Background

CD19 CAR T immunotherapy has been successful in achieving durable remissions in some patients with relapsed/refractory B cell malignancies, but disease progression and loss of CAR T cell persistence remains common. Interleukin 15 (IL-15) is known to support T cell proliferation and survival, and therefore may enhance CAR T cell efficacy, however, utilizing native IL-15 is challenging due to its unfavorable pharmacokinetics and poor tolerability in the clinic. NKTR-255 is a polymer-conjugated IL-15 that retains binding affinity to IL15Rα and exhibits reduced clearance, providing sustained pharmacodynamic activity.

Methods

CD19 CAR T cells incorporating 4-1BB costimulation were generated from CD8 and CD4 T cells isolated from healthy donors. For in vitro studies, CAR T cells were incubated with NKTR-255 or native IL-15 with and without NKTR-255. STAT5 phosphorylation, CAR T phenotype and dosedilution were assessed by flow cytometry and cytokine production by Luminex. For in vivo studies, NSG mice received 5x10^6 Raji lymphoma cells IV on day 0 and a subtherapeutic dose (0.8x10^6) of CAR T cells (1:1 CD4:CD8) on D0. To determine optimal start date of NKTR-255, mice were treated weekly with NKTR-255 starting on D1, D7, or 14, and post CAR T cell infusion. Tumors were assessed by bioluminescence imaging. Tumor-free mice were rechallenged with Raji cells. For necropsy studies mice received NKTR-255 every 7 days following CAR T cell infusion and were euthanized on D8, 11, 14, 21, and 28 post CAR T cell infusion.

Figure 1: CD19 CAR construct

Figure 2: CAR T cells exhibit dose-dependent phosphorylation of STAT5 and proliferation to NKTR-255. CD8 and CD4 CAR T cells were generated from healthy donors and assayed on day 15. (A) CAR T cells were stimulated with various concentrations of NKTR-255 or IL-15 for 20 minutes. Phosphorylation of STAT5 was measured by flow cytometry. (B) CAR T cells were incubated with CFSE and incubated with various concentrations of NKTR-255 or IL-15 for 5 days without antigen. Proliferation was assayed by flow cytometry.

Results

Figure 3: Treatment with NKTR-255 increases antigen specific CD8 CAR T cell cytokine production. Expansion and enhances survival. CD8 CAR T cells were generated from healthy donors. On day 15, CAR T cells were co-cultured with irradiated K562-CD19- or K562-CD19+ cells. (A) IFNγ and TNFα production was assessed by Luminex 24 hours after co-culture. CAR T cell numbers (B) and intracellular expression of bcl-2 and activated caspase 3 were determined after 5 days of co-culture. Expression of bcl-2 and activated caspase 3 were determined by flow cytometry.

Figure 4: Raji lymphoma bearing NSG mice treated with NKTR-255 and CAR T cells have decreased tumor burden and increased survival. (A) Raji bearing NSG mice received CAR T cell infusions on D0 followed by 0.3mg/kg of NKTR-255 starting on D1, D7 or D14 then weekly (5xmg/kg). (B) Tumor burden was assessed by weekly bioluminescent imaging. (C) Tumor radiance. (D) Survival.

Figure 5: Characterization of CAR T cells in bone marrow of lymphoma-bearing NSG mice. NSG mice received Raji lymphoma cells on D-7, CAR T cells on D0 and weekly injections of NKTR-255 (0.3mg/kg) starting on D7. Mice were euthanized on D8, 11, 14, 21 and 28 post CAR T cell infusions. Single cell suspensions were made from bone marrow. (2um and 2.5um par mesas). (A) Absolute number of CAR T cells in bone marrow (B) Ki-67 expression was assessed by intracellular staining. (C) Bcl-2 expression in CAR T cells on shown as histograms on D8 post infusion (gray + CAR T cell only mice, red and blue = CAR T cells with NKTR-255) and bar graphs. (D) Expression of memory markers CD45RA and CCR7. All protein expression assessed by flow cytometry. Graphs show mean ± SEM.

Conclusions

- CAR T cells treated with NKTR-255 demonstrate increased proliferation and survival both in vitro and in vivo which may in part be due to increased expression of bcl-2.
- Tumor bearing mice treated with NKTR-255 and CAR T cells have decreased tumor burden and increased survival compared to mice treated with CAR T cells alone.
- Tumor-bearing mice previously treated with NKTR-255 and CAR T cells are able to reject tumor rechallenge supporting persistence of functional CAR T cells.
- Administration of NKTR-255 in combination with CD19 CAR T cells leads to improved antitumor efficacy making NKTR-255 an attractive candidate for enhancing CAR T cell therapy in the clinic.
- First in human dose escalation NKTR-255 monotherapy trial is actively enrolling at selected sites (NCT04130768).

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