Anchored IL-12 synergizes with an epigenetic modulator to promote immune remodeling and overcome anti-PD1-refractory murine tumors

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ABSTRACT

Background Most patients with solid malignancies harbor innate or acquired resistance to immune checkpoint blockade (ICB), prompting the need for novel therapeutic strategies. Interleukin-12 (IL-12) is a promising cytokine for cancer therapy due to its ability to bridge innate and adaptive immunity. However, a narrow therapeutic index limits the use of systemic IL-12 therapy. Here, we investigated the tumor-suppressive effects and mode of action of intra-tumorally delivered murine Interleukin-12 anchored to aluminum hydroxide (referred as mANK-101)[1,2] in combination with the class I HDAC inhibitor Entinostat, in various ICB-refractory murine tumor models, including CT26 (colorectal) and MOC-1 (HPV16^{neg}). We hypothesized that combining Entinostat with an anchored form of IL-12 could overcome systemic toxicity while maintaining anti-tumor activity.

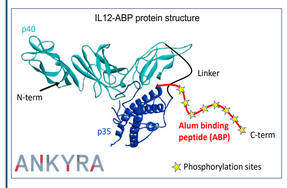
Methods Entinostat and intra-tumoral mANK-101 were administered to mice bearing well-established αPD-1-refractory CT26 (colorectal) and MOC-1 (HPV16^{neg}) tumors. Antitumor activity, survival, and protective memory upon tumor rechallenge were evaluated. Comprehensive proteomic and immune cell analysis was performed in MOC-1 tumors, tumor-draining lymph node (tdLN), and periphery. Tumor-specific T cell responses were examined.

Results We demonstrate that intra-tumoral mANK-101 synergizes with Entinostat to suppress multiple αPD1-refractory tumors, resulting in significant tumor eradication (62-88%), survival benefit ($P < 0.0001$), and protective memory, including CT26 (colon, Kras G12D^{mut}) and MOC-1 (oral, HPV16^{neg}). Analysis of MOC-1 tumor-bearing mice demonstrated these effects to be associated with peripheric activation of CD8$^+$ and NK lymphocytes, augmented polyfunctional IFNγ$^+$/TNFα$^+$- producing CD8$^+$ T cells, CD8$^+$ T cell effector memory, and tumor-specific T cell responses. Significant decrease in CD4$^+$ Tregs and increased CD8/Treg ratio were also observed. Ongoing functional studies, proteomic and immune cell analysis at the tumor site, tdLN, and periphery, including single cell transcriptomics and epigenetic studies, will allow for a deeper understanding of the synergistic effect of mANK-101 with the epigenetic modulator Entinostat.

Conclusions Collectively, these findings form a rationale for the clinical combination of intralesional delivery of ANK-101 with Entinostat for patients with ICB-refractory malignancies, including colorectal and HPV16^{neg} head and neck cancers.

MATERIALS AND METHODS

Reagents. mANK-101 and Entinostat were kindly provided by Ankyra Therapeutics and Syndax under Cooperative Research and Development Agreements (CRADA), respectively. Entinostat was formulated into a low-fat diet of 35% sucrose for a target daily dose of 6mg/kg (Research Diets).



Tumor studies and treatments (in brief). MOC-1 and CT26 tumor cells were implanted subcutaneously (s.c.) into the right or the right and left flank of C57Bl/6 (MOC-1) or Balb/c (CT26) mice for respective studies. Mice were randomized to receive Entinostat (p.o.) or intra-tumoral (i.t.) injections of mANK-101 as depicted in designated Figures. A sub-optimal dose of ANK-101 (2ug) has been chosen to explore the combination potential of ANK-101 with Entinostat. Analysis of spleen and tumor immunomes was performed by flow cytometry. Cytokines and chemokine proteins were examined in the TME and sera.

Statistics. One-way ANOVA with Tukey's multiple comparisons were used for data presented in violin plots. Two-way ANOVA was used to analyze tumor growth curves. Survival was analyzed using Log-rank (Mantel-Cox) test. All tumor growth data are representative of 2 independent experiments. Statistical significance was set at *p < 0.05, **p < 0.005, ***p < 0.001.

RESULTS

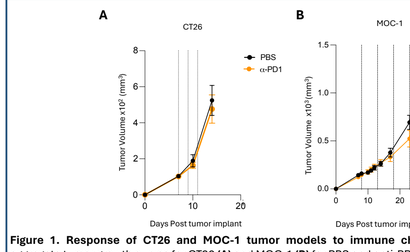


Figure 1. Response of CT26 and MOC-1 tumor models to immune checkpoint blockade. Respective aggregate tumor growth curves for CT26 **(A)** and MOC-1 **(B)** for PBS and anti-PD1 treatment groups.

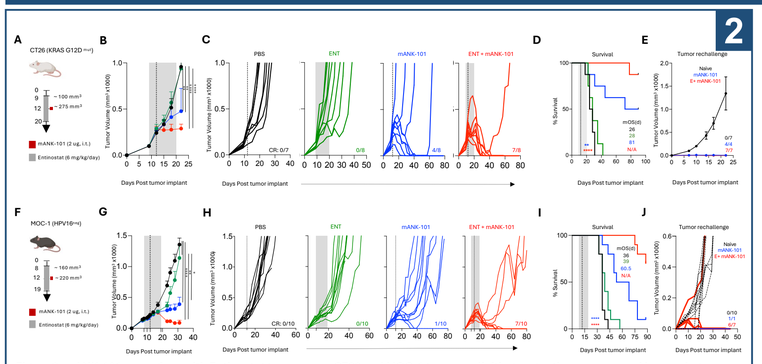


Figure 2. mANK-101 synergizes with Entinostat to suppress CT26 and MOC-1 tumors, eliciting protective memory and increasing survival. Study schematic for **(A)** CT26 and **(F)** MOC-1 tumor models. Respective aggregate **(B, G)** and individual tumor growth curves with cure rates (CR) **(C, H)** for each treatment group. **(D, I)** Graphs displays % survival and median overall survival (mOS) for tumor-bearing mice. Graphs show tumor growth curves of naïve or CT26 **(E)** and MOC-1 **(J)** cured mice rechallenged with matching tumor cells. *Dotted lines: mANK-101 dosing; grey bar: Entinostat diet.*

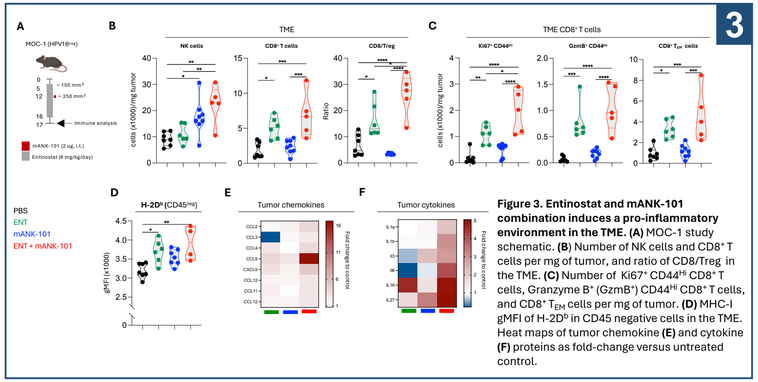


Figure 3. Entinostat and mANK-101 combination induces a pro-inflammatory environment in the TME. (A) MOC-1 study schematic. **(B)** Number of NK cells and CD8$^+$ T cells per mg of tumor, and ratio of CD8/Treg in the TME. **(C)** Number of Ki67$^+$ CD44^{Hi} CD8$^+$ T cells, Granzyme B$^+$ (GzmB$^+$) CD44^{Hi} CD8$^+$ T cells, and CD8$^+$ T_{EM} cells per mg of tumor. **(D)** MHC-I gMFI of H-2D^b in CD45 negative cells in the TME. Heat maps of tumor chemokine **(E)** and cytokine **(F)** proteins as fold-change versus untreated control.

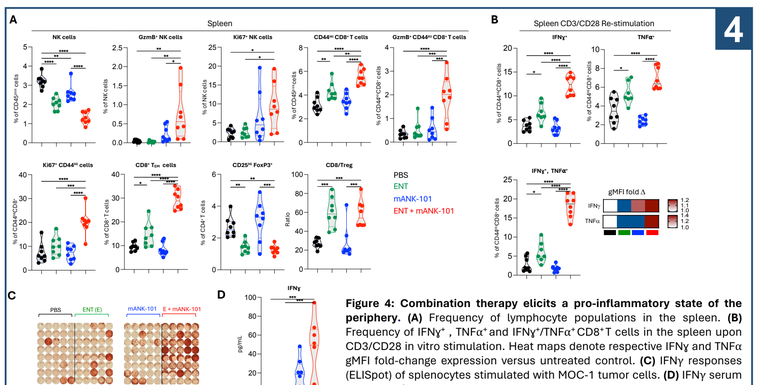


Figure 4: Combination therapy elicits a pro-inflammatory state of the periphery. (A) Frequency of lymphocyte populations in the spleen. **(B)** Frequency of IFNγ$^+$, TNFα$^+$ and IFNγ$^+$/TNFα$^+$ CD8$^+$T cells in the spleen upon CD3/CD28 in vitro stimulation. Heat maps denote respective IFNγ and TNFα gMFI fold-change expression versus untreated control. **(C)** IFNγ responses (ELISpot) of splenocytes stimulated with MOC-1 tumor cells. **(D)** IFNγ serum concentration.

RESULTS

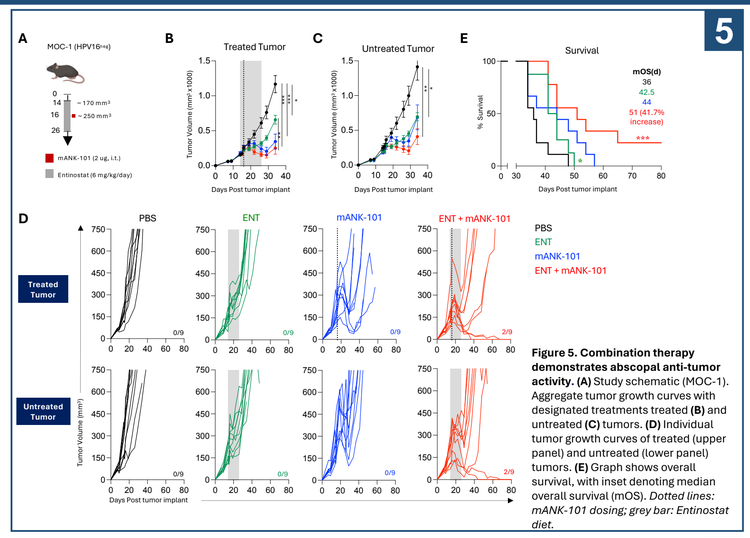


Figure 5. Combination therapy demonstrates abscopal anti-tumor activity. (A) Study schematic (MOC-1). Aggregate tumor growth curves with designated treatments treated **(B)** and untreated **(C)** tumors. **(D)** Individual tumor growth curves of treated (upper panel) and untreated (lower panel) tumors. **(E)** Graph shows overall survival, with inset denoting median overall survival (mOS). *Dotted lines: mANK-101 dosing; grey bar: Entinostat diet.*

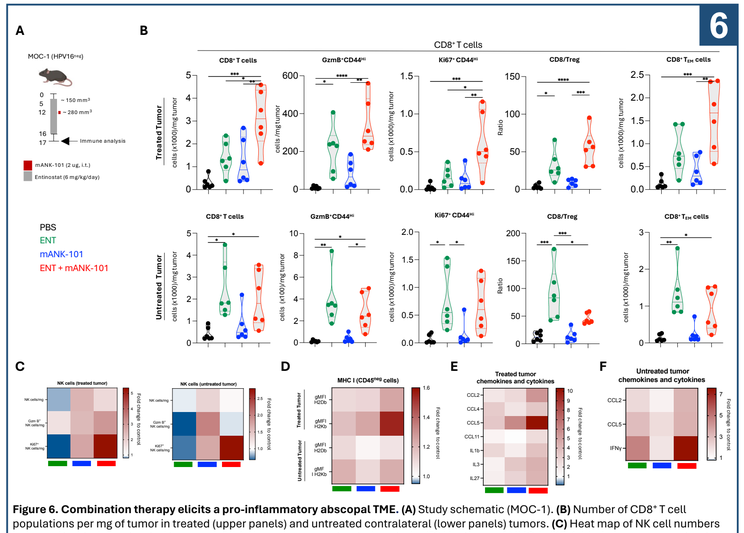


Figure 6. Combination therapy elicits a pro-inflammatory abscopal TME. (A) Study schematic (MOC-1). **(B)** Number of CD8$^+$ T cell populations per mg of tumor in treated (upper panels) and untreated contralateral (lower panels) tumors. **(C)** Heat map of NK cell numbers per mg of treated or untreated tumors as fold-change versus untreated controls. **(D)** Heat map of MHC-I gMFI fold-change versus untreated controls in CD45^{neg} cells in the TME of treated and untreated tumors. Heat maps of tumor chemokines and cytokine proteins in treated **(E)** and untreated **(F)** tumors as fold-change versus untreated controls.

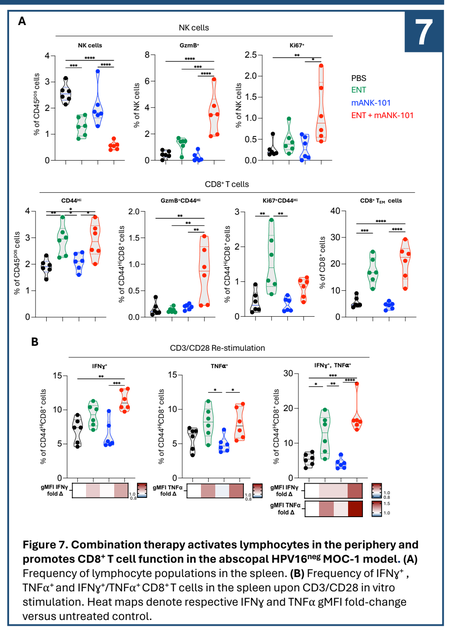


Figure 7. Combination therapy activates lymphocytes in the periphery and promotes CD8$^+$ T cell function in the abscopal HPV16^{neg} MOC-1 model. (A) Frequency of lymphocyte populations in the spleen. **(B)** Frequency of IFNγ$^+$, TNFα$^+$ and IFNγ$^+$/TNFα$^+$ CD8$^+$ T cells in the spleen upon CD3/CD28 in vitro stimulation. Heat maps denote respective IFNγ and TNFα gMFI fold-change versus untreated control.

SUMMARY

- Combination therapy with mANK-101 and Entinostat synergized to elicit significant anti-tumor effects and prolonged survival in CT26 (colorectal) and MOC-1 (HPV16^{neg}) tumors relative to single agent therapies.
- Tumor suppression was associated with:
  - Increased activation of CD8$^+$ T cells in the periphery and the TME.
  - Augmented CD8$^+$ T cell function.
  - Increased cytokines and chemokines in the TME.
  - Augmented tumor-specific memory responses.
- Abscopal studies demonstrated tumor suppression of untreated tumors.
- Future proteomic and single-cell transcriptomic investigations will allow for a deeper understanding of the mechanisms by which combination therapy suppresses tumors.
- Collectively, these data support the use of ANK-101 in combination with Entinostat for the treatment of patients with ICB-refractory malignancies, including colorectal and HPV16^{neg} head and neck cancers.

ACKNOWLEDGMENTS

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REFERENCES

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