A cryopreserved allogeneic anti-BCMA CAR-NK cellular therapy exhibits both innate and CAR-mediated MM cell killing in vitro and in vivo

1322

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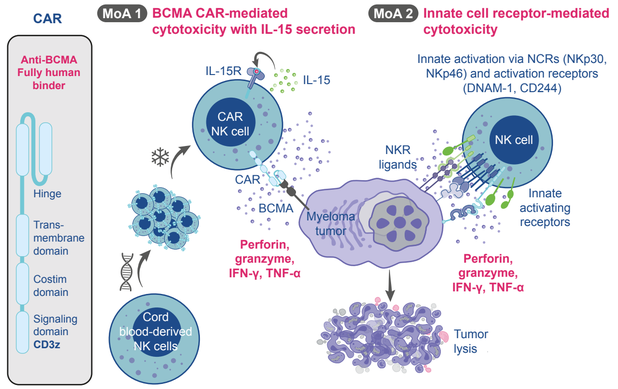
Background

- MM is the second most prevalent hematopoietic malignancy, with an overall 5-year survival rate of 59.8%.[1,2]
- BCMA is selectively expressed on normal and malignant plasma cells, making it an attractive MM target.[3]
- Anti-BCMA CAR T-cell therapy and T-cell engagers have shown promise as treatment options for certain patients with MM; however, potential toxicities associated with these anti-BCMA therapies, such as cytokine release syndrome, have limited their broader utilization.[4]
- NK cells are cytolytic, much like T cells, and have demonstrated an innate capacity to reduce tumor burden while exhibiting a more favorable safety profile in clinical testing.[5,6]
- To that end, we developed a cryopreserved allogeneic cell therapy comprised of genetically modified human umbilical CBNK cells transduced with a gammaretroviral vector, which incorporates genes for an anti-BCMA CAR and soluble human IL-15 (referred to hereafter as anti-BCMA CAR-NK).
- Anti-BCMA CAR-NK demonstrates a dual mechanism of action (**Figure 1**).
- Herein, we evaluated the in vitro and in vivo activity of anti-BCMA CAR-NK against established MM tumors.

Figure 1: Mechanism of action of anti-BCMA CAR-NK



Methods

- CBNK cells were isolated from donor cord blood units, propagated using feeder cells, transduced with a gammaretroviral vector to express an anti-BCMA CAR and soluble IL-15, and propagated further before harvest and cryopreservation (**Figure 2**).
- Donor-equivalent UTD-NK cells were generated via the same process but without the transduction step.
- Cryopreserved anti-BCMA CAR-NK and UTD-NK cells were used for in vitro studies, including short-term killing, as well as for evaluation of activity against established MM tumors in vivo using the MM.1S-Luc4 model.

Question

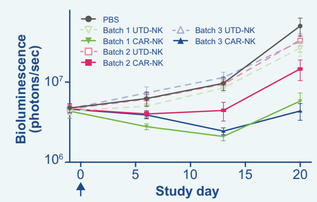
What are the preclinical findings related to the activity of cryopreserved allogeneic anti-BCMA CAR-NK cellular therapy in MM tumor models?

Methods

Figure 2: Overview of CAR design components and expansion/transduction process for anti-BCMA CAR-NK therapy

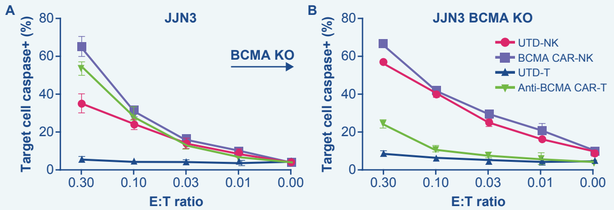
Results

Figure 3: In vivo anti-tumor activity following anti-BCMA CAR-NK treatment



Mean bioluminescence over time in female NSG mice bearing MM.1S-Luc4 human MM xenograft tumors administered a single dose (indicated by arrow) of anti-BCMA CAR-NK or UTD-NK cells.

Figure 4: Cytotoxic activity of anti-BCMA CAR-NK and anti-BCMA CAR-T against MM tumor cells



Target cells were labeled with CellTracker™ deep red dye and seeded in 384-well plates. Two hours later, effector cells (T cells or NK cells) were added at different E:T ratios. Target cells and effector wells were co-cultured for 20 hours and cells were then incubated with caspase-3/7 reagent and submitted for FACS analysis. The percentage of target cells with positive caspase-3/7 staining was used to report the cytotoxicity of effector cells.

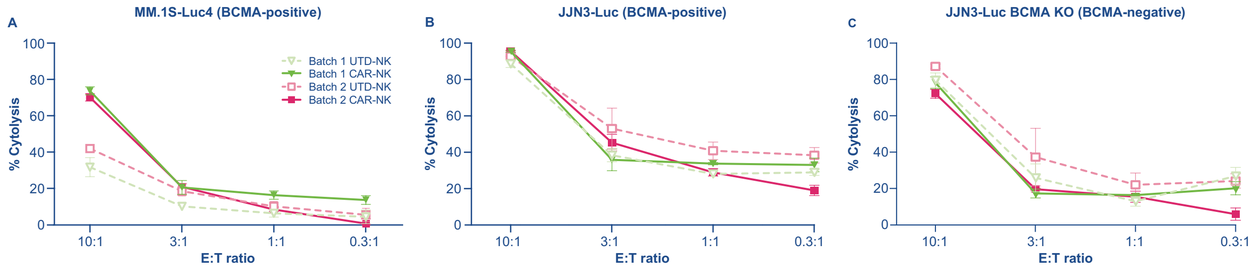
- Single low dose of anti-BCMA CAR-NK showed anti-tumor activity in a mouse model of MM (**Figure 3**).
- Compared to anti-BCMA CAR-T, anti-BCMA CAR-NK was cytotoxic to MM tumor cells even post–BCMA loss (**Figure 4**).

Key Takeaway

Anti-BCMA CAR-NK exhibited both innate NK- and CAR-mediated killing in vitro and single low-dose activity against established MM tumors in vivo

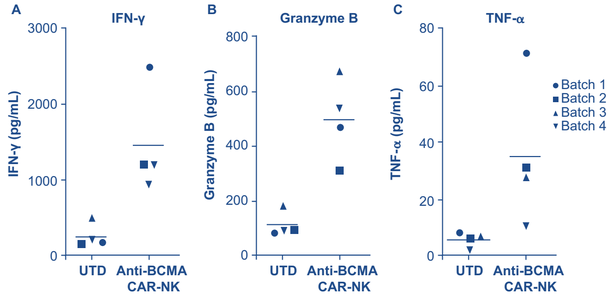
Results

Figure 5: Anti-BCMA CAR-NK exhibits both innate NK- and CAR-mediated tumor cell killing in vitro



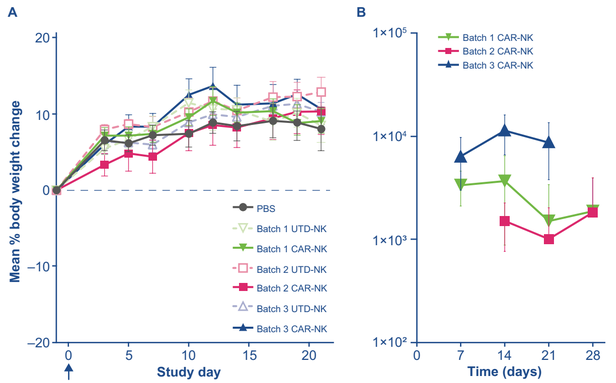
Effector cells were incubated with target cells at different E:T ratios for 20 hours. One Glo reagent was then added to the co-culture to measure the % cytolysis of target cells.

Figure 6: Anti-BCMA CAR-NK enhances cytokine production upon engagement of target cells



Four batches were assessed for IFN-γ, granzyme B, and TNF-α secretions by co-culturing with MM.1S-Luc4 BCMA-positive cell line at fixed E:T ratio for 22 hours. Supernatant samples were collected from each well. 20 uL of sample was used in each well coated with specific antibodies and linkers. Detection antibody of each cytokine was added, and levels of cytokine secreted were assessed using a Meso Scale Discovery assay.

Figure 7: Body weight data show no loss in body weight and cellular kinetics show systemic maintenance of CAR+ NK cells across the duration of the study



(A) Mean % change in body weight of mice over time after a single dose (indicated by arrow) of anti-BCMA CAR-NK. (B) Cellular kinetics of CAR-NK cells in circulation over time after a single dose of anti-BCMA CAR-NK.

Conclusions

- A cryopreserved allogeneic anti-BCMA CAR-NK cellular therapy exhibited a dual mechanism of action comprising both innate and CAR-mediated killing in vitro in MM tumor models.
- A single dose of anti-BCMA CAR-NK elicited in vivo activity and persistence.

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Abbreviations

BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; CBNK, cord blood natural killer; CD, cluster of differentiation; Costim, costimulation; E:T, effector-to-target; FACS, fluorescence-activated cell sorting IFN, interferon; IL, interleukin; KO, knockout; LTR, long terminal repeat; Luc, luciferase; MM, multiple myeloma; MoA, mechanism of action; NCR, natural cytotoxicity receptors; NK, natural killer; NKR, NK cell receptors; NSG, NOD scid gamma; sBCMA, soluble BCMA; TM, transmembrane; TNF, tumor necrosis factor; UTD, untransduced.

Disclosures

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