INTRATUMORAL ELECTROPORATION OF PLASMID-ENCODED IL-12 AND MEMBRANE-BOUND ANTI-CD3 INCREASES TUMOR IMMUNOGENICITY AND AUGMENTS THE FUNCTION OF T CELL SUBSETS

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Poster No: LB-390

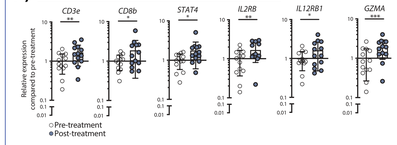
Abstract

Intratumoral (IT) delivery of plasmid IL-12 (tavokinogene telseplasmid; tavo) via electroporation (EP), collectively referred as IT-tavo-EP, generates immunologically-relevant levels of localized IL-12, triggering regression of both treated and distant tumors with minimal toxicity in preclinical and clinical studies. Our previous clinical trial data from melanoma patients treated with IT-tavo-EP identified a treatment-related increase of infiltrating T cells and transcripts related to immune activation, as well as a significant increase in the IFN-γ score of patients with a clinical benefit, suggesting that $CD3^+$ tumor-infiltrating lymphocytes (TIL) may be critical in maximizing the anti-tumor effects of IT-tavo-EP. Furthermore, in-house biomarker data have identified an abundance of non-tumor reactive TIL that, if mobilized could additionally contribute to a clinical response. Accordingly, a plasmid-encoded membrane-bound polyclonal T cell-stimulating anti-CD3 (αCD3) hybrid antibody (scFv) was developed and used in combination with tavo (IT-tavo-αCD3-EP) to broaden the scope and depth of the T cell response. We previously demonstrated that membrane expression of αCD3 on neoplastic and stromal cells could activate $CD3^+$ TIL, driving enhanced proliferation and cytotoxicity in a B16-OVA murine model.

Here, using immune profiling of the tumor microenvironment (TME), we have demonstrated that this membrane-bound αCD3 therapeutic can significantly upregulate frequencies of $CXCR3^+CD8^+$ T cells and short-lived effector T cells, while reducing PD-1 expression on $CD8^+$ T cells in vivo. Critically, naïve T cells, Treg cells, and exhausted T cells (subsets not typically associated with strong anti-tumor responses) displayed enhanced effector function (IFN-γ and granzyme B release) with engagement of membrane-bound αCD3 and IL-12. Furthermore, we found that this therapeutic approach could equally enhance proliferation of T cells regardless of the affinity for their cognate peptide:MHC, suggesting a TCR independent mechanism. Collectively, these observations demonstrate that IT-tavo-αCD3-EP can mobilize broad subsets of T cells beyond dominant anti-tumor effectors demonstrated. Thus, while enhanced cytolytic function is associated with this therapy, inclusion of additional atypical anti-tumor T cell subsets may also promote reshaping of the TME by production of effector cytokines upon engagement of surface-bound αCD3. Moreover, functional restoration of TIL isolated from a melanoma patient with active clinical progression on anti-PD-1 therapy, was possible with engagement of membrane-bound αCD3 in the presence of IL-12. Collectively, these data continue to support the utility of IT-tavo-αCD3-EP as a promising therapeutic approach for patients with melanoma and other accessible solid tumors.

Background

A) Immune activation with IT-tavo-EP treatment



B) Detection of TILs

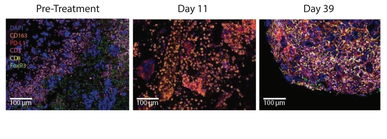
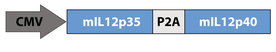


FIGURE 1: In a previous study[1], we reported that patients with stage III/IV melanoma treated with IT-tavo-EP demonstrate significantly increased expression levels of immune activation associated modules **(Panel A)**. Moreover, more TILs were detected post treatment (Day 39; **Panel B**) even if a patient was progressing on IT-tavo-EP. While induction of adaptive resistance may play a role in the lack of clinical response, the composition of the lymphocytic population may also be relevant. Specifically, different types of TILs are generally detected on-treatment, including low avidity T cell clones, "partially exhausted", or senescent T cells and regulatory T cells. These non-activated T cells likely do not exert anti-tumor effects, thereby "wasting" the potential impact of localized IL-12 expression. Here, we report a novel strategy to co-express a potent T cell stimulator with IL-12 via electroporation to enhance local as well as systemic T cells activation.

[1]A. Algazi, S. Bhatia, et al.. Intratumoral delivery of tavokinogene telseplasmid yields systemic immune responses in metastatic melanoma patients. Annuals of Oncology. 2020;31(4):532-540.

Multicistronic P2A-linked plasmid IL-12 and membrane-bound anti-CD3 construct is functional when expressed in vitro and in vivo

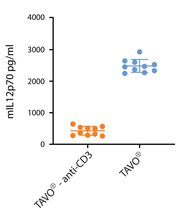
A) P2A-linked mIL12, TAVO®



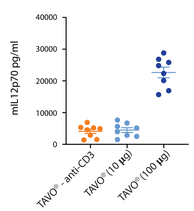
B) anti-CD3 scFv

C) TAVO®- anti-CD3 scFv

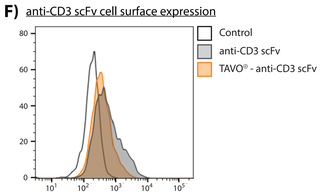
D) *in vitro* mIL12 expression



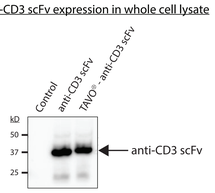
E) *in vivo* mIL12 expression



F) anti-CD3 scFv cell surface expression



G) anti-CD3 scFv expression in whole cell lysate



H) anti-CD3 scFv expression on cell membrane

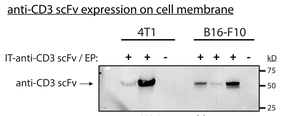


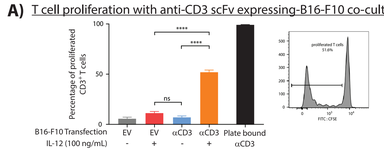
FIGURE 2: A) Schematic of multicistronic P2A-linked murine IL-12 plasmid (TAVO®, mIL12p35-P2A-mIL12p40). **B)** Schematic of anti-mouse CD3 (αCD3; clone: 2C11) single-chain variable fragment (scFv). **C)** Novel multicistronic plasmid incorporating murine anti-CD3 scFv with P2A-linked murine IL-12 (TAVO®-anti-CD3 scFv). **D)** ELISA of mIL-12p70 in conditioned media from HEK293T cells transfected with TAVO® or TAVO®-anti-CD3 scFv plasmid for 72 hours (n=10). **E)** ELISA of mIL-12p70 from whole cell lysates of B16-F10 tumor xenografts 48-hours post-electroporation with TAVO® or TAVO®-anti-CD3 scFv plasmid (n=8). **F)** Flow cytometry analysis of anti-CD3 scFv surface expression on HEK293T cells transfected with pUMVC3 empty vector (EV), anti-CD3 scFv, or TAVO®- anti-CD3 scFv plasmid for 72 hours. **G)** Western blot analysis of anti-CD3 scFv expression from whole cell lysates of HEK293T cells transfected with EV, anti-CD3 scFv, or TAVO®-anti-CD3 scFv plasmid for 72 hours. **H)** Western blot analysis of anti-CD3 scFv from the cell membrane fraction of lysates of B16-F10 and 4T1 tumor xenografts 48-hours post-electroporation with anti-CD3 scFv plasmid.

Combination of IL-12 and TCR stimulation yield T cell proliferation and effector cytokines from both regulatory and naïve T cells

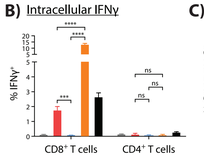
For the following graphs:

B16-F10 Transfection	EV	EV	αCD3	αCD3	Plate bound αCD3
IL-12 (100 ng/mL)	-	+	-	+	

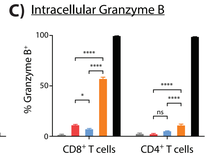
A) T cell proliferation with anti-CD3 scFv expressing-B16-F10 co-culture



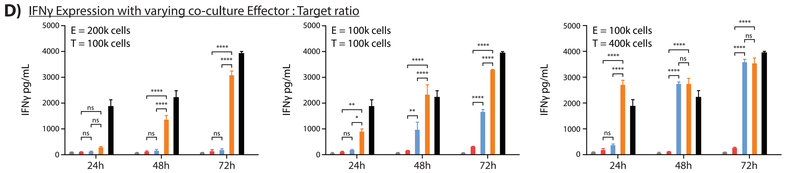
B) Intracellular IFNγ



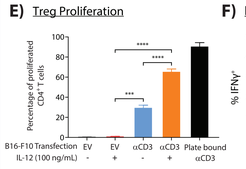
C) Intracellular Granzyme B



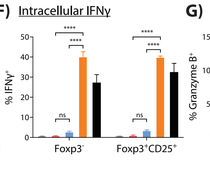
D) IFNγ Expression with varying co-culture Effector : Target ratio



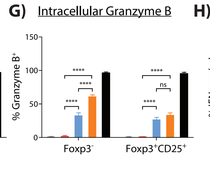
E) Treg Proliferation



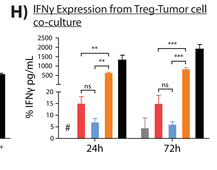
F) Intracellular IFNγ



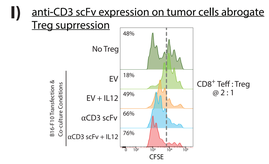
G) Intracellular Granzyme B



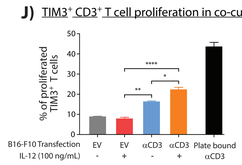
H) IFNγ Expression from Treg-Tumor cell co-culture



I) anti-CD3 scFv expression on tumor cells abrogate Treg supression



J) $TIM3^+$ $CD3^+$ T cell proliferation in co-culture



K) IFNγ expression from $TIM3^+$ $CD3^+$ T cell

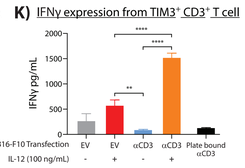


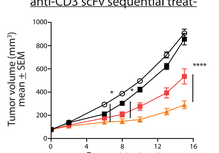
FIGURE 3: A) Percentage of proliferating $CD3^+$ T cells after 4 days of co-culture with B16-F10 cells, which were transfected with EV or anti-CD3 scFv plasmid, with or without 100 ng/ml mIL12. Co-cultures were initiated with similar numbers of $CD3^+$ T cells and B16-F10 cells. $CD3^+$ T cells were cultured with plate bound anti-CD3 as positive control (n=3). **B-C)** Flow cytometric analysis of intracellular IFNγ and Granzyme B expression in $CD8^+$ and $CD4^+$ T cells after 3 days of co-culture with B16-F10 cells, which were transfected in various conditions as described in (A) (n=3). **D)** ELISA measuring IFNγ expression at different effector (E; $CD3^+$ T cells) and target (T; transfected B16-F10) ratios (n=3) with the effector cell number fixed. **E)** Percentage of proliferating $CD4^+Foxp3^+$ Treg cells after 3 days of co-culture B16-F10 tumors, which were transfected in various conditions as described in (A). (n=3). **F-G)** Flow cytometric analysis of intracellular IFNγ or Granzyme B expression in $Foxp3^-$ or $Foxp3^+$ $CD4^+CD25^+$ T cells (n=3). **H)** ELISA measuring IFNγ expression in conditioned media from Treg, B16-F10 co-cultures (n=3). # - below limit of detection. **I)** Polarized *in vitro* Treg suppression assay after 24 hours of co-culture with B16-F10 tumors, which were transfected in various conditions as described in (A). Representatitve image of $CD8^+$ T cells proliferation shown. **J)** Percentage of proliferating $Tim3^+$ $CD3^+$ TILs, derived from B16-F10 tumors, after 3 days of co-culture with B16-F10 cells, which were transfected in various conditions as described in (A) (n=3). **K)** ELISA of IFNγ expression in conditioned media from $Tim3^+$ T cells, B16-F10 co-cultures (n=3). Statistical significance determined via two-way ANOVA for (b, c, d, f, g) and determined via one-way ANOVA for (a, e, j, k). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

Membrane-bound anti-CD3 scFv augments local and systemic TAVO⊕ anti-tumor immunity in a metastatic 4T1 tumor model

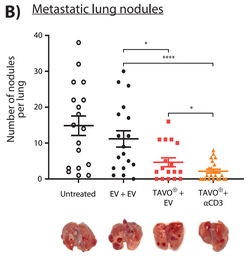
For the following graphs:

- Untreated
- EV + EV
- TAVO® + EV
- TAVO® + anti-CD3 scFv

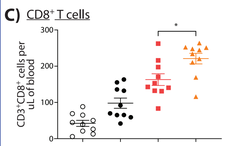
A) Tumor volume with TAVO® and anti-CD3 scFv sequential treat-



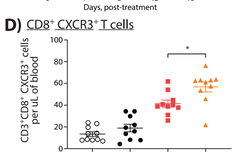
B) Metastatic lung nodules



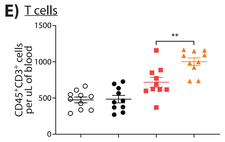
C) $CD8^+$ T cells



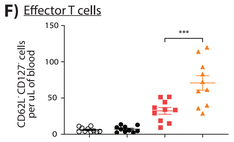
D) $CD8^+$ $CXCR3^+$ T cells



E) T cells



F) Effector T cells



G) Effector memory T cells

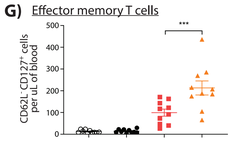
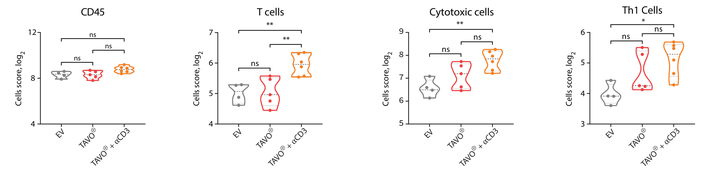


FIGURE 4 : A) Tumor volumes of 4T1 lesions electroporated with 50 µg of TAVO® or EV plasmid on Day 0 followed by subsequent electroporations on days 3 and 5 with 50 µg of EV plasmid (red) or 50 µg of anti-CD3 scFv plasmid (black). **B)** Quantitation of spontaneous metastatic lung nodules derived from 4T1 tumors electroporated as described in (A). **C-G)** Quantitation of PBMC: $CD8^+$ T cells (**C**), $CD8^+CXCR3^+$ T cells (**D**), $CD3^+$ T cells (**E**), $CD3^+CD62L^-CD127^-$ effector T cells (**F**), and $CD3^+CD62L^-CD127^-$ effector memory T cells (**G**) from B16-F10 tumor bearing mice 6 days post-electroporation with EV, TAVO® or TAVO® - anti-CD3 scFv plasmid. Statistical significance determined via one-way ANOVA for all experiments except (B) which used two-tailed Student's *t* test. All experiments were repeated three times with similar results $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

Transcriptomic analysis suggests membrane-bound anti-CD3 augments critical anti-tumor immune pathways triggered by TAVO⊕

A) Cellular phenotyping gene expression signatures



B) Signaling gene expression signatures

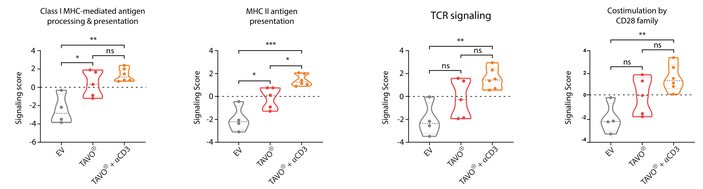
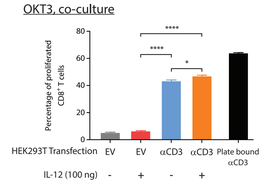


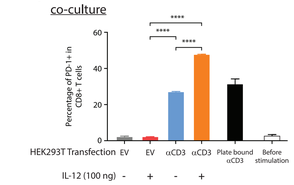
FIGURE 5: B16-F10 tumor bearing mice were electroporated with EV, TAVO®, or mTAVO®- anti-CD3 scFv plasmid and Nanostring nCounter (mouse PanCancer IO360 panel) analyses were performed on tumors isolated 48-hours post-electroporations. **A)** Gene expression signature scores associated with CD45, T cells, cytotoxic cells, and Th1 cells were analyzed. **B)** Gene expression signature scores associated with Class I MHC, Class II MHC, TCR signaling, and co-stimulation by CD28 family were also analyzed. Statistical significance determined via one-way ANOVA. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

TILs isolated from patients actively progressing on anti-PD-1 CPI demonstrate recovery of immune function when cultured with IL-12 and anti-CD3-transfected cells

A) Melanoma TILs proliferate with anti-CD3 scFv, OKT3, co-culture



B) PD-1 expression with anti-CD3 scFv co-culture



C) IFNγ expression with ant-CD3 scFv co-culture

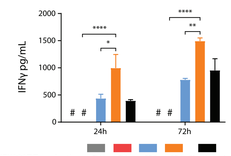
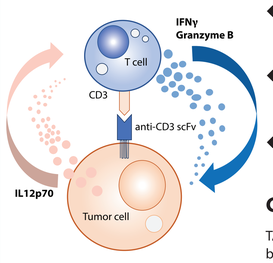


FIGURE 6: A) Percentage of TILS (derived from a patient with melanoma actively progressing on an anti-PD-1 CPI regimen) proliferating after 3 days of co-culture with HEK293T cells transfected with EV or human anti-CD3 scFv (clone: OKT3) plasmid with or without 100 ng/ml hIL12; tumor infiltrated T cells cultured with plate bound anti-human CD3 set as positive control (n=3). **B)** Percentage of PD-1 expression on these $CD8^+$ TILs after 3 days of co-culture with HEK293T cells transfected in various conditions as described in (A). **C)** ELISA measuring human IFNγ in the conditioned media of co-cultures of the TILS and HEK293T cells transfected in various conditions as described in (A). # - below limit of detection. Statistical significance determined via one-way ANOVA. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$.

Summary and Conclusion

Proposed Mechanism of Action:
Intratumoral IL12/anti-CD3 scFv



Summary

- Compared to IT-tavo-EP, addition of TAVO⊕- anti-CD3 scFv augments both T cell phenotype and function *in vivo*.
- Immune-profiling of TAVO⊕- anti-CD3 scFv-treated mice identified increased numbers of effector and effector memory T cells and activated T cells in peripheral blood.
- Intratumoral treatment TAVO⊕- anti-CD3 scFv demonstrated significant increases in antigen-specific cytotoxicity and corresponding reduction in metastatic (untreated) tumor burden
- Combination of IL-12 and membrane-bound anti-CD3 scFv induced ex vivo functional restoration of TILs isolated from a melanoma patient progressing on anti-PD-1mAb.

Conclusion

TAVO⊕- anti-CD3 is a promising novel therapy that can significantly reshape the TME by increasing immunogenicity, limiting Treg function, all the while engaging typically non-reactive T cell subsets (bystander/exhausted/naïve), yielding deep systemic anti-tumor responses.

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