employing neoadjuvant treatment permit integrated assessments of pathologic response along with assessments of treatment effects and mechanisms of resistance in the tumor microenvironment. Resistance mechanisms including immune exclusion and myeloid suppression have been shown to be regulated by semaphorin 4D (SEMA4D) in preclinical and clinical studies.

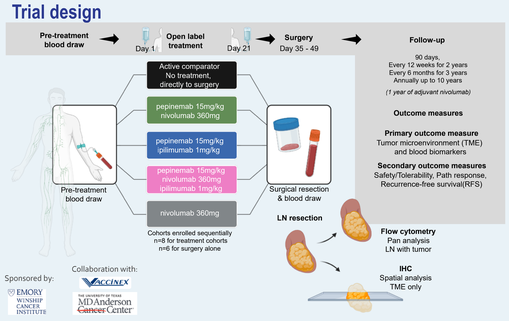
A neoadjuvant window of opportunity study was designed to evaluate activity and efficacy of pepinemab, SEMA4D blocking antibody, combined with nivolumab (nivo) and/or ipilimumab (ipi) in patients with surgically resectable metastatic melanoma.

## METHODS

Consenting patients with resectable stage III melanoma received neoadjuvant nivo alone, pepinemab/nivo, pepinemab/ipi, or pepinemab/nivo/ipi (NCT03769155). Patients underwent six weeks of immunotherapy, followed by surgical resection one year of adjuvant nivolumab. In separate studies conducted at University of Texas MD Anderson Cancer Center, patients received neoadjuvant treatment with nivo or nivo/ipi and underwent surgical resection following six weeks of immunotherapy[1]. Surgical tissue underwent high dimensional analysis using multiparametric flow cytometry, multiplex immunohistochemistry and Nanostring RNAseq and GeoMX imaging to evaluate biomarkers of immune response to treatment. Nanostring and flow cytometry was performed using entire biopsy tissue. Quantification from multiplex IHC was restricted to pathologist defined tumor area (include language as defined in manscript). GeoMx analysis was restricted to areas of TLS. Circulating lymphocytes and soluble factors were also evaluated in blood at baseline and day of surgery.

Building on prior clinical experience, we hypothesized that pepinemab could be safely administered in the neoadjuvant setting to melanoma patients in combination with ICIs. We further proposed that blocking SEMA4D would enhance ICI efficacy through distinct and complementary immunomodulatory mechanisms.

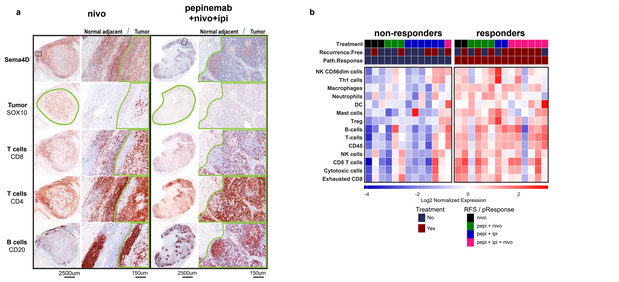
### Trial design




## RESULTS

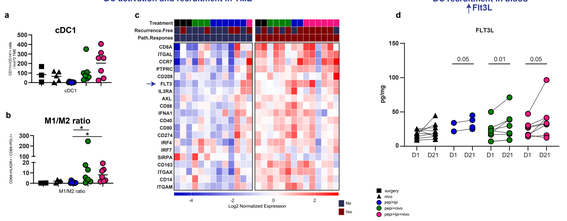
### Pepinemab represents a novel strategy to boost dendritic cell infiltration, activation, and organization of functional TLS to overcome limitations of immune checkpoint inhibitors (ICI).

#### 1 Pepinemab overcomes immune exclusion to enhance response to ICI




**Immune cells are excluded from tumor bed in non-pepinemab containing treatments.** In contrast, pepinemab treatment enables penetration of immune cells deep within the tumor with pepinemab treatments (a) 1x and 20x magnification of representative images from non-pepinemab (nivolumab) and pepinemab-containing (pepinemab/nivolumab/ipilimumab) post treatment surgical resections. Pepinemab (anti-SEMA4D), nivolumab (anti-PD-1), ipilimumab (anti-CTLA-4). (b) Nanostring analysis of resected tissue demonstrates increase in immune cells within responders by immune Cell Type abundance profiling based on Rosalind Cell Type analysis (mean of log2 normalized expression markers). Pathological response includes PR, mPR and CR. Recurrence Free rates defined as >1year.

#### 2 Pepinemab recruits and reprograms dendritic cells (DC)



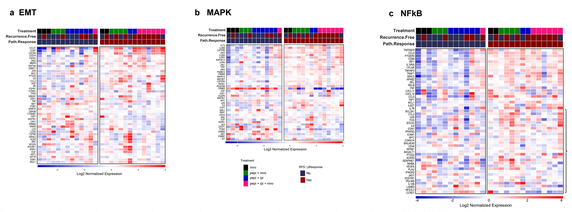

**Pepinemab containing treatments recruit and activate DC within the TME and circulating blood.** (a) cDC1 abundance and (b) quantification of M1/M2 ratio (CD68+HLA-DR+/CD68+PD-L1+) in melanoma tumors, as determined by multiplex IHC, demonstrates increased levels of APC in tumors of patients treated with pepinemab combination therapies, as compared to those receiving nivolumab or no immunotherapy (surgery). Data analyzed by ordinary one-way ANOVA. Pepinemab (pepi), nivolumab (nivo), ipilimumab (ipi).(c) Nanostring analysis of dendritic cell markers, cytokines and transcription factors based on Rosalind Cell Type analysis (mean of log2 normalized expression markers). Pathological response includes PR, mPR and CR. Recurrence Free rates defined as >1year. (d) Quantification of plasma levels of FLT3L comparing baseline(D1) to Day 21 (D21) for each treatment group demonstrates increase in FLT3L in pepinemab containing treatment arms relative to nivolumab. Data analyzed by paired T-test.

#### 3 Pepinemab-containing regimens elicit immune gene signatures in metastatic melanoma tumors are associated with clinical benefit:

immune cell recruitment

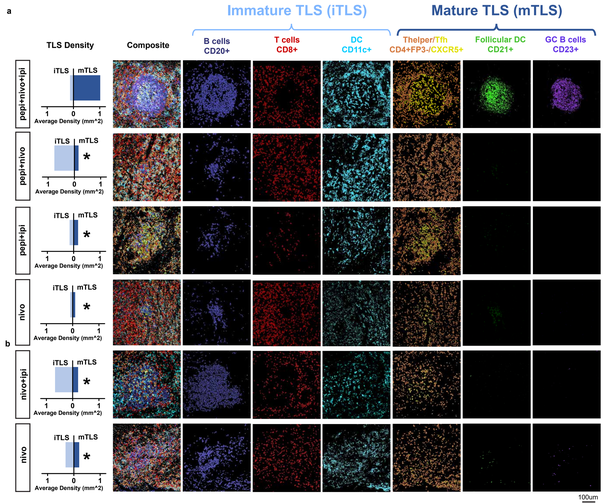
antigen presenting cell mobility and polarization

shift to memory B cell response

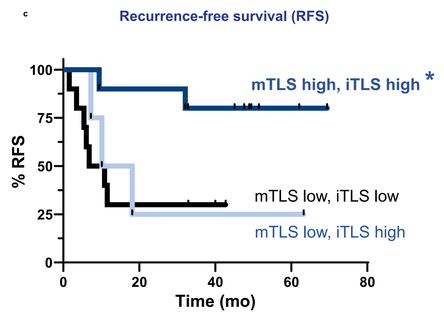



**Pepinemab + nivo reveals enrichment in transcriptional genes from bulk tumor in pathways related to EMT, MAPK, and NF-kB signaling when compared with nivo alone.** Heatmaps of genes enriched in pathways with the greatest number of differential genes (a) EMT, (b) MAPK, and (c) NF-kB were also associated with clinical benefit. Differential expression of EMT and MAPK related genes, including strong upregulation of FLT3, CXCR4, CCL21, CCR7, associated with immune cell recruitment, as well as MET, HGF, GREM1, CCL2, which influence mobility and polarization of antigen presenting cells. Triple combination of pepinemab + nivo + ipi drives a shift towards non-canonical NF-kB signaling suggesting a strong memory B cell response. Pathways stratified by treatment group and pathological response.

#### 4 Pepinemab and ICI therapy drive formation of immune aggregates in tumors. Presence of mature TLS in triple combination therapy correlates with positive clinical outcome.



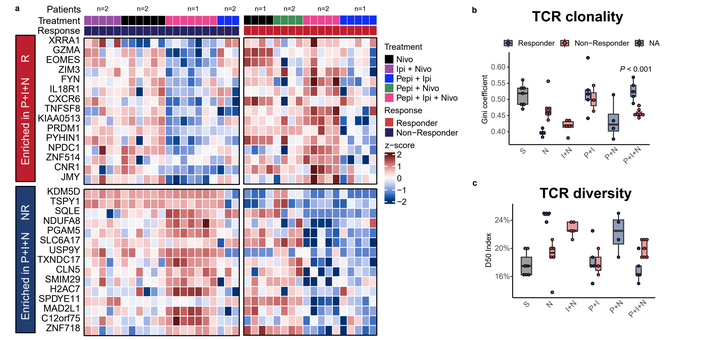

**Mature tertiary lymphoid structures correlate with improved recurrence free survival in patients treated with pepinemab and ICI** (a) Quantification of average TLS density within the tumor bed categorized by maturity: Immature (iTLS) defined as CD21+/- CD23- CD20+ aggregates. Mature (mTLS) defined as CD21+ CD23+ CD20+ aggregates. Representative mIHC images depicting composition of iTLS and mTLS in Emory melanoma patients and (b) biologic comparator cohorts from MDACC. Asterisk denotes a significant increase ($p<0.05$) in mTLS compared to the pepi + nivo + ipi cohort. ANOVA with Tukey's multiple comparison test was used to calculate P values. (c) Kaplan-Meier survival analysis stratified by mTLS high correlated with longer RFS relative to mTLS low, regardless of iTLS abundance. Log-rank test was used to calculate P values. Recurrence-free survival (RFS), mature TLS (mTLS), immature TLS (iTLS), pepinemab (pepi), nivolumab (nivo), ipilimumab (ipi).




#### 5 Pepinemab + ICI increases clonal expansion of cytotoxic T cells within TLS

**Addition of pepinemab to ipi + nivo treatment overcomes immunosuppression and ICI resistance by enrichment of genes associated with cell proliferation and GPCR signaling**

**Responding patients to pepinemab containing treatments demonstrated clonal expansion and reduced diversity of cytotoxic T cell signatures**




**Nanostring GeoMx was employed to determine differentially expressed genes within intratumoral TLS.** Responders treated with pepinemab/nivolumab/ipilimumab were enriched for cell proliferation and GPCR signaling along with TCR clonal expansion relative to treatment with nivolumab/ipilimumab (a) Heatmap showing the expression of the top 15 genes that were specifically up- and down-regulated in responders vs non-responders receiving Pepi + Ipi + Nivo (FDR < 0.05), with the expression patterns displayed across all treatment groups (except surgery). (b) Boxplot illustrating Gini coefficients as a measure of TCR clonality in TLS. (c) Boxplot showing D50 indices as a measure of diversity in TLS. TLS, tertiary lymphoid structures; MDACC, MD Anderson Cancer Center; pepi (P), pepinemab; Ipi (I), ipilimumab; nivo (N), nivolumab; TMA, tissue microarray; ROI, regions of interest; R, responder; NR, non-responder.

## CONCLUSIONS

Pepinemab in combination with nivo and/or ipi significantly increased tumor-infiltrating B cells and CD4+ T cells within TME, compared to nivo alone.

Significant changes in DC activation markers including Flt3 and cDC2 marker CD163, correlated with pepinemab treatment and pathologic response.

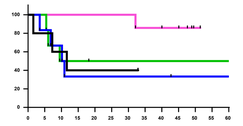
Tertiary lymphoid structures (TLS) detected in biopsies of patients treated with pepinemab in combination with ICI were larger and more mature, containing high density of activated DCs and follicular DCs, follicular B cells, and Tfh cells expressing CXCR5, characteristic of germinal centers. Mature TLS were significantly increased in the triple combination compared to doublets or nivo alone. High mTLS within the tumor bed following treatment correlates with prolonged recurrence-free survival.

Increased density and maturity of TLS and increased T cell clonality correlated with pepinemab treatment and clinical benefit. All patients receiving triple combination therapy (8/8) were recurrence-free at 24 months with mature TLS in the resected tumors (6/6).

### Pathologic response rates

| Cohort | Therapy | N | pCR | mPR |
|---|---|---|---|---|
| A | pepinemab / nivolumab | 8 | 25.0% | 37.5% |
| B | pepinemab / ipilimumab | 8 | 12.5% | 12.5% |
| C | pepinemab / ipilimumab / nivolumab | 8 | 62.5% | 75.0% |
| D | nivolumab | 8 | 25.0% | 37.5% |

### Reccurence-free survival

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### References

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