

COLLAGEN-ANCHORED IL-2 AND IL-12 CYTOKINES POTENTIATE ANTI-TUMOR IMMUNITY IN ADVANCED CANINE CANCERS

¹Jordan A Stinson*, ²Matheus Moreno M Passos Barbosa, ¹Allison Sheen, ²Jordan Hampel, ¹Noor Momin, ¹Elizabeth Fink, ²Rebecca Kamerer, ²Jonathan Samuelson, ²Rebecca Bernstein, ²Timothy Fan, ³K Dane Wittrup. ¹Massachusetts Institute of Technology, Cambridge, MA, USA; ²University of Illinois at Urbana-Champaign, Urbana, IL, USA; ³Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA, USA

Background The successful clinical translation of novel immunotherapies begins with evaluation in animal models that accurately recapitulate the complex tumor-immune interactions observed in human cancers. Naturally-occurring tumors in pet dogs present an under-utilized opportunity to assess the safety and efficacy of investigational immunotherapies, with particular advantages over mouse models including patient characteristics (e.g. immune senescence, weight, age), tumor heterogeneity (e.g. mutational burden, metastasis, immune infiltration), and dosing considerations (e.g. biodistribution, metabolic rate). Here, we examine the anti-tumor responses driven by engineered IL-2 and IL-12 cytokines in canine soft tissue sarcomas and oral melanomas.

Methods We have previously reported on our strategy to recombinantly express IL-2 and IL-12 cytokines as fusion proteins to a collagen-binding protein domain and albumin, which enable anchoring within the tumor microenvironment following intratumoral administration.¹ Given the promising activity and tolerability observed in syngeneic murine tumor models, we canine-ized these cytokine fusion proteins for evaluation in canine cancers. Pet dogs with soft tissue sarcoma were enrolled for treatment and received cytokines at different intervals prior to surgical tumor excision. Tumor tissue was then profiled by immunohistochemistry and Nanostring for cytokine-driven changes to the tumor microenvironment. Pet dogs with advanced oral melanoma were enrolled in a dose-escalation trial, receiving a single 9 Gy dose of radiation, followed by intratumoral cytokines every two weeks for 6 total doses. Serum was collected for analysis of systemic cytokine/chemokine response, with serial blood count/chemistry profiles and CT scans taken for the assessment of safety and radiologic response, respectively.

Results Intratumoral dosing of collagen-binding cytokines was well tolerated by tumor-bearing dogs at the lowest 3 investigational doses, with manageable cytokine release syndrome observed at the highest tested dose (57 µg/kg IL-2; 6.8 µg/kg IL-12). Transient elevation of systemic IFN-γ and IL-10 was observed following treatment in both tumor types. Analysis of treated sarcoma tissue revealed enhanced infiltration by T-cells, corroborated by increases in gene expression programs associated with cytotoxic immune function.² Robust anti-tumor responses were observed in melanoma patients, including 6/13 PR and 3/13 CR by RECIST criteria. Two dogs have remained tumor-free nearly 2 years after completing treatment.

Conclusions This data supports the safety and activity of collagen-anchored IL-2 and IL-12 after intratumoral delivery in tumor-bearing dogs, combining with standard-of-care radiation therapy or resection. This work highlights the utility of comparative oncologic evaluation in canine tumors alongside murine models to accelerate the build-test-learn design cycle for novel immunotherapies and/or treatment combinations.

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Ethics Approval All mouse studies were conducted under approval of the MIT Committee on Animal Care in accordance with federal, state, and local guidelines. The study protocol for the treatment of oral melanoma and soft tissue sarcomas in pet dogs was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign. All pet dog owners provided written consent before enrollment in the trial.

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