ABSTRACT #721

Intratumoral immunotherapy with aluminum hydroxide-tethered IL-12 induces potent local and systemic immunity with minimal toxicity

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Abstract

Interleukin-12 (IL-12) is a potent pro-inflammatory cytokine that promotes Th1 skewing, IFNγ expression, and T- and NK-cell activation(1). In preclinical models, recombinant IL-12 treatment can elicit robust anti-tumor responses through activation of both innate and adaptive immunity. However, clinical translation of IL-12 has been hindered by significant immune-related toxicity when delivered systemically, necessitating low doses that are often insufficient for efficacy. Intratumoral (IT) administration can expand the therapeutic window of IL-12 by increasing the local tumor concentration relative to systemic exposure(2) but is in turn limited by rapid vascular and lymphatic clearance of injected drug from the tumor and corresponding systemic accumulation(3). Ankyra has developed a proprietary drug delivery platform for locally retaining cytokines and other immune modulators following IT administration by complexing them with the common vaccine adjuvant aluminum hydroxide (Alhydrogel®) through a novel phosphopeptide linkage (Figure 1). Here we describe how this approach can be applied to expand the therapeutic window of IL-12 by forming an extended intratumoral cytokine depot that induces a potent anti-tumor response without systemic toxicity.

Single-chain human IL-12 was genetically fused at its c-terminus to a short alum-binding peptide (IL-12-ABP) that is specifically phosphorylated on multiple serines when co-expressed with the kinase Fam20C. Phosphorylated IL-12-ABP proteins are stably complexed with a 10x mass excess of Alhydrogel® through a naturally occurring ligand exchange reaction between the phosphoserines in the ABP and surface hydroxyl groups on the aluminum hydroxide to form the complex ANK-101. Since human IL-12 is not active in murine systems, a surrogate complex containing the mouse IL-12 sequence (mANK-101) was generated and tested in multiple syngeneic tumor models including MC38, CT26, 4T1, and B16F10 following IT administration.

Human and mouse IL-12-ABP proteins are phosphorylated on multiple sites when co-expressed with Fam20C and stably retained on Alhydrogel® particles *in vitro* under elution conditions containing phosphate and serum. Alum-bound IL12-ABP remains active in cellular assays with a 3-4 fold increase in EC50 compared to free protein. Following IT administration in mice, mANK-101 complexes have significantly extended tumor retention compared to unmodified mIL-12, leading to potent local immune activation for >1 week. One or two doses of IT administered mANK-101 is sufficient to induce robust monotherapy efficacy in diverse syngeneic tumor models including cold tumors resistant to checkpoint blockade and other immunotherapies. Locally administered mANK-101 is further able to prime a systemic immune response leading to efficacy against non-injected tumors and spontaneous metastases. Doses required for optimal efficacy are well tolerated in mice with no significant weight loss or other evidence of systemic toxicity.

Ankyra Platform for Intratumoral Cytokine Retention

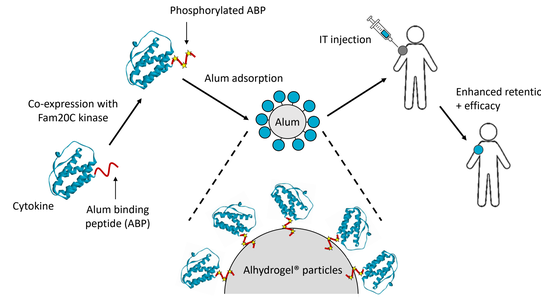


Figure 1: Cytokines and other immune agonists are genetically fused to a proprietary alum binding peptide (ABP) that is specifically phosphorylated on multiple sites following co-expression with the kinase Fam20C. Phosphorylated cytokine fusions are complexed with Alhydrogel® (alum) particles which are locally retained at the injection site due to their size and charge. Cytokine/Alhydrogel® complexes are administered IT where they form a depot that promotes potent and long-lasting immune activation

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

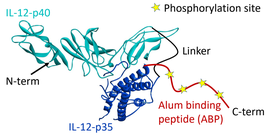


Figure 2: Single-chain human IL-12 was genetically fused at its C-terminus to an alum-binding peptide to form IL-12-ABP and complexed with a 10x mass excess of Alhydrogel® to form ANK-101. Since human IL-12 is not active in murine systems, a surrogate molecule containing the mouse IL-12 sequence (mIL-12-ABP) was also generated and complexed with Alhydrogel® under identical conditions to form mANK-101.

IL-12-ABP is phosphorylated on multiple sites and retained on Alhydrogel® particles

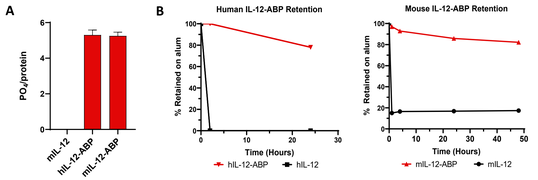


Figure 3: (A) Human and mouse IL-12-ABP are phosphorylated on multiple serines following co-expression with Fam20C kinase as measured by malachite green assay (B) Human (left) or mouse (right) IL-12-ABP or IL-12 control proteins were complexed with a 10x mass excess of Alhydrogel® then diluted in elution buffer containing 1 mM phosphate and 20% serum. At various times, samples were centrifuged to pellet Alhydrogel® particles and free protein quantified in the supernatant by ELISA. IL-12-ABP proteins are retained longer on Alhydrogel® than IL-12 controls lacking the phosphorylated ABP

ANK-101 Complexes Retain IL-12 Activity *In Vitro*

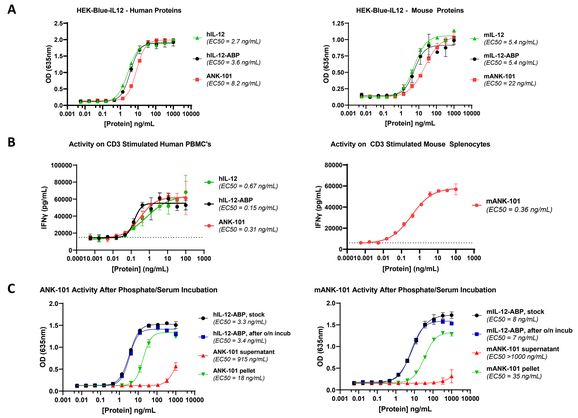


Figure 4: Functional potency of human (left) or mouse (right) IL-12-ABP as free protein or complexed with a 10x mass excess of Alhydrogel® to form ANK-101 / mANK-101 was assessed in multiple cellular assays in comparison to unmodified IL-12 controls. (A) Activity in HEK-Blue-IL12 assay with alkaline phosphatase reporter under control of a pSTAT4 inducible promoter (B) IFNγ production from primary human PBMC's or mouse splenocytes incubated for 3 days with test agent and 100 ng/mL anti-CD3 antibody (C) HEK-Blue-IL12 assay with ANK-101 or mANK-101 following overnight incubation in 1 mM phosphate, 20% serum and centrifugation to separate eluted IL-12-ABP protein in the supernatant from intact complex in the pellet. All experiments performed in triplicate and presented as mean +/- SD.

Enhanced Monotherapy Efficacy After 1 or 2 IT Doses

Curative single-shot efficacy in CT26 model associated with increased intratumoral CD8/Treg ratio

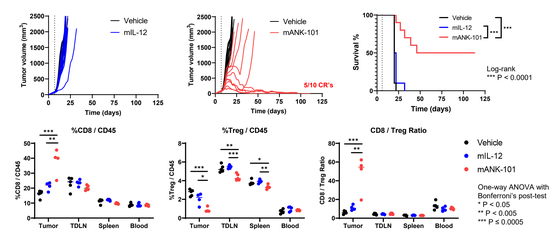


Figure 5: BALB/c mice (10/group) bearing CT26 tumors were administered a single IT injection of vehicle, 5 µg mIL-12, or 5 µg mANK-101 on Day 7 when mean tumor volume was ~80 mm³ (A) Tumor volume (TV) and survival. Mice were euthanized when TV > 2000 mm³ (B) Mice (5/group) were euthanized on Day 7 after IT injection and FACS immunophenotyping performed on tumor, lymph node, spleen, and blood. mANK-101 treatment significantly extends survival and increases the intratumoral CD8⁺/Treg ratio compared to vehicle or mIL-12 control treated mice

Efficacy in cold B16F10 model associated with prolonged intratumoral IFNγ and myeloid cell activation

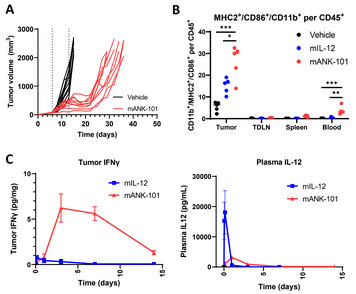


Figure 6: (A) C57BL/6 mice (10/group) bearing B16F10 tumors were injected IT on Day 6 and 13 with vehicle or 7.7 µg mANK-101 (B) B16F10 tumor bearing mice (5/group) were given a single IT injection of vehicle, 5 µg mIL-12, or 5 µg mANK-101 and FACS immunophenotyping performed on Day 7 for tumor, lymph node, spleen, and blood (C) B16F10 tumor bearing mice (5/group) were given a single IT injection of 5 µg mIL-12 or 5 µg mANK-101 and euthanized at various times to collect tissues. Murine IFNγ and IL-12 were measured in tumor lysate and plasma by Meso Scale Discovery (MSD) assay. Data presented as mean +/- SEM

Combination with Checkpoint Blockade

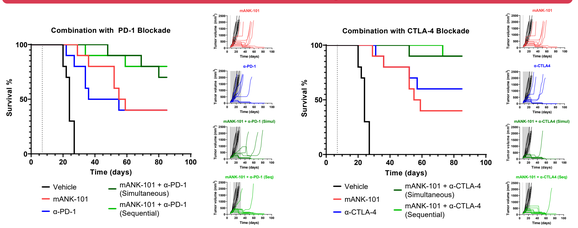
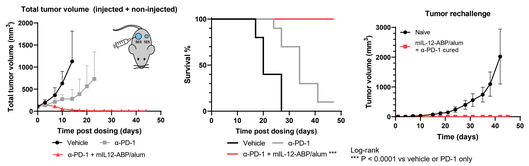


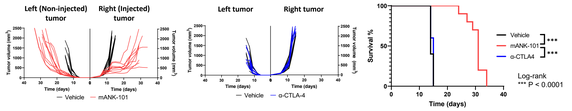
Figure 7: BALB/c mice (10/group) bearing CT26 tumors were administered a single intratumoral injection of 5 µg mANK-101 on Day 7 alone or in combination with systemic PD-1 or CTLA-4 inhibition. Tumor volume and body weight were monitored, and mice were euthanized when TV > 2000 mm³. 200 µg anti-PD-1 clone 29F.1A12 was administered IP BIW for 4 weeks starting on Day 7 (simultaneous) or BIW for 3 weeks starting on Day 14 (sequential). 200 µg anti-CTLA-4 clone 9D9 was administered IP BIW for 3 weeks starting on Day 7 (simultaneous) or 2 weeks starting on Day 14 (sequential)

Activity Against Non-Injected Lesions

Single shot of mIL12-ABP/alum + systemic PD1 blockade regresses injected and non-injected MC38 tumors and induces immune memory against subsequent MC38 tumor challenge



mANK-101 monotherapy delays growth of injected and non-injected B16F10 tumors



Single IT injection of mANK-101 reduces spontaneous lung metastases from orthotopic 4T-1 tumor

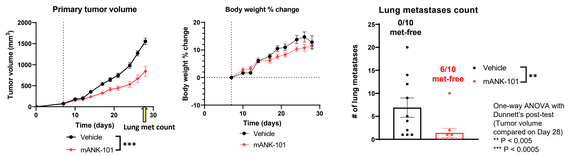


Figure 8: (Top) C57BL/6 mice (10/group) were inoculated with 5x10⁵ MC38 cells in the right flank and 1x10⁵ MC38 cells in the left flank. When right tumors reached 100 mm³, mice were treated with 200 µg α-PD-1 clone 29F.1A12 IP on Day 0,3,6,9 with or without IT injection of 5 µg mIL-12-ABP/alum complex in the right tumor only on Day 0. Mice that cleared both injected and non-injected tumors were rechallenged with 5x10⁵ MC38 cells in the right flank. (Middle) C57BL/6 mice (10/group) were inoculated with 1x10⁶ B16F10 cells in the right flank on Day 0 and 1x10⁶ B16F10 cells in the left flank on Day 3. Mice were treated with 5 µg mANK-101 in the right tumor only on Day 6 and 16. As a control group, mice were treated IP with 200 µg anti-CTLA4 clone 9D9 BIW for 3 weeks. (Bottom) BALB/c mice (10/group) bearing orthotopic 4T-1 tumors were injected IT on Day 7 with vehicle or 5 µg mANK-101. On Day 28, animals were sacrificed and lung metastases counted. Data presented as mean +/- SEM

Conclusions

- Ankyra's proprietary platform improves the therapeutic window of cytokines and other immune agonist drugs and reduces the need for repeat injections through formation of an extended intratumoral drug depot mediated by high affinity complexation to the common vaccine adjuvant aluminum hydroxide
- 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
- 1 or 2 intratumoral injections of a murine surrogate of ANK-101 (mANK-101) induces complete regressions in CT26 syngeneic tumors and significant delays in cold B16F10 tumors unresponsive to other IO agents; wild-type IL-12 has no activity when administered IT at identical doses
- Tumor regressions are associated with robust intratumoral immune activation including extended IFNγ production, increased CD8⁺/Treg ratio, and activation of myeloid cells
- Local administration of mANK-101 primes a systemic immune response that reduces growth of non-injected tumors and formation of micrometastases in mice
- Combination with systemic checkpoint blockade further enhances complete response rate after single mANK-101 injection
- IND enabling studies for ANK-101 initiated with an IND planned in 2022
- Multiple early-stage programs in progress to apply platform to other immune-relevant drugs

References – *1. Lasek et al. Cancer Immunol Immunother, 2014; 2. Nguyen et al. Front Immunol, 2020; 3. Kwong et al. Biomaterials, 2011*

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