Background Although the presence and activity of tumor infiltrating lymphocytes (TILs) have been shown to be important factors for survival and response to immunotherapy for multiple cancer types, the benefits of immunotherapy in soft tissue sarcomas (STSs) have been limited, and novel approaches are needed. In this study, we sought to characterize the phenotype and function of tumor infiltrating natural killer (NK) and T cells in STS patients and to evaluate clinically relevant strategies to augment TIL function.

Methods Using both prospectively collected blood and tumor tissue from STS patients undergoing surgical resection (n = 21) and archived specimens (n = 45), we performed flow cytometry and immunohistochemistry to evaluate the extent of peripheral and intratumoral CD3+CD56+ NK and CD8+ T cell phenotype and function as predictors of outcome. We also analyzed TCGA data and the peripheral blood of dogs with spontaneous osteosarcoma receiving inhaled IL-15 on a clinical trial to evaluate the association of CD3-NKp46+ NK and CD8+ T cell activation as well as TIGIT upregulation with outcome. Finally, we stimulated patient PBMCs and TILs ex vivo with IL-15 and a novel human anti-TIGIT antibody to assess the impact of combination therapy on NK and T cell phenotype and function. Parametric and non-parametric statistical tests were used where appropriate. Univariable and multivariate survival analyses were performed by Cox proportional hazards models.

Results Compared to peripheral expression, intratumoral NK and T cells showed an activated and exhausted phenotype by CD69 and TIGIT, respectively. Ex vivo TIL stimulation with IL-15 further increased markers of activation and function including CD69, Ki67, IFNγ, and granzyme B, while increasing expression of exhaustion marker TIGIT. Analysis of a retrospective STS cohort and TCGA STS gene expression confirmed the association of TILs with improved prognosis. Dogs with metastastic osteosarcoma receiving inhaled IL-15 exhibited upregulation of activation markers and TIGIT. In vitro, IL-15 and TIGIT blockade of both peripheral and intratumoral NK cells increased cytotoxicity against sarcoma cell lines and increased expression of degranulation marker CD107a compared to IL-15 alone.

Conclusions TILs are associated with improved survival in STS, and tumor infiltrating NK and T cells show features of both increased activation and increased exhaustion. Tumor infiltrating NK and T cells respond to IL-15 stimulation, but simultaneously further upregulate TIGIT with the combination of IL-15 and TIGIT blockade showing greatest cytotoxic effects. Overall, our data suggest that the combination of IL-15 and TIGIT blockade is a promising clinical strategy in STS.

Ethics Approval All experiments involving human and canine patients were approved by the respective Institutional Review Boards at the University of California, Davis, Schools of Medicine (Protocol #218204-9) and Veterinary Medicine (IACUC #20179).

REFERENCES
5. Tumor infiltrating lymphocytes in soft tissue sarcomas upregulate the exhaustion marker TIGIT and are reinvigorated by IL-15 stimulation and TIGIT blockade

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