Background Acute myeloid leukemia (AML) incidence increases with age. Five year survival for those over 65 is less than 11%, highlighting the need for safe interventions to improve outcomes. Adoptive natural killer (NK) cell products have achieved success as a ‘bridge to transplant’ in refractory leukemia and lymphoma, inducing remission to a point where patients are eligible for stem cell transplantation. Multiplexed-engineered induced pluripotent stem cells (iPSCs) are a reproducible source of highly functional NK cells (iNK) for on-demand treatment and broad patient access. Clinical trials are currently testing iNK cells with therapeutic antibodies for the treatment of leukemia and lymphoma (NCT04023071, NCT04614636, NCT04714372). We have developed a protocol for the production of highly functional iNK cells, engineered for greater anti-tumor effect. However, we hypothesized that performing NK cell lineage commitment under physiological oxygen conditions found in bone marrow (5%) would create a niche that could support the generation of a more functional cell product.

Methods iPSCs are matured into CD34+ precursors, then differentiated into iNK cells that are subsequently expanded to clinically-relevant quantities (figure 1A). We have previously published on iNK cells that consist of three unique edits: high-affinity non-cleavable CD16, membrane bound IL-15 and knockout of CD38. Using these cells, we performed stage specific differentiation from CD34+ precursors in 5% oxygen (physoxic iNK) or conventional 20% oxygen, with subsequent expansion in 20% oxygen. iNK cells were compared for their phenotype (CyTOF), proliferation (flow cytometry), cytotoxicity (live cell imaging), metabolic stability (reactive oxygen species staining by flow cytometry) and ability to control tumor (xenograft mouse model).

Results CyTOF analysis revealed a more naive phenotype in physiological oxygen conditions that persisted after expansion. However, these cells were equally capable of natural cytotoxicity and antibody-dependent cellular cytotoxicity. In a xenograft model of AML (NSG mice with HL60-GFP/luciferase; figure 1B) there was greater persistence of physoxic iNK cells in blood and bone marrow (figure 1C), correlating with greater tumor control within the bone marrow (figure 1D) and across the whole animal (figure 1E). When exposed to oxidative stress, physoxic iNK cells were more resilient, with lower reactive oxygen species detected in their mitochondria, suggesting greater tumor control arose from greater persistence within the animal, rather than better cytotoxicity.

Conclusions These data suggest that manufacturing therapeutic NK cells in a physiological environment at a unique stage of lineage commitment can generate resilient cells with a greater durability for anti-tumor activity.

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Abstract 365 Figure 1 Differentiating NK cells in 5% oxygen, compared to 20% oxygen, improves their persistence and control of tumor in a xenograft model of AML. (A) The manufacturing process for iNK cells. iPSC-derived iNK cells were differentiated in 5% or 20% oxygen, with subsequent expansion in 20% oxygen. iNK cells were compared for their phenotype (CyTOF), proliferation (flow cytometry), cytotoxicity (live cell imaging), metabolic stability (reactive oxygen species staining by flow cytometry) and ability to control tumor (xenograft mouse model). (B) Schematic representation of xenograft mouse study for AML. A single dose of iNK cells was delivered intravenously on day 1. Blood was taken to analyze circulating cells by flow cytometry; bioluminescent imaging tracked tumor load for HL60-luciferase/GFP cells; bone marrow and spleens were harvested on day 21 and analyzed by flow cytometry (C) Physiologic iNK cells are found in mouse bone marrow in greater numbers than conventional iNK cells three weeks after intravenous injection. Bars show the median and interquartile range. Mann Whitney test. (D) Less HL60 xenograft tumor is found in mouse bone marrow when mice are treated with physiologic iNK cells compared to conventional iNK cells (day 21). Bars show the median and interquartile range. Mann Whitney test. (E) Less HL60 xenograft tumor is detected by bioluminescent imaging when mice are treated with physiologic iNK cells compared to conventional iNK cells. Bars show the mean. Each dot represents an animal. Two way ANOVA with Dunnett’s multiple comparison test. One of two independent experiments is shown. * p<0.05, ** p<0.01, *** p<0.001.

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