1159 USE OF SMALL MOLECULE STING AGONIST IMMUNOTHERAPY FOR CANINE SOFT TISSUE SARCOMA: A CROSS-SPECIES ANALYSIS

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Background Soft tissue sarcomas are rare connective tissue malignancies that are highly resistant to traditional systemic therapies.[1] Sarcomas are relatively common in dogs, yet few studies have previously investigated anti-sarcoma immunotherapies in this species.[2,3] In this study, we sought to investigate several small molecule STING (STimulator of INterferon Genes) agonists' dose toxicities and pathway inductions via canonical cytokine responses in canine macrophage and sarcoma lines, as compared to murine and human macrophage and sarcoma lines. We further aimed to assess the efficacy of a selected small molecule STING agonist without species specificity (ADU-S100) as an intra-tumourally administered drug in two canine patients with soft tissue sarcoma.

Methods To assess the cytotoxicity of STING agonists DMXAA, ADU-S100, and MSA-2, an in-vitro MTT cell viability assay was used. Murine, canine, and human macrophages and sarcoma cells were exposed to the following conditions: media, vehicle control, or 0.1, 1, 10, and 100μg/mL of treatment (up to 200μg/mL of treatment for ADU-S100). 6, 12, and 24-hours post-exposure to the conditions, cell viability was assessed via formazan absorbance values. STING-pathway induced interferon-dependent cytokine production (IFN-β, TNF-?, CXCL-10) in cells was assessed via the Luminex cytokine assay. All cells were treated with 177nmol/L of the STING agonists, and cytokine release 2- and 6-hours post-exposure were quantified. Clinical efficacy of ADU-S100 was further evaluated in vivo for two canine STS patients (1 hindlimb, 1 forelimb). Serial intra-tumoural doses further ranged from 200μg to 2.0mg of ADU-S100. Tumour volumes were calculated from caliper measurements of tumour length, width, and depth.

Results DMXAA and ADU-S100 were not cytotoxic below 100 and 200μg/mL, respectively, and MSA-2 was cytotoxic above 10μg/mL. All STING agonists effectively stimulated the interferon-dependent STING pathway in murine macrophage cells. In addition, ADU-S100, MSA-2, and E7766 stimulated the canine and human interferon-dependent STING pathways. Our subsequent in vivo pilot study demonstrated that the intra-tumoural administration of ADU-S100 led to a 3.5-fold and 2.3-fold reduction of canine patients' tumour volumes over the course of 6-weeks, respectively (from 594cm^3 to 172cm^3 for patient 1, and from 420cm^3 to 180cm^3 for patient 2).

Conclusions Overall, our findings suggest that STING agonists – in particular, ADU-S100 – possess potential as a novel and effective therapeutic approach for canine STS. As sarcomas are highly metastatic and commonly fatal in dogs, further evaluating STING agonist therapy in canines may provide therapeutic insights into similar challenges for treating human disease using a comparative biology approach.

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Ethics Approval All experiments were approved by the canine patients' respective owners and their informed consent, along with the approval and expertise of veterinary medicine specialists who assisted with conducting the trials.

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