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

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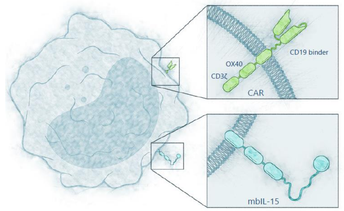
nkarta THERAPEUTICS

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.



NKX019 express a CAR composed of proprietary CD19 binder, OX40 costimulatory domain and the CD3ζ signaling moiety, co-expressed with membrane-bound IL-15.

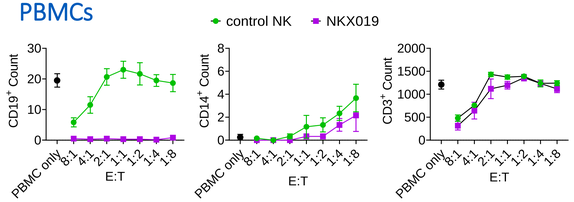
Methods

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

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

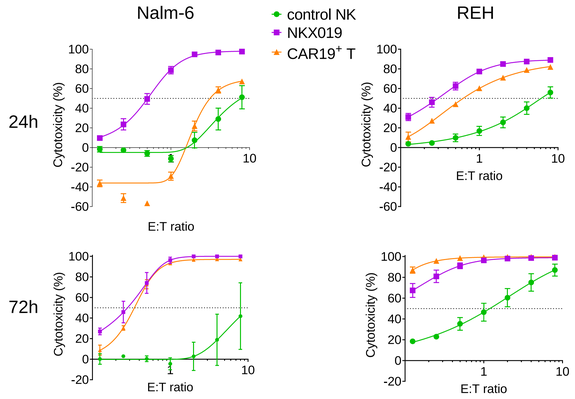
C Female NSG mice (JAX) bearing Nalm-6.fluc (Nalm6, 2x10^5, i.v.) tumor were treated with NKX019 or control NK cells. In-life analysis of tumor-bearing and naïve NSG mice include: 1) bioluminescence imaging, 2) clinical observations, 3) serum cytokines by Luminex and 4) CAR+ NK cell persistency by flow cytometry.

NKX019 enhanced cytotoxicity is limited to CD19$^+$ PBMCs



Surface markers were detected by flow cytometry from PBMCs co-cultured with allogeneic NKX019 or control NK cells for 4 days. Data represents mean ± SEM. N=3 replicates per PBMC donor, 3 PBMC donors/E:T

NKX019 in vitro cytotoxicity is more rapid than CAR19$^+$ T cells

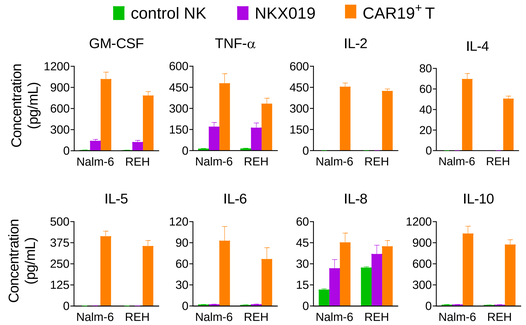


Target cells growth was measured by Incucyte when co-cultured with effector cells from 1:8 to 8:1 E:T ratio for up to 72h. Cytotoxicity was calculated as percent of target cells growth inhibition compared to reference wells containing the target cells only. Data represents mean ± SEM analyzed by Richard's five-parameter non-linear regression. Dotted line indicates EC_{50}. N=3 PBMC donors/E:T

EC_{50}	At 24h		At 72h	
	Nalm6	REH	Nalm6	REH
Control NK	7.83	6.11	10.58	1.19
NKX019	0.51	0.30	0.28	<0.1
CAR19$^+$ T	3.14	0.62	0.35	<0.1

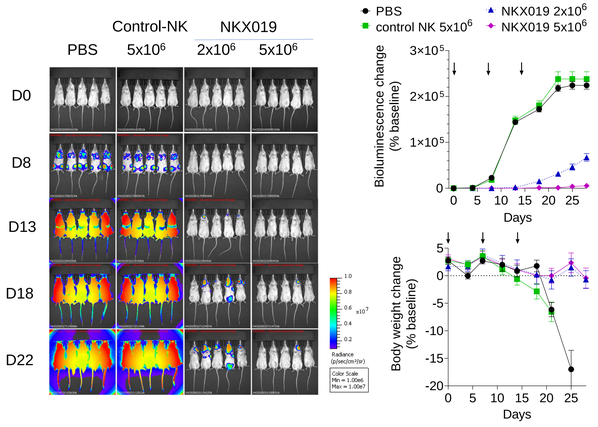
EC_{50} values were interpolated from Richard's five-parameter non-linear regression. The lower the EC_{50} value, the higher the potency

NKX019 cytokine responses to tumor cells are limited relative to CAR19$^+$ T cells



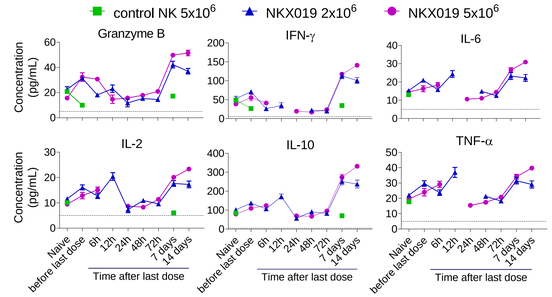
Effector cells were co-cultured at a 1:1 E:T ratio with target cells for 24h. Cytokines levels (mean ± SEM) were measured from culture media by Luminex. N=3 independent NK donors

NKX019 inhibition of Nalm-6 growth in vivo is well tolerated and dose-dependent



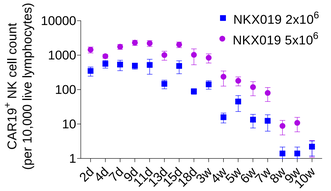
Bioluminescence images, bioluminescence change and body weight change compared to baseline (measured prior to artlcle dosing) from Nalm-6 (2x10^5 cells/animal, i.v., day -1)-bearing NSG mice treated with NKX019 or control NK (2 or 5x10^6 cells/animal, i.v., day 0, 7 and 14). Images represent 5 out of 14-17 animals/group. PBS and control-NK treated animals reached tumor burden endpoint on D22 or D25; their last BLI value recorded was carried over to subsequent time points. The arrows indicate article dosing. Data represents mean ± SEM

NKX019 generates moderate in vivo levels of key cytokines in response to Nalm-6 lymphoma cells



Mean cytokine concentration (± SEM) from Nalm-6 (2x10^5 cells/animal, i.v., day -1)-bearing NSG mice treated with NKX019 or control NK (2 or 5x10^6 cells/animal, i.v., day 0, 7 and 14). Serum was collected before Nalm-6 ("naïve"), before and after the last NK dose. The dash line represents quantification limit; values below this limit resulted in discontinuous data line. N=6/group/timepoint.

NKX019 exhibits better pharmacokinetic parameters than control NK cells



Pharmacokinetic parameters		
Treatment	AUC (±SEM)	Half-life (days)
Control NK 5x10^6	8622 (±660)	9.7
NKX019 2x10^6	19276 (±1804)	17.4
NKX019 5x10^6	48290 (±4635)	28.4

Naïve NSG mice received NKX019 or control NK (2 or 5x10^6 cells/animal, i.v., day 0, 7 and 14). Whole blood was collected at different timepoints (d=days, w=weeks) after the last NK dosing and analyzed by flow cytometry. Left: NKX019 detection is represented as mean CAR19$^+$ CD56$^+$ 7-AAD$^-$ counts ± SEM per 10,000 7-AAD$^-$ lymphocytes. Right: AUC and half-life from NKX019 and control NK cells detected as CD56$^+$ 7-AAD$^-$ counts per 10,000 7-AAD$^-$ lymphocytes. N=5/group/timepoint.

Conclusion

NKX019 treatment results in enhanced cytotoxicity against CD19-expressing target, longer in vivo half-life and increased exposure than control NK cells. NKX019 also exhibited advantages compared to CAR19$^+$ T cells including faster cytotoxic kinetics and limited production of cytokines potentially associated with CRS[2]. A first-in-human trial of NKX019 in B cell malignancies is planned for 2021.

References

1. Liu et al. 2020 NEJM; 2. Klinger et al, 2012 Blood.

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