Title: NT-I7, a long-acting recombinant human interleukin-7, enhances T cell reconstitution following total body irradiation

Yujing Zou[1,*], Yiqun Jiao[1,*], Alexandra A. Wolfarth[2], Sara Ferrando-Martinez[2], Byung Ha Lee[2], and Benny J. Chen[1]. [1]Division of Hematologic Malignancies and Cellular Therapy, Duke University, Durham, NC; [2]NeoImmuneTech, Inc, Rockville, MD (*contributing equally)

Summary:
NT-I7 is a novel long-acting recombinant human interleukin-7. In this study, we found that NT-I7 promotes systemic T cell recovery after total body irradiation through both thymic-dependent and -independent mechanisms. Importantly, NT-I7 treatment preserves T cell effector function and proliferation capacity.

Abstract:
Total body irradiation (TBI) causes profound suppression of hematopoiesis and T cell depletion, increasing chances of infection. Currently, therapeutic options for improving recovery of the T cell compartment following radiation exposure are not available. Exogenous administration of IL-7 has been shown to increase peripheral blood (PB) T cell levels in mice and humans. However, this effect can be offset by the short half-life (<10 hours) of IL-7 in circulation. NT-I7 (efineptakin alfa) is a long-acting recombinant human IL-7 fused with a hybrid Fc fragment. In the clinical setting, NT-I7 has a significantly longer half-life and has been shown to significantly and persistently increase T cell counts while being safe and well tolerated.
In mice exposed to TBI (5 Gy), total T cell count in the NT-I7 treated group recovered back to normal much quicker than that in the vehicle control group (Figure 1A). NT-I7 also enhanced T cell recovery across various subsets, including naïve (T_N), central memory (T_{CM}), and effector memory (T_{EM}) T cells. To determine whether NT-I7 also stimulates systemic T cell recovery, we established a T cell-specific luciferase reporter strain, CD4-Cre x $Luc^{flox/flox}$ (T-Luc), to monitor T cell recovery in vivo. Chimeras were generated by transferring T-Luc bone marrow (BM) cells to lethally irradiated (10.5 Gy) syngeneic white C57BL/6 mice to improve imaging resolution. Robust T cell recovery was observed in NT-I7-treated animals following TBI (Figures 1B). Notably, the effect on T cell recovery persisted after the last injection and was readily detected in the thymus, lymph nodes, spleen, and the gastrointestinal tract. These findings were subsequently validated by significantly increased absolute T cell counts after TBI (NT-I7 vs. vehicle, $P < 0.05$, CD8 PB and BM T cells; $P < 0.001$, PB CD4 T cells and spleen CD8 T cells, day 14). T cell subset analyses revealed significant increase in both CD8 T_N and T_{EM} T cells in NT-I7 group. In BM (a niche for memory T cells), T_{CM}, which account for the majority of CD8 T cells, were increased in NT-I7-treated mice. Imaging analyses showed that both irradiated BM cells and remaining peripheral T cells contributed to rapid induction of T cell recovery by NT-I7 through thymic-dependent and -independent mechanisms. Interestingly, thymocyte development showed that NT-I7 preferentially enhanced CD8 fate commitment (SP4, NT-I7 (10.51 ± 1.81%) vs. vehicle (17.02 ± 2.09%), $P < 0.001$; SP8, NT-I7 (7.70 ± 1.44%) vs. vehicle (3.56 ± 0.39 %), $P < 0.001$;) which may be critical for mounting cytotoxic response against pathogen challenges. Furthermore, functional assays suggested that expression of both Granzyme B, a cytotoxic mediator and proinflammatory cytokines, IFN-γ and TNF-α, were preserved in NT-I7 group. Furthermore, proliferation capacity in response to TCR stimuli was also preserved in NT-I7-treated T cells.
Altogether, we have demonstrated that NT-I7 enhances rapid induction of systemic T cell regeneration and preserves effector T cell functions. These findings indicate that NT-I7 as a promising therapeutic for significantly accelerating and enhancing T cell recovery after radiation exposure.