Evaluation of Alum-Anchored IL-12 (JEN-101) Immunotherapy in Canine Melanoma: Safety and Efficacy Across Weight-Based and Tumor Volume Dosing Strategies

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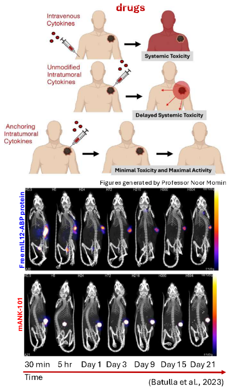
BACKGROUND

- Melanoma is an aggressive cancer in dogs similar to humans. We developed an anchored IL-12 immunotherapy (**JEN-101**) linked to aluminum hydroxide and conducted a phase I trial to assess safety, activity, and immune responses [1-3].
- Using a 3+3 design, dogs received intratumoral JEN-101 at 1–20 µg/kg (weight-based) every three weeks for up to eight doses, followed by an expansion cohort using a tumor volume–based dosing approach. JEN-101 was well tolerated, causing only mild fever, lethargy, and transient liver enzyme elevations.
- Weight-based dosing produced stronger immune activation and more consistent IL-12, IFN-γ, and IL-10 responses, while the tumor volume–based approach further confirmed safety and biological activity.
- JEN-101 was safe, immunologically active, and showed therapeutic potential, supporting further development and translational relevance (NCT06171750).

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



Figures generated by Professor Noor Momin

(Batulla et al., 2023)

ANKYRA'S CANINE PLATFORM: JEN-101

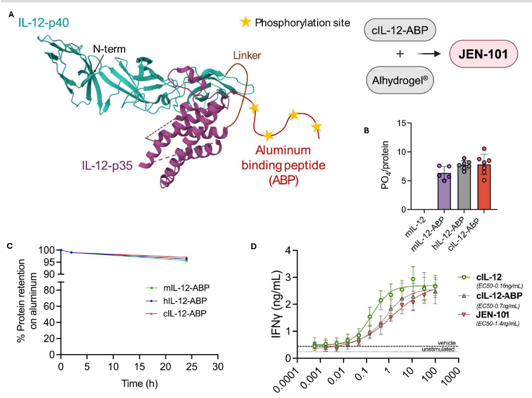


Figure 1. *In vitro* characterization and biological activity of cIL-12-ABP and JEN-101. (A) Ribbon diagram and schematic illustration of JEN-101 components. **(B)** Recombinant murine IL-12 (mIL-12; control), mouse (mIL-12-ABP), human (hIL-12-ABP), and canine (cIL-12-ABP) proteins are comparably phosphorylated at multiple serines following co-expression with Fam20C kinase as measured by malachite green assay (mean ± SD, n=2 replicates). **(C)** cIL-12-ABP, mIL-12-ABP and hIL-12-ABP were complexed with Alhydrogel® and incubated in elution buffer containing 1 mM phosphate and 40% serum. Free IL-12-ABP proteins in the supernatant were measured at various times by ELISA. >90% of cIL-12-ABP protein was bound to Alhydrogel® at 24 hours (mean ± SD, n=2 replicates), comparable to human and mouse IL-12-ABP constructs. **(D)** IFNγ production from canine PBMCs stimulated for 3 days with SEB (500 ng/ml) and test agents. The functional potency of free cIL-12-ABP and the JEN-101 complex were similar in both assay systems (mean ± SD, n=2 replicates).

CANINE MALIGNANT MELANOMA - JEN-101 PHASE 1 STUDY

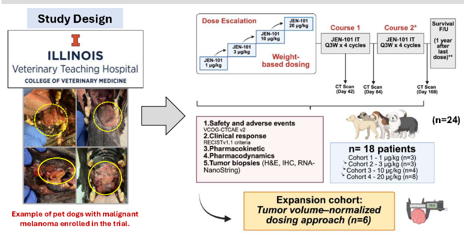


Figure 2. Schematic of the clinical trial design. Eligible dogs received JEN-101 IT every 21 days for 4 cycles. Nineteen dogs were treated at cohorts of 1, 3, 10, and 20 µg/kg and 6 dogs were treated using our tumor-volume normalized dosing approach. Eligible dogs also received a second course of JEN-101 IT every 21 days for 4 cycles. Dogs were assessed for clinical responses, pharmacokinetic, pharmacodynamic, immunologic, and CT imaging assessments.

Patient demographics and assessment of clinical responses

Table 1. Patient demographics and baseline characteristics.

	Cohort 1 1 µg/kg (n=3)	Cohort 2 3 µg/kg (n=3)	Cohort 3 10 µg/kg (n=4)	Cohort 4 20 µg/kg (n=8)	Volumetric Cohort (n=6)	Total (n=24)
Breed [n (%)]						
Purebred	3 (100%)	1 (33%)	2 (50%)	5 (57%)	3 (50%)	14 (58.4%)
Crossbred	0 (0%)	2 (67%)	2 (50%)	3 (43%)	3 (50%)	10 (41.6%)
Age (years)						
Median	9	9	9	8	12	10
Min, Max	9, 12	5, 10	4, 11	7, 16	11,16	4, 16
Gender [n (%)]						
Male	1 (33%)	1 (33%)	1 (25%)	5 (62%)	3 (50%)	11 (45.8%)
Female	2 (67%)	2 (67%)	3 (75%)	3 (38%)	3 (50%)	13 (54.2%)
Baseline ECOG [n (%)]						
0	3 (100%)	3 (100%)	4 (100%)	8 (100%)	6 (100%)	24 (100%)
1	0	0	0	0	0	0
Baseline weight (kg)						
Median	33.6	25.4	33.3	25.6	23.3	26.2
Min, Max	26.5, 40.2	22.1, 37.2	16.1, 36.8	12.7, 37.2	7.2, 36.7	7.2, 40.2
Baseline WHO Stage[1] [n (%)]						
I	0	1 (33%)	2 (50%)	3 (37%)	2 (33%)	8 (33%)
II	0	1 (33%)	0	1 (13%)	3 (50%)	5 (21%)
III	2 (67%)	0	2 (50%)	0	1 (17%)	5 (21%)
IV	0	1 (33%)	0	4 (50%)	0	5 (21%)
NA	1 (33%)	0	0	0	0	1 (4%)

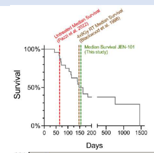


Figure 3. Kaplan-Meier survival curve for JEN-101 treated dogs across all dosing cohorts with a median survival time of 156 days (green dotted line) as August 2025. Comparator historical studies for untreated (red dotted line) or palliative radiation (brown dotted line) treated dogs with oral malignant melanoma with a median survival times of 65 days or 147 days, respectively.

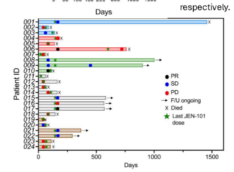
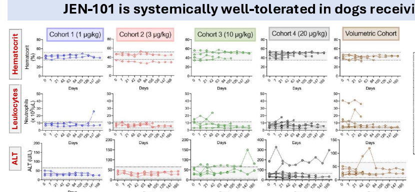


Figure 4. Swimmer's plot of individual subject response and durability as August 2025 (arrow denotes alive).

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



Incidence of JEN-101 adverse events in 2 or more dogs.

2 grade 3 JEN-101-related AEs at 10µg/kg dose (fever and lethargy);

3 grade 3 JEN-101-related AEs at 20µg/kg dose (fever, lethargy, elevated serum ALP).

All other events were ≤grade 2.

JEN-101 was well tolerated with adverse events being fever, lethargy, and isolated elevated liver enzymes.

Figure 5. Safety hematological and biochemical profile of JEN-101 treatment. Representative longitudinal laboratory measurements of hematocrit (%), alanine aminotransferase (ALT) (U/L), and leukocytes (x 10^3/µL) shown for all groups. Dotted upper and lower lines define normal reference ranges.

Pharmacokinetics and pharmacodynamics of JEN-101

Transient and consistent increase in serum cIL-12-ABP, IFN-γ, and IL-10 levels were detected in all cohorts following JEN-101 treatment. Tumor volume cohort had lower levels of circulating IFN-γ and IL-10.

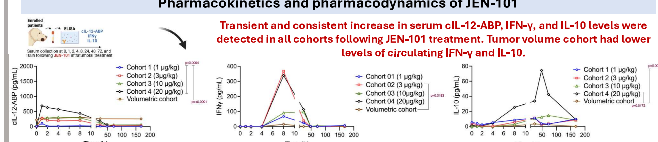


Figure 6. Serial serum samples were obtained at timepoints shown according to the figure and circulating cIL-12-ABP, IFNγ, and IL-10 levels were measured by ELISA. Data is shown by treatment cohorts following first dosing and the median for all cohorts following the first dosing. Transient and consistent increase in serum cIL-12-ABP, IFNγ, and IL-10 levels were detected in all dogs following JEN-101 treatment (mean ± SD, n=2 replicates).

CANINE MALIGNANT MELANOMA – JEN-101 PHASE 1 STUDY

Case studies, clinical, and immunological responses of patients with JEN-101 treatment

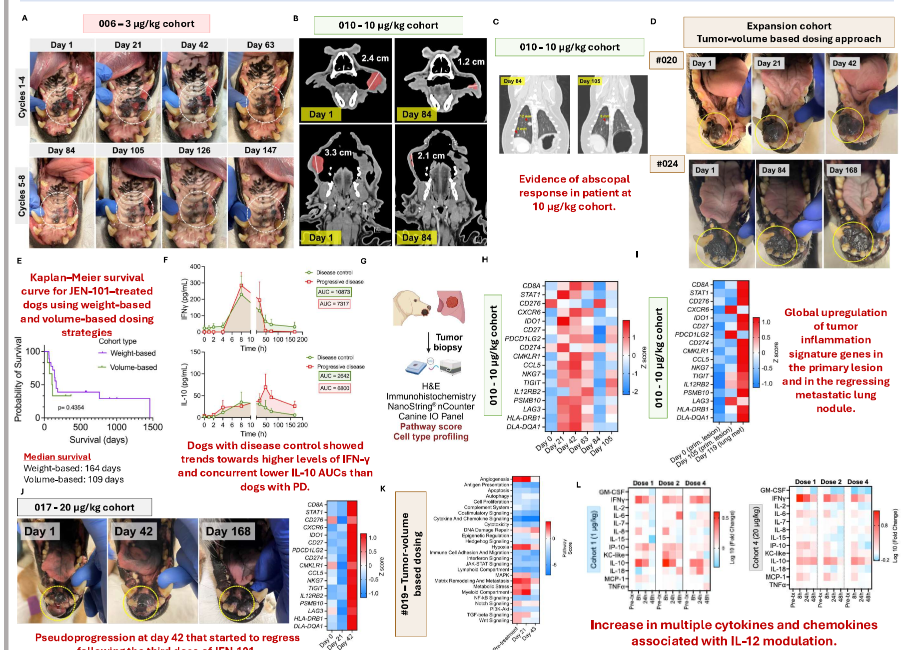


Figure 7. (A) Dog 006 (3 µg/kg JEN-101) showed partial tumor response after 8 cycles. **(B)** Dog 010 (10 µg/kg) exhibited significant lip melanoma regression confirmed by CT (day 1 vs. day 84). **(C)** Dog 010 also showed an abscopal effect with partial regression of untreated lung metastases. **(D)** Dogs 020 and 021 from tumor-volume dosing approach. **(E)** Kaplan-Meier survival curve for weight-based and volumetric-based approaches. **(F)**) 72-hour IFN-γ and IL-10 AUC levels in dogs with disease control (green) vs. no response (red). **(G)** Experimental design schematic. **(H)** NanoString analysis of Dog 010's primary tumor biopsies revealed strong immune activation. **(I)** NanoString analysis of Dog 010's regressing lung metastasis showed a tumor inflammation signature at the distant site. **(J)** Dog 017 (20 µg/kg) displayed partial regression in mandibular tumor and biopsies showed inflammatory gene upregulation before tumor regression. **(K)** NanoString analysis of Dog 019's primary tumor showed a lack of strong immune activation **(L)** Representative images of cytokine profiling by Milliplex showed increased IL-12-associated markers following JEN-101. (mean ± SD, n=2 replicates).

CONCLUSIONS AND FUTURE DIRECTIONS

- JEN-101 anchored IL-12 immunotherapy was safe, well-tolerated, and induced local and systemic immune activation in dogs with advanced melanoma.
- Preliminary analysis suggest that weight-based dosing produced stronger therapeutic and immune responses than tumor volume–based delivery, however further exploration of this dosing regimen is warranted.
- Future research should assess JEN-101 as monotherapy and in combination with radiation or checkpoint inhibitors, as well as other solid tumors.
- These findings support continued development of anchored IL-12 immunotherapy and highlight the translational value of canine models (**NCT06171750**).

REFERENCES

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