Improved immunologic and therapeutic activity of anchored interleukin (IL)-12 immunotherapy in combination with cytotoxic chemotherapy and immune checkpoint inhibitor in a head and neck cancer model

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Abstract

Background. Head and neck squamous cell carcinoma is a heterogeneous group of cancers associated with high rates of locoregional recurrence and metastatic disease despite chemoradiation therapy. These tumors have exhibited limited responses to immune checkpoint inhibitors. Murine ANK-101 (mANK-101) is a novel anchored form of IL-12 that persists in the tumor microenvironment (TME) for up to 30 days without systemic toxicity. We tested the hypothesis that local IL-12 could potentiate the therapeutic response of chemotherapy or immune checkpoint blockade for head and neck cancers.

Methods. In C57BL/6 mice, we used established murine oral carcinoma (MOC1) tumors as a model for non-HPV-related head and neck carcinoma with the treatments commencing when the tumors were 100-200 mm^3. Treatment with cisplatin (5 mg/kg, intraperitoneal (i.p.) injection once weekly for 3 weeks), murine ANK-101 (mANK-101; 5 μg intratumoral (i.t.)), anti-PD-1 (200μg, i.p. once weekly for 3 weeks), or various combinations were evaluated. Tumors were measured with calipers and tumor size one week after last treatment was used for comparisons of tumor growth. Mice were also followed for survival. A subset of tumors was collected for immunohistochemistry and flow cytometry 5 days after ANK-101 treatment, when the tumors were regressing. Tumor size was compared using Student's t-test between groups and multiple comparisons utilized two-way ANOVA testing.

Results. In the MOC1 model, anti-PD-1 and cisplatin alone had no impact on tumor growth. mANK-101 alone, however, was associated with a significant delay in tumor growth. The addition of cisplatin or anti-PD-1 to mANK-101 treatment did not improve the anti-tumor effect when compared to mANK-101 monotherapy. On the other hand, triple combination therapy with mANK-101, cisplatin, and anti-PD-1 further delayed tumor growth and resulted in the highest number of animals cured. The combination of mANK-101, cisplatin, and anti-PD-1 was associated with increased $CD8^+$ T cell recruitment to the tumor microenvironment. Furthermore, mANK-101 monotherapy and triple combination therapy skewed the macrophage population from M2 to M1.

Conclusions. Anchored IL-12 improves therapeutic responses to cisplatin chemotherapy and immune checkpoint blockade in the murine PD-1-refractory MOC1 head and neck cancer model. These preliminary data demonstrate that combining local IL-12 with cytotoxic chemotherapy and/or immune checkpoint blockade merits investigation in other tumor models.

Introduction

- Head and neck squamous cell carcinomas (HNSCC) account for 90% of all head and neck cancers and develop from the mucosal epithelium of the oral cavity, pharynx, and larynx.
- Risk factors for HNSCC include tobacco use, excess alcohol consumption, and human papilloma virus (HPV) infection.
- The KEYNOTE-048 study (NCT02358031) showed evidence that treatment with pembrolizumab plus platinum- and fluorouracil-based chemotherapy is an appropriate first-line therapy for patients with metastatic or recurrent HNSCC.
- MOC1 is a syngeneic murine model of oral cavity squamous cell carcinoma (OCSCC) derived from carcinogen-induced primary tumors.
- IL-12 is a pleiotropic cytokine that
  - Increases the proliferation and cytotoxicity of $CD8^+$ CTLs, NK cells, and NKT cells
  - Drives Th1 differentiation
  - Promotes IFNγ production by NK and T cells
  - Upregulates chemokines that induce T cell infiltration
- Early phase I clinical trials of systemic administration of recombinant IL-12 resulted in sub-optimal anti-tumor efficacy, which is often associated with high toxicity that is partially due to the over-stimulation of lymphocytes in healthy tissues. Localized delivery of IL-12 can potentially lower systemic toxicity and immune-related adverse events.
- ANK-101 is a complex composed of human IL-12 that is genetically fused to an alum-binding peptide (ABP) mixed with a 10-fold mass excess of Alhydrogel® (alum; Figure 1). For pre-clinical studies, a surrogate complex containing mouse IL-12 sequence was developed.
- Intratumoral administration of mANK-101 demonstrated enhanced tumor retention and monotherapy efficacy in diverse syngeneic tumor models.

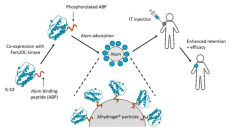

Figure 1: Schematic of anchored IL-12. IL-12 is genetically fused to a proprietary ABP and co-expressed with the kinase Fam20C that phosphorylates multiple target serine residues within the peptide. Phosphorylated IL-12-ABP is complexed with 10X Alhydrogel® (alum) particles that are administered intratumorally, where they form a stable depot promoting sustained immune activation and thereby enhanced anti-tumor efficacy.

Results

Standard-of-care therapy for HNSCC resulted in limited efficacy in the MOC1 tumor model

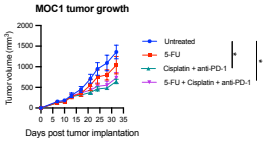

Figure 2. C57BL/6 mice bearing MOC1 tumors were treated with 5-fluorouracil (5-FU; 25 mg/kg, i.p.), cisplatin (5mg/kg, i.p.), and anti-PD-1 (200 μg, i.p.) once weekly for 3 weeks, starting on Day 10. Tumor volumes were measured and presented as mean tumor growth. Repeated measures (RM) two-way ANOVA was used to compare groups. Treatment with cisplatin + anti-PD-1 combination resulted in comparable tumor growth suppression as the standard-of-care regimen (5-FU + cisplatin + anti-PD-1).

Combination of mANK-101 with cisplatin and PD-1 improved therapeutic efficacy in MOC1 tumor model

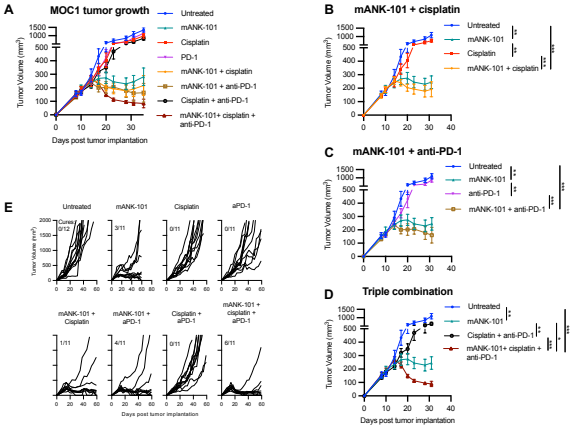

Figure 3. C57BL/6 mice bearing MOC1 tumors were administered with a single intratumoral injection of mANK-101 (5 μg, i.t.) on Day 10, cisplatin (5 mg/kg, i.p.) on Days 10, 17, and 24 and anti-PD-1 (200 μg, i.p.) on Days 10, 17, and 24. Tumor volumes were measured and presented as (A-D) mean tumor growth or (E) single mouse tracks. RM two-way ANOVA was used to compare groups. Tumors that regressed from >200mm^3 and remained <100mm^3 in volume for at least 7 days before end point were considered cured. mANK-101 monotherapy had significant anti-tumor effect which was further improved by triple combination with mANK-101 + cisplatin + anti-PD-1.

Combination therapy with mANK-101, cisplatin, and PD-1 promotes $CD8^+$ T cell infiltration and effector T cell activity in the TME

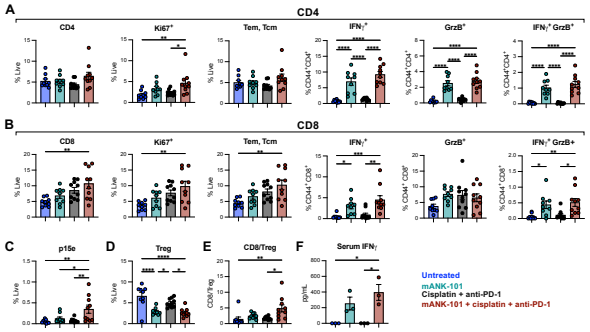

Figure 4. On Day 10 post tumor inoculation, C57BL/6 MOC1 tumor-bearing mice were treated with mANK-101 (5 μg, i.t.), cisplatin (5 mg/kg, i.p.) and anti-PD-1 (200 μg, i.p.) and on Day 15, tumors and sera were collected. Tumor infiltrating lymphocytes (TILs) were assessed via flow cytometry. (A) $FoxP3^-$ $CD4^+$ and (B) $CD8^+$ T cells were evaluated for memory phenotype and expression of Ki67, IFNg, and granzyme B. (C) Tetramer staining was performed to identify p15e-specific $CD8^+$ TILs. (D) $FoxP3^+$ $CD4^+$ Tregs were also identified and (E) CD8/Treg ratio in the TME was calculated. (F) IFN_γ levels in the serum were evaluated via ELISA. One-way ANOVA was used to compare groups. Triple combination promoted the infiltration of $CD8^+$ T cells and improved CD8/Treg ratio in the TME. mANK-101 monotherapy and triple combination decreased Tregs and enhanced cytokine expression in effector $CD4^+$ and $CD8^+$ TILs.

mANK-101, alone or in combination, skewed macrophage population in the TME from M2 to M1 phenotype

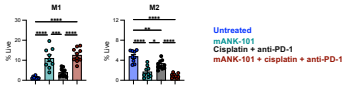

Figure 5. On Day 10 post tumor inoculation, C57BL/6 MOC1 tumor-bearing mice were treated with mANK-101 (5 μg, i.t.), cisplatin (5 mg/kg, i.p.) and anti-PD-1 (200 μg, i.p.). On Day 15, tumors were collected and macrophage ($CD11b^+F4/80^+$) populations were assessed for M1 (CD38+CD206-) and M2 (CD38-CD206+) phenotypes via flow cytometry. One-way ANOVA was used to compare groups. mANK-101 monotherapy and the triple combination therapy promoted M1 populations while decreasing M2 populations.

$CD4^+$ and $CD8^+$ T cells are critical for the anti-tumor activity of the mANK-101 + cisplatin + anti-PD-1 combination

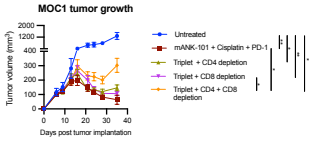

Figure 6. MOC1-tumor bearing C57BL/6 mice were administered with mANK-101 (5 μg, i.t.) on Day 10, cisplatin (5 mg/kg, i.p.) on Days 10, 17, and 24, anti-PD-1 (200 μg, i.p.), CD4 depleting antibodies (100 μg, i.p.) on Days 6, 7, 8, 15, 22, and 29, and CD8 depleting antibodies (100 μg, i.p.) on Days 6, 7, 8, 15, 22, and 29. RM two-way ANOVA was used to compare groups. Depletion of both $CD4^+$ and $CD8^+$ T cells abrogated the anti-tumor effect of the mANK-101 + cisplatin + anti-PD-1 triple combination.

mANK-101, alone or in combination, suppressed distal untreated tumor lesions

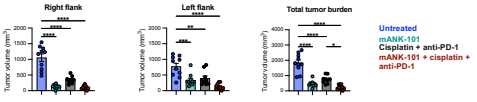

Figure 7. C57BL/6 mice were implanted with $5x10^6$ MOC1 tumor cells on both the right and the left flanks. On Day 10, the tumor on the right flank was treated intratumorally with 5 μg mANK-101. On Days 10, 17, and 24, intraperitoneal injections of cisplatin (5mg/kg) and anti-PD-1 (200 μg) were administered. One-way ANOVA was used to compare tumor volumes on Day 30, when the untreated group has reached ethical limits. mANK-101 monotherapy and the triple combination induced abscopal regression of distal (left) tumors and reduced overall tumor burden. The triple combination also significantly reduced total tumor burden compared to systemic treatment with cisplatin + anti-PD-1.

Summary and Future Directions

- Standard-of-care therapy for HNSCC has significant but limited anti-tumor effect in the MOC1 tumor model.
- The combination of mANK-101 with a standard-of-care therapy (cisplatin + PD-1) resulted in synergistic anti-tumor activity in the MOC1 tumor model.
- Similarly, combination therapy with mANK-101 + cisplatin + PD-1 suppressed the growth of the more aggressive MOC2 murine OCSCC tumor model.
- Triple combination with mANK-101 + cisplatin + anti-PD-1 promoted the infiltration of $CD8^+$ T cells, including p15e-specific populations, in the TME.
- Triple combination decreased Tregs and improved CD8-to-Treg ratios.
- mANK-101 monotherapy and triple combination upregulated the expression of IFN_γ and granzyme B in effector $CD4^+$ and $CD8^+$ T cells.
- mANK-101 monotherapy and triple combination skewed the macrophage population from an M2 to an M1 phenotype.
- mANK-101 monotherapy and triple combination promoted the regression of distal, untreated MOC1 tumors.
- The role of natural killer cells in the therapeutic effect of mANK-101 + cisplatin + anti-PD-1 will be investigated.
- scRNAseq analysis of TILs and comprehensive serum cytokine analysis are currently underway.

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