Intratumoral IL-12 in combination with HDAC inhibition overcomes checkpoint-refractory tumors

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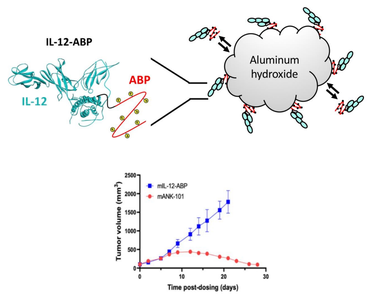
BACKGROUND

Background: Clinical resistance to immune checkpoint blockade (ICB) is prevalent across solid malignancies, thus prompting the need for novel therapies. Interleukin-12 (IL-12) is an important cytokine for cancer immunotherapy due to its ability to bridge innate and adaptive immunity[1,2]. However, the narrow therapeutic index and toxicity associated with systemic IL-12 administration has hampered its clinical development. ANK-101 is an alum-anchored human IL-12 providing tumor retention upon intra-tumoral delivery[3]. In this study, we investigated the anti-tumor activity and mechanism of action of murine ANK-101 (mANK-101) delivered intra-tumorally in combination with the class I histone deacetylase (HDAC) inhibitor entinostat, in different ICB-refractory murine tumor models, including CT26 (colorectal) and MOC-1 (HPV16^{neg} head and neck).



MATERIALS AND METHODS

Reagents: Entinostat and mANK-101 were kindly provided by Syndax and Ankyra, respectively, under Cooperative Research and Development Agreements (CRADA) with the NCI. A sub-optimal dose of mANK-101 (2µg) has been chosen to explore the combination potential of mANK-101 with entinostat. Low-fat diet of 35% sucrose was enriched with entinostat for a target daily dose of 6 mg/kg (Research Diets). Anti-mouse CD8α (clone 2.43), CD4 (GK1.5), and NK1.1 (PK136) antibodies were purchased from BioXCell.

Methods (in brief): Mice receiving entinostat diet and/or mANK-101 were monitored for anti-tumor activity, survival and protective memory. The contribution of CD8$^+$, CD4$^+$, and NK lymphocytes to antitumor effects were investigated via immune depletion. Analysis of tumor-specific T cell responses and comprehensive proteomic, transcriptomic, and tumor architecture analysis of the immunome was performed in MOC-1 tumors, tumor-draining lymph node (tdLN), and spleen. In addition, functional analysis of CD8$^+$ T cells in the periphery were performed.

Statistics: Bar graphs and violin plots show mean ± SEM with one-way ANOVA and Tukey's multiple comparisons; tumor volumes show two-way ANOVA with multiple comparisons; survivals show Mantel-cox; all data except scRNAseq is representative of 2 or 3 independent experiments. ns=not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

All schemas were created with BioRender.com.

RESULTS

1 mANK-101 synergizes with Entinostat to suppress CT26 and MOC-1 tumors, eliciting protective memory and increasing survival

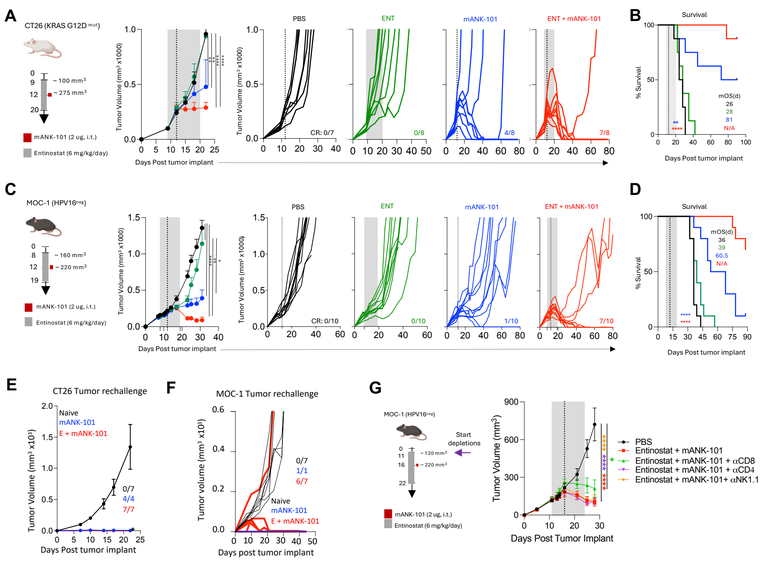


Figure 1. **(A)** Aggregate and individual tumor growth curves with cure rates (CR) in CT26 tumor-bearing mice. **(B)** Graph displaying % survival and median overall survival (mOS) for CT26 tumor-bearing mice. **(C)** Aggregate and individual tumor growth curves with CR in MOC-1 tumor-bearing mice. **(D)** Graph displaying % survival and mOS for MOC-1 tumor-bearing mice. **(E)** Tumor growth curves of naïve or CT26 cured mice rechallenged with matching tumor cells. **(F)** Tumor growth curves of naïve or MOC-1 cured mice rechallenged with matching tumor cells. **(G)** MOC-1 depletion study schematic and associated tumor growth curves for mANK-101 plus entinostat combination-treated mice with and without depletion antibodies. *Dotted lines: mANK-101 dosing; grey bar: Entinostat diet; mOS = Median overall survival (days).*

2 Combination therapy elicits a pro-inflammatory environment in the periphery and induces abscopal anti-tumor activity in MOC-1 tumors

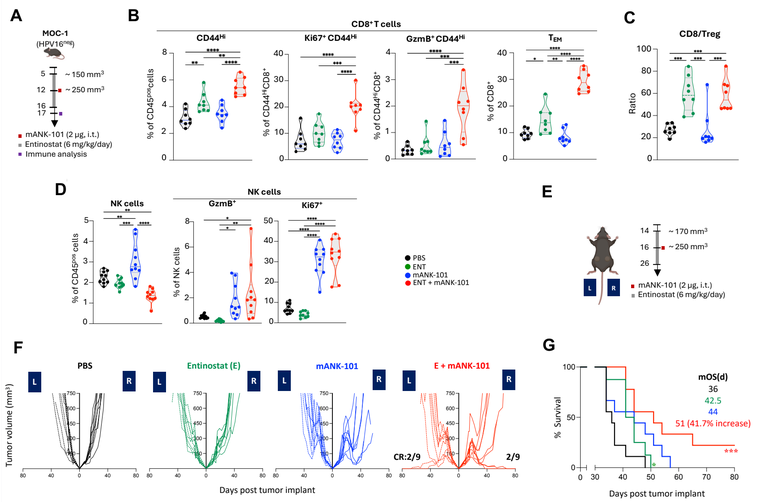


Figure 2. **(A)** MOC-1 tumor-bearing mice were treated as shown and sacrificed on Day 17 for immune analysis. **(B)** Quantification of activated CD8$^+$T cells that are proliferative (Ki67$^+$) and cytolytic (GzmB$^+$) in the spleen. **(C)** Quantification of IFNg in sera. **(D)** Number of NK cells populations in the spleen. **(E)** Schematic of MOC-1 HPV16^{neg} abscopal study. **(F)** Individual tumor growth curves from treated (Right, R) and untreated (Left, L) tumors, with cure rates (CR). **(G)** Graph displaying % survival and median overall survival (mOS) for MOC-1 abscopal study. *Dotted lines: mANK-101 dosing; grey bar: Entinostat diet*

3 Combination therapy creates a pro-inflammatory environment in the TME increasing CD8$^+$T cell infiltration and activation

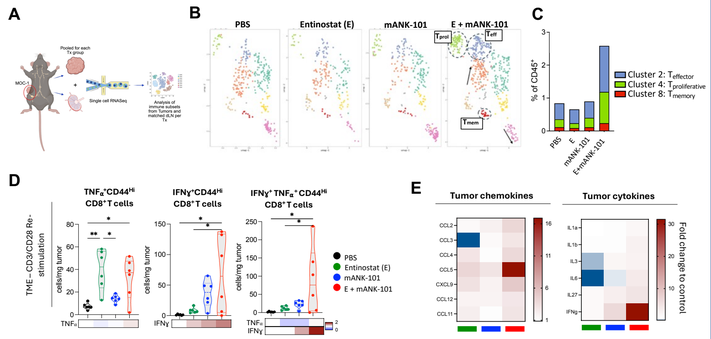


Figure 3. MOC-1 tumor bearing mice were treated as in Figure 2A and sacrificed for scRNAseq analysis. **(A)** scRNAseq was carried out for matched tdLN and tumors pooled per treatment and analyzed. **(B)** Representative UMAPS of tumor CD8$^+$ T cells per treatment. **(C)** Clusters of effector, proliferation, and memory CD8$^+$ T cells in TME as percent of CD45^{pos} cells. **(D)** Frequency of TNFα+, IFNγ+, and IFNγ+/TNFα+ CD8$^+$ T cells in the tumor upon CD3/CD28 *ex vivo* stimulation. Heat maps denote respective IFNγ and TNFα gMFI fold-change versus untreated control. **(E)** Heatmaps show tumor chemokines and cytokines as fold changes from control (PBS) group.

4 Tertiary lymphoid structures form in response to combination therapy

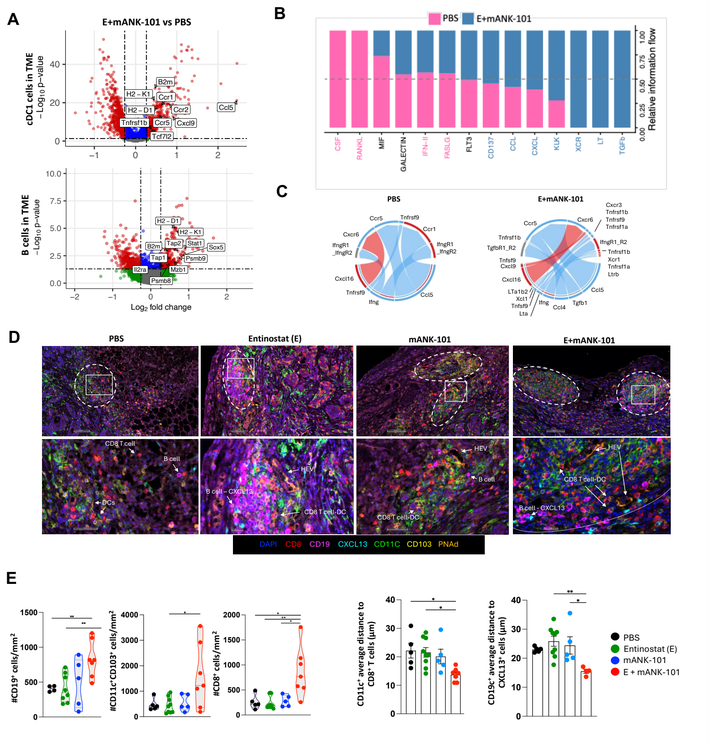


Figure 4. **(A)** Volcano plots show significantly expressed genes in cDC1s (top) and in B cells (bottom) in the TME. Volcano plots show Entinostat (E)+mANK-101 versus PBS. Cell chat inference was applied to B, CD8 and cDC1 cells from scRNAseq. RankNet algorithm was utilized to visualize pair-wised ranked pathways **(B)** and genes **(C)** in PBS and E+mANK-101. **(D)** MOC-1 HPV16^{neg} tumor-bearing mice treated as in Figure 2A were examined at day 17 (4 days after mANK-101 treatment) by multiplex immunofluorescence (mIF) staining and imaging. **(E)** Quantification relative to figure 4D.

5 Entinostat and mANK-101 combination therapy induce stemness in CD8$^+$ T cells

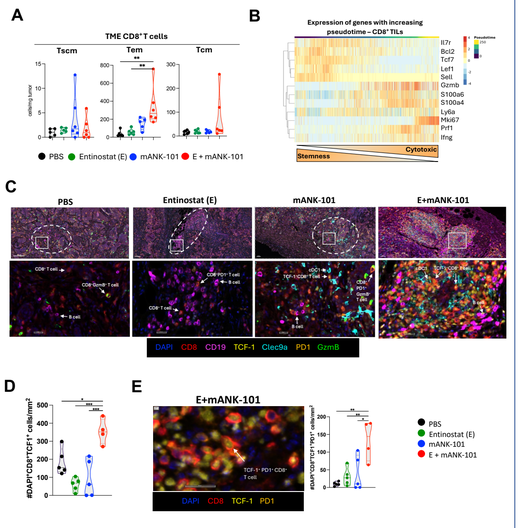


Figure 5. **(A)** Frequencies in the tumor of *CD62L$^+$CD44^{neg}CD127$^+$TCF1$^+$* T stem like memory (Tscm), *CD8$^+$CD62L^{neg}CD44$^+$CD127$^+$* T effector memory (Tem) and *CD8$^+$CD62L$^+$CD44$^+$CD127$^+$TCF1$^+$* T central memory (Tcm) CD8$^+$ T cells. **(B)** Pseudotime analysis of CD8$^+$ T cells progression within MOC-1 tumor. **(C)** Multiplex immunofluorescence (mIF) staining of MOC-1 HPV16^{neg} tumors. **(D)** Quantification of mIF shown in 5C. **(E)** mIF staining of MOC-1 HPV16^{neg} tumor treated with E+mANK-101 combination therapy, and quantification.

SUMMARY

1. mANK-101 plus Entinostat modulates the tumor immune microenvironment of CPB resistant MOC-1 HPV16^{neg} murine tumors for robust anti-tumor activity driven by CD8$^+$ T cells.
2. Combination therapy increases tumor and peripheral pro-inflammatory cytokine milieu.
3. Increased effector and stem-like TCF1$^+$CD8$^+$ T cells combined with cDC1s, B cells, and pro-inflammatory milieu form TLS and stem-immunity hubs.

FUTURE DIRECTIONS:

1. Role of other immune cells noted increased in TME including NK, and M1 macrophage cells.
2. Role of increased stem-like cells in TME.

References

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