ADDITIONAL 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/m² 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/m² 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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