1016 IL-2 EXPANDED TUMOR INFILTRATED NATURAL KILLER (TINK) CELLS FROM CANINE SARCOMAS POSSESS POTENT ANTI-TUMOR CYTOTOXICITY

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Background Tumor infiltrating NK (TINK) cells are present and linked to prognosis in many solid tumors.[1] While these cells may have potent antitumor effector function, they are thought to be dysfunctional in the tumor microenvironment (TME).[2] Because it is difficult to culture potent TINKs from human tumors, relatively little is known about their interactions with various TME components or their potential for therapeutic applications. Here we report reproducible culture of highly potent TINKs from naturally occurring canine sarcomas for use in modeling the human TME.

Methods Canine sarcoma tumor specimens were cut into fragments and cultured individually in 24-well plates with medium supplemented with 6000 IU/ml of rhIL-2. Expanded wells were maintained separately and autologous tumors were cultured separately. Expanded TINKs and TILs were characterized by flow cytometry and co-cultured with autologous and allogeneic tumor cells at a E:T ratio of 5:1; cytotoxicity of TILs was monitored by tumor confluence in the Incucyte system.

Results Ninety-six individual fragments were derived from soft tissue sarcomas (STS) and 120 fragments were derived from osteosarcoma. 88 (92%) of fragments from STS tumors and 23(19%) from Osteosarcoma were expanded sufficiently for additional analysis. While most TINKs and TILs were able to kill autologous tumor and resulted in increased Granzyme B expression, we surprisingly found that the number of TINKs (NKp46+) was positively corelated with tumor killing, with pure cultures of NKp46+ cells resulting in complete and efficient tumor elimination. Furthermore, the percentage of CD5 + T cells was negatively corelated with tumor killing. By flow cytometry, we found that NKp46+ TINKs were CD5(dim) TCRαβ− TCRγδ− CD4− B220- CD1d-, with some NKp46+ subpopulations co-expressing CD3 or CD8. Compared with the less potent TIL cultures composed of mainly CD5+ T cells, NKp46+ TINKs secreted significantly higher T1 cytokines (IFN-γ, TNF-α and GM-CSF) after co-cultured with tumor. Tumor stimulation preferentially activated the secretion of TNF-α over IFN-γ from TINKs, with concentration levels of TNF-α about 20 folds higher than IFN-γ, suggesting impotent role of TNF-α in TINK mediated tumor killing.

Conclusions Our results demonstrated that functional TINKs can be efficiently expanded from canine sarcoma tumor tissue with little NK cell infiltration seen by immunohistochemistry. Future studies using canine TINKs will explore therapeutic potential for targeting, manipulating, and transferring these cells in human.

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REFERENCES

1. Cózar B, Greppi M, Carpentier S, Narni-Mancinelli E, Chiossone L, Vivier E. Tumor-infiltrating natural killer cells. *Cancer Discov*. 2021;**11**(1):34–44.
2. Nersesian S, Schwartz SL, Grantham SR, MacLean LK, Lee SN, Pugh-Toole M, Boudreau JE. NK cell infiltration is associated with improved overall survival in solid cancers: a systematic review and meta-analysis. *Transl Oncol*. 2021;**14**(1):100930.

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