Inhibition of Semaphorin 4D induces lymphoid aggregates, correlating with clinical outcomes when combined with immune checkpoint therapy

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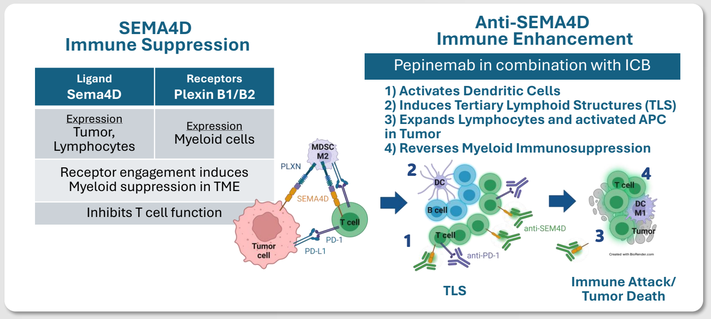
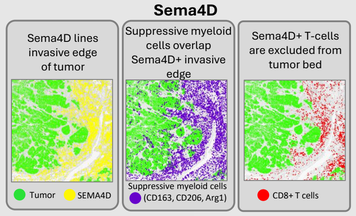
Summary

Evidence from multiple clinical studies suggests that pepinemab treatment led to increased penetration of immune cells organized into lymphoid aggregates of progressive maturity. This effect was further amplified when combined with immune checkpoint therapies (ICB), even in immunologically cold TME observed in PD-L1 low and HPV-negative HNSCC. Spatial analysis revealed an increase in lymphoid aggregates comprised of B cell clusters mixed with dendritic cells (DC) and a T cell zone including CD8 and CD4 T helper cells following treatment. Mature aggregates identified in biopsies of patients treated with pepinemab and ICB combinations were larger and contained follicular dendritic cells, follicular B cells, and Tfh cells expressing CXCR5, characteristic of germinal centers (GC). Increased density and maturity of lymphoid aggregates correlated with disease control and longer progression-free survival (PFS) / recurrence-free survival (RFS).

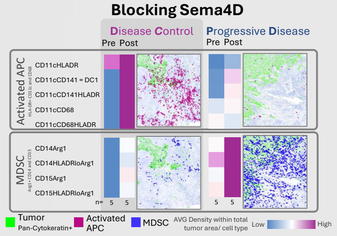


Background

Myeloid cells have a crucial role in suppressing adaptive immunity. Preclinical studies have shown that SEMA4D signaling through its cognate receptors (Plexin B1/B2, CD72) promotes myeloid cell recruitment and suppressive function within the tumor microenvironment (TME) (Clavijo PE et al. Cancer Immunol Res. 2019 (2):282-291). When SEMA4D is blocked from binding to its receptors, suppression is reduced, leading to increased penetration and organization of antigen presenting cells (APC) and B and T lymphocytes in the tumor microenvironment. It is expected that this would facilitate interaction and communication among these cell populations, and lead to improved immune responses in otherwise "cold" tumors.



Sema4D regulates myeloid suppression and immune exclusion. Pro-inflammatory cells are excluded from tumor and accumulate at the invasive edge. CD8 and CD4 T cells are excluded from the tumor bed and many express Sema4D. Suppressive myeloid cells express receptors for SEMA4D and restrict T cells from entering tumor at the invasive edge. *Human HNSCC tumor*[2]



Antibody blockade of SEMA4D with pepinemab alters the balance from suppressive myeloid cells to antigen-presenting cells. Combination treatment with pepinemab and pembrolizumab appears to reverse the immunosuppressive tumor microenvironment in patients who experienced clinical benefit, compared to those with progressive disease. Human HNSCC tumor[1]

Methods

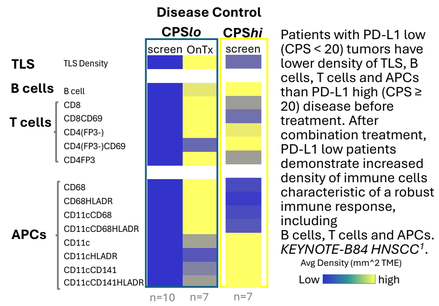
Screening and on-treatment tumor biopsies were collected from several clinical trials:

Trial	Inclusion	Treatment Cohorts	End-points	Status
KEYNOTE-B84 (NCT04815720)[1]	First-line, IO naïve, R/M HNSCC	pepinemab + pembrolizumab	Safety and efficacy	Ongoing N=
Biomarker neoadjuvant HNSCC (NCT03690986)[2]	surgically resectable HNSCC	pepinemab pepinemab + nivolumab Pepinemab + ipilimumab nivolumab alone or ipilimumab alone	Biomarker analysis	Ongoing N=6 per cohort
Biomarker neoadjuvant Melanoma (NCT03769155)[3]	Metastatic melanoma, surgically resectable	pepinemab + nivolumab + ipilimumab pepinemab +nivolumab pepinemab + ipilimumab nivolumab alone	Biomarker analysis	Manuscript in progress N=8 per cohort

Biomarker analyses included the evaluation of tumoral immune cells using multiplex immunohistochemistry (mIHC) assessing up to 36 markers per biopsy. Unbiased algorithms were used to identify co-localized markers for advanced cell phenotyping, density, spatial, and proximity analysis using Visiopharm image analysis software. The biomarker results were then stratified by clinical outcome measures.

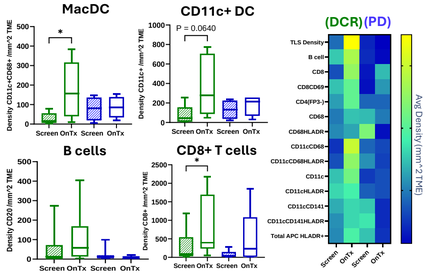
Pepinemab, a Semaphorin 4D (SEMA4D) blocking antibody, in combination with immune checkpoint therapy (ICB) appears to convert "Cold" tumors to "Hot" by inducing organized lymphoid aggregates.

Combination therapy reprograms Cold tumors to Hot tumors[1]



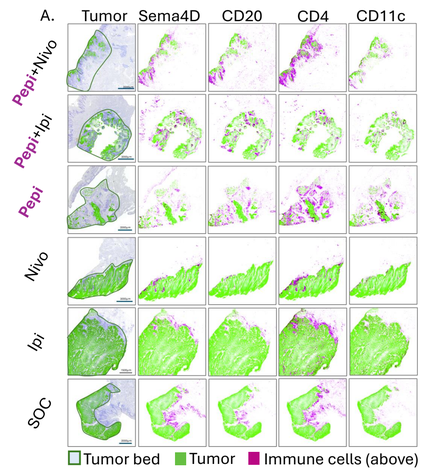
Patients with PD-L1 low (CPS < 20) tumors have lower density of TLS, B cells, T cells and APCs than PD-L1 high (CPS ≥ 20) disease before treatment. After combination treatment, PD-L1 low patients demonstrate increased density of immune cells characteristic of a robust immune response, including B cells, T cells and APCs. *KEYNOTE-B84 HNSCC*[1].

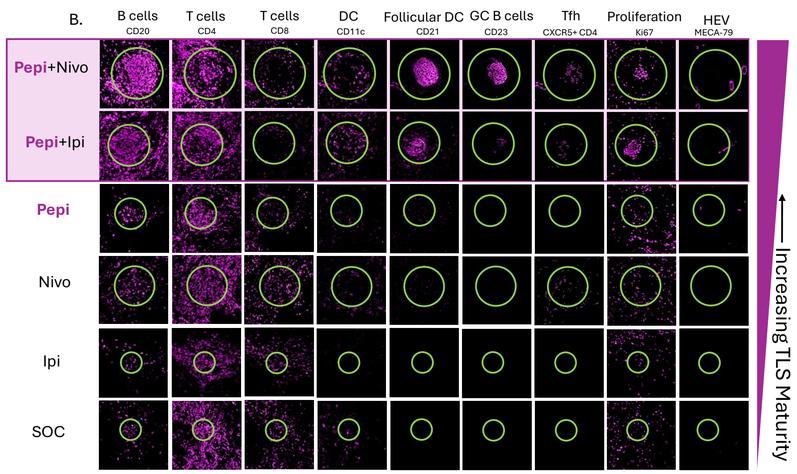
Increase in key immune cells within the TME correlates with Disease Control in HNSCC patients [1]



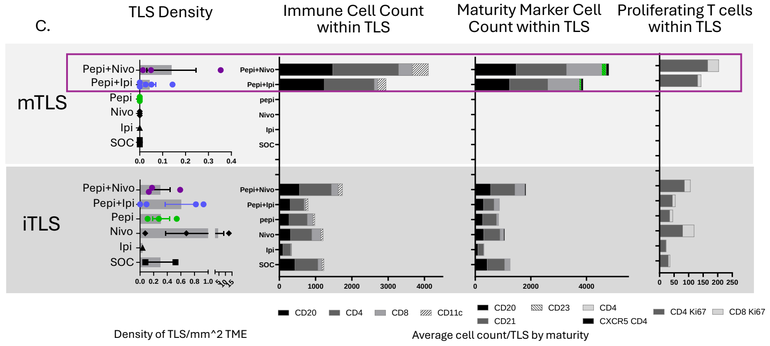
Tumors from patients benefitting from disease control (DCR include: CR, PR, SD) show an increase in important inflammatory immune cell subsets over those with progressive disease (PD) following treatment with with pepinemab plus pembrolizumab). MacDc and CD8 T cells had the most significant increase with treatment. *Statistical analysis: Two tailed unpaired t test, P<0.05. KEYNOTE-B84 HNSCC*[1]

Pepinemab treatment overcomes suppressive barrier allowing penetration and organization of key immune cells into the TME with enhanced maturity of TLS when combined with ICB in HNSCC patients[2]



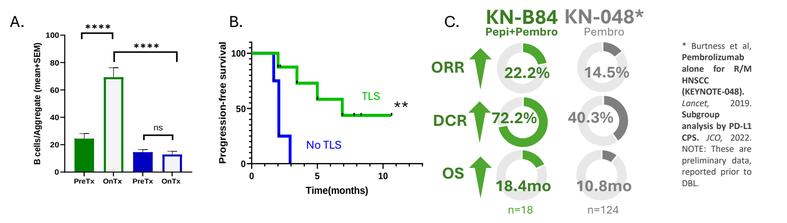


A. Increased immune cell penetration into the tumor bed and reduced tumor burden following neoadjuvant treatment that includes pepinemab. Representative images of HNSCC on-treatment biopsies[2] show Sema4D+ T cells lining the invasive edge of tumors in biopsies without Sema4D blocking antibody. In contrast, following pepinemab treatment, alone and in combination with ICB, an increase in penetration and organization of B cells, T cells and DCs within the tumor beds was observed. **B. A higher frequency of mature TLS were observed in tumors treated with pepinemab in combination with either Nivolumab or Ipilimumab over single immune therapies.** Enhanced maturity stratified using TLS maturity markers: B cell aggregates >20 cells, T cell zone, mature DC, follicular DC, germinal center (GC) B cels (mTLS) T follicular helper, and high endothelial venules (HEV). Proliferation (Ki67+) within TLS suggests biological activity. **C. Quantification of biomarkers,** showing increased density of mature TLS (mTLS) with Pepi in combination with ICB. Average immune cell and maturity marker cell densities by treatment group. Biological activity shown using Ki67+ proliferating B and T cells within the TLS. Note: Analysis shown for HPV- patient biopsies, which tend to lack or have vastly reduced TLS and immune infiltration, relative to HPV+ HNSCC. *Neoadjuvant HNSCC*[2]



Mature lymphoid aggregates correlate with improved clinical outcomes

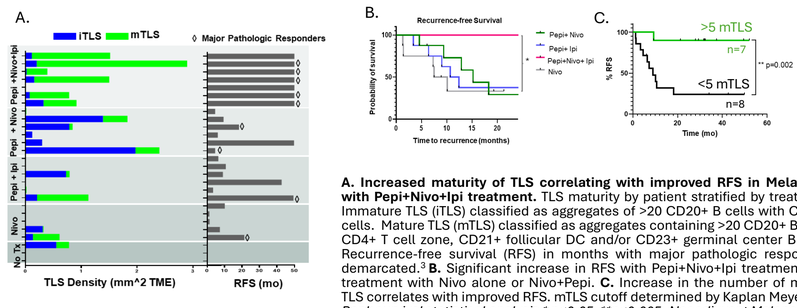
Treatment induces mature immune aggregates and correlates with Disease Control in HNSCC[1]



* Burtness et al, **Pembrolizumab alone for R/M HNSCC (KEYNOTE-048).** *Lancet,* 2019. **Subgroup analysis by PD-L1 CPS.** *JCO,* 2022. NOTE: These are preliminary data, reported prior to DBL.

A. Patients who experience clinical benefit (Disease Control) during treatment with pepinemab and pembrolizumab have a higher frequency of mature immune aggregates with a high density of B cells in their on-treatment biopsy (n=7) compared to their pre-treatment biopsies (n=16), p<0.0001. One-way ANOVA, **** p<0.0001; ns = not significant, p≥0.05. **B. B cell aggregates correlate with PFS**. On-treatment 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. **C. Increased ORR, DCR, and OS in Hard-to-treat PD-L1 low patients (CPS 1-19).** *KEYNOTE-B84 HNSCC*[1]

Combination treatment induces mature immune aggregates and correlates with Recurrence-Free survival in Melanoma[3]



A. Increased maturity of TLS correlating with improved RFS in Melanoma with Pepi+Nivo+Ipi treatment. TLS maturity by patient stratified by treatment. Immature TLS (iTLS) classified as aggregates of >20 CD20+ B cells with CD4+ T cells. Mature TLS (mTLS) classified as aggregates containing >20 CD20+ B cells, CD4+ T cell zone, CD21+ follicular DC and/or CD23+ germinal center B cells. Recurrence-free survival (RFS) in months with major pathologic responders demarcated.[3] **B.** Significant increase in RFS with Pepi+Nivo+Ipi treatment over treatment with Nivo alone or Nivo+Pepi. **C.** Increase in the number of mature TLS correlates with improved RFS. mTLS cutoff determined by Kaplan Meyer. Log *Rank survival statistical analysis *p<0.05, **p<0.005. Neoadjuvant Melanoma*[3]