ABSTRACT# 6347

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Preliminary Results of an Exploratory Phase I Clinical Trial of Anchored Canine Interleukin-12 (cANK-101) in Dogs with Advanced Oral Malignant Melanoma

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ABSTRACT

Malignant melanoma is the most common form of oral cancer in canines with a median survival of 3 months for dogs with stage III or IV disease. Currently, there are limited effective systemic treatment options for these patients with advanced disease. We have developed an anchored immunotherapy approach in which canine interleukin-12 is stably linked to aluminum hydroxide (Alhydrogel®) to form the complex cANK-101. The anchored IL-12 forms a stable, functional depot of IL-12 and is expected to increase therapeutic responses with limited systemic toxicity (1-3). We report the preliminary results of an exploratory Phase I study of cANK-101 in dogs with advanced melanoma.

The clinical study was approved by the University of Illinois IACUC, conducted at the College of Veterinary Medicine, and all pet owners provided written, informed consent. The primary objective of the trial was to determine the safety and tolerability of cANK-101 in dogs with advanced melanoma. A standard 3+3 dose-escalation design was used with three dose levels (1, 3 and 10 µg/kg) of cANK-101 given by intratumoral injection every three weeks for 4 cycles. In the absence of overt clinical progression, dogs were allowed to receive a second course of four cycles. Dogs were monitored for adverse events via VCOG-CTCAE and clinical responses were measured using RECISTv1.1. Serum was collected for pharmacokinetic (PK) and immunogenicity analyses. In addition, serial tumor biopsy and lymph node cytology were performed. Serum cytokines were assayed via ELISA and MILLIPLEX® assays, while immunophenotyping of PBMC and lymph node aspirates were assessed by flow cytometry. Tumor-infiltrating lymphocyte (TIL) analysis was performed by immunohistochemistry (IHC) and gene expression profiling (Nanostring). Descriptive statistics were used for analyses.

As of April 10th, 2023, 9 (purebred and crossbred) dogs have been dosed and 3 dogs remain on study. All 9 dogs had at least one treatment-emergent AE (TEAE), with 4 dogs having TEAEs considered related to cANK-101 treatment (Table 2). No DLTs or SAEs were reported. One dog had a Grade 3 AE of elevated potassium which was not considered clinically significant, or treatment related. The most reported TEAEs were hypoalbuminemia, diarrhea and pain (n=3 each). Treatment was associated with increases in serum IFNγ, IP-10 and IL-10, increases in tumor infiltration of $CD3^+$ T cells as confirmed by IHC as well as increases in peripheral $CD8^+$/Treg ratio as measured by flow cytometry. PK and gene expression data are in process. Thus far, cANK-101 appears to be safe and tolerable in dogs with advanced melanoma. Data from this trial will help inform human clinical trials and may represent a new therapeutic option for dogs with advanced melanoma and perhaps other solid tumors. Our experience further suggests that companion animal trials could serve as relevant translational models for early immuno-oncology drug development.

BACKGROUND

Ankyra's 'Anchored Immunotherapy' platform improves the therapeutic window of potent immune-agonist drugs

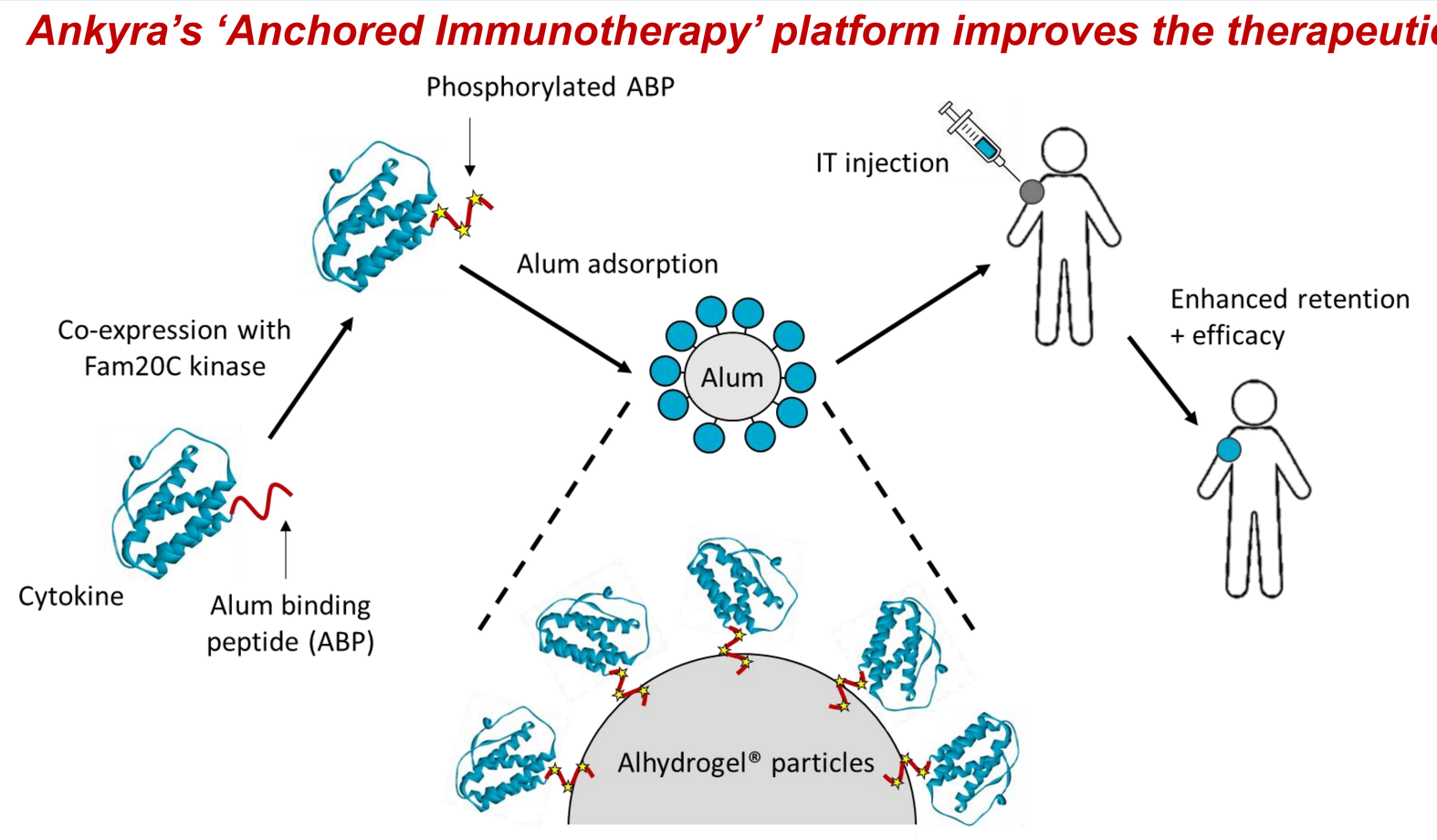

Figure 1. Schematic of **'Anchored Immunotherapy'** platform: A cytokine or protein of interest is genetically fused with a proprietary alum binding peptide (ABP) and co-expressed with FAM20C kinase to phosphorylate the peptide at multiple serine residues. The phosphorylated fusion proteins are then mixed with Alhydrogel® to form a stable complex. This mixture is intratumorally administered at the primary tumor site which forms a local depot that intensely drives efficacy in the tumor microenvironment yet decreases systemic toxicity of drug payloads with resultant improved therapeutic windows.

mANK-101 has increased tumor retention compared to free IL-12-ABP with corresponding increase in efficacy

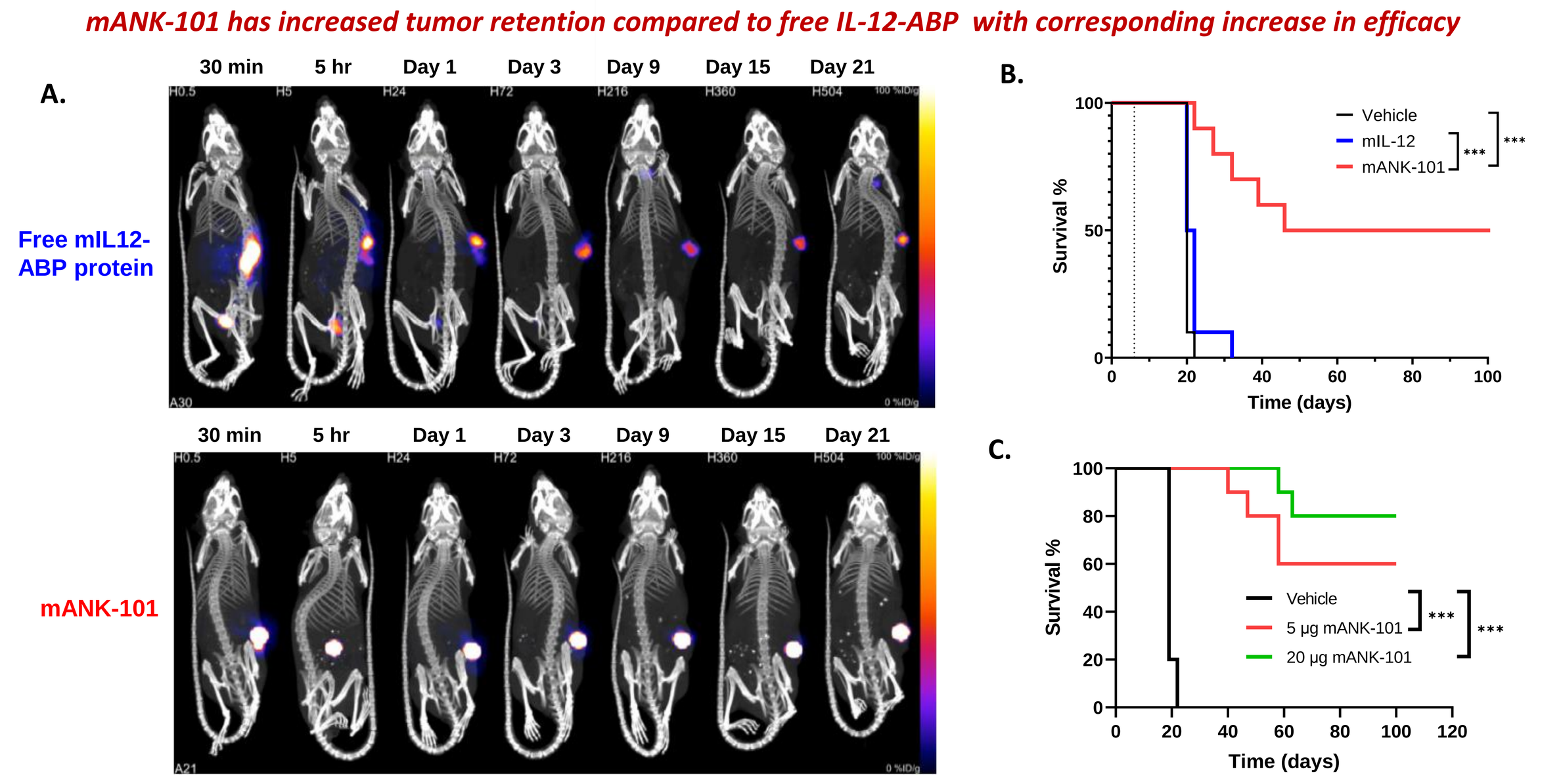

Figure 2. Biodistribution and efficacy studies in murine models: (A) 5µg of ^{125}I labeled murine IL-12-ABP (mIL-12-ABP) was injected intratumorally in BALB/c mice bearing CT26 tumors (n = 3) either as free protein or complexed with Alhydrogel® to form mANK-101 complex, and the animals were imaged by Single-Photon Emission Computed Tomography (SPECT) over time. Survival curves of BALB/c mice (10/group) bearing ~80 mm^3 CT26 tumors treated with a single IT dose of vehicle, 5 µg mIL-12, or 5 µg mANK-101, **(B)** or BALB/c mice bearing ~300 mm^3 CT26 tumors administered two IT doses of vehicle, 5 µg or 20 µg mANK-101 ten days apart **(C)**. mANK-101 displayed efficacy in both small and large tumor sizes with minimal effects on body weight (data not shown). (***P < 0.0001, log rank test)

CANINE ANK-101

cANK-101= cIL-12-ABP + Alhydrogel®

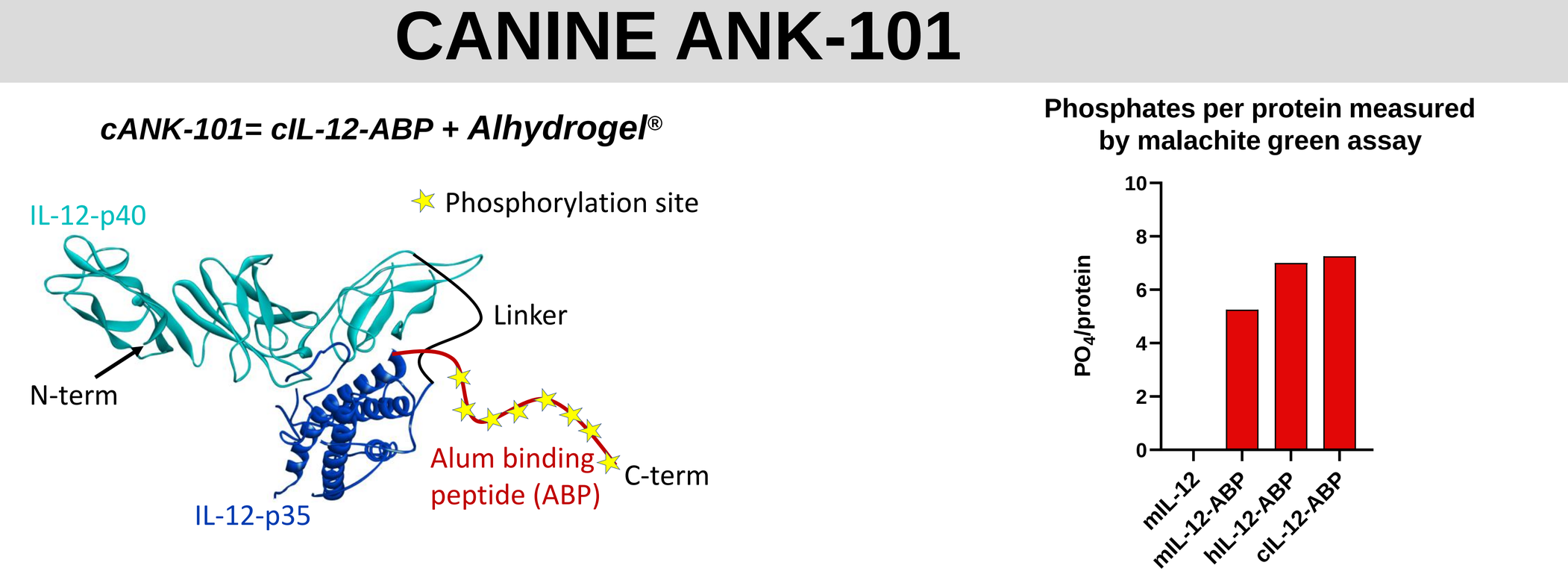

Figure 3. (left) Single-chain canine IL-12 with wild-type p40 and p35 sequences was genetically fused at its C-terminus to an alum-binding peptide to form cIL-12-ABP and complexed with a 10X mass excess of Alhydrogel® to form cANK-101. **(right)** Mouse (mIL-12-ABP), human (hIL-12-ABP), and canine (cIL-12-ABP) proteins are comparably phosphorylated at multiple serines following co-expression with Fam20C kinase as measured by malachite green assay.

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

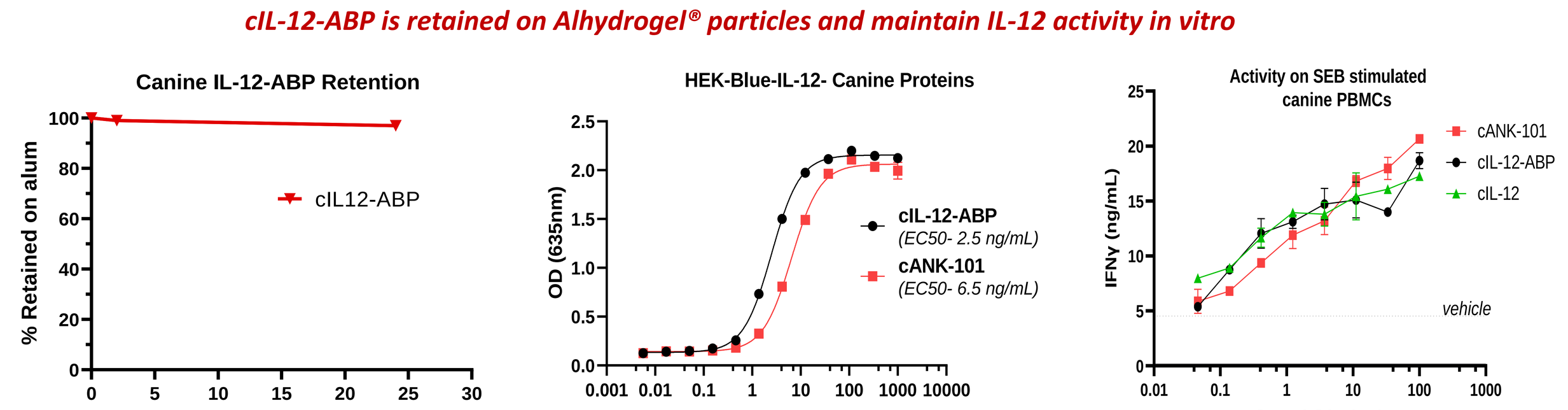

Figure 4. (**A**) ***In vitro* characterization of cIL-12-ABP and cANK-101:** cIL-12-ABP was complexed with Alhydrogel® and incubated in elution buffer containing 1 mM phosphate and 20% serum. Free cIL-12-ABP protein in the supernatant was measured at various times by ELISA. >90% of cIL-12-ABP protein was bound to Alhydrogel® at 24 hours. (**B**) Comparable activities of cIL-12-ABP and cANK-101 in HEK-Blue-IL-12 assay with pSTAT4 inducible promoter **(C)** IFNγ production from canine PBMCs stimulated for 3 days with SEB (10µg/ml) and test agents. The functional potency of free cIL-12-ABP and the cANK-101 complex are similar in both assay systems.

CANINE MALIGNANT MELANOMA

Study Design

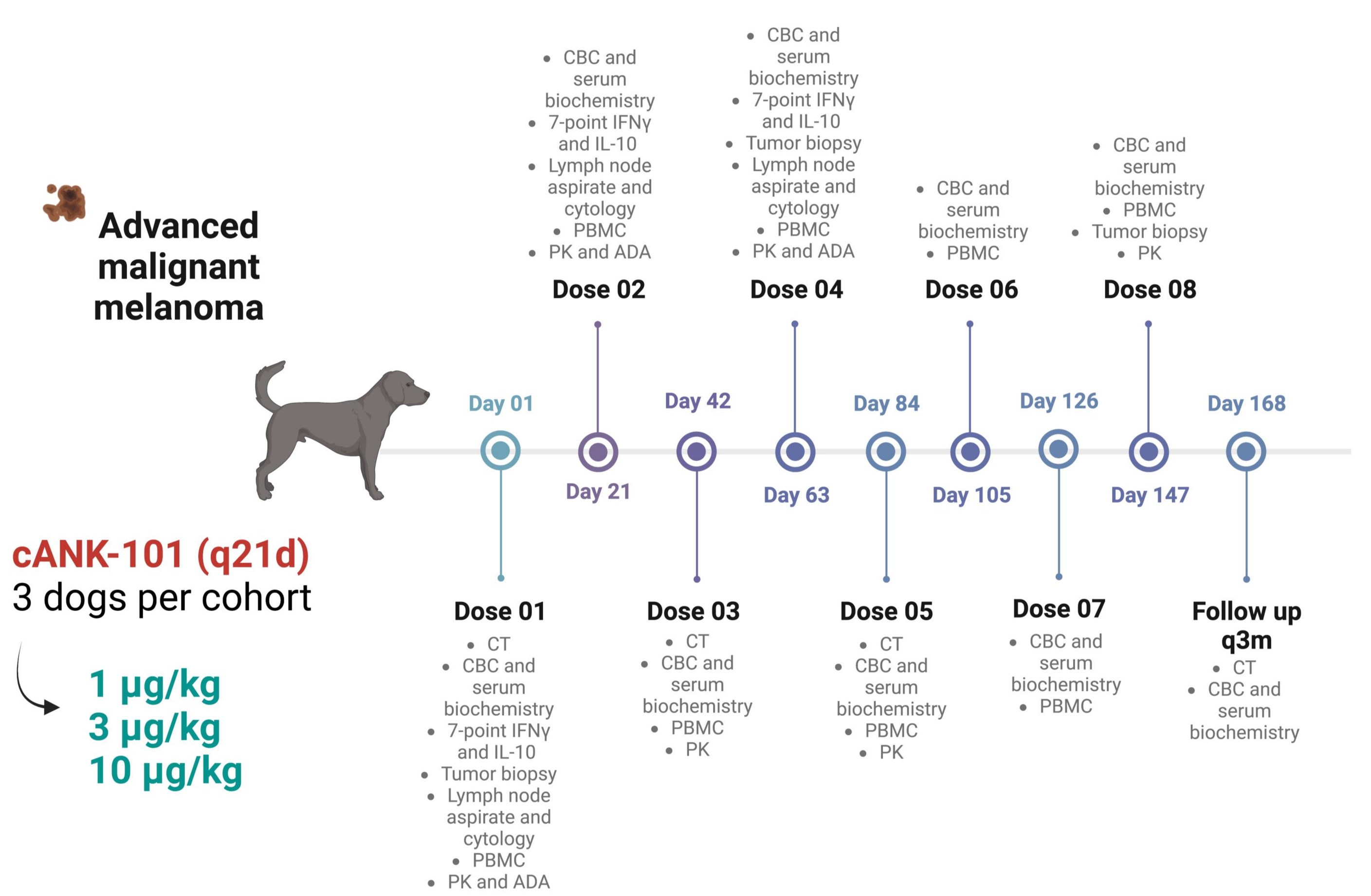

Figure 4. Exploratory canine clinical trial design for identifying optimal biologic dosing of cANK-101 in dogs with malignant melanoma. Through the inclusion of pet dogs, serial clinical, pharmacokinetic, pharmacodynamic, immunologic, and radiologic assessments are generated, and study findings could be used to guide future studies in human patients.

Patient demographics and clinical tolerability

cANK-101 Phase 1 Canine Dose Escalation Study in Dogs with Oral Malignant Melanoma[1][2]
Table 1: Demographic + Baseline Characteristics and cANK-101 Dosing Summary

	Cohort 1 (n=3)	Cohort 2 (n=3)	Cohort 3 (n=3)	Total (n=9)
Demographics and Baseline Characteristics				
Breed [n (%)]				
Purebred	3 (100%)	1 (33%)	1 (33%)	5 (56%)
Golden Retriever	1	0	0	1
Labrador Retriever	2	1	1	4
Crossbred	0 (0%)	2 (67%)	2 (67%)	4 (44%)
Golden Retriever/Standard Poodle	-	0	1	1
Pit bull/Chinese Shar Pei	-	1	0	1
Pit Bull/Beagle	-	0	1	1
Shetland Sheepdog/German Shepherd		1	0	1
Primary site of oral melanoma [n (%)]				
Cutaneous	1 (33%)	0	0	1 (11%)
Lingual	0	0	1 (33%)	1 (11%)
Lip	0	1 (33%)	0	1 (11%)
Mandible	1 (33%)	0	1 (33%)	2 (22%)
Maxilla	0	1 (33%)	0	1 (11%)
Hard Palate	0	1 (33%)	0	1 (11%)
Soft Palate	1 (33%)	0	1 (33%)	2 (22%)
Age (years)				
Median (min, max)	9 (9 – 12)	9 (5 – 10)	8 (4 –10)	9 (4 –12)
Gender [n (%)]				
Male	1 (33%)	1 (33%)	1	3 (33%)
Female	2 (67%)	2 (67%)	2 (100%)	6 (67%)
Baseline ECOG [n (%)]				
0	3 (100%)	3 (100%)	3 (100%)	9 (100%)
1	0	0	0	0
Baseline weight (kg)				
Median (min, max)	33.6 (26.5, 40.2)	25.4 (22.1, 37.2)	34.5 (32.0, 36.8)	33.6 (22.1, 40.2)
Baseline WHO Stage[3] [n (%)]				
I	0	0	2 (67%)	2 (22%)
II	0	2 (67%)	0	2 (22%)
III	3 (100%)	1 (33%)	1 (33%)	5 (56%)
Baseline number of RECIST target lesions				
Median (min, max)	3 (1, 4)	1 (1, 2)	1 (1, 1)	1 (1, 4)
Baseline SLD of RECIST target lesions				
Median (min, max)	7.6 (3.5, 8.3)	2.7 (1.9, 7.9)	1.5 (1.0, 7.1)	3.5 (1.9, 8.3)
# cANK-101 doses administered per patient				
Median (min, max)	7 (4, 8)	8 (5, 8)	2 (2, 7)	7 (2, 8)
Total # injection visits in cohort	19	21	11	51

[1] Data cut from 10APR2023. Data cleaning ongoing.
[2] Percentages may not add to 100% due to rounding.
[3] WHO stage calculated retrospectively based on entered baseline EDC data.
SLD=sum of longest diameters (or short axis for lymph nodes)

Table 1. Baseline Characteristics and Demographics (10April2023)

cANK-101 Phase 1 Canine Dose Escalation Study in Dogs with Oral Malignant Melanoma[1][2]
Table 2: Incidence of Treatment-Emergent Adverse Events (TEAEs)

	Cohort 1 (n=3)	Cohort 2 (n=3)	Cohort 3 (n=3)	Total (n=9)
At least one TEAE	3 (100%)	3 (100%)	3 (100%)	9 (100%)
At least one DLT	0	0	0	0
At least one SAE	0	0	0	0
At least one Grade 3 or higher TEAE	0	1 (33%)	0	1 (11%)[3]
At least one treatment-related TEAE	1 (33%)	2 (67%)	1 (33%)	4 (44%)[4]
TEAEs Reported in 2 or More Patients Overall [# (%) patients]				
Hypoalbuminemia	0	2 (67%)	1 (33%)	3 (33%)
Diarrhea	2 (67%)	0	1 (33%)	3 (33%)
Pain	1 (33%)	2 (67%)	0	3 (33%)
Increased alkaline phosphatase	0	1 (33%)	1 (33%)	2 (22%)
Increased CPK	0	1 (33%)	1 (33%)	2 (22%)
Lethargy/fatigue	1 (33%)	1 (33%)	0	2 (22%)
Decreased hemoglobin	1 (33%)	1 (33%)	0	2 (22%)
Hypophosphatemia	0	1 (33%)	1 (33%)	2 (22%)
Thrombocytopenia	0	0	2 (67%)	2 (22%)

[1] Data cut from 10APR2023. Data cleaning ongoing.
[2] Percentages may not add to 100% due to rounding.
[3] Dog 004 had a Grade 3 AE of elevated potassium on the day of Dose 5 that was not considered clinically significant or treatment related.
[4] Related TEAEs (9 AEs recorded in 4 patients – all considered not treatment related; *note: some recorded AEs need to be split into multiple AEs):*
- Dog 003: localized right sided facial swelling (3 days after dose 3); localized right sided facial & neck swelling + pain/hesitant to eat (2 days after dose 6)
- Dog 004: inflammation of mass per owner at least doubled in size and pain (between doses 1 and 2)
- Dog 005: lethargy/fatigue (2 weeks post dose 1); lethargy/fatigue + pain/lack of appetite (3 days after dose 4)
- Dog 008: increased alkaline phosphatase (2+ weeks post dose 1)

TEAE=treatment-emergent adverse event; DLT=dose-limiting toxicity; SAE=serious adverse event

Table 2. Incidence of Treatment-Emergent Adverse Events (TEAEs)

cANK-101 is systemically well-tolerated in dogs receiving intratumoral treatment

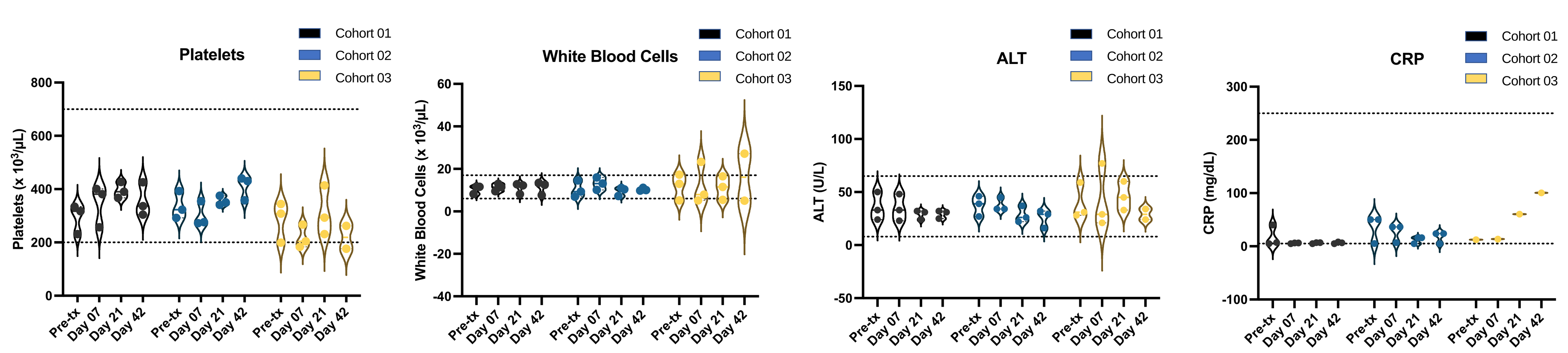

Figure 5. Serial samples were collected from dogs for CBC and serum biochemical profile as per the clinical protocol. cANK-101 treatment demonstrated to be safe and well-tolerated in dogs. Representative longitudinal laboratory measurements of platelets, white blood cells, alanine aminotransferase (ALT), and c-reactive protein (CRP) shown for dose cohorts. Dotted upper and lower lines define normal reference ranges.

cANK-101 treatment leads to enhanced tumor cell immune infiltration and elevated circulating INFγ

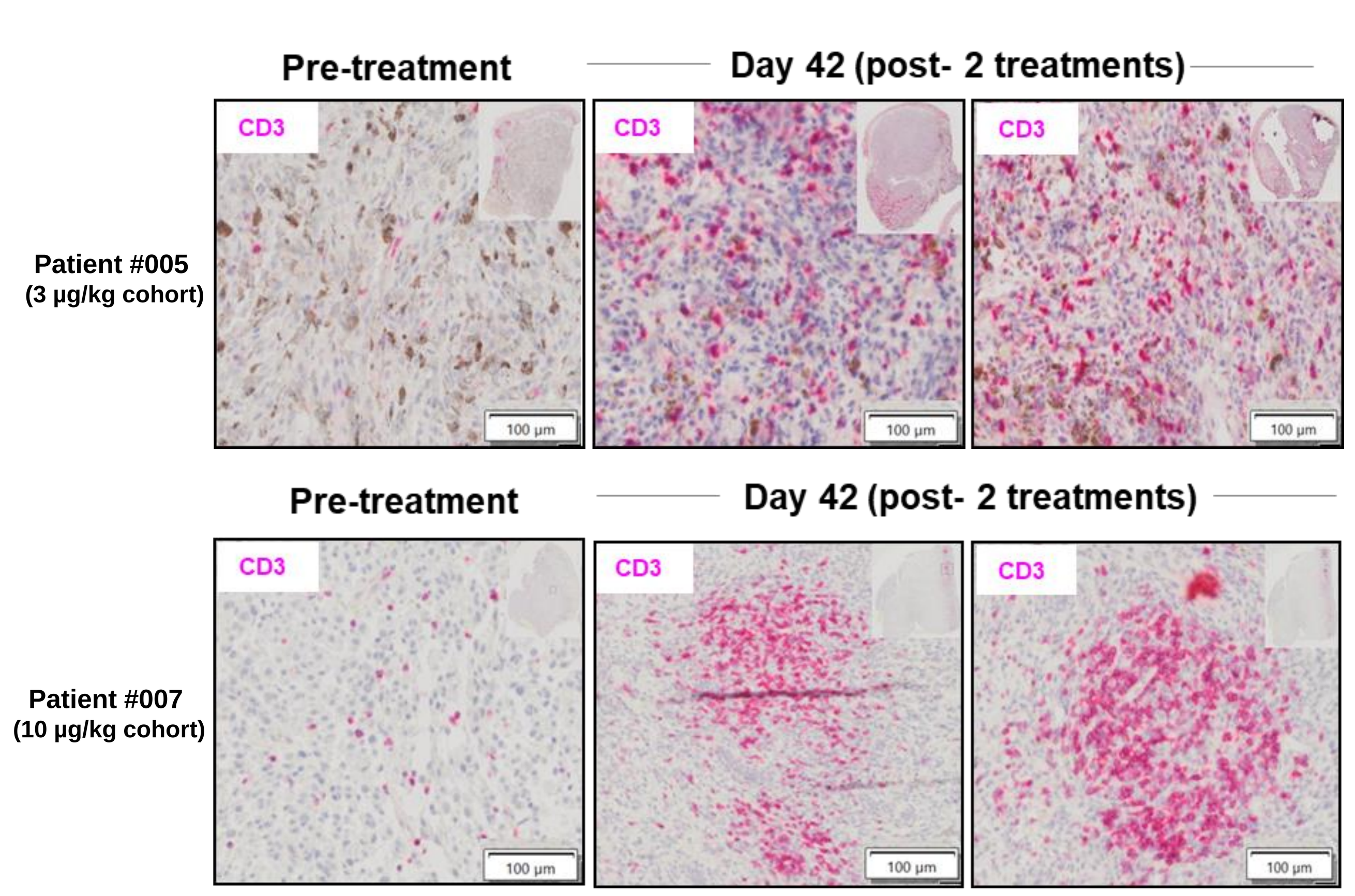

Figure 6. Tumor biopsies were collected as per the clinical protocol and FFPE oral melanoma tissue biopsies were immunostained for $CD3^+$ T-cells (clone CP215C). An increase in $CD3^+$ tumor infiltrating lymphocytes (TIL) was observed in response to cANK-101 treatment. Representative images of tissue sections from baseline (pre-treatment) and on day 42 post-treatment in patient #005 (3 µg/kg cohort) and patient #007 (10 µg/kg cohort).

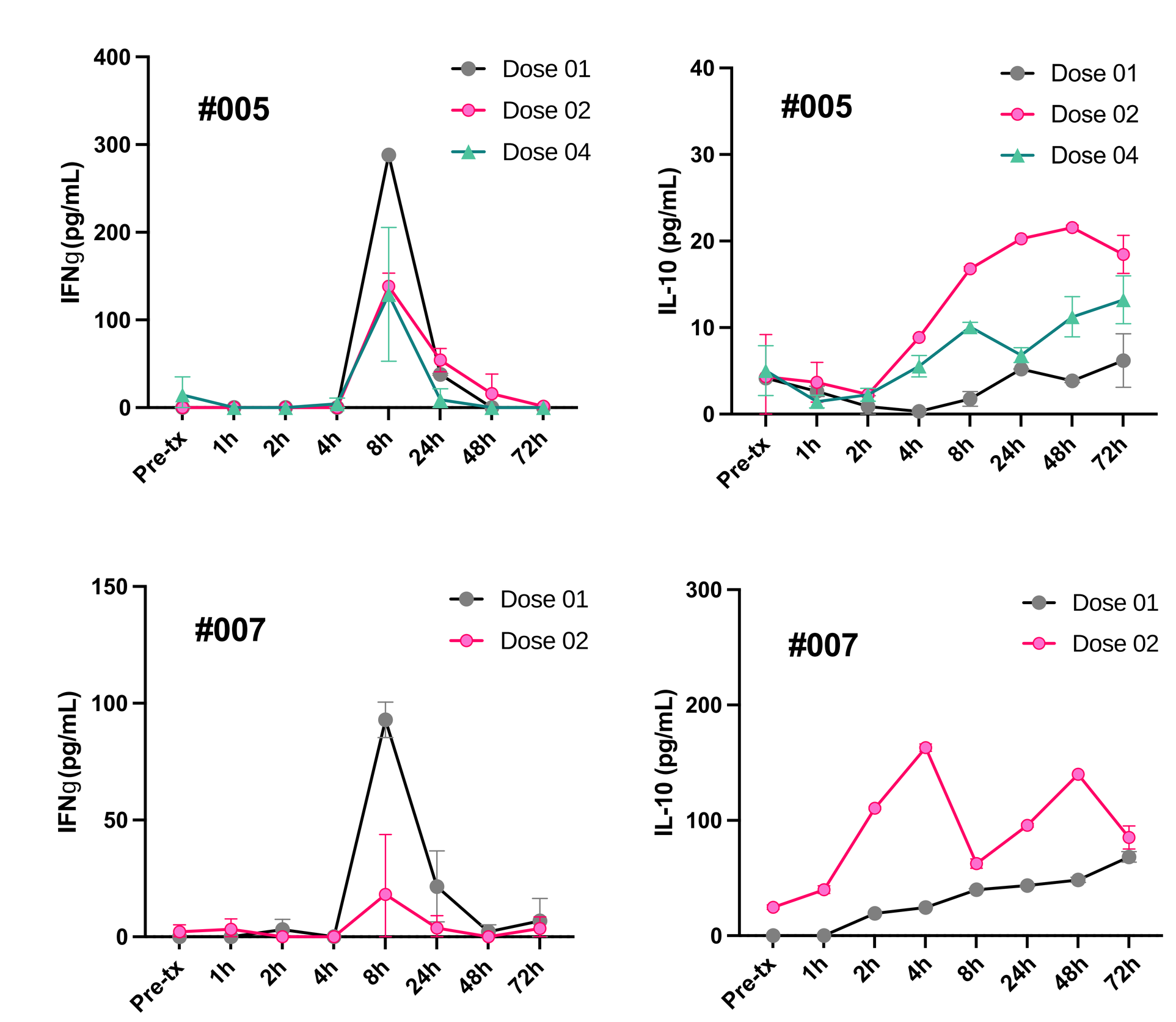

Figure 7. Serial serum samples were collected as per the clinical protocol and analyzed by ELISA for IFNγ and IL-10 measurements. The top panel represents cytokines from patient #005 (3 µg/kg cohort) and the bottom panels represent cytokines from patient #007 (10 µg/kg cohort). A transient elevation in serum IFNγ and IL-10 is indicative of immune modulation with cANK-101 treatment.

Clinical objective response with cANK-101

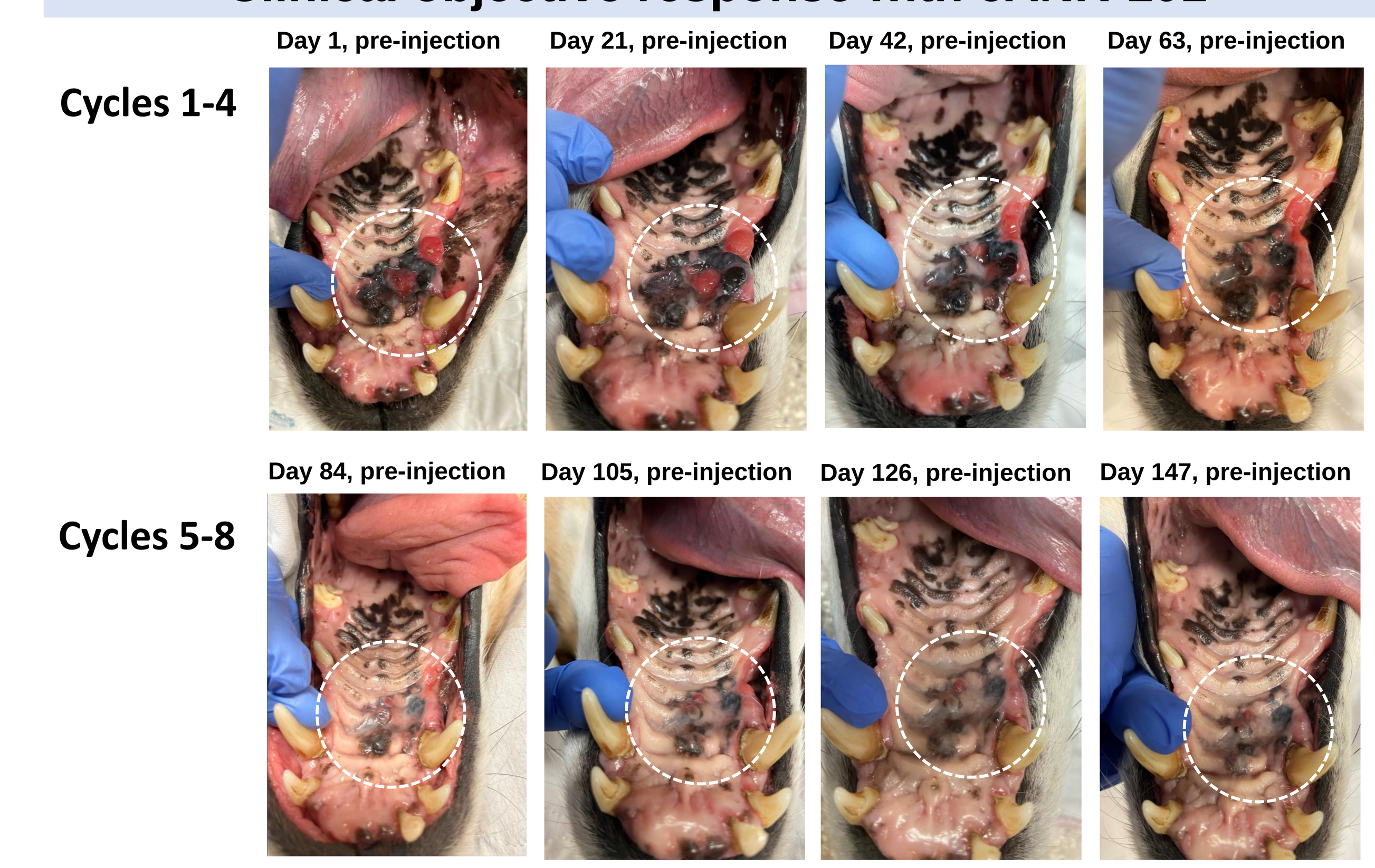

Figure 8. Strong partial response achieved in patient #006 with cANK-101 at 3 µg/kg over 8 cycle treatment period.

Correlative immune responses with cANK-101

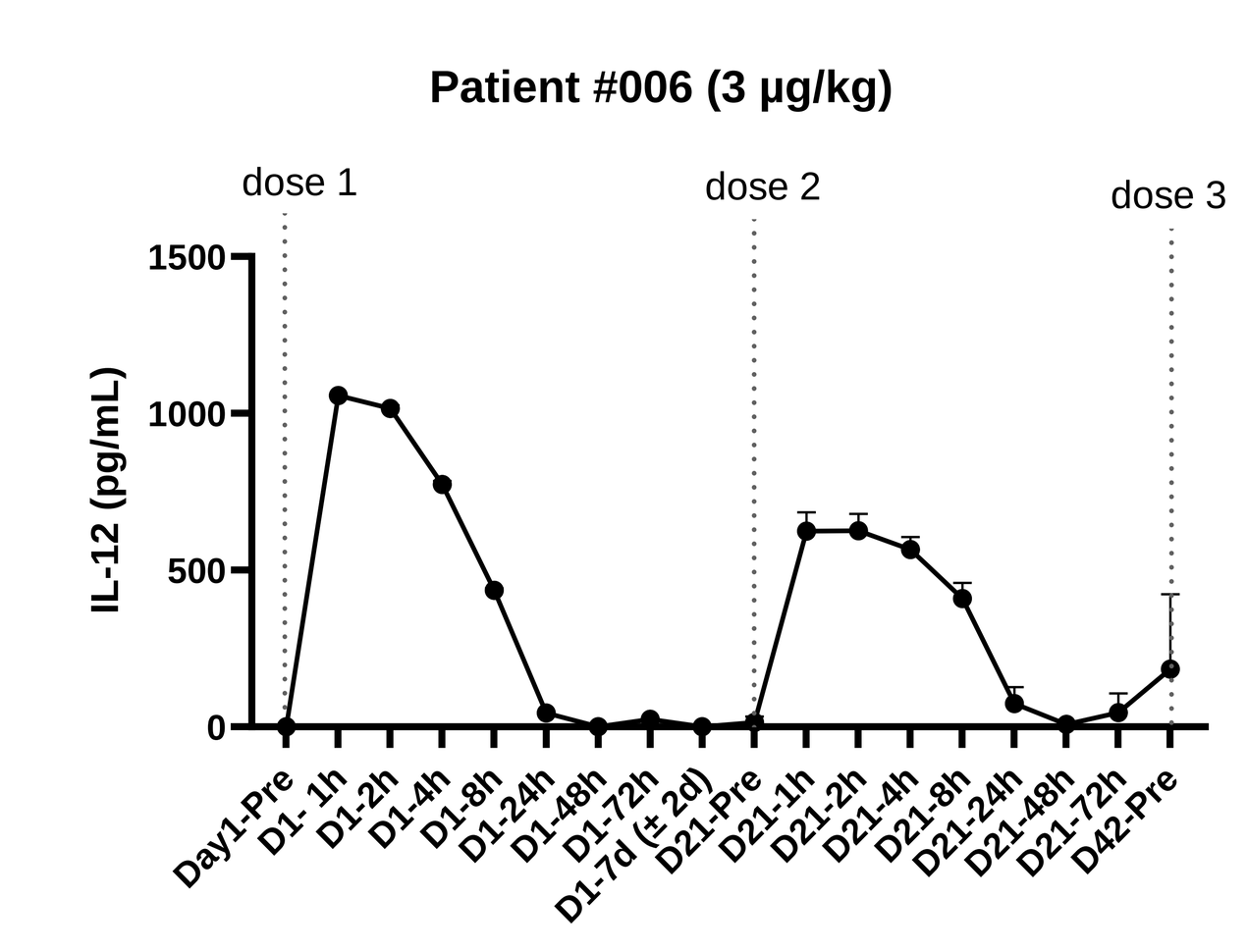

Figure 9. Pharmacokinetic assessments: Serial serum samples were collected as per the clinical protocol and serum IL-12 levels were measured by ELISA. A transient increase in serum cIL-12 levels were detected post-treatment with cANK-101.

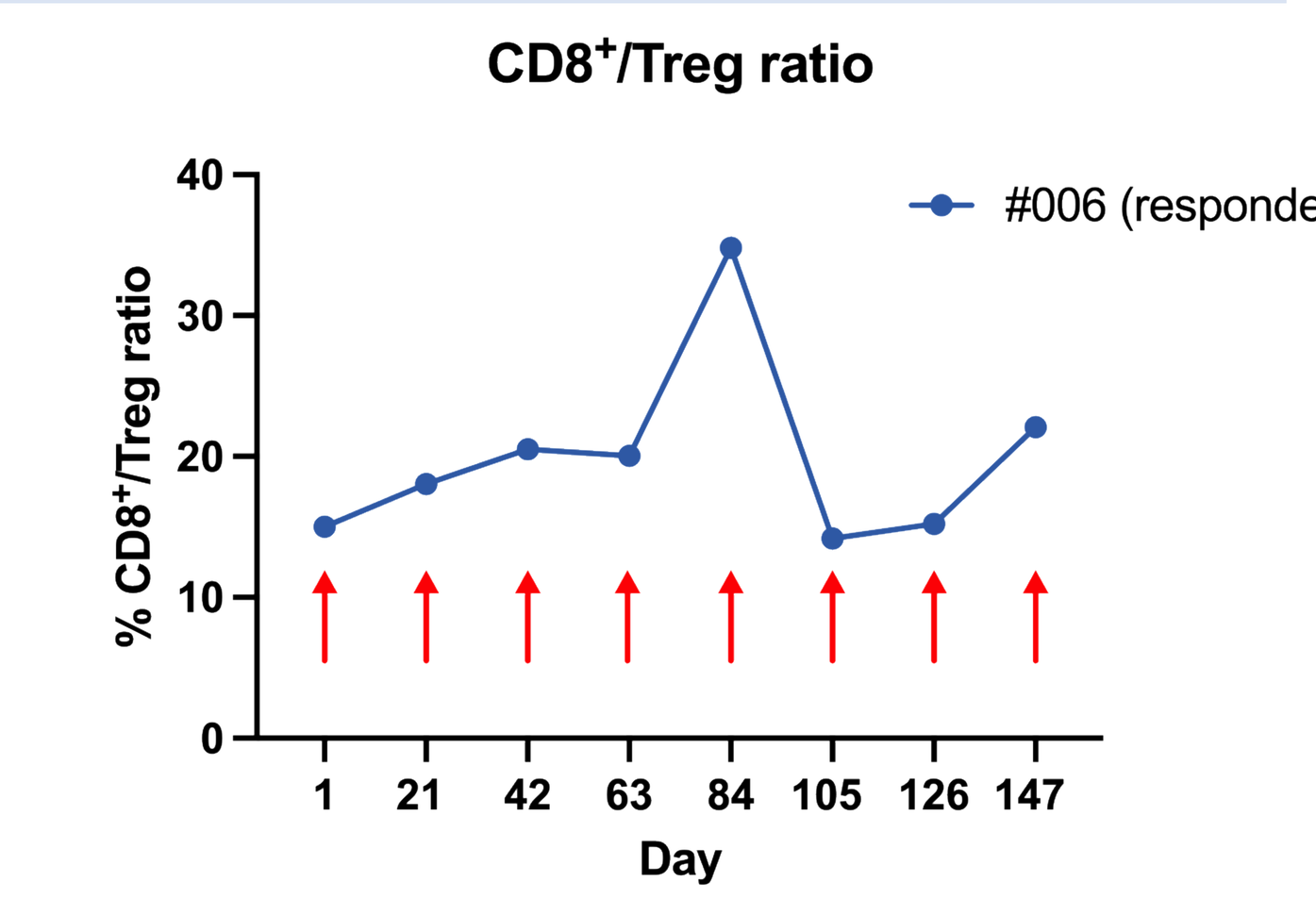

Figure 10. PBMCs were collected at various timepoints as per the clinical protocol and immunophenotyping was performed by flow cytometry analysis. A favorable increase in $CD8^+$/Treg ratio temporally correlated with the onset of objective clinical response in patient #006. Red arrows indicate treatment with cANK-101.

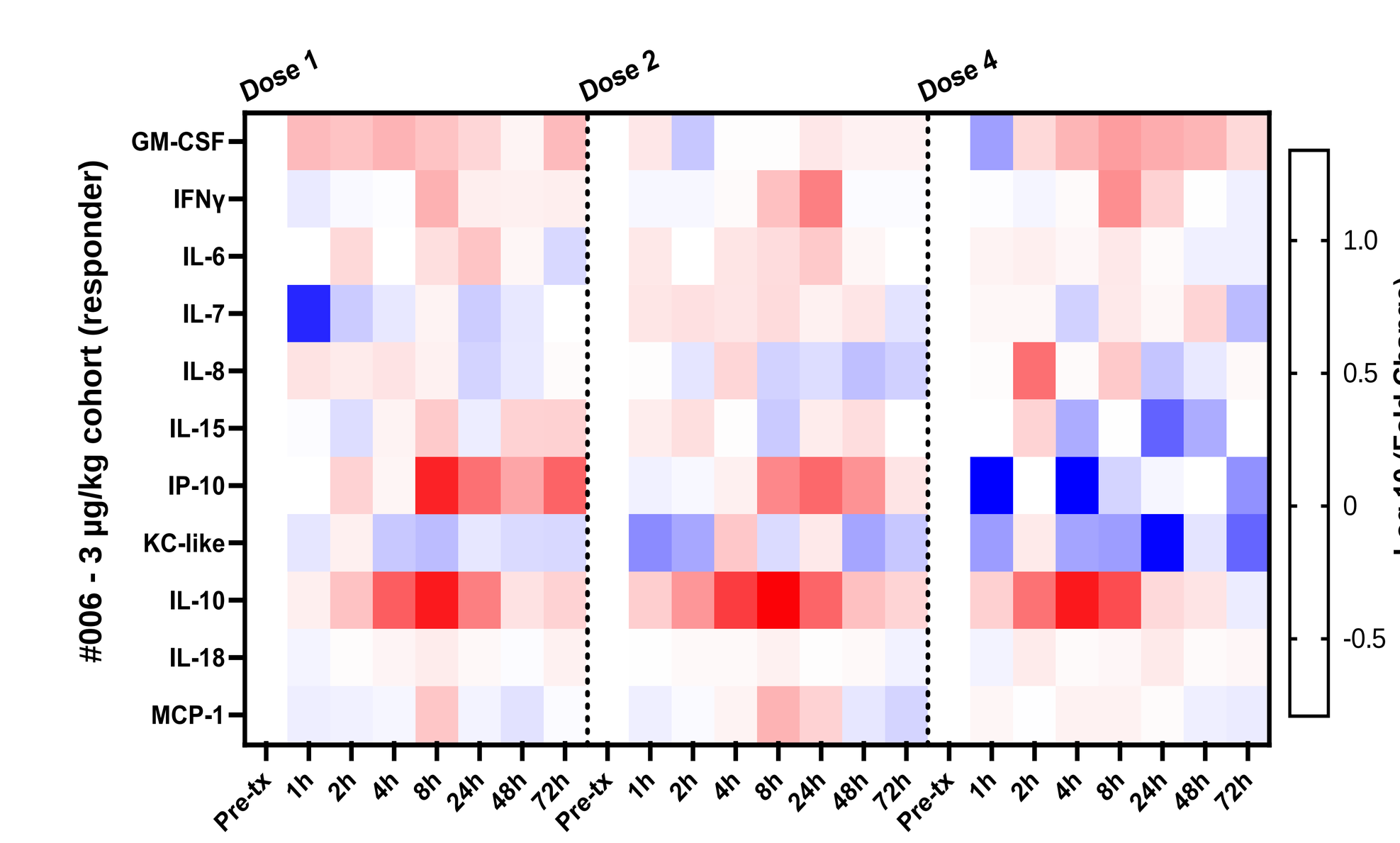

Figure 11. In Patient #006, serial serum samples were collected as per the clinical protocol and serum cytokine and chemokine profiling was performed using Milliplex kit (Millipore, Cat. No: CCYTOMAG-90K). With cANK-101 treatment, an elevation in a panel of cytokines and chemokines such as IFNγ, IL-10, IP-10, and MCP-1, which are known to be associated with the biology of IL-12 modulation was observed.

CONCLUSIONS & FUTURE DIRECTIONS

- Ankyra's proprietary 'Anchored Immunotherapy' platform utilizes the FDA-approved vaccine adjuvant Alhydrogel® (aluminum hydroxide/alum) as a scaffold to locally retain potent cytokine drugs and is designed to improve the therapeutic window of potent immunotherapy approaches.
- A canine surrogate molecule cANK-101 (cIL-12-ABP + Alhydrogel®) is being explored for its use as a therapeutic option for pet dogs with cancer.
- cIL-12-ABP is retained on Alhydrogel® particles and maintains IL-12 activity *in vitro.*
- Results from this on-going clinical trial suggest that cANK-101 is safe and well-tolerated in dogs with advanced melanoma.
- cANK-101 treatment is associated with increases in serum IFNγ, IP-10 and IL-10, increases in tumor infiltration of $CD3^+$ T cells as confirmed by IHC, as well as increases in peripheral $CD8^+$/Treg ratio as measured by flow cytometry.
- Based on the emerging safety profile, the study will expand cANK-101 to an additional cohort at 20 µg/kg.
- Data from this trial will help inform human clinical trials and may represent a new therapeutic option for dogs with advanced melanoma and possibly other solid tumors.
- Companion animal trials could serve as relevant translational models for early immuno-oncology drug development.

ACKNOWLEDGMENTS

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REFERENCES

1. *Lasek et al. Cancer Immunol Immunother, 2014*
2. *Agarwal et al. Nat Biomed Eng., 2022*
3. *Wittrup KD et.al., Expert Opin Drug Del. 2002; 1–8*