NK-92® (aNK™) Whole Cell Lysate Exerts Potent Cytotoxic and Anti-Proliferative Activity on Tumor Cells

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ABSTRACT

Lysates from human cells represent biofluids that are used in the biotechnology field for a number of applications, such as biomarker identification and antibody detection. Lysate from human blood platelets is widely used in the clinical setting to control bleeding.
Here, we have generated whole cell lysate from NK-92® cells, and erIL-2 - engineered variant haNK® cells, by repeated freeze/thaw cycles in a balanced salt solution (BSS) buffer containing no detergent, protease inhibitors, or EDTA. The presence of Perforin, Granulysin, a full spectrum of Granzymes and various chemokines and cytokines, as well as conservation of Granzyme B activity before and after cryopreservation, was confirmed in the whole cell lysates. Incucyte® SX3 live cell imaging assays showed that fresh lysate displays cytotoxic and anti-proliferative activity against human and canine cancer cells, suggesting cross-species specificity. Intra-tumor injection of cryopreserved haNK lysate into intradermal tumors in immunocompetent C57BL/6 mice provided tumor control and clearance in two-thirds of the animals. Strikingly, when re-challenged with the same tumor cells 6-8 weeks later, no tumor growth occurred suggesting a vaccine-like effect of the haNK lysate.

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INTRODUCTION

- Natural killer (NK) cells play a critical role in the innate immune response, and have the ability to kill infected or tumor cells. NK cells have been studied as a type of cell therapy in cancer, but use of a patient's own NK cells has been found to have limited feasibility and efficacy.
- The NK-92[1] cell line was isolated from a lymphoma patient decades ago, and has been shown to have highly active NK cell activity, but with much broader and greater cytotoxicity.
- NK-92 cells overcome the challenges of obtaining and expanding NK cells from donor or patient blood, and more than 100 patients with cancer have been treated with NK-92 cells or modified variants, demonstrating their safety and efficacy.
- One variant is the high-affinity Fc-receptor expressing NK-92 (haNK)
- Here, to assess the therapeutic potential of NK-92 and haNK cell lysates, rather than whole cells, we characterize the components of these lysates and their biological activity *in vitro* and *in vivo.*

METHODS AND MATERIALS

- **Cells and media** NK-92 and haNK grown in X-Vivo™ 10 medium + 5% human serum, supplemented or not with 500 IU/mL rhIL-2. SKBR-3, SKOV-3 (ATCC) grown in McCoys' 5A medium + 10% FBS, MC-38 grown in DMEM + 10% FBS. CTAC (Millipore Sigma) and OSCA-40 (Kerafast, Inc) grown in RPMI + 10% FBS. Primary lung fibroblast cells and HUVEC (Sigma-Aldrich) cultured in fibroblast and endothelial growth medium (Cell Applications).
- **Lysate preparation** Cells harvested by centrifugation and resuspended in BSS buffer. Cells were subjected to 3 cycles of freeze/thaw and clarified by centrifugation. Used fresh, or cryopreserved at -80°C, until needed.
- **Cytotoxicity assays** performed with Incucyte SX3 Live-Cell imaging System with Nuclight-Red-labeled target cells, co-incubated with lysate for 15 mins at 37°C, and monitored for cell death (Cytox green reagent, Sartorius) and cell proliferation.
- **In vivo assays** Eight-week-old C57BL/6J mice (Jackson Laboratory) were inoculated intradermally with 3 x 10^5 MC-38 tumor cells. Mice received six intra-tumor injections once a day, every other day. Mice that exhibited complete clearance of the primary tumor were re-challenged on Day 50 with 3 x 10^5 MC-38 cells.

REFERENCES

1. Klingemann H. The NK-92 cell line-30 years later: its impact on natural killer cell research and treatment of cancer. *Cytotherapy*. 2023;25(5):451-457. https://doi.org/10.1016/j.jcyt.2022.12.003. Epub 2023 Jan 6.
2. Jochems C, Hodge JW, Fantini M, et al. An NK cell line (haNK) expressing high levels of granzyme and engineered to express the high affinity CD16 allele. *Oncotarget*. 2016;7(52):86359-86373. https://doi.org/10.18632/oncotarget.13411. PMCID: PMC5341330

RESULTS

Figure 1. MSD analysis using the V-Plex Plus Cytokine Panel 1 Human kit and Proinflammatory Panel 1 Human kit shows levels of IL-8, IL-10, IL-16, IFN-γ, TNF-β, MIP1-α and MIP1-β are significantly elevated in NK-92 and haNK cell lysates. Immunoblot analysis confirmed the presence of Perforin, an array of Granzymes, Granulysin, and both precursor and active forms of IL-16 in NK-92 and haNK cell lysates.

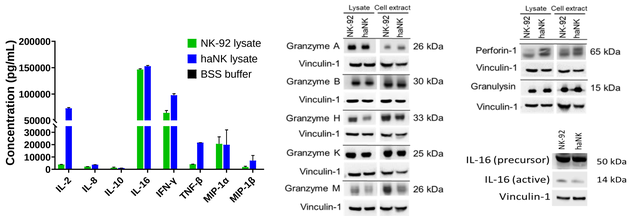


Figure 2. A short, 15 minute exposure of human SKOV-3 and SKBR-3, and canine OSCA-40 and CTAC cancer cell lines to haNK cell lysate induces anti-proliferative and cytotoxic effects, as revealed by real-time live cell analysis using Incucyte SX3. *This effect was not observed in normal primary cells controls.*

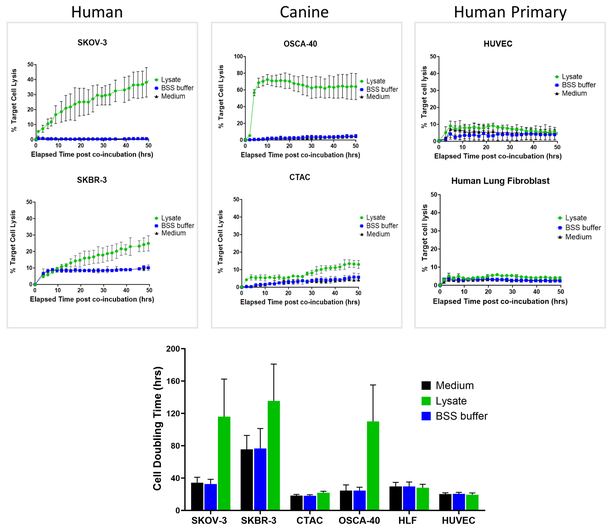


Figure 3. Cryopreservation of the lysate at -80°C does not affect the cytotoxic or anti-proliferative effects the lysate, or Granzyme B activity.

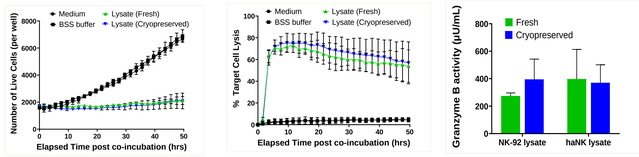
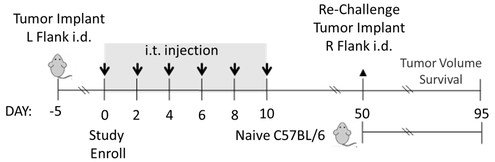
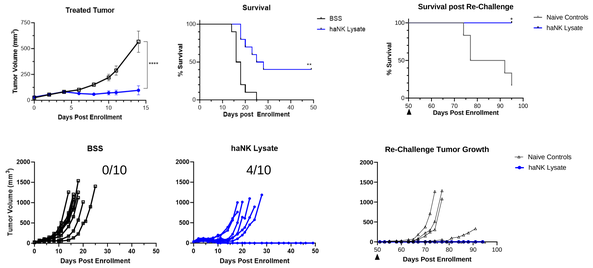


Figure 4. Intra-tumor injection of cryopreserved haNK lysate into intradermal MC-38 tumors in C57BL/6 mice significantly reduced tumor growth relative to BSS controls, with 4/10 of animals exhibiting complete tumor clearance. No tumor re-growth was observed when surviving mice were re-challenged with the same tumor cells 6 weeks later.





CONCLUSIONS

- **We have developed a simple, scalable method to prepare bioactive NK-92 and haNK cell lysates that contain Perforin, Granzymes, and immune-active cytokines/chemokines.**
- **Cryopreserved haNK lysate has anti-tumor effects in mice, preventing tumor growth after re-challenge with cancer cells.**
- **NK-92/haNK lysate holds potential as a therapeutic modality for certain human and canine cancers.**