lymphomas in vitro and in vivo in immunodeficient NSG mouse models.

**Conclusions** Collectively, these data identify promising combinations of AFM13 with cytokine-activated adult blood or cord blood NK cells against CD30+ hematoic malignancies, warranting clinical trials with these novel combinations.

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**Immune cell types**

533 CROSS-SPECIES IMMUNOGENOMIC ANALYSIS IDENTIFIES PATHWAYS OF CANINE NATURAL KILLER CELL RESPONSE TO CYTOKINE THERAPY, AND REVEALS CONVERGENCE OF ACTIVATED DOG AND HUMAN NATURAL KILLER TRANSCRIPTOMES

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**Background** Natural killer (NK) cells are key effectors of the innate immune system, but major differences between human and murine NK cells impede translation. Outbred dogs offer an important link for NK-based cancer immunotherapy studies. We compared gene expression profiles of dog NK signatures in vitro and from a phase I clinical trial of inhaled IL-15, and analyzed dog, mouse and human NK cells using a novel orthologous transcriptome.

**Methods** We performed differential gene expression (DGE) using resting healthy donor CD5dim NK populations and following ex vivo activation using recombinant human (rh) IL-15 or co-culture with irradiated feeder cells. Eight dogs with naturally-occurring pulmonary metastases were enrolled on a Phase I clinical trial of inhaled rhIL-15 using a 3 + 3 cohort design with escalating doses of inhaled rhIL-15. Blood was collected from study dogs before, during, and after therapy. We compared DGE among healthy and cancer-bearing dogs and then across mouse, dog and human NK cells in resting and activated states using −7000 1:1 orthologous genes.

**Results** DGE revealed distinct transcriptional profiles between the ex vivo resting, IL-15 and co-cultured canine NK cells. Among treated patients, hierarchical clustering revealed that in vivo NK cell transcriptional signatures grouped by individual dog, and not amount of time exposed to treatment. PCA showed in vivo profiles of the clinical responders were distinctly separate from the non-responding patients (PC1 38%, PC2 12%). Patient in vivo NK cell transcription profiles most closely resembled those of ex vivo resting NK cells and not IL-15 treated or co-culture activated (PC1 43%, PC2 19%), likely reflecting key differences in activation. In cross-species analysis, PCA showed within-species spatial clustering of resting NK cells. After activation, variance between dog and human NK cells decreased, while variance between human and mouse NK cells increased (PC1 40%, PC2 28%).

**Conclusions** In this first transcriptomic sequencing of dog NK cells, we demonstrate distinct gene profiles of ex vivo activated NK cells from healthy donors compared to circulating NK cells from dogs receiving inhaled rhIL-15 on a clinical trial. Baseline in vivo NK cell profiles appear to predict response to therapy more than changes over time. We also show distinct gene profiles of NK cells across the most commonly used mouse, dog, and human NK populations, with convergence of dog and human NK cells after activation. By defining the canine NK cell DGE signatures, these data fill a gap in translational NK studies.

**Ethics Approval** The canine clinical trial study was approved by IACUC and Clinical Trials Review Board (Inhaled IL-15 Immunotherapy for Treatment of Lung Metastases, Protocol #20179).

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