Background Natural killer (NK) cells are highly effective and fast-acting cytolytic cells capable of eradicating target cells with limited adverse effects such as cytokine release syndrome (CRS) or graft-versus-host disease. Chimeric antigen receptors (CARs)-engineered NK cells have been recently used against leukemia with encouraging clinical outcomes. The surface antigen CD19, expressed by B-lymphoblasts, represents an ideal CAR target against B cell acute lymphoblastic leukemia (B-ALL). We developed a highly potent CD19-directed CAR NK cell therapy, NKX019, with an extended in vivo half-life aimed at killing CD19-expressing target.

Methods NK cells isolated from healthy PBMCs were expanded in the presence of NKSTIM cells, IL-2, IL-12, IL-18 and transduced with both a CD19-targeted CAR construct and a membrane-bound form of IL-15 (mIL-15). Control (non-engineered) NK cells were produced in parallel. Cytotoxic activity of NKX019 against CD19+ B-ALL cell line (REH), pre-B ALL cell line (Nalm-6), allogeneic PBMCs was assessed using Incucyte® or flow cytometry. NSG mice bearing either Nalm-6.fluc (Nalm6) or REH.fluc (REH) tumor received different concentrations of NKX019 or control NK cells. In-life analysis of tumor-bearing and naïve NSG mice include: 1) bioluminescence imaging, 2) clinical observations, 3) serum cytokines and 4) CAR+ NK cell persistency.

Results NKX019 showed enhanced cytolytic activity against REH and Nalm-6 tumor cells compared to control NK cells and CAR19+ T cells. The superiority of NKX019 over CAR19+ T cells was more pronounced at the earlier time point (24 hours) with near identical calculated EC50 observed at 72 hours for both cell types. Increased cytolytic activity of NKX019 was limited to CD19+ cells in bulk PBMCs. Consistent with our in vitro observations, NKX019 controlled Nalm-6 and REH tumor growth in doses as low as 2 × 106 cells/kg for up to 30 days with no apparent decrease in cytokines commonly associated with CRS. Increased Nalm-6 tumor growth coincided with an apparent decrease in measureable NKX019 in the periphery. In tumour-naïve NSG mice, NKX019 was detectable in the blood for up to 9 weeks post-infusion consistent with its extended half-life.

Conclusions NKX019 expresses mIL-15 and is produced in the presence of IL-12 and IL-18, resulting in enhanced in vitro expansion and longer in vivo half-life than non-engineered NK cells. NKX019 also exhibited advantages compared to CAR19+ T cells including faster cytotoxic kinetics and limited production of cytokines associated with CRS. A first-in-human trial of NKX019 in B cell malignancies is planned for 2021.

Ethics Approval The animal procedures described in this abstract were conducted in accordance with Explora BioLabs Animal Care and Use Protocol approved by Explora BioLabs Institutional Animal Care and Use Committee.

REFERENCE
1. Liu, et al. 2020 NEJM

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