A long-acting pharmacological grade interleukin-7 molecule logarithmically accelerates UCAR-T proliferation, differentiation, and tumor killing.

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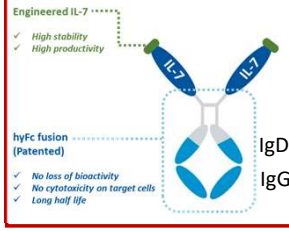
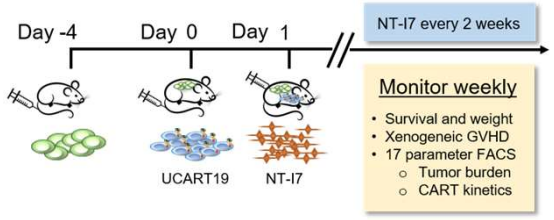
Background: Chimeric antigen receptor T cell (CAR-T) therapy is revolutionizing modern cancer therapy with two anti-CD19 CAR-Ts FDA-approved for relapsed/refractory B cell lymphoma/leukemia and many other CAR-Ts undergoing clinical trials. Our group recently developed a multiplexed CRISPR/Cas9 gene-editing "off-the-shelf" CAR-T for the treatment of T cell malignancies without inducing fratricide or causing GVHD[1]. In current clinical practice, suboptimal CAR-T persistence permits tumor cell escape and, ultimately, disease relapse. We hypothesized that a pro-lymphoid growth factor such as the long-acting form of recombinant human interleukin-7 fused with hybrid Fc (rhIL-7-hyFc, NT-I7, NeoImmuneTech, Inc.) could promote CAR-T persistence and increase efficacy.



Human Ig isotypes	IgG1	IgG4	IgD
Hinge flexibility	++	+	++++
Binding of FcR of phagocytes (ADCC)	++++	++	+
Activation of C1q (CDC)	++	-	-
Binding of FcRn	++++	++++	-
In vivo serum half life (days)	21	21	3

Methods: To create anti-CD19 universal CAR-T (UCART19), we activated human T cells with anti-CD3/CD28 beads, electroporated the T cells with Cas9 mRNA and a TRAC-targeted gRNA, and virally transduced an anti-CD19 3rd generation CAR containing a 2A-cleaved truncated human CD34 allowing for both purification and tracking of CAR-T in vivo. Residual TRAC+ cells were depleted using magnetic selection. For xenograft tumor modeling in vivo, we injected NSG mice with 5x10^5 Ramos$^{CBR-GFP}$ cells four days prior to UCART19 (2x10^6 cells) infusion. Mice were treated with NT-I7 (10mg/kg SC) on days +1, +15 and +29 post UCART19 infusion.



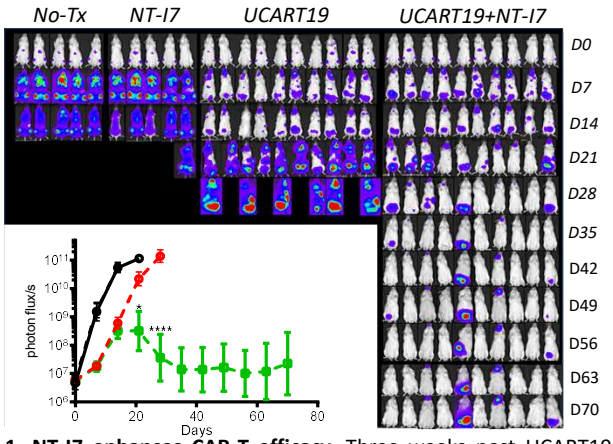


Fig 1. NT-I7 enhances CAR-T efficacy. Three weeks post UCART19 infusion, bioluminescent imaging (BLI) revealed minimal tumor signal in *UCART19+NT-I7* treated mice (10^8 vs. 10^{10} photon flux/s, p<0.05, *UCART19+NT-I7* vs. *UCART19)* and near-undetectable photon flux/s at four weeks (10^7 vs 10^{11} photon flux/s, p<0.0001, *UCART19+NT-I7* vs. *UCART19*).

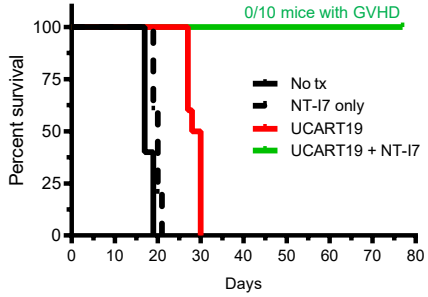


Fig 2. NT-I7 prolongs survival of UCART19 treated mice. Ramos$^{CBR-GFP}$ mice treated with UCART19 alone (*UCART19* group) survived 29 days, 100% of Ramos$^{CBR-GFP}$ mice treated with UCART19 and NT-I7 (*UCART19+NT-I7* group) were alive at 80 days with no mouse showing signs of xenogeneic GVHD (p<0.0001, *UCART19+NT-I7* vs. *UCART19*).

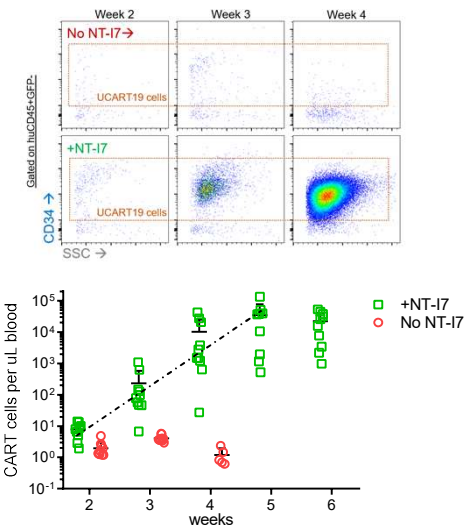


Fig 3. NT-I7 enhances CAR-T expansion and persistence. Quantitative 17-parameter flow cytometric analyses of blood reveals logarithmic expansion of UCART19 cells in mice receiving NT-I7.

Discussion: CAR-T cells engineered to express either interleukin-7, or transgenic IL-7 receptors have demonstrated enhanced efficacy against hematologic and solid organ malignancies in vivo[2,3]. However, genetically engineered potentiation strategies lack "off-switches" and may induce severe toxicities in human clinical trials. Here, we demonstrate that the use of pharmacological grade, long-acting interleukin-7 agonist can potentially mitigate toxicity problems induced by engineered Il-7/Il-7R CAR-T, allowing NT-I7 dosing and timing to be adjusted. NT-I7 can dramatically enhance gene modified T-cell proliferation, persistence, and tumor killing in vivo, resulting in enhanced survival and providing a tunable clinic-ready adjuvant for reversing suboptimal CAR-T activity in vivo.

References

1 Cooper et al, *Leukemia*, 2018
2.ShuM et al, Cancer Discovery 2017
3. Adachi et al, *Nature Biotechnology*, 2018

Funding

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