**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 is well tolerated in tumor-bearing dogs.

**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.

**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.

**Acknowledgements** This material was supported by Merit Review Award 101 BX003916 from the Biomedical Laboratory Research and Development Service of the United States (U.S.) Department of Veterans Affairs, P50026787 (NIH/NIDCR), the University of Wisconsin Carbone Cancer Center (UWCCC) Support Grant (P30 CA014520, NIH/NIH), the Barbara A. Suran Comparative Research Endowment, melanoma research gifts to the UWCCC, and use of facilities at the William S. Middleton Memorial Veterans Hospital, Madison, WI. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government. The hu14.18-IL2 IC was provided by Apeiron Inc. of Vienna Austria. This animal protocol was reviewed and approved by the following: School of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC), Protocol ID V006037, and William S. Middleton Memorial Veterans Hospital IACUC, Protocol ID MRA0001-1. Consent forms for each part of the study and language in the ACORP state that “The owner must provide written, informed consent prior to enrolling the dog in the study.”

**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.”