Effects 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

Immunotherapy with CD19 CAR T cells achieves complete or partial remission in a fraction of patients with B cell malignancies, but disease progression remains common. IL-15 promotes T cell proliferation and survival, and may enhance CAR T cell efficacy. However, exploiting native IL-15 is challenging due to its unfavorable pharmacokinetics and tolerability. In contrast, NKTR-255 is a polymer-conjugated IL-15 that retains binding affinity to IL-15Rα, maintaining full spectrum of IL-15 biology. NKTR-255 also exhibits improved pharmacokinetics thereby providing sustained pharmacodynamic responses without the need for daily dosing. 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.

Methods

T cells expressing a CD19/4-1BB/CD3ζ CAR were generated from healthy donors. Briefly, CD4 and CD8 T cells were isolated and separately transduced with CD19 CAR lentiviral vector (Fig.1), transduced cells were sorted then expanded with LCL (lymphoblastoid B-cell line) cells for 14-16 days. For *in vitro* studies, CAR T cells were incubated with NKTR-255. STAT5 phosphorylation and CFSE dilution were assessed by flow cytometry. 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. NKTR-255 (0.03, 0.1 or 0.3 mg/kg) was infused IV weekly starting on D-1, 7, or 14. Tumor-free mice were rechallenged with Raji cells. Tumors were assessed by bioluminescence imaging.

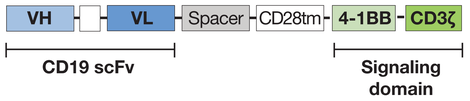

Figure 1: CD19 CAR construct

Results

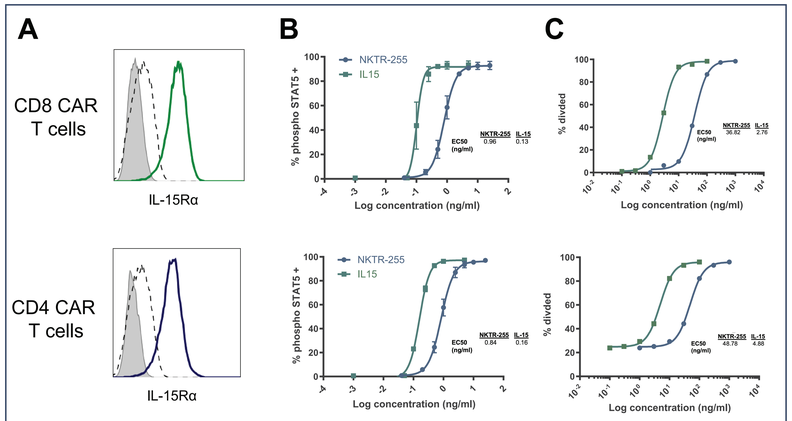

Figure 2: CAR T cells express IL-15Rα and exhibit dose-dependent phosphorylation of STAT5 and proliferation in response to NKTR-255. CD8 and CD4 CAR T cells were generated from healthy donors and assayed on day 15. (A) IL-15Rα expression was measured by flow cytometry. CD8 (green line), CD4 (blue line), FMO (filled gray) and isotype control (dashed line) are shown. (B) 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. (C) CAR T cells were labeled with CFSE and incubated with various concentrations of NKTR-255 of IL15 for 4 days. Proliferation was assayed by flow cytometry.

Results

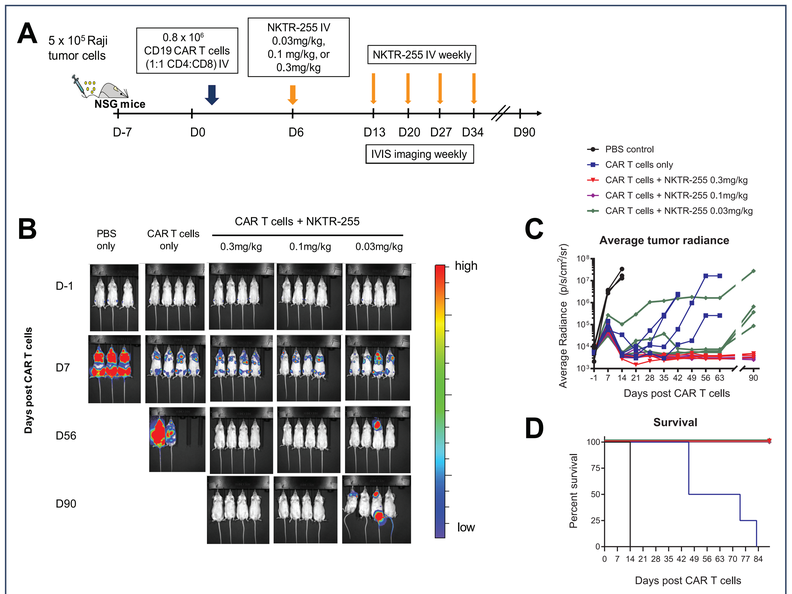

Figure 3: NKTR-255 treatment at 0.1 mg/kg and 0.3 mg/kg in combination with CAR T cells leads to decreased tumor burden and eradication of Raji lymphoma in NSG mice compared to CAR T cells alone. (A) Raji bearing NSG mice received a subtherapeutic dose of CAR T cells on D0 followed by 0.03, 0.1 or 0.3 mg/kg of NKTR-255 starting on D6 then weekly. Mice were imaged weekly and tumor bioluminescence images for representative timepoints are shown in (B) and average radiance in (C). Survival is shown in (D).

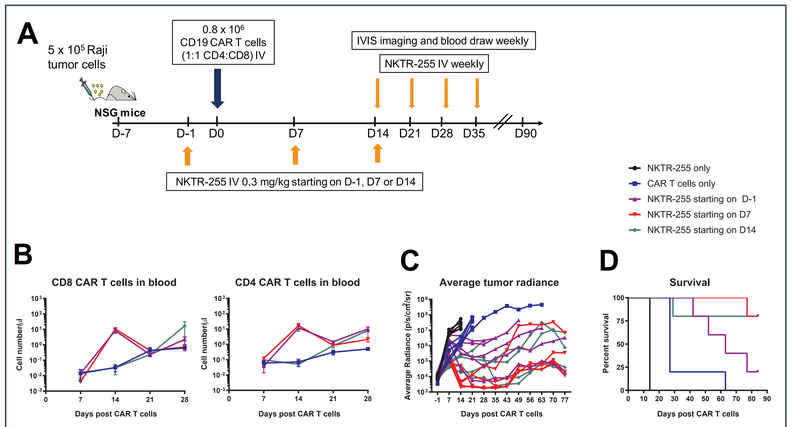

Figure 4: NKTR-255 in combination with CAR T cells results in increased CAR T cells in the blood, decreased tumor burden and increased survival of Raji lymphoma bearing NSG mice. (A) Raji bearing NSG mice received CAR T cell infusions on D0 followed by 0.3mg/kg of NKTR-255 starting on D-1, D7 or D14 then weekly (5 mice/group). (B) Mice were bled weekly and CD8 and CD4 CAR T cells were identified by flow cytometry. Tumor burden was assessed by weekly bioluminescent imaging shown in (C) and survival in (D).

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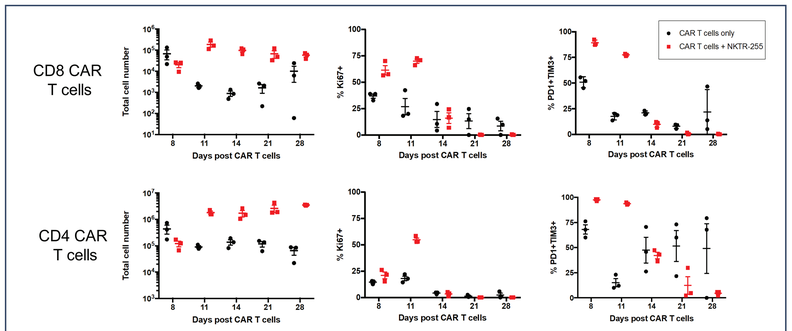

Figure 5: Mice treated with NKTR-255 have increased absolute number of CAR T cells in the bone marrow. NSG mice received Raji lymphoma cells on D-7, CAR T cells on D0 and weekly injections of NKTR-255 (0.3mg/kg) starting on D7. Mice were euthanized on D8, 11, 14, 21 and 28 post CAR T cell infusion. Single cell suspensions were made from bone marrow and CAR T cells, Ki67, PD1 and TIM3 expression were assessed by flow cytometry. Graphs show mean + SEM.

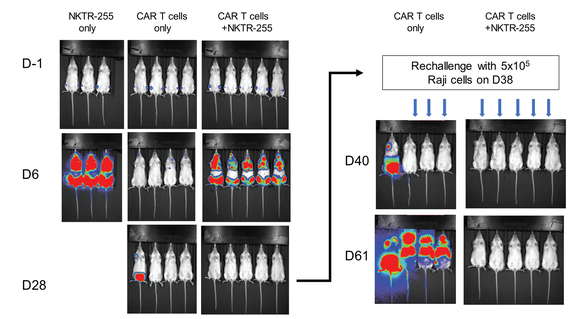

Figure 6: Mice previously treated with NKTR-255 and CAR T cells are able to reject Raji tumor rechallenge. NSG mice were inoculated with Raji tumor cells on D-7 and received 0.3 mg/kg of NKTR-255 starting on D-1 then weekly. Mice received CAR T cell infusions on D0. Tumor-free mice (depicted by arrows)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

Conclusions

- CD8 and CD4 CAR T cells express IL-15-Rα and NKTR-255 induces STAT5 phosphorylation and proliferation in a dose-dependent manner.
- NKTR-255 in combination with CAR T cells decreases tumor burden and increases survival of NSG mice in a Raji lymphoma model compared to CAR T cells alone.
- NKTR-255 treatment resulted in increased accumulation and proliferation of CAR T cells in the bone marrow of Raji bearing NSG mice and decreased prolonged dual expression of PD1 and TIM3.
- Tumor-bearing mice previously treated with NKTR-255 and CAR T cells are able to reject tumor rechallenge supporting CAR T cell persistence.
- NKTR-255 administration improves the antitumor efficacy and kinetics of CD19 CAR T cells, supporting development of clinical trials of combination therapy with NKTR-255 and approved CD19 CAR T cell products.