502 THE CO-EXPRESSION OF TWO IMMUNE COMPLEX MOLECULES, VISTA AND TIGIT, DEFINE A DYSFUNCTIONAL CYTOTOXIC T CELL SUBSET

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Background Cancer immunotherapies have proven, over the last decade, to be of extreme importance for long term survival of patients. Specifically, immune checkpoint inhibitors such as anti-CTLA-4 and anti-PD-1/PD-L1 have had tremendous clinical success treating many cancers including melanoma, lung, breast, colon, and bladder cancer. The low response rate (~30%) of these drugs suggest a mechanism of resistance within the tumor microenvironment, and it demonstrates the immense need to study and develop alternative routes to long-term anti-tumor immunity.[1]V-domain Immunoglobulin Suppressor of T-cell Activation (VISTA) has been shown to be a suppressive molecule in the tumor microenvironment in preclinical models and VISTA's expression is correlated with poor patient outcome across several cancers.[2]T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) is a functional receptor expressed on NK cells and T cells that contributes to a suppressive tumor microenvironment by acting on T cells, NK cells, and antigen presenting cells.[3]

Methods We use flow cytometry to visualize single cell expression of VISTA and TIGIT on CD8+ tumor infiltrating lymphocytes in pre-clinical models. Functional studies include cytotoxic assays as well as intracellular cytokine staining after cell sorting.

Results Here we show the expression of these two immune checkpoint molecules, VISTA and TIGIT, across several preclinical models, and how their co-expression subsets a distinctly dysfunctional population of cytotoxic T cells.

Conclusions Our data provides foundation to study the rejuvenation of this subset of T cells to restore cytotoxic function and therefore, anti-tumor immunity.

Trial Registration NA

Ethics Approval All in vivo studies were reviewed and approved by Institutional Animal Care and Use Committee (Approval number 2019-2142).

Consent NA

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503 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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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 (STS) 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. Univariate 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, IFNg, 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 metastatic 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).

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