ABSTRACT# 4065

Intratumoral delivery of aluminum hydroxide-tethered IL-12 induces potent anti-tumor immune response

Sailaja Battula[1], Gregory Papastoitsis[1], Howard Kaufman[1], Darrell Irvine[2], K. Dane Wittrup[2], Michael M Schmidt[1]

[1]Ankyra Therapeutics, Boston, MA, USA; [2]Massachusetts Institute of Technology, Cambridge, MA, USA

Abstract

IL-12 is a potent cytokine that can mediate anti-tumor immune responses (1), but its clinical development has been hindered by systemic toxicity. Intratumoral (IT) delivery can improve the therapeutic window of cytokine drugs but is in turn limited by rapid clearance of injected drug from the tumor which reduces efficacy, necessitates frequent administration, and increases systemic accumulation (2,3). Ankyra has developed a novel drug delivery platform for locally retaining cytokines and other immune agonists following IT administration through formation of a stable linkage with the approved vaccine adjuvant aluminum hydroxide (Alhydrogel®) (4). Here, we describe the therapeutic and immune effects of Ankyra's intratumoral IL-12 complex using relevant in vitro and in vivo murine models.

Single-chain human IL-12 was genetically fused at its c-terminus to a proprietary, phosphorylated alum-binding peptide (IL-12-ABP) that forms a high affinity complex with Alhydrogel®. Human IL-12-ABP proteins were mixed with a 10-fold mass excess of Alhydrogel® to form the therapeutic complex, ANK-101. Since human IL-12 is not active in mice, a surrogate complex containing the mouse IL-12 sequence (mANK-101) was also generated. The functional potency of ANK-101 and mANK-101 was assessed in multiple cellular assays in comparison to their respective unmodified IL-12 controls. The therapeutic efficacy of mANK-101 was evaluated in syngeneic mouse tumor models including MC38, CT26, A20, 4T1, and B16F10. The intratumoral retention of mANK-101 after a single IT dose was analyzed by IVIS imaging studies. Furthermore, mechanistic studies were performed to understand the mode of action of ANK-101 including transcriptional profiling by Nanostring and measurement of intratumoral immune infiltration by immunohistochemistry (IHC). Statistical analyses were done using Student's t-test for comparison between groups and Kaplan-Meier method for survival.

ANK-101 complexes retained IL-12 activity as demonstrated by concentration dependent increases in IFNγ production by anti-CD3 stimulated peripheral blood mononuclear cells (PBMCs). IVIS studies conducted in tumor bearing mice showed that the labeled mANK-101 complexes were retained in the tumor for >21 days while free mIL-12-ABP protein was cleared in <24 hours. One or two doses of IT administered mANK-101 induced tumor regressions in diverse syngeneic tumor models, including complete responses either alone or in combination with approved checkpoint blockers targeting PD-1 or CTLA-4. Efficacious doses were well tolerated in mice with no significant weight loss compared to vehicle treated animals. Tumor transcriptional profiling showed that mANK-101 induced robust and extended activation of multiple functional immune pathways compared to mice treated with vehicle or unanchored mIL-12. In addition, IHC analysis of tumors showed increased infiltration of CD8⁺ T cells, macrophages and elevation in PD-L1 expression.

In conclusion, Ankyra's platform is a novel approach that expands the therapeutic window of IL-12 and other intratumoral, immunomodulatory drugs. ANK-101 is currently in IND-enabling studies and will be tested in a phase I clinical trial.

Anchored Immunotherapy for Intratumoral Drug Delivery

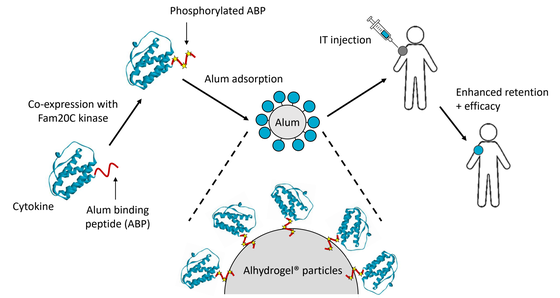


Figure 1: Schematic of anchored Immunotherapy platform: Cytokines or other immune agonists of interest are genetically fused to a proprietary alum binding peptide (ABP) and co-expressed with the kinase Fam20C that specifically phosphorylates multiple target serine residues within the peptide. Phosphorylated cytokine fusions are complexed with 10X Alhydrogel® (alum) particles and are administered IT, where they form a stable depot promoting sustained immune activation and thereby enhanced anti-tumor efficacy.

ANK-101: IL-12-ABP Complexed with Alhydrogel®

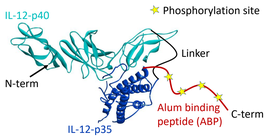


Figure 2: Single-chain human IL-12 with wild-type p40 and p35 sequences was genetically fused at its C-terminus to an alum-binding peptide to form IL-12-ABP and complexed with a 10x mass excess of Alhydrogel® to form ANK-101. Since human IL-12 is not active in murine systems, surrogate molecule mANK-101 with mouse IL-12 p40 and p35 sequences was used for preclinical studies

IL-12-ABP is retained on Alhydrogel® particles and complexes maintain IL-12 activity in vitro

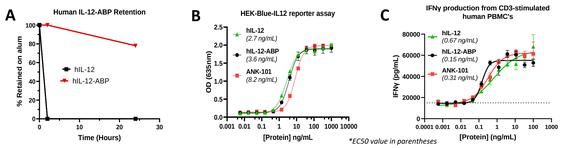
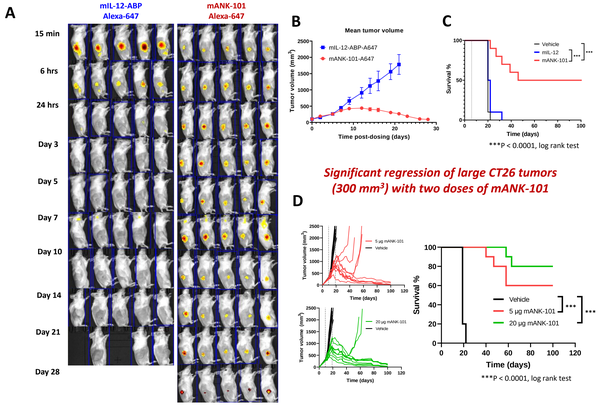


Figure 3: (A) Human IL-12-ABP or IL-12 control proteins were complexed with Alhydrogel® then incubated in elution buffer containing 1 mM phosphate and 20% serum. Free IL-12 protein in the supernatant was measured at various times by ELISA. IL-12-ABP proteins are retained longer on Alhydrogel® compared to unmodified IL-12 controls. (B) Activity in HEK-Blue-IL12 assay with pSTAT4 inducible promoter (C) IFNγ production from human PBMC's stimulated for 3 days with α-CD3 antibody (100ng/ml) and test agents. The functional potency of free IL-12-ABP and the ANK-101 complex are similar to native IL-12 in both assay systems. The mANK-101 surrogate molecule displayed similar functional activity in experiments using anti-CD3 stimulated mouse splenocytes. (data not shown)

Enhanced Tumor Retention and Monotherapy Efficacy



Significant regression of large CT26 tumors (300 mm³) with two doses of mANK-101

Figure 4: (A) BALB/c mice (10/group) bearing CT26 tumors were administered a single IT injection of 5 µg Alexa-647 labeled IL-12-ABP either as free protein or complexed with Alhydrogel® to form mANK-101. Animals were imaged at various timepoints by IVIS (PerkinElmer, IVIS Lumina Series III). The un-complexed protein cleared from the tumor within 24 hours whereas extended tumor localization of mANK-101 was observed. (B) Mean tumor volumes of animals pictured in A. (C) Survival of Balb/C mice bearing ~80 mm³ CT26 tumors that were administered a single IT injection of vehicle, 5 µg mANK-101, or an equimolar concentration of native mIL-12. (D) Tumor growth curves and survival of Balb/C mice bearing ~300 mm³ CT26 tumors administered two IT doses of vehicle, 5 µg or 20 µg mANK-101 ten days apart. Mice were euthanized when TV > 2000 mm³. ANK-101 is well tolerated and showed significant anti-tumor activity compared to Veh (***P < 0.0001, log rank test)

mANK-101 is Efficacious in Diverse Syngeneic Tumor Models

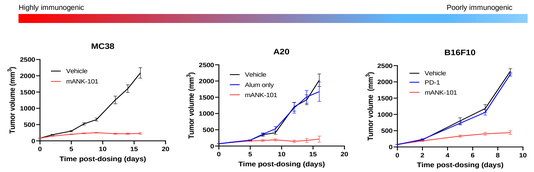


Figure 5: mANK-101 (5µg) was tested for anti-tumor response in various syngeneic tumor models. A single IT dose was tested in MC38 model whereas two IT doses spanning 7 days was tested in A20 and B16F10 models. Alum alone has minimal effect on reducing tumor burden. ANK-101 is effective in PD-1 resistant B16F10 model.

mANK-101 is Well-Tolerated Without Body Weight Loss

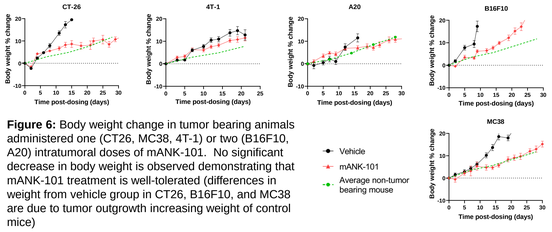


Figure 6: Body weight change in tumor bearing animals administered one (CT26, MC38, 4T-1) or two (B16F10, A20) intratumoral doses of mANK-101. No significant decrease in body weight is observed demonstrating that mANK-101 treatment is well-tolerated (differences in weight from vehicle group in CT26, B16F10, and MC38 are due to tumor outgrowth increasing weight of control mice)

mANK-101 Elicits Robust Immune Infiltration

Efficacy is associated with sustained immune activation (IFNγ) and increased TIL infiltration

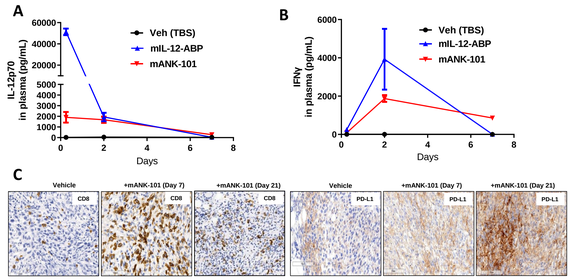


Figure 7: (A) C57BL/6 mice bearing MC38 tumors (100mm³) were given one IT dose of 5µg of IL-12-ABP either free or complexed with alum. Serial plasma collections were carried out for IL-12p70 and IFNγ measurements. Alum bound IL-12-ABP treatment resulted in sustained IL-12p70 without sharp systemic elevations, and at the same time maintained IFNγ levels up to 7 days post treatment.
(C) In a similar study, FFPE tumors were stained by IHC for CD8 T-cells and PD-L1 expression. An increase in CD8+ tumor infiltrating lymphocytes (TIL) was observed in mANK-101 treated animals within day 7 post-treatment (Representative images of tissue sections stained for CD8 (left) and PD-L1 (right) harvested at day 7 and day 21 post-treatment).

mANK-101 Modulates both Innate and Adaptive Immunity

Activation of predominant IL-12 mediated immune pathways with mANK-101

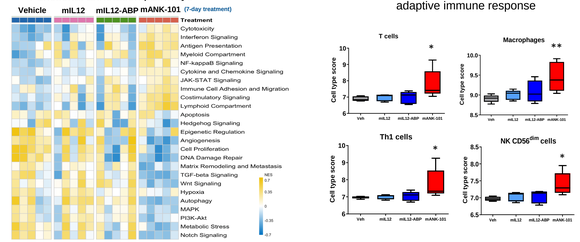


Figure 8: C57Bl/6 mice bearing MC38 tumors were administered a single intratumoral injection of 5 µg of mIL-12, free mIL-12-ABP protein, or mANK-101 complex, and tumors were harvested after 7 days. RNA was isolated and transcriptional analysis was performed by Nanostring (PanCancer IO 360 panel) and data were analyzed using the nSolver analysis software with advanced analysis. (A) Heatmap representing changes in functional annotation pathways with treatment by normalized enrichment score (NES) (B) Cell type's abundance is measured as the average log-scale expression of its characteristic genes based on the Nanostring analysis user manual. Cell type score for T cells (probe set: Cd3d, Cd3e, Cd3g, Cd6, Sh2d1a and Trat1), macrophages (probe set: Cd163, Cd68, Cd84 and Ms4a4a), Th1 cells (Probe set: TBX21), NK CD56^{dim} cells (probe set: IL21R, KIR2DL3, KIR3DL1, KIR3DL2) in response to treatment, *p<0.05, **p<0.01, One-Way Anova with Dunnett's.

Combination with Checkpoint Blockade

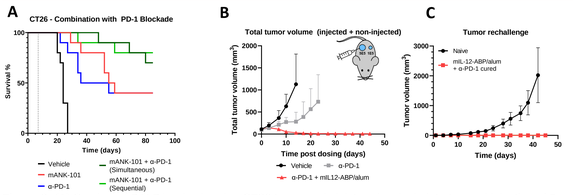


Figure 9: (A) Survival of BALB/c mice bearing CT26 tumors administered a single intratumoral injection of 5 µg mANK-101 on Day 7 alone or in combination with 200 µg anti-PD-1 clone 29F.1A12 administered IP BIW for 4 weeks starting on Day 7 (simultaneous) or BIW for 3 weeks starting on Day 14 (sequential). (B) C57BL/6 mice (10/group) were inoculated with 5x10^5 MC38 cells in the right flank and 1x10^5 MC38 cells in the left flank. When right tumors reached 100 mm^3, mice were treated with 200 µg α-PD-1 IP on Day 0,3,6,9 with or without IT injection of 5 µg mIL12-ABP/alum in the right tumor only on Day 0. (C) Mice that cleared both injected and non-injected tumors were rechallenged with 5x10^5 MC38 cells in the right flank. 10/10 mice in the combo group cleared both tumors and established protective immune memory

Conclusions

- Ankyra's Anchored Immunotherapy platform forms an extended intratumoral depot that improves the therapeutic window of cytokines and other immune agonist drugs, and reduces the need for repeat injections
- ANK-101, a stable complex of human IL-12-ABP with Alhydrogel®, potently activates IL-12 receptor signaling in reporter cell lines and primary immune assays
- A single IT dose of an ANK-101 surrogate molecule induced durable anti-tumor responses in several mouse cancer models with diverse immunogenic potential without decreases in body weight
- Tumor regressions are associated with sustained IFNγ production, increased intratumoral immune infiltration, and robust modulation of both innate and adaptive immune response.
- Combination with systemic PD-1 blockade further enhances ANK-101 anti-tumor activity and supports generation of robust, systemic immune memory
- IND enabling studies for ANK-101 initiated with an IND planned in 2023
- Multiple early-stage programs in progress to apply platform to other immune-relevant drugs and modalities

References – *1. Lasek et al. Cancer Immunol Immunother, 2014; 2. Nguyen et al. Front Immunol, 2020; 3. Kwong et al. Biomaterials, 2011; 4. Agarwal et al. Nat Biomed Eng., 2022*

Ankyra Therapeutics
83 Newbury Street, 4th Floor
Boston, MA 02116
www.ankyratx.com