CANINE NK CELL EXPANSION AND NOVEL XENOGRAFT MODEL FOR ADOPTIVE IMMUNOTHERAPY OF OSTEOSARCOMA

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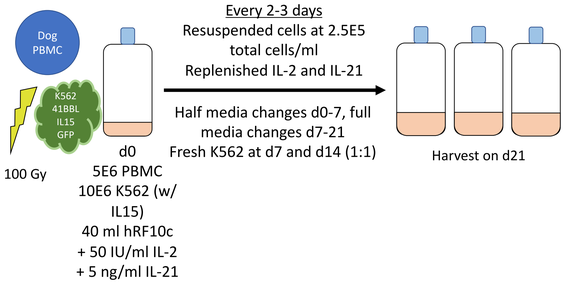
BACKGROUND

- Osteosarcoma is the most common bone cancer in both canine and human patients, but dogs develop osteosarcomas with an incidence of twenty times that of people.
- In both species, metastatic and relapsed disease have poor survival with current chemotherapy and surgical treatments.
- The usage of ex vivo activated natural killer (NK) cells as an adoptive immunotherapy is a promising approach for osteosarcoma treatment, but xenograft models of canine osteosarcoma are not yet available.

OBJECTIVES

- Develop a methodology to expand and activate canine NK cells ex vivo
- Develop an *in vivo* xenograft model of canine osteosarcoma using immunodeficient mice
- Demonstrate cytotoxicity of canine NK cells vs canine osteosarcoma cells *in vitro*

METHODS



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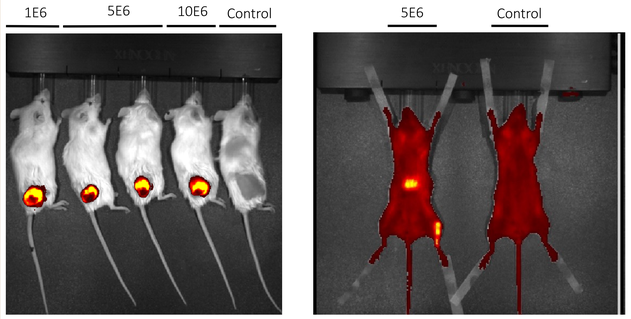
CONCLUSIONS:

Canine natural killer cells can be expanded and activated using feeder cells and cytokines readily available in human NK cell trials and show **potent cytotoxicity against osteosarcoma *in vitro*.** The canine D17 mKate2 xenograft model recapitulates human osteosarcoma growth locally and metastasizes, making it a **viable platform for testing adoptive immunotherapy with NK cells.**

RESULTS

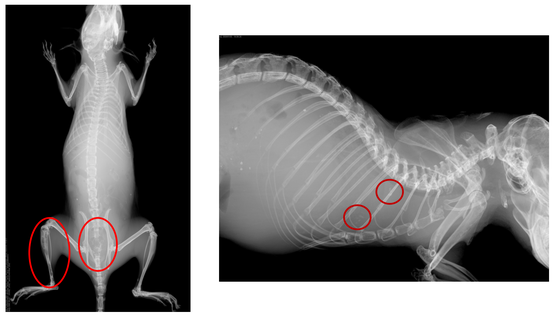
Canine osteosarcoma tumor growth and metastases can be monitored via fluorescence in vivo



A B

Fluorescent images were taken via IVIS on day 90 post injections of D17 mKate2 canine osteosarcoma cells. **A**, Lateral view of mice receiving right flank subcutaneous injections of 1-10E6 D17 mKate2 cells versus control. **B**, Anteroposterior view of mouse receiving tail vein IV injection of 5E6 D17mKate2 cells versus control.

Canine osteosarcoma shows bony tumor formation as well as lung and bone metastasis in a xenograft model

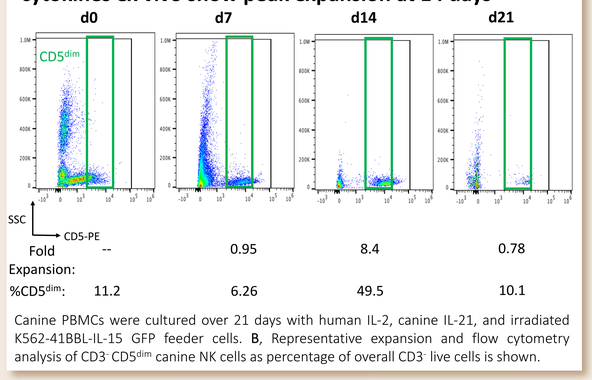


A B

NSG mice were injected intravenously with 5E6 D17 mKate2 osteosarcoma cells. Faxitron images were taken on day 90 of representative mouse. **A**, Posteroanterior view, red circles show tumor formation in left tibia and bony destruction of spine. **B**, Lateral view, red circles show lung metastases in the same animal.

RESULTS

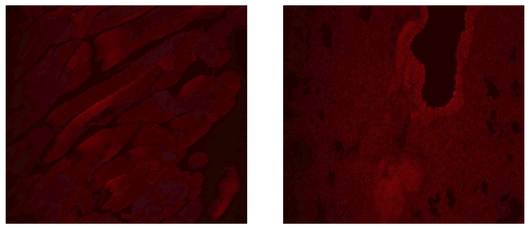
Canine natural killer cells cultured with feeder cells and cytokines ex vivo show peak expansion at 14 days



Canine PBMCs were cultured over 21 days with human IL-2, canine IL-21, and irradiated K562-41BBL-IL-15 GFP feeder cells. **B**, Representative expansion and flow cytometry analysis of $CD3^-$ $CD5^{dim}$ canine NK cells as percentage of overall $CD3^-$ live cells is shown.

RESULTS

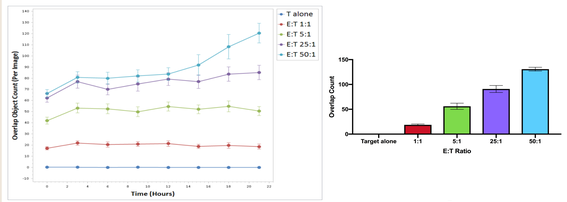
Canine osteosarcoma cells can be detected via immunofluorescence in tissue samples



A B

On day 90 following intravenous injection of D17 mKate2 osteosarcoma cells, leg and lung tissue samples were harvested from mice on day 90. Immunofluorescence was used to detect D17 mKate2 tumor cells in **A,** leg and **B,** lung. Samples analyzed on a fluorescence microscope at 200x magnification.

Canine NK cells demonstrate cytotoxicity against canine osteosarcoma cells *in vitro*



A B

Cytotoxicity of effector canine NK cells vs target canine osteosarcoma (d17 mKate2) cells using Incucyte. Cells were plated at E:T ratios of 1:1- 50:1. **A,** overlap counts of target mKate2 and caspase-3 488nm signals over 22 hours. **B,** Overlap count at 24 hours