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. Intra-tumoural injection of small molecule STING (STimulator of INterferon Genes) receptor agonists generates a profound immune response anti-tumour effect in pre-clinical murine soft tissue sarcoma models. Sarcomas are relatively common in dogs, yet few studies have previously investigated anti-sarcoma immunotherapies in this species.

Questions/Purposes: In this study, we sought to i) evaluate the dose toxicity of STING agonists DMXAA, ADU-S100, and MSA-2, ii) measure the STING-related cytokine production post-exposure to the three drugs in canine cell lines, as compared to murine and human macrophage and sarcoma cell lines. We further aimed to assess efficacy of a selected small molecule STING agonist without species specificity (ADU-S100) as an intra-tumoural 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-α, 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 ranged from 200µg to 2.0mg of ADU-S100. Tumour volumes were calculated from caliper measurements of tumour length, width, and depth. Continuous data was analyzed using an unpaired student’s t-test.

Results: DMXAA was not cytotoxic below 100 μg/mL (p ≤ 0.05) and ADU-S100 was not cytotoxic below 200μg/mL (p ≤ 0.05), respectively. However, MSA-2 was cytotoxic above 10μg/mL across the multiple cell lines (p ≤ 0.01). DMXAA, ADU-S100, and MSA-2 effectively stimulated the interferon-dependent STING pathway in murine macrophage cells. In addition, ADU-S100 and MSA-2 effectively stimulated both the canine and human interferon-dependent STING pathway. Our subsequent in vivo pilot study demonstrated that intra-tumoural administration of ADU-S100 led to a significant reduction both patients’ tumour volumes over the course of 6-weeks. Patient 1 had a reduction in tumour volume from 594cm³ to 172cm³ (3.5-fold reduction) and patient 2 had a reduction from 420cm³ to 180cm³ (2.3-fold reduction). An improvement in ambulation was observed in one of the patients, which may have been secondary to decreased mass effect on the sciatic nerve.

Conclusion: Overall, our findings suggest that STING agonists possess potential as a novel, low-cost, and effective therapeutic approach for canine STS. Notably, ADU-S100 effectively activated the STING interferon-dependent pathway in murine, canine, and human cell lines, and demonstrated in vivo efficacy against 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. Further investigation is ongoing to evaluate intra-tumoural phenotypic changes induced by STING therapy.