Background Natural killer (NK) cells are cytotoxic immune cells capable of recognizing heterogeneous cancer targets without prior sensitization, making them promising prospects for use in cellular immunotherapy. Previously, CD5 depletion of peripheral blood mononuclear cells (PBMCs) has been used in dogs to isolate and expand a CD5dim-expressing NK subset prior to co-culture with an irradiated feeder line, but this can limit the yield of the final NK product. This study aimed to assess NK activation, expansion, and preliminary clinical activity in first-in-dog clinical trials using unmanipulated PBMCs without CD5 depletion to generate our NK cell product.

Methods Starting populations of CD5-depleted cells and PBMCs from 12 matched healthy beagle donors were co-cultured with irradiated K562-C9-mIL21 cells and 100IU/mL rhIL-2 for 14 days. Phenotype, cytotoxicity, and cytokine secretion were measured, and samples were sequenced using the 3'-Tag-RNA-Seq protocol for gene profiling. In addition, two first-in-dog feasibility clinical trials were performed in dogs with melanoma (MEL, N=5) and osteosarcoma (OSA, N=9) using autologous and allogeneic NK cells, respectively, expanded from unmanipulated PBMCs.

Results Calculated cell counts, overall fold change, and viability in NK expansions displayed higher means at day 14 using PBMCs versus CD5-depleted cells, reaching a peak mean of 677x10^6 cells from 5 x10^6 starting cells (P=NS). Flow analysis showed similar upregulation of NKp46 and Granzyme B expression in both groups, reaching >90%. Killing assays against M5 (MEL) and OSCA78 (OSA) canine tumor targets demonstrated comparable percent killing >50% among both subsets of day 14 NK cells (P=NS). Median production of canonical NK cytokines, IFN-γ and GM-CSF, at day 14 was over 5-fold greater in PBMC-expanded (IFN-γ=316.7pg/mL, GM-CSF=267.0pg/mL) compared to CD5-depleted NK cells (IFN-γ=59.6pg/mL, GM-CSF=48.7pg/mL) (P=NS). Sequencing data showed principal component sample variance based on time points and upregulation of NK pathways related to activation, crosstalk, and glycolytic function in both groups. PBMC-expanded NK cells for first-in-dog clinical trials showed sufficient expansion for multiple NK cell transfers at 7.5 x 10^6 cells/kg with no serious adverse events. We also observed preliminary data for efficacy, particularly in the allogeneic setting where peripheral blood gene expression significantly changed post-transfer and one dog survived 445 days post-treatment.

Conclusions Overall, the use of unmanipulated PBMCs appears safe and potentially effective for canine NK immunotherapy, with equivalent or superior results to CD5 depletion in NK expansion, activation, and cytotoxicity. Our pre-clinical and clinical data support further evaluation of this technique as a novel platform for optimizing NK immunotherapy in dogs.

Ethics Approval Clinical trials involving dog patients were IACUC and Clinical Trials Review Board-approved (Protocols #21620 and #22157). Dog owners gave informed consent before taking part.