AMPLIFICATION OF THE CXCR3 / CXCL9 AXIS VIA INTRATUMORAL ELECTROPORATION OF CXCL9 SYNERGIZES WITH IL-12 GENE THERAPY (TAVO) TO ELICIT ROBUST ANTI-TUMOR IMMUNITY

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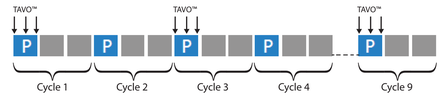
Abstract

Clinical and preclinical studies have demonstrated that plasmid IL-12 (tavokinogene telseplasmid; TAVO) delivered intratumorally via electroporation drives IFN-γ expression and recruits T cells to the tumor microenvironment, ultimately yielding durable systemic T cell responses. Interrogation of longitudinal biomarker data from our IL-12/anti-PD-1 clinical trial has identified that clinical responses are closely tied to intratumoral CXCR3 levels. While all patients had a similar frequency of activated $CD8^+$ T cells in the periphery, responding patients had a significant increase of intratumoral CXCR3 transcripts post-treatment ($p=0.03$) compared to nonresponding patients ($p=0.4$), underscoring the clinical relevance of tumor-infiltrating $CXCR3^+$ immune cells. Since the IFN-γ/CXCL9/CXCR3 axis is known to increase sensitivity to anti-PD-1 therapies, we hypothesized that combining intratumoral TAVO with a DNA-encoded locally secreted CXCL9 (cognate ligand for CXCR3) would further augment/restore this axis and 'license' a robust anti-PD-1 response beyond the treated lesion.

An appropriate CXCL9 gradient can productively modulate frequencies of tumor infiltrating tumor-reactive $CXCR3^+$ T cells. We have previously demonstrated that intratumoral electroporation of plasmid IL-12 and CXCL9 elicits a robust anti-tumor immune response evidenced by increased systemic antigen-specific $CD8^+$ T cells and improved regression of both treated and contralateral CT26 tumors. In the current study, we demonstrate that an enhanced CXCL9 gradient via intratumoral electroporation leads to efficient trafficking of $CXCR3^+$ $CD8^+$ T cells into CT26 tumors. We further explored how DNA-encodable IL-12/CXCL9 can work together to improve checkpoint inhibitor response. We demonstrated that intratumoral TAVO rapidly drives a population of $CXCR3^+$ $CD8^+$ T cells into the lymph node and importantly, depletion of these $CXCR3^+$ immune cells abrogated an IL-12-mediated anti-tumor response. Furthermore, upon electroporation of IL-12 and CXCL9, transcriptomic analysis of the tumor microenvironment revealed an enrichment of genes associated with immune-related pathways (IFN-γ signaling, interleukin signaling, GPCR signaling), antigen presentation machinery, and TCR signaling, indicating that this combination therapy augments anti-tumor immunity. Lastly, leveraging the partially responsive anti-PD-1 CT26 tumor model, we demonstrated that intratumoral electroporation of plasmid IL-12 with CXCL9 significantly improved anti-PD-1 response, providing a strong rationale for filing an Investigational New Drug application based on this intratumoral DNA-encodable combination approach.

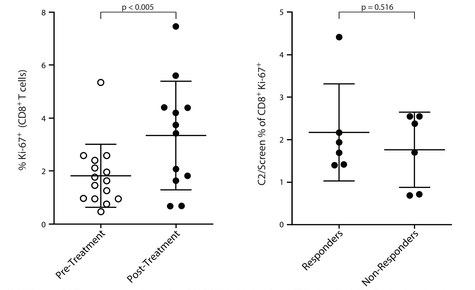
Clinical response to TAVO/Pembrolizumab combination therapy with intratumoral CXCR3 expression

A) Treatment Regimen for OMS-I102



Patients are treated with:
- 0.5 mg/mL TAVO™ to the accessible lesions on days 1, 5, and 8 every 6 weeks, and
- 200 mg IV pembrolizumab on day 1 of each 3-week cycle for 27 weeks.

B) Significant increase in proliferating $CD8^+$T cells post-treatment in both responders and non-responders



C) Increase in intratumoral CXCR3 transcript levels only in responders

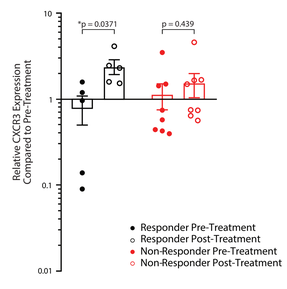
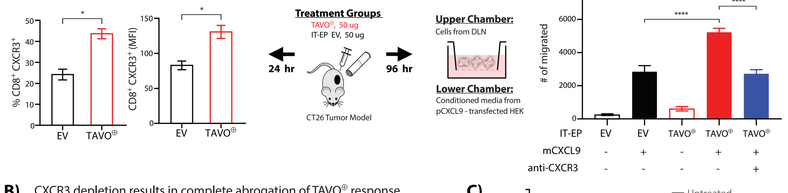


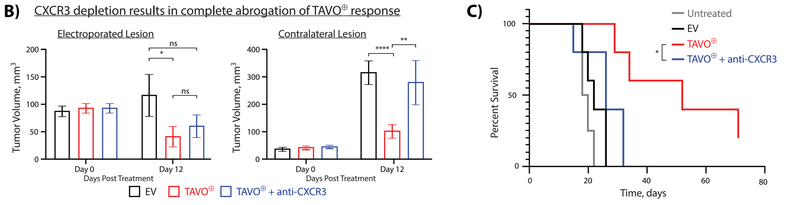
FIGURE 1: A) Treatment regimen for OMS-I102 Clinical Trial (TAVO=pIL12/EP, P= i.v Pembrolizumab). TAVO™ is injected at a dose volume of ~¼ of the calculated lesion volume, with a minimum dose volume/lesion of 0.1 mL. An applicator with a hexagonal array of 6 microneedles is placed into and around the injected tumor, with the tip co-localized at the site(s) and depth of plasmid injection. Six pulses at a field strength of 1500 volt/cm and pulse width of 100 µs at 300-msec intervals are delivered. **B)** Flow cytometric analysis of peripheral PBMCs. **Left Panel:** %Ki67$^+$ lymphocytes (Gating strategy Singlets< Live<CD3<CD8) at pre-treatment (Screen) vs post-treatment (Cycle 2), and in responding vs non-responding patients (**Right Panel**, fold-change over screen, n=6 per group). p-value for unpaired Mann-Whitney *t* test is shown. **C)** Comparison of post-treatment and pre-treatment levels of CXCR3 mRNA transcript between R (n=5) and NR (n=8). *$p < 0.05$ for paired Wilcoxin *t* tests are shown.

CXCR3-mediated signaling is required for intratumoral pIL12-P2A/EP (TAVO⊕) therapy in a CT26 murine model.

A) Intratumoral pIL12-P2A/EP (TAVO⊕) results in an increase in $CXCR3^+$ lymphocytes in the draning lymph node



B) CXCR3 depletion results in complete abrogation of TAVO⊕ response

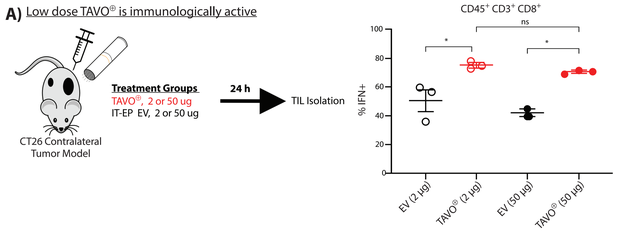


C)

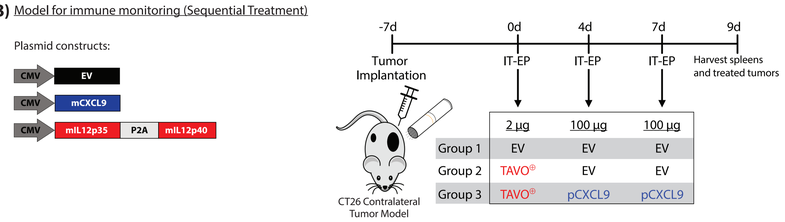
FIGURE 2: A) Left Panel: Frequency and MFI of $CXCR3^+$ expression on CD8 cells from draining lymph nodes collected 24 hours post IT/EP of CT26 tumors with empty vector (EV) or TAVO⊕. Cells were gated on live<singlets<$CD45^+CD3^+$<$CD8^+CD4^-$. Statistical significance determined with Welch's correction unpaired *t* test, *$p < 0.02$. **Middle Panel:** Chemotaxis schematic of draining lymph node cells collected 4 days post-IT-EP with EV or TAVO⊕ (50 µg per treatment). **Right Panel:** Transfection-derived mouse CXCL9 (250 ng/mL) induced chemotaxis through polycarbonate membranes with 5.0-micron pores (Costar 3421). Number of observed chemotactic cells after 2 hours at 37°C was abrogated with the pre-incubation of cells with anti-mCXCR3 monoclonal antibody (BioXCell BE0249). **B)** CT26 contralateral tumor model were intratumorally electroporated with TAVO⊕ with or without concomitant anti-CXCR3 antibody treatment. Growth of primary electroporated **(Left Panel)** and untreated contralateral **(Right Panel)** CT26 lesions after IT-EP with EV, TAVO⊕ + isotype control (red), and TAVO⊕ + anti-mCXCR3 (blue) are shown. (n = 10, data shown is a cumulative plot of two experiments with 5 animals in each group) Statistical significance determined using two-way ANOVA with Bonferroni correction, *$p < 0.02$, **$p < 0.006$, ***$p < 0.0008$, ns: not significant). **C)** Kaplan–Meier curve showing the survival difference between IT-EP TAVO⊕ + anti-mCXCR3 and TAVO⊕ groups (*$p < 0.05$; log-rank (Mantel-Cox) test). 50 µg of EV or TAVO⊕ and 150 µg of anti-CXCR3 was used for treatment.

Intratumoral-pCXCL9/EP and TAVO⊕ combine to increase tumor immunogenicity by modulating the TME and expanding the pool of antigen-specific $CD8^+$ T cells

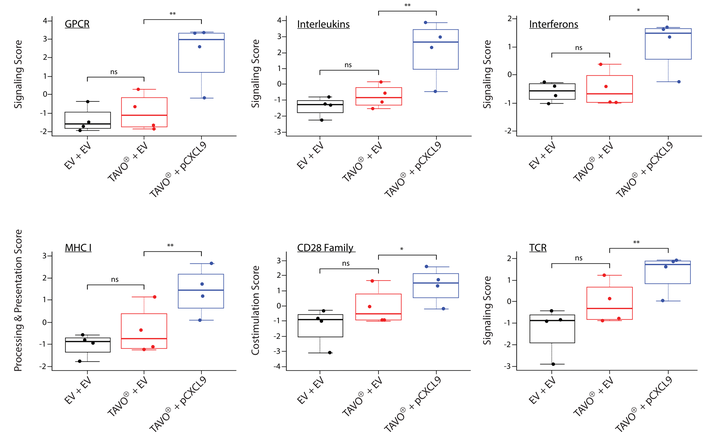
A) Low dose TAVO⊕ is immunologically active



B) Model for immune monitoring (Sequential Treatment)



C) Modulation of intratumoral immune-transcriptome upon IT-pCXCL9/EP and TAVO⊕ combination treatment



D) Antigen-specific $CD8^+$ T cells

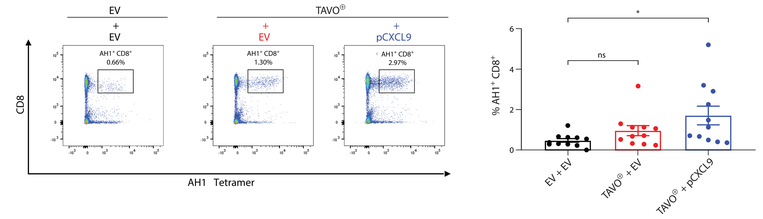
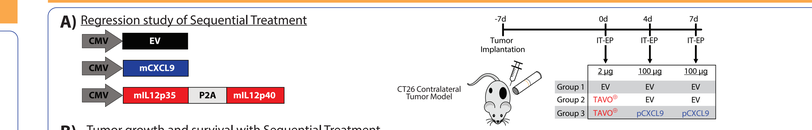


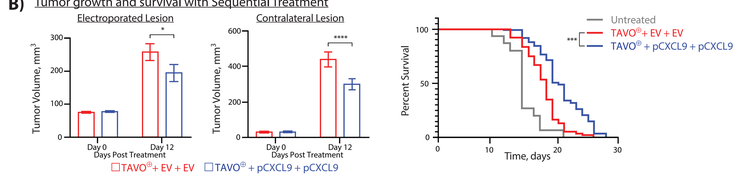
FIGURE 3: A) CT26 contralateral tumor mouse models intratumorally electroporated with 2 or 50 µg of pIL12. Tumor resident $CD8^+$ T cells were isolated and stained for intracellular IFN-γ expression. Intratumoral IFN-γ expression by $CD8^+$ T cells similar regardless of plasmid input amount. **B)** Schematic showing sequential treatment regimen to monitor immune response. CT26 tumor bearing mice were intratumorally electroporated with TAVO⊕ (2 µg, Day 0) followed by IT-EP of 100ug of either pCXCL9 or EV (days 4 and 7). Tumor and splenocytes were harvested 2 days after last EP (i.e. Day 9) for NanoString and flow-based analysis. **C)** Gene expression changes in electroporated CT26 lesions were assessed by NanoString nCounter® technology (mouse PanCancer IO360 panel) with pathway scores. Pathway scores follow the assumptions of equal variance and normal distribution of *t* scores. Ordinary One-way ANOVA was used to calculate significance compared to the empty vector treated group (n=4 per group, *$p < 0.05$, **$p < 0.009$). **D) Left Panel:** Representative plots demonstrating enrichment of antigen specific CD8 T cells ($AH1^+CD8^+$) in mice treated with TAVO®+ pCXCL9 when compared to TAVO® alone or EV on FSC vs SSC<Live<Singlets<$CD4^-$ splenocytes. **Right Panel:** Significant increase in the percentage of $AH1^+CD8^+$ T cells compared to empty vector control (N=3, 3-5 animals/group/experiment; *$p < 0.05$, One way ANOVA).

Intratumoral pCXCL9/EP augments IL-12 mediated abscopal anti-tumor response

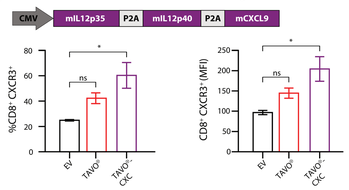
A) Regression study of Sequential Treatment



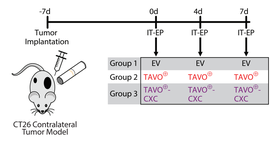
B) Tumor growth and survival with Sequential Treatment



C) Intratumoral CXCR3 expression with Multigene Plasmid (TAVO⊕-CXC) Treatment



D) Regression study for Multigene Plasmid Treatment



E) Tumor growth and survival with Multigene Plasmid Treatment

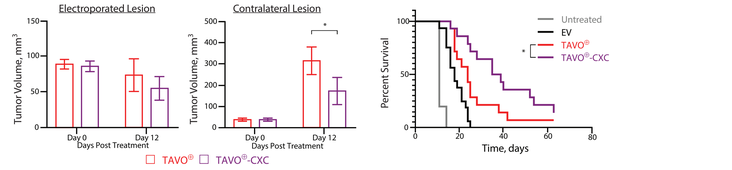
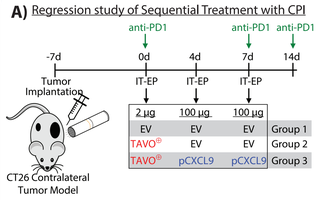


FIGURE 4: A) Schematic illustrating treatment regimen for sequential therapy to assess tumor regression. CT26 contralateral tumor model were intratumorally electroporated with TAVO® (2 µg on Day 0) followed by either IT-EP of pCXCL9 or EV (100 µg, days 4 and 7). Tumor volumes were measured three times a week for regression and survival studies. **B)** Growth of primary electroporated **(Left Panel)** and untreated contralateral CT26 lesions **(Middle Panel)** after IT-EP of TAVO® + EV (red) and TAVO® + pCXCL9 (blue) are shown. (n = 5; 5-10 animals/experiment) statistical significance determined using 2-way ANOVA *$p < 0.05$, ****$p < 0.0001$. Kaplan–Meier curve **(Right Panel)** comparing the survival of the TAVO® + pCXCL9 cohort to that of TAVO® + EV. ***$p < 0.0001$ by log-rank (Mantel-Cox) test. **C)** Schematics of the bicistronic TAVO®-CXC plasmid construct, coding for both IL12 and CXCL9. Frequency and MFI of $CXCR3^+$ expression on CD8 cells from tumors collected 24 hours post IT/EP with EV, TAVO®, or TAVO®-CXC. Cells were gated on live<singlets<$CD45^+CD3^+$<$CD8^+CD4^-$ *$p < 0.05$. **D)** Schematic of the treatment regimen to assess tumor regression of CT26 contralateral tumor model upon treatment with EV, TAVO® or TAVO®-CXC. The dose of TAVO® plasmid was normalized to the amount of mIL12 produced from the TAVO®-CXC plasmid by ELISA. Measurements for day 0 (pre-treatment), and day 14 (post-treatment) are shown. **E)** At 12 days post-treatment, contralateral tumors from the TAVO®-CXC cohort were significantly smaller **(Middle Panel)**. No significant difference was observed in primary tumor growth **(Left Panel)**. (n = 2; 7-10 animals/experiment, statistical significance determined using 2-way ANOVA, *$p < 0.05$). Kaplan–Meier curve **(Right Panel)** showing the survival of the TAVO®-CXC cohort to be significantly better than that of TAVO® (*$p < 0.05$; log-rank (Mantel-Cox) test).

Intratumoral electroporation of IL-12 and CXCL9 improves anti-PD1 therapy

A) Regression study of Sequential Treatment with CPI



B) Tumor growth and survival

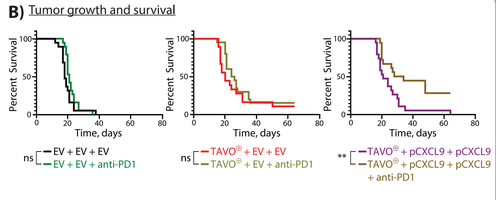
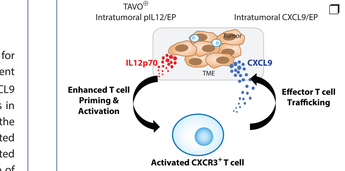


FIGURE 5: A) Schematic showing treatment regimen for combination therapy to assess tumor regression. CT26 tumor bearing mice (two tumor contralateral model) were intratumorally electroporated on Day 0 with EV, or TAVO® followed by 100 µg of either pCXCL9 or EV on days 4 and 7. Each group received 3 weekly i.p. injections of 150 ug of either isotype control (Rat IgG2a) or anti-mouse PD1. Tumor volumes were measured three times a week for regression and survival studies. **B)** Kaplan–Meier curve comparing the survival of the TAVO® + pCXCL9 + anti-PD1 cohort to that of TAVO® + pCXCL9 + isotype (**Right Panel**; **$p < 0.001$; log-rank (Mantel-Cox) test).

Summary and Conclusion

Proposed Mechanism of Action:

Intratumoral IL12/CXCL9



- ❒ IL-12 response is dependent on CXCR3/Chemokine axis in the CT26 tumor model
- ❒ Intratumoral CXCL9/EP when combined with intratumoral IL12/EP (TAVO⊕):
  - » Modulates immune contexture
  - » Results in generation of antigen-specific $CD8^+$ CTL ($AH1^+CD8$ in CT26 model)
  - » Augments abscopal response of TAVO⊕ (CT26 contralateral tumor model).
  - » Significantly improves anti-PD1 response

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