Combination of NKTR-255, a polymer conjugated human IL-15, on efficacy of CAR T cell immunotherapy in a preclinical lymphoma model

Cassie K. Chou[1,2], Simon Frassle[3], Rachel N. Steinmetz[1], Reed M. Hawkins[1], Tinh-Doan Phi[1], Dirk H. Busch[3], Takahiro Miyazaki[4], Mario Marcondes[4], Stanley R. Riddell[1,5], Cameron J. Turtle[1,5]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, [2]Division of Pediatric Hematology/Oncology, Seattle Children's Hospital, Seattle, WA, [3] Technical University of Munich, Munich, Germany, [4]Nektar Therapeutics, San Francisco, CA, [5]Department of Medicine, University of Washington, Seattle, WA

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 responses. We investigated the effects of NKTR-255 on human CD19 CAR T cells both *in vitro* and in an *in vivo* xenogeneic B cell lymphoma model and found improved survival of lymphoma bearing mice receiving NKTR-255 and CAR T cells compared to CAR T cells alone. Here, we extend upon these findings to further examine potential mechanisms underlying improved anti-tumor efficacy.

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 CD19 antigen. STAT5 phosphorylation, CAR T phenotype and CFSE dilution were assessed by flow cytometry and cytokine production by Luminex. For *in vivo* studies, NSG mice received 5x10^5 Raji lymphoma cells IV on day (D)-7 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 D-1, 7, or 14 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

Results

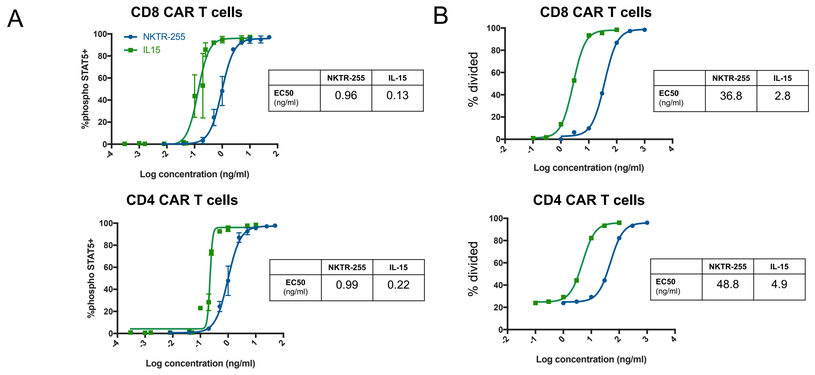

Figure 2: CAR T cells exhibit dose-dependent phosphorylation of STAT5 and proliferation to NKTR-255. CD8 and CD4 CAR T cells were generated from healthy donors and assayed on day 15. (A) CAR T cells were stimulated with various concentrations of NKTR-255 or IL-15 for 20 minutes. Phosphorylation of STAT5 was measured by flow cytometry. (B) CAR T cells were labeled with CFSE and incubated with various concentrations of NKTR-255 of IL15 for 5 days without antigen. Proliferation was assayed by flow cytometry.

Funding Support

This work was supported by an NIH T32 pediatric hematology/oncology training grant (CKC) and Nektar Therapeutics.

Results

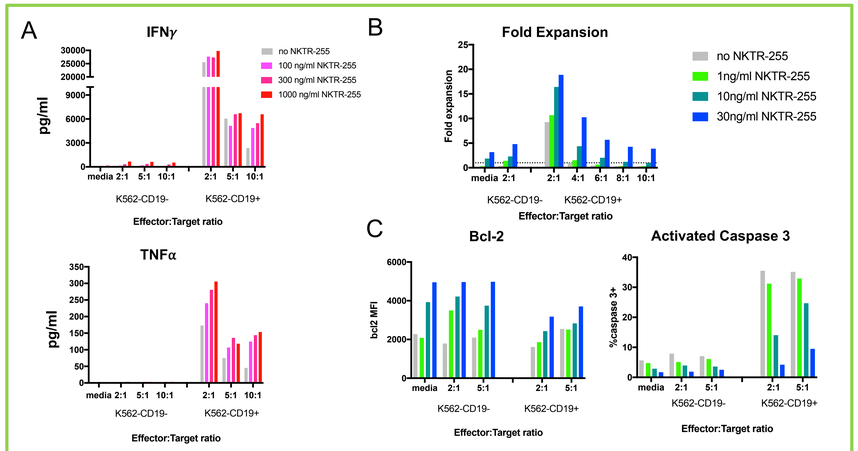

Figure 3: Treatment with NKTR-255 increases antigen specific CD8 CAR T cell cytokine production, expansion and enhances survival. CD8 CAR T cells were generated from healthy donors. On day 15 CAR T cells were co-cultured with irradiated K562-CD19+ or K562-CD19- cells. (A) IFNγ and TNFα production was assayed 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.

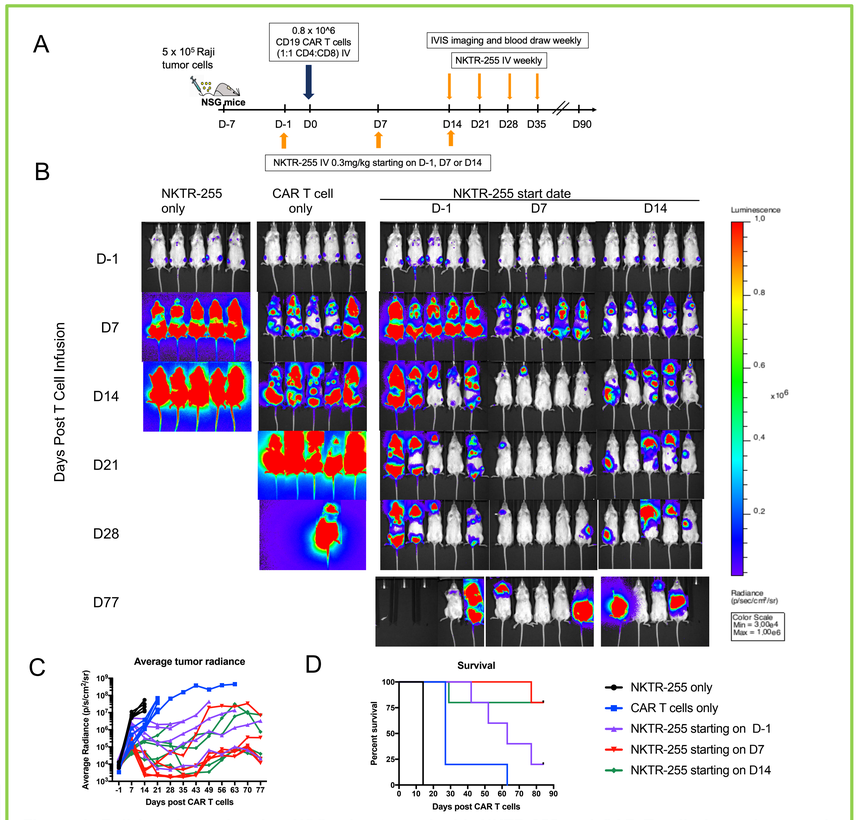

Figure 4: Raji lymphoma bearing NSG mice treated with NKTR-255 and CAR T cells have decreased tumor burden and increased survival. (A) Raji bearing NSG mice received CAR T cell infusions on D0 followed by 0.3mg/kg of NKTR-255 starting on D-1, D7 or D14 then weekly (5 mice/group). (B) Tumor burden was assessed by weekly bioluminescent imaging. (C) Tumor radiance. (D) Survival.

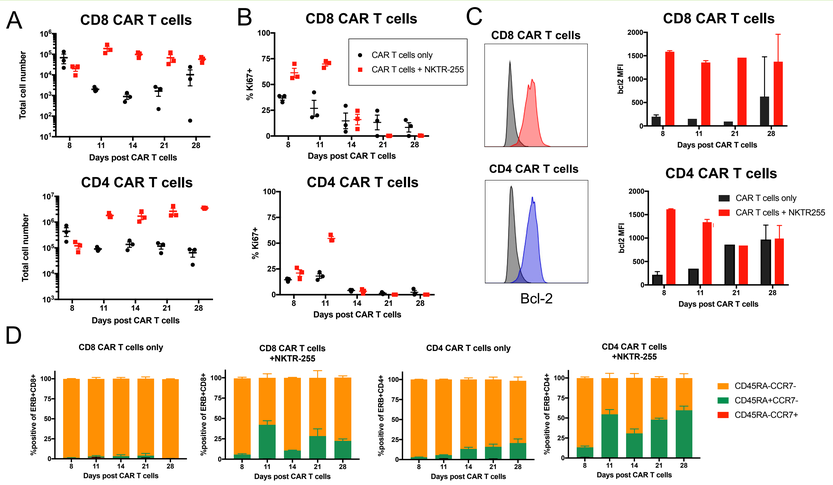

Figure 5: Characterization of CAR T cells in bone marrow of lymphoma bearing NSG mice. NSG mice received Raji lymphoma cells on D-7, CAR T cells on D0 and weekly injections of NKTR-255 (0.3mg/kg) starting on D7. Mice were euthanized on D8, 11, 14, 21 and 28 post CAR T cell infusion. Single cell suspensions were made from bone marrow (2 femur and 2 tibia per mouse). (A) Absolute number of CAR T cells in bone marrow (B) Ki-67 expression was assayed by intracellular staining. (C) Bcl-2 expression in CAR T cells on shown as histograms on D8 post infusion (grey = 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 assayed by flow cytometry. Graphs show mean ± SEM.

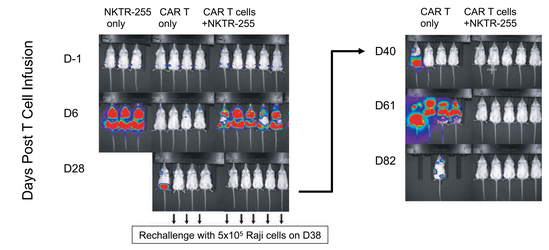

Figure 6: Mice previously treated with NKTR-255 and CAR T cells are able to reject Raji tumor rechallenge. NSG mice were inoculated with Raji tumor cells on D-7 and received 0.3mg/kg of NKTR-255 starting on D-1 then weekly. Mice received CAR T cell infusions on D0. Tumor-free mice (depicted by arrows) were rechallenged with Raji tumor cells on D38 and imaged weekly to assess for tumor burden.

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 (NCT04136756).