ABSTRACT #999

Alum-anchored CD40 antibody drug conjugate (ANK-201) promotes local anti-tumor immunity and therapeutic activity without systemic toxicity in preclinical tumor models

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Abstract

Background

CD40, a TNFR family member, is a critical mediator of humoral and cellular immunity and agonist CD40 monoclonal antibodies (CD40mAb) have shown therapeutic benefit in preclinical tumor models. However, clinical development of traditional CD40mAb has been hampered by systemic toxicity. We hypothesized that alum anchoring of CD40 agonist mAbs would promote receptor clustering needed for T cell activation and result in an improved therapeutic window. ANK-201 is a next-generation CD40 monoclonal agonistic antibody structured to reduce toxicity and improve efficacy.

Methods

CD40mAb was genetically fused at its C-terminus to a short alum-binding peptide (ABP) and co-expressed with the kinase Fam20C. Phosphorylation of selected ABP amino acids was measured by malachite green assay. The phosphorylated CD40-ABP was complexed with alum, and the complexes were characterized for in vitro potency in reporter assays and a primary human dendritic cell (DC) activation assay; and in vivo for safety (body weight and liver enzymes), immune responses, and efficacy in hCD40 C57BL/6 Transgenic mice using a bilateral flank MC38 tumor model. Various doses were tested by local delivery either one or two doses separated by 7 days. Tumor and blood samples harvested at various time points from independent experiments were analyzed for PK. Tumor size was compared using Student's t-test between groups and multiple comparisons utilized two-way ANOVA testing. For survival studies, log-rank (Mantel–Cox) tests were used.

Results

CD40-ABP is phosphorylated when co-expressed with Fam20C and the level of phosphorylation is proportional to the number of ABP repeats. Addition of ABP attenuated signaling compared to parental CD40mAb in a phosphate-level dependent manner. However, anchoring of CD40mAb-ABP fusions to alum potentiated CD40 signaling, leading to enhanced signaling vs. parental mAb suggesting the role of alum anchoring in promoting receptor clustering. CD40-ABP complexed to alum activated primary human DCs, and interestingly, showed minimal IL-6 secretion. Two doses of IT administered CD40-ABP alone or in complexation with alum is sufficient to induce robust monotherapy efficacy in syngeneic MC38 mouse model in hCD40 C57BL/6 mice (p<0.01 by day 9 post first dose). Locally administered CD40-ABP is further able to prime a systemic immune response leading to abscopal effects. At these doses, non-anchored antibody induced transient elevations in AST/ALT, whereas ANK-201 did not.

Conclusions

This is the first report of anchoring a humanized fully length mAb to alum. ANK-201 was associated with enhanced CD40 signaling in vitro, generation of anti-tumor immunity, and an improved therapeutic window. These findings validate alum anchoring as a novel delivery strategy and position ANK-201 as a promising candidate for clinical translation.

Ankyra's Platform for Intratumorally Retained CD40 Antibody

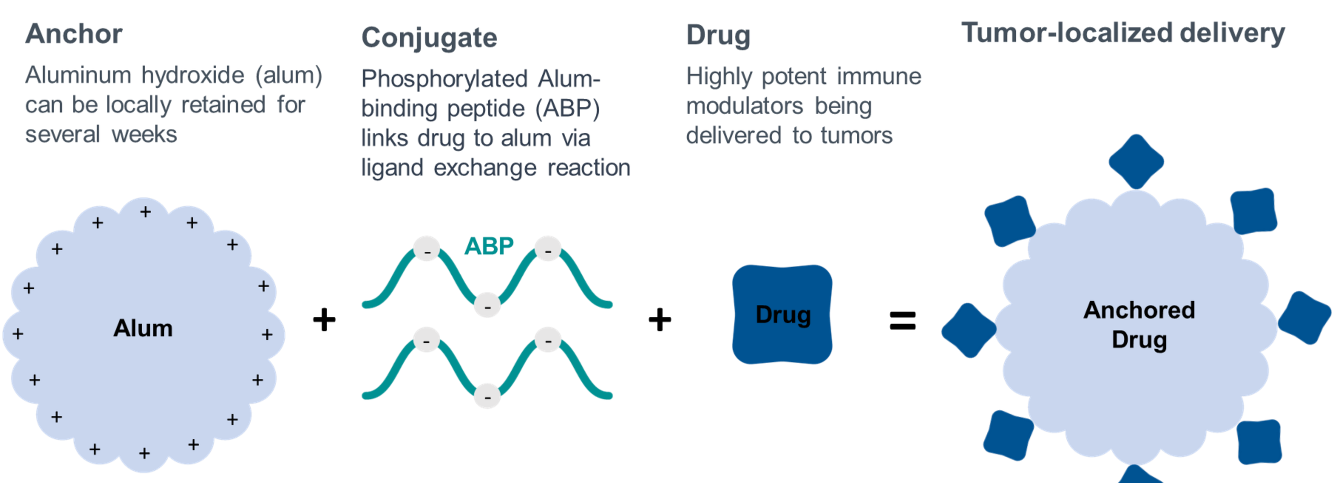

Figure 1: Ankyra's platform to generate alum-anchored CD40 antibody (ANK-201). Cytokines and antibodies (Drug) are genetically fused to a proprietary alum-binding peptide (ABP) and co-expressed with Fam20C kinase to phosphorylate the peptide at multiple serine residues. The phosphorylated fusion proteins are mixed with the FDA-approved vaccine adjuvant aluminum hydroxide (alum) to form a spontaneous complex through a phosphoserine mediated ligand exchange reaction. Drug/Alum complexes are administered locally at the tumor site where they are retained due to their size and charge, leading to a drug depot that promotes potent and long-lasting local immune activation.

CD40-ABP Shows Stable Retention on Alum

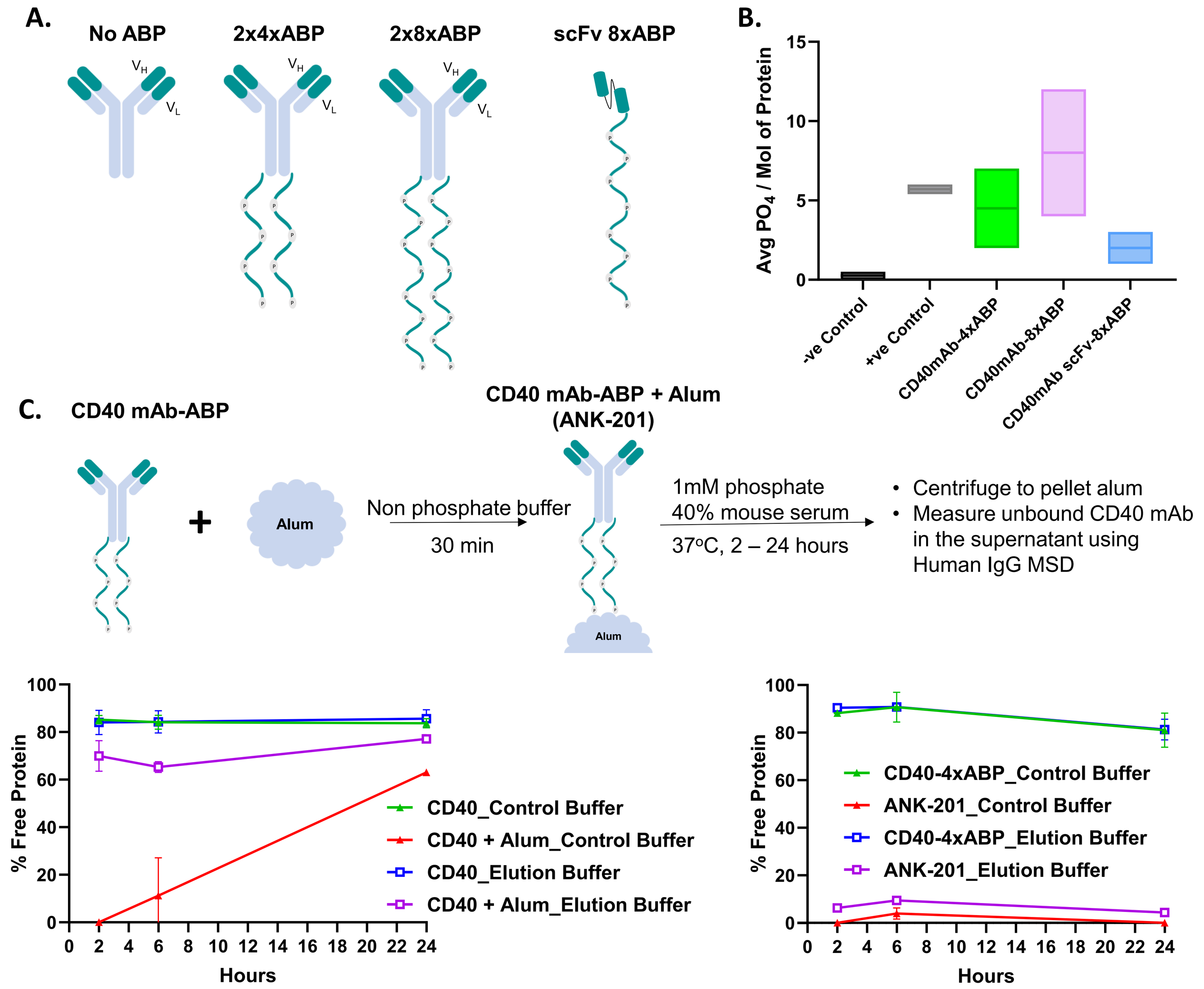

Figure 2: Phosphorylated ABP peptide allows stable retention of CD40 antibody on Alum. **(A)** Human CD40 antibody is engineered with varying repeats of ABP domain. **(B)** The ABP peptide is phosphorylated at multiple serines following co-expression with Fam20C kinase as measured by malachite green assay. **(C)** The native CD40 antibody and CD40-4xABP are complexed with alum then incubated in elution buffer containing 1 mM phosphate and 40% mouse serum or a non-phosphate containing buffer control. Free human IgG in the supernatant is measured at various times by MSD.

CD40-ABP + Alum (ANK-201) is Functionally Active In Vitro

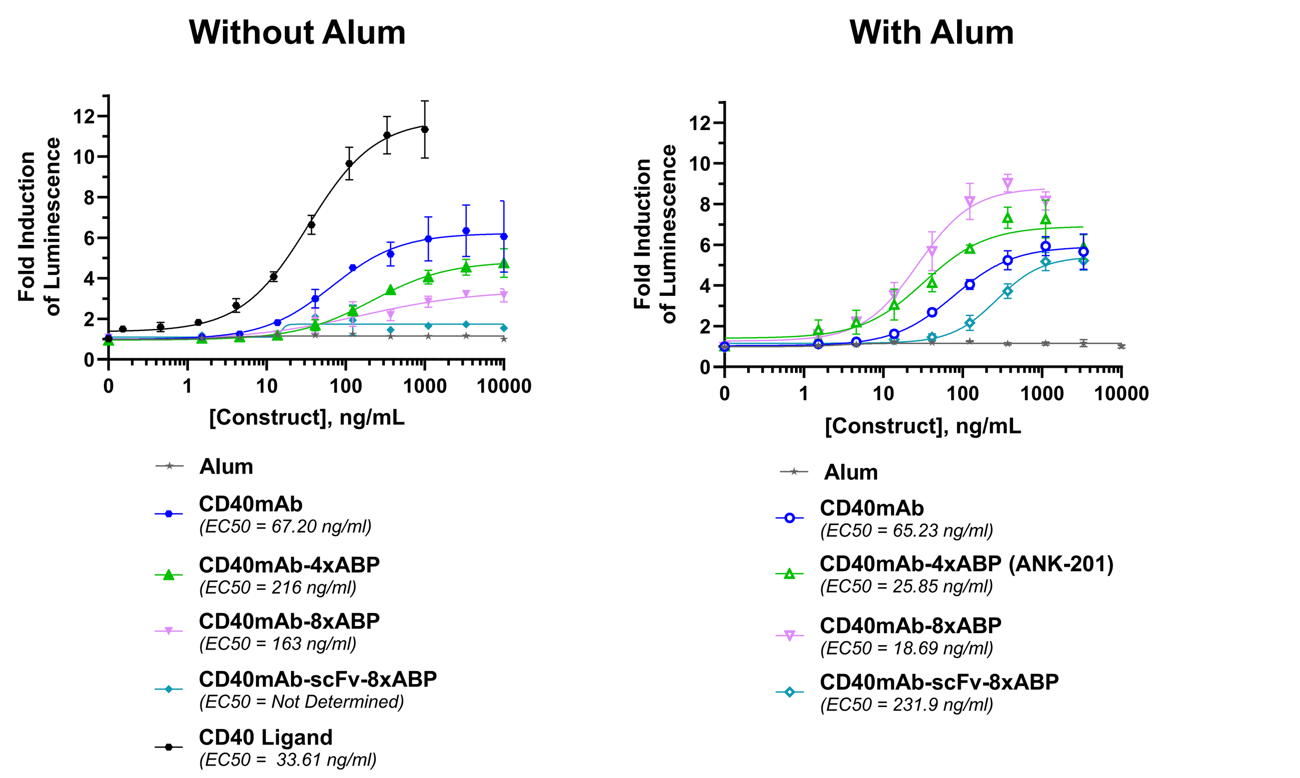

Figure 3: Functional activity of ANK-201 is confirmed by CD40 potency assay. Signaling activity of ANK-201 is assessed in vitro using the CD40 Bioassay from Promega. Genetically engineered CD40 Effector Cells expressing human CD40 and a luciferase reporter are stimulated with a dose range of ANK-201 or CD40 ligand control. The addition of CD40 ligand, or CD40-ABP antibody with and without alum induces the CD40 pathway-activated luminescence, which is detected in a dose-dependent manner by addition of Bio-Glo™ Reagent.

ANK-201 Activates Dendritic Cells In Vitro; IL-6 Secretion Low

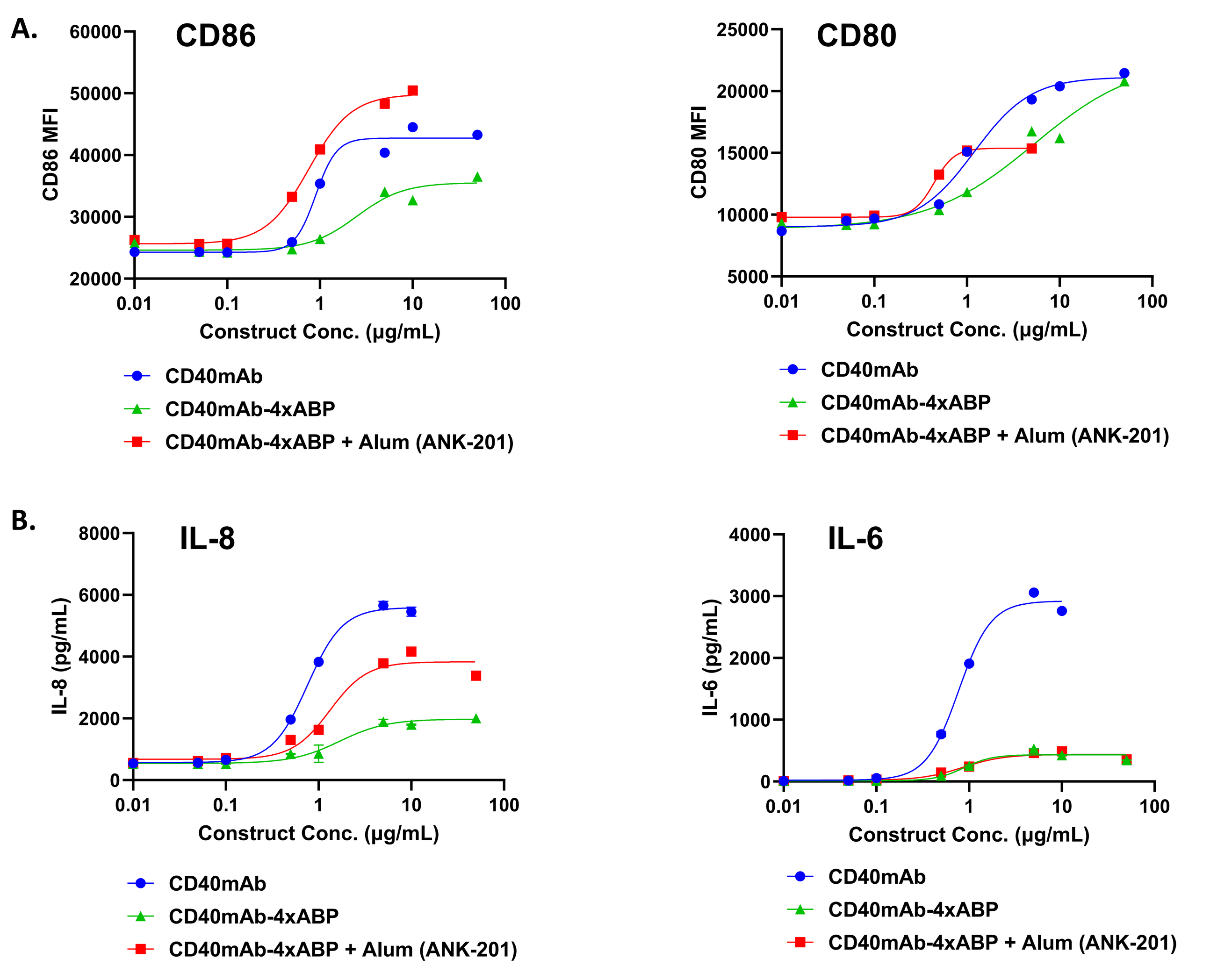

Figure 4: ANK-201 activates primary human dendritic cells in vitro. Frozen primary human monocyte derived mature dendritic cells are stimulated with increasing doses of native CD40 antibody, or CD40-ABP antibody with and without alum. **(A)** Expression of the DC activation markers CD80 and CD86 are analyzed by flow cytometry and **(B)** the levels of IL-6 and IL-8 secreted are measured by MSD.

Minimal Systemic Release of ANK-201 in hCD40Tg mice

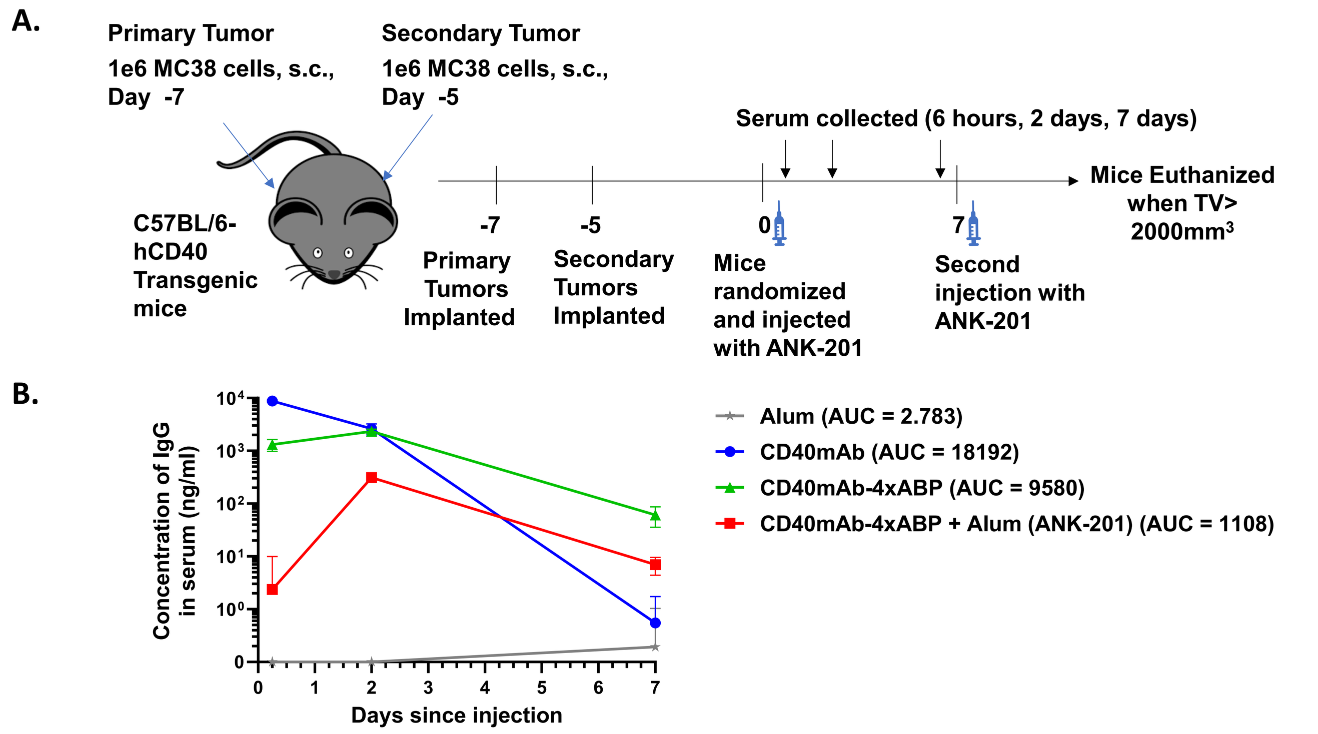

Figure 5: Alum retains CD40 antibody within the tumor in humanized CD40 transgenic mice. **(A)** C57BL/6-$Cd40^{tm1(CD40)Bcgen}$/Bcgen mice from Biocytogen harboring a humanized CD40 extracellular domain are injected with $1e^6$ MC38 tumor cells on both flanks, two days apart. Once the primary tumors reach 80 mm^3 size, mice are administered two intra-tumoral (IT) doses seven days apart of Vehicle (TBS), alum alone, 19µg CD40 antibody, 20µg CD40-4xABP, or 20µg ANK-201 (CD40-4xABP + Alum). **(B)** Peripheral blood is collected from the mice 6 hours, 2 days, and 7 days post first injection. Serum PK levels are analyzed through an MSD assay detecting human IgG. The AUC (ng/ml*Days) for each treatment is mentioned in the figure legend.

ANK-201 Prevents Liver Toxicity and Inhibits MC38 Tumor Growth

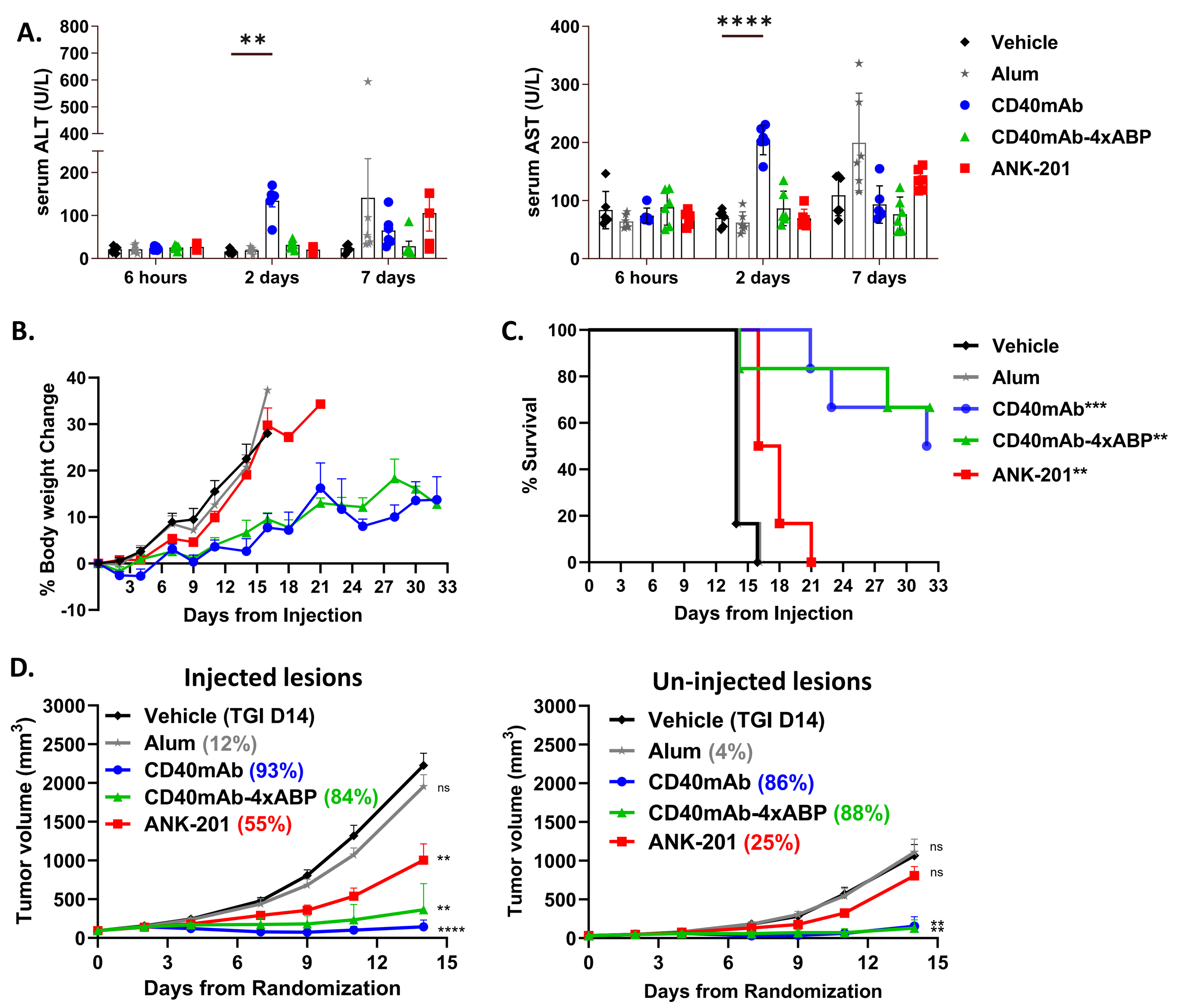

Figure 6: Anchoring CD40 antibody to alum prevents liver toxicity, decrease in body weight, and MC38 tumor growth in humanized CD40 transgenic mice. Liver enzymes, body weight, and tumor volumes are measured for C57BL/6-$Cd40^{tm1(CD40)Bcgen}$/Bcgen mice inoculated with MC38 tumors on both flanks and administered with two IT doses of Vehicle (TBS), alum alone, 19µg CD40, 20µg CD40-4xABP, or 20µg ANK-201 (CD40-4xABP + Alum) on Days 0 and 7. **(A)** Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) liver enzymes are measured 6 hours, 2 days, and 7 days post first injection of the test constructs. Addition of ABP tag to the CD40 antibody prevents increase in AST and ALT liver enzymes and **(B)** prevents drop in body weight. **(C and D)** MC38 tumor growth and Kaplan Meier survival curves are plotted. Alum alone has no effect on reducing tumor burden. ANK-201 inhibits MC38 tumor growth. (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). % Tumor Growth Inhibition (%TGI) on Day 14 is in the legend for tumor volume graphs.

Conclusions

- Ankyra's Anchored-Drug Conjugate platform is suitable for local delivery and retention of fully human monoclonal antibodies, including against human CD40.
- ABP-tagged CD40 antibody shows robust phosphorylation levels and stable retention on alum for up to 24 hours in vitro.
- ANK-201 is functionally active in CD40 reporter assays. Alum provides the scaffold for CD40 clustering required for maximal activity.
- ANK-201 activates primary monocyte-derived dendritic cells in vitro. There is a dose-dependent increase in CD86 expression and secretion of IL-8. Notably, treatment with CD40 antibody conjugated to alum minimizes secretion of IL-6.
- ANK-201 displays minimal systemic release in vivo with 1000-fold lower levels in serum in comparison to parent CD40 antibody at 6 hours post injection.
- ANK-201 is efficacious without any liver toxicity or body weight loss in MC38 tumor model.
- This is the first in vivo proof-of-concept for intratumoral delivery of antibodies conjugated to alum through Ankyra's proprietary platform.
- We are actively exploring novel methods for further improving the efficacy of anchored immunotherapy.

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