862 ADMINISTRATION OF INTRATUMORAL HU14.18-IL2 IMMUNOCYTOKINE AND LOCAL RADIATION THERAPY TO ACTIVATE IMMUNE REJECTION OF SPONTANEOUS CANINE MELANOMA

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Background Canine malignant melanoma provides a clinically relevant, large animal model to study the GD2-reactive hu14.18-IL2 immunocytokine (IC) as it is similar to human melanoma and expresses GD2. Murine preclinical studies have shown that intratumoral (IT) injection of IC (IT-IC) in combination with local radiation therapy (RT) can convert the injected tumor into an effective *in situ* tumor vaccine. We previously reported that IT-IC at 12 mg/m^2 on 3 consecutive days is well tolerated in tumor-bearing dogs.

Methods Twelve dogs (6 dogs/arm) with locally advanced or metastatic melanoma were randomized to receive a single 8 Gy fraction (Arm A) or three 8 Gy fractions delivered over 1 week (Arm B) to the primary site and regional lymph nodes (when clinically involved) with the single or last fraction 5 days prior to IT-IC at 12 mg/m^2 on 3 consecutive days. Tumor biopsies and peripheral blood mononuclear cells (PBMC) were obtained for immune monitoring at pre-treatment and various times post-treatment.

Results All 12 dogs completed protocol treatment and none experienced significant or unexpected adverse events. Antitumor activity includes 3 dogs with partial response at day (D) 30 and 4 dogs with mixed responses. Eleven dogs ultimately experienced progressive disease and 1 is currently alive in immune partial response 5 months post treatment initiation. Hematoxylin and eosin (H&E) stains of 5 serial biopsies (pre-treatment, D1, D10, D17, D24) show a variably timed increase in intratumoral lymphocytic inflammation post-therapy in 6/6 dogs in Arm A while 4/6 also show at least focal tumor necrosis post-treatment. In 2/6 dogs in Arm B, a clear increase in intratumoral lymphoid infiltrate occurred post-treatment. However, 5/6 of Arm B dogs also showed tumor necrosis with 2/6 with no viable tumor by D24. Immunohistochemistry (IHC) staining for CD3, CD8, and FOXP3 (pre-treatment, D1, D10, D17) show the large majority of intratumoral lymphoid cells to be CD3+ T cells, with only a minority staining for CD8 or FOXP3. A 9-marker multi-color immunophenotyping panel for flow cytometry (CD3, CD5, CD4, CD8, CD14, CD21, CD25, FoxP3, and PD-1) was optimized using cryopreserved healthy canine PBMC and will be used to assay PBMC from pre and post-treatment timepoints for the protocol treatment dogs.

Conclusions IT-IC in combination with local RT in canine melanoma is safe and has antitumor activity with potential to inform clinical development of IT-IC in melanoma patients.

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Ethics Approval Our animal protocol was reviewed and approved by the following: School of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC), Protocol ID V006037. William S. Middleton Memorial Veterans Hospital IACUC, Protocol ID MRA0001-1. We have consent forms for each part of the study and language is included in the ACORP that "The owner must provide written, informed consent prior to enrolling the dog in the study."

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