Regulating myeloid cells to promote mature tertiary lymphoid structures and enhance anti-tumor immunity

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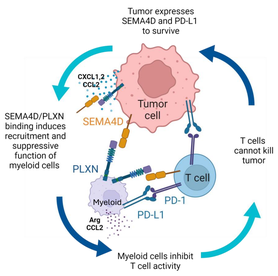
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Abstract

Strategies to recruit and activate DC have the potential to enhance cancer immunotherapy. Semaphorin4D (SEMA4D) binding to PLXNB1/B2 receptors on myeloid cells drives immune exclusion and myeloid suppression. Pepinemab, humanized IgG4 SEMA4D-blocking antibody, reprogrammed myeloid contexture and promoted activated APC and T cells in the TME in preclinical and clinical studies.



- SEMA4D ligand is expressed by both Tumor and T cells.
- PlexinB1/B2 receptors
- are expressed on myeloid cells.

➢ Receptor engagement induces suppressive functions of myeloid cells.

SEMA4D accumulates at the tumor border providing a mechanism that drives immune exclusion and immunotherapy resistance by inhibiting T cell penetration into the tumor bed.

Pro-inflammatory cells are excluded from tumor and build up at the invasive edge
CD8 and CD4 T cells align with SEMA4D at the invasive edge of the tumor. Most of these excluded T-cells express SEMA4D. SEMA4D is also cleaved to generate soluble SEMA4D in TME.
Suppressive myeloid cells express receptors for SEMA4D and restrict T cells from entering tumor at the invasive edge. *Biopsy of human HNSCC tumor, in collaboration with Emory University (NCT)*

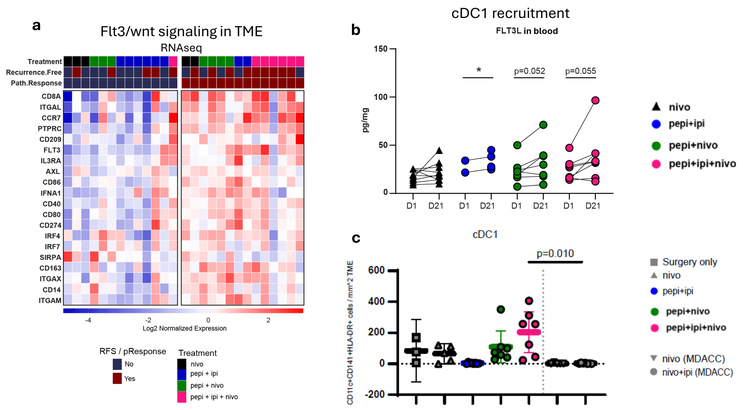


Clinical Trials: Pepinemab neoadjuvant treatment in metastatic melanoma and HNSCC

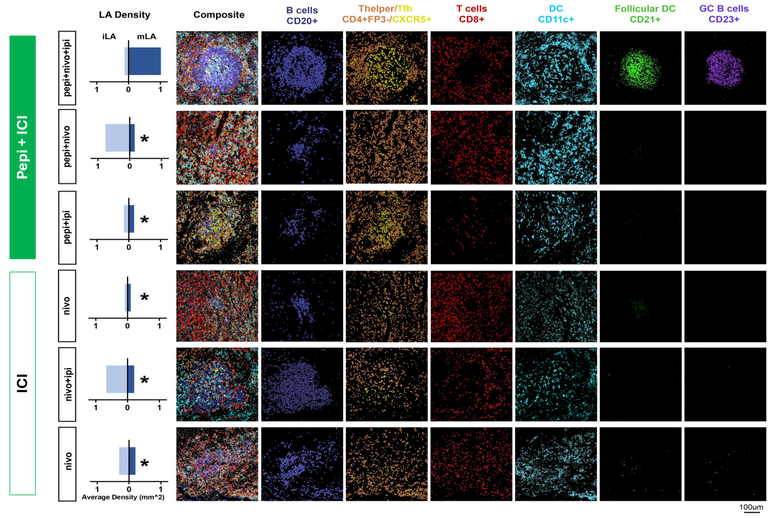
In two separate neoadjuvant treatment studies of surgically resectable metastatic melanoma (NCT03769155) and HNSCC (NCT03690986), treatment with immune checkpoint inhibitors (ICI), nivolumab and/or ipilimumab, and combinations with pepinemab were employed to investigate effects on tumor immunity. Tumor biopsies at screening and on-treatment were characterized using multiplex immunohistochemistry, RNAseq and spatial transcriptomics. In patients receiving pepinemab-containing combinations, TME demonstrated increased density of cDC1 and improved ratio of activated HLA-DR+ to suppressive myeloid cells, compared to ICI alone. Lymphoid aggregates (LA) were larger and more mature, containing follicular CD21+ DC and CD23+ germinal center B cells, and CXCR5+ Tfh cells characteristic of germinal centers. Spatial transcriptomics of mature LA revealed more clonal, less diverse TCR repertoires, and significant changes in genes associated with antigen presentation and follicular organization. Mature LA were associated with clinical benefit. Tumor expression of FLT3-associated genes and circulating FLT3L protein, both linked to cDC1 and TLS development and function, were elevated in patients receiving pepinemab-containing therapy.

Metastatic Melanoma: Neoadjuvant Pepinemab + Immune Checkpoint Therapy

SEMA4D blockade with pepinemab + ICI was associated with increased cDC1 infiltration and mature lymphoid aggregates

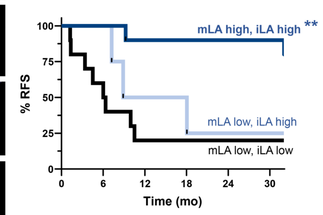


Neoadjuvant trial in resectable Stage III Melanoma, collaboration with Emory University (NCT03769155) Patients received 2 neoadjuvant immunotherapy treatments followed by surgery on day 35-49, followed by 1 year adjuvant nivolumab treatment (a) Nanostring analysis of bulk tumor biopsy shows enrichment of dendritic cell (DC) genes, including FLT3, in responders receiving pepinemab treatment. (b) Quantification of soluble factors in plasma using Eve Technologies 96-plex human cytokine/chemokine Array Discovery Assay shows an increase in Flt3L in pepinemab treated patients from day 1 (D1) to day 21(D21). (c) Multiplex IHC shows an increase in density of cDC1 ratio within tumor in pepinemab + nivo treatment groups. **Ruffin, A., Mallow, C. et al, 2026, Nature Communications**



Melanoma (NCT03769155) in collaboration with Emory University and MD Anderson Cancer Center (MDACC). (Left) Bar graphs show cohort-average of immature lymphoid aggregates (iLA) (light blue) and mature LA (mLA) (dark blue) density for each treatment group, quantified from spatial multiplex IHC (mIHC) analysis of entire tumor area. (Right) Representative pseudo-colored mIHC images of lymphoid aggregates (LA). Images were selected from tumors with iLA and mLA densities approximating the cohort mean. Cell populations characterizing LA include clusters of CD20+ B cells, CD4+ and CD8+ T cells, CD11c+ dendritic cells. mLA also include presence of CD4+FoxP3-CXCR5+ T helper/T follicular helper cells, CD21+ follicular dendritic cells, and CD23+ germinal center B cells.

High density of mLA was associated with longer recurrence-free survival (RFS))



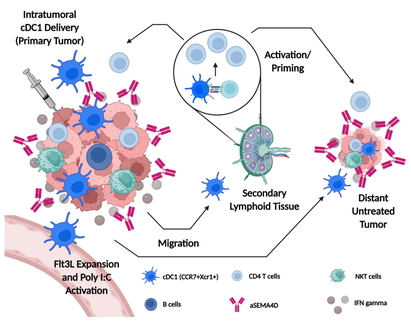
Conclusions

- **SEMA4D blockade remodels the suppressive myeloid microenvironment** promoting a more activated, antigen-presenting state and improved dendritic-cell trafficking.
- **Pepinemab enhances immune organization within tumors** increasing T-cell infiltration and promoting mature tertiary lymphoid structures associated with improved anti-tumor immune responses.
- **Across clinical and preclinical studies, SEMA4D blockade complements checkpoint immunotherapy** supporting a mechanism in which myeloid reprogramming enables more coordinated local and systemic anti-tumor immunity.
- **SEMA4D blockade increases DC migration and interactions with T cells** DC expansion strategies coupled with SEMA4D blockade represent an alternative or complementary approach in settings where ICI is not effective or feasible.

Ex vivo DC expansion and adoptive transfer

-OR-

In vivo DC expansion with Flt3L



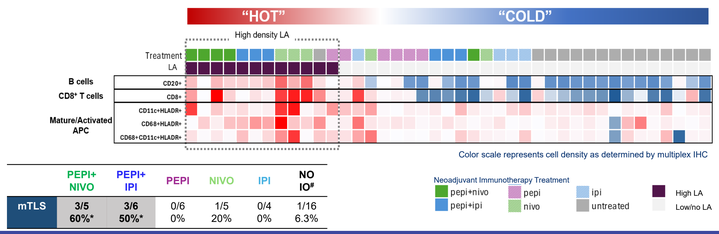
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HNSCC: Neoadjuvant Pepinemab + ICI Therapy

SEMA4D blockade with pepinemab + ICI was associated with increased immune infiltration and mTLS in "cold" HNSCC tumors

Neoadjuvant trial in resectable Head and Neck Cancer, collaboration with Emory University (NCT03690986) Patients received 1 neoadjuvant immunotherapy treatment followed by surgery between days 17-36. (a) Tumors with high density B cells, CD8+ T cells, and mature/activated APC were more frequent in patients that received pepinemab + ICI versus ICI alone. (Quantification of multiplex IHC, cell density in TME)
(b) High density of mature TLS observed with Pepinemab + ICI



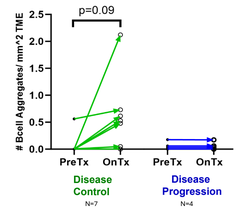
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	PEPI+ NIVO	PEPI+ IPI	PEPI	NIVO	IPI	NO IO#
mTLS	3/5 60%*	3/6 50%*	0/6 0%	1/5 20%	0/4 0%	1/16 6.3%

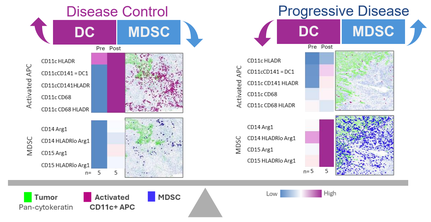
Recurrent/Metastatic HNSCC (KEYNOTE-B84) Patients who experience clinical benefit (Disease Control: CR, PR, SD) during treatment with pepinemab and pembrolizumab have a higher frequency of mature LA with a high density of B cells in their on-treatment biopsy relative to their pre-treatment biopsies. Quantification of matched pre/on-treatment biopsies from same patient. One-way ANOVA.

R/M HNSCC KEYNOTE-B84: Pepi + Pembro

TLS are induced following treatment of pepinemab + pembrolizumab



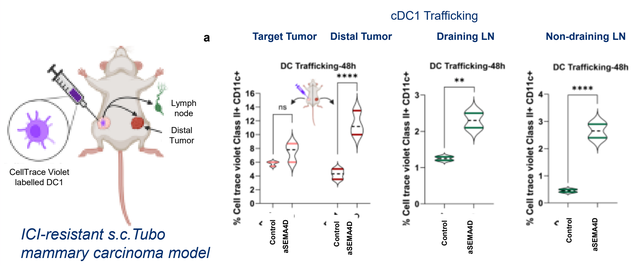
Pepinemab shifts the balance from suppressive myeloid cells to favorable activated DC/antigen presenting cells in the TME



Pre-clinical: SEMA4D blockade enhances abscopal effects of DC therapy

Ex vivo DC expansion

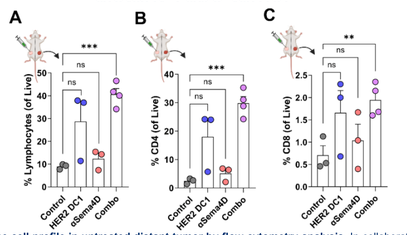
SEMA4D blockade improved DC trafficking to distant tumor and lymph nodes



ICI-resistant s.c.Tubo mammary carcinoma model

Treatment of tumor-bearing mice with SEMA4D blocking antibody promotes active migration of primed cDC1 to lymph nodes and distant tumor sites → improved ABSCOPAL tumor regression. In collaboration with Brian J. Czerniecki at the H. Lee Moffitt Cancer Center and Research Institute. Balb/c mice were subcutaneously injected with HER2+ TUBO breast cancer cells into the left (target) and right (non-target) flank. Antigen-pulsed cDC1 (HER2 DC1) were injected into target tumor; SEMA4D neutralizing monoclonal antibodies (anti-Sema4D) was administered systemically (IP). (a) anti-SEMA4D treatment promotes trafficking of labeled cDC1 into distal tumor and lymph nodes after 48 hours. * Two-way ANOVA (b) anti-Sema4D inhibited tumor growth and tumors were eliminated with the combination of anti-SEMA4D and Her2 DC1 compared to single agents in both targeted and non-targeted tumors. * One-way ANOVA. **Garg et al., 2026, Cell Reports 45, 117050**

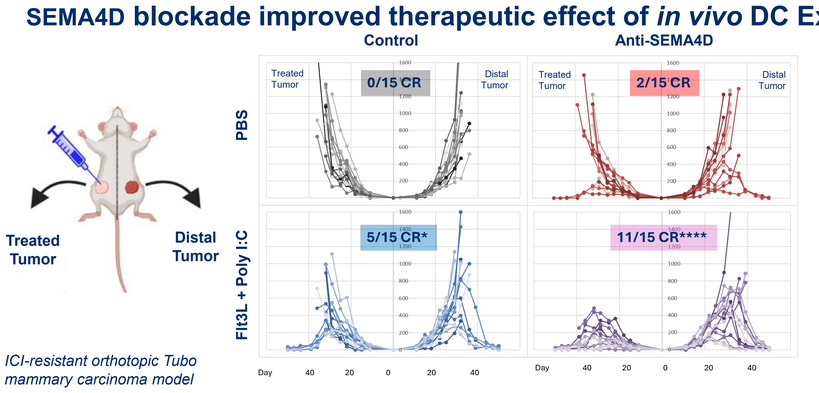
Increased immune infiltration in the distant Tubo tumor



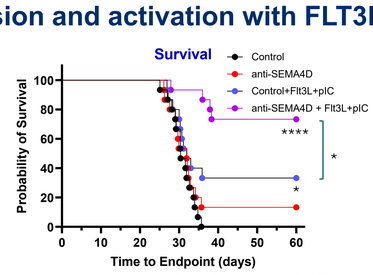
Immune cell profile in untreated distant tumor by flow cytometry analysis. In collaboration with Brian J. Czerniecki at the H. Lee Moffitt Cancer Center and Research Institute. Balb/c mice were subcutaneously injected with HER2+ TUBO in the mammary fat pad. Antigen-pulsed cDC1 (HER2 DC1) were injected into target tumor; SEMA4D neutralizing monoclonal antibodies (anti-Sema4D) was administered systemically (IP). TIL were quantified using flow cytometry. Data are represented as mean +/- SEM. Non-significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by t-test.

In vivo DC expansion

SEMA4D blockade improved therapeutic effect of *in vivo* DC Expansion and activation with FLT3L + pIC

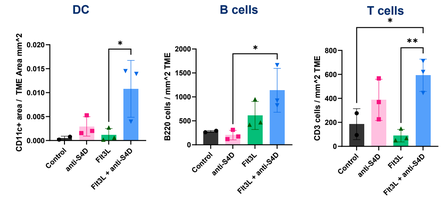


ICI-resistant orthotopic Tubo mammary carcinoma model



Flt3L expansion of DC and activation with Poly I:C combined with SEMA4D antibody blockade improves control of distant tumors. TUBO tumor cells were implanted orthotopically into the mammary fat pad bilaterally on day 0. Only one tumor (Target tumor) received intratumoral (i.t.) injections of Poly I:C (pIC) or PBS; Antibodies (aSEMA4D or Control Ig) and Flt-3 ligand (Flt3L) were administered systemically i.p. (a) Treatment arms included; control mab (control), anti-SEMA4D mab, Control + Flt3L+pIC and anti-SEMA4D+ Flt3L + pIC. Flt3L was administered daily from day 7-13 and day 18-24. Anti-SEMA4D treatment and Poly I:C treatment were on day 12 and 23. Mice were humanely sacrificed if either tumor burden exceeded 800-1000 mm^3. Individual tumor volumes shown, CR = complete tumor regression; Fisher's exact test. Survival calculated up to day 60; Log-rank test. * p<0.05; **** p<0.0001

Increased immune infiltration in Colon26 model



SEMA4D antibody blockade enhanced infiltration of DC, B and T cells into colon tumor. Colon26 tumor cells were implanted subcutaneously on day 0. Treatment arms included; control mab (control), anti-SEMA4D mab (anti-S4D), Flt-3 ligand (Flt3L) or a combination of anti-S4D + Flt3L. Tumors were harvested and prepared for immunohistochemistry on day 16. Quantification of immune cell densities by IHC of three mice per group including dendritic cells (DC), B cells (B220), and T cells (CD3). *One-way ANOVA Tukey's multiple comparison test.