Preclinical evaluation of NKX019, a CD19-targeting CAR NK Cell


Introduction

Natural killer (NK) cells are highly potent 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 receptor (CAR)-engineered NK cells have been recently used in patients with relapsed or refractory CD19-positive cancers with encouraging clinical outcomes (1). We describe here the development of NKX019, a highly potent CD19-directed CAR NK cell therapy with an extended in vivo half-life.

Methods

NK cells isolated from healthy PBMCs were expanded and engineered to express a CD19-targeted CAR and a membrane-bound form of IL-15 (mIL-15). Control (non-engineered) NK cells were produced in parallel. Cryopreserved NKX019 and control NK cells were used for the experiments described herein.

Cytotoxic activity of NKX019 and control NK cells against CD19+ B-ALL cell line (REH), pre-B ALL cell line (Nalm-6), and autologous PBMCs were assessed at different effector-to-target ratios (E:T) using Incucyte® or flow cytometry.

Female NSG mice (5x14, i.e., B6; 12-14 weeks of age) were treated with Nalm-6 or control NK cells. In-life analysis of tumor-bearing and naïve NSG mice include: 1) bioluminescence imaging, 2) clinical observations, 3) serum cytokines by Luminox, and 4) CAR+ NK cell persistency by flow cytometry.

Figure 1: NNX019 enhanced cytotoxicity is limited to CD19+ PBMCs

Surface markers were detected by flow cytometry from PBMCs co-cultured with autologous NKX019 or control NK cells for 4 days. Data represents mean ± SEM. No replicates per PBMC dose. 3 PBMC donors/E.

Figure 2: NNX019 in vitro cytotoxicity is more rapid than CAR19+ T cells

Target cell growth was measured by Incucyte® when co-cultured with effector cells from 1:1 to 8:1 E:T ratios for up to 72h. Cytotoxicity was calculated as percent of target cells growth inhibition compared to random wells containing the target cells only. Data represents mean ± SEM analyzed by Richard’s five-parameter non-linear regression. Dotted line indicates EC50, NNX019 PBMC donor E.

Figure 3: NNX019 inhibition of Nalm-6 growth in vivo is well tolerated and dose-dependent

Figure 4: NNX019 generates moderate in vivo levels of key cytokines in response to Nalm-6 lymphoma cells

Figure 5: NNX019 exhibits better pharmacokinetic parameters than control NK cells

Conclusion

NKX019 treatment results in enhanced cytotoxicity against CD19-expressing target, longer in vivo half-life and increased exposure than control NK cells. NNX019 also exhibited advantages compared to CAR19+ T cells including faster cytokine kinetics and limited production of cytokines potentially associated with CRS. A first-in-human trial of NKX019 in B cell malignancies is planned for 2021.

References


Contact

James Trager, PhD
jtrager@nkartax.com
www.nkartax.com