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rhIL-7-hyFc (efineptakin alfa; NT-I7) enhances the anti-tumor response when combined with hIL-2/TCB2c complex

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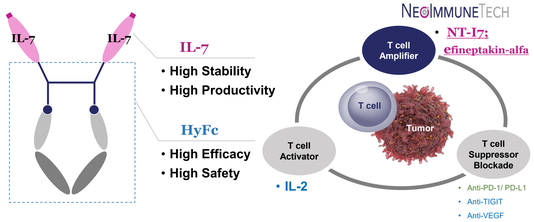
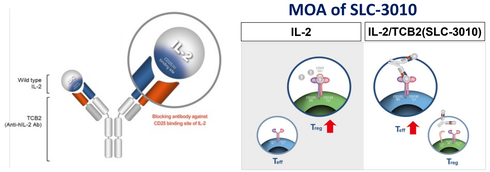
Abstract

rhIL-7-hyFc (efineptakin-alfa; NT-I7) is a potent T cell amplifier, with a homodimeric interleukin-7 (IL-7) fused to the hybridizing IgD/IgG4 immunoglobulin domain. Previous work has shown that in mice, NT-I7 dramatically increases tumor-infiltrating CD8+ T cells while reducing the frequency of PD-1$^+$ CD8$^+$ T cells in the tumor. There is also significant expansion of Central Memory (CM)-phenotype CD8$^+$ T cells (CD62L$^+$CD44$^+$) in the tumor and tumor-draining lymph node (TDLN). Here, we investigated the anti-tumor effect of NT-I7 in combination with a T cell activator, SLC-3010 (hIL-2/TCB2c complex), in MC38 tumor-bearing mice. Because TCB2 is an antibody specific for IL-2 that blocks interaction of IL-2 and IL-2Rα (CD25), SLC-3010 can selectively activate T cells while disfavoring Treg activation. Mice were administered a single dose of NT-I7 or SLC-3010 via intramuscular or intravenous injection, respectively. The combination of NT-I7 with SLC-3010 enhanced the anti-tumor response with increased number and frequency of CD8$^+$ T cells as well as granzyme B expression in the tumor. The number of CD8+ T cells peaked at day 4 and day 7 by SLC-3010 and NT-I7, respectively. The number of Tregs in the tumor was slightly increased by NT-I7 and SLC-3010, but it was not statistically significant. Interestingly, NT-I7, but not SLC-3010, increased the frequency of PD-1$^+$TCF-1$^+$TOX$^-$ stem-like CD8$^+$ T cells in the draining lymph node. Meanwhile, SLC-3010 significantly increased the number of PD-1$^+$ CD8$^+$ T cells in the tumor. Our data suggests that NT-I7 can be applied in combination with other immunotherapies such as IL-2 to enhance the anti-tumor response.

rhIL-7-hyFc (efineptakin-alfa; NT-I7)

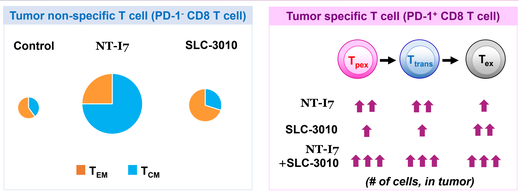


IL-2/TCB2 Complex (SLC-3010)



** SLC-3010 (IL-2/TCB2) was provided by Selecxine, South Korea.*

Schematic Hypothesis



Anti-tumor synergy

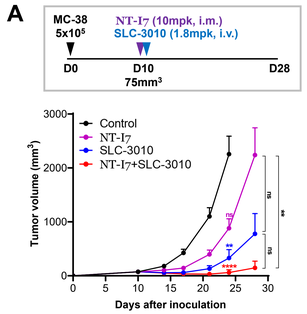
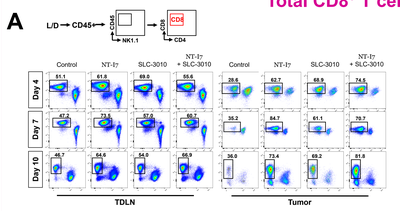


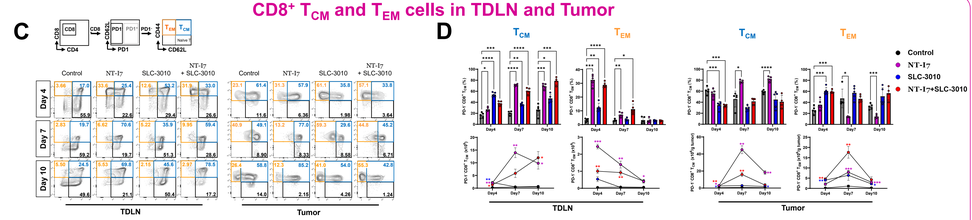
Figure 1. NT-I7 combined with SLC-3010 inhibits tumor growth in MC38-bearing mice. (A) The experimental scheme (upper) and mean tumor growth curves (lower). Data were statistically analyzed with Kruskal-Wallis compared groups at day 24 and day 28. Data are Mean±SEM. (n=9 per group). Statistical analysis was performed by Two-way ANOVA with Dunnett post hoc test.

NT-I7 and SLC-3010 increase CD8$^+$ T cells in the tumor and TDLN

Total CD8$^+$ T cells in TDLN and Tumor



CD8$^+$ T_{CM} and T_{EM} cells in TDLN and Tumor



CD8$^+$/Treg cells in Tumor

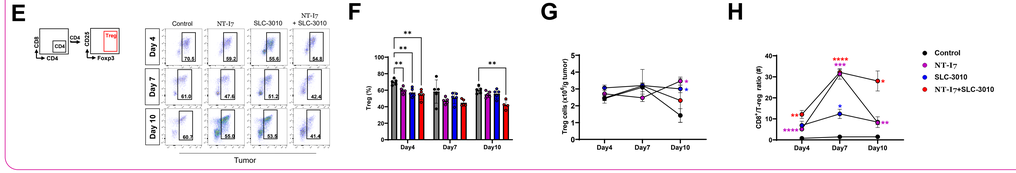


Figure 2. NT-I7 combined with SLC-3010 increases frequency and number of CD8$^+$ T cells. (A) FACS analysis strategy (upper) and representative plots (lower) for the frequency of total CD8$^+$ T cells. (B) Increased numbers of total CD8$^+$ T cells in NT-I7 and NT-I7 + SLC-3010 co-treated group in tumor-draining lymph node (TDLN) and tumor. (C) FACS analysis strategy (upper) and representative plots (lower) for the frequency of CD8$^+$ T_{CM} and T_{EM} cells. (D) Frequency (upper) and cell numbers (lower) of CD8$^+$ T_{CM} and T_{EM} cells in TDLN and tumor. Significant expansion of T_{CM} cells treated by NT-I7. (E) FACS analysis strategy (left) and representative plots (right) for the frequency of Treg cells in tumor. (F) Decreased frequency of Treg cells treated with NT-I7. (G) Numbers of Treg cells in tumor. (H) Ratio of CD8$^+$ T/Treg cell numbers in the tumor. Data are Mean±SD (frequency) and Mean±SEM (cell number) and representative of 2 or 3 independent experiments (n = 3~5 per group per experiment) *(*p≤0.05; **p ≤ 0.001; ***p ≤0.0001; ****p ≤0.00001)*. Statistical analysis was performed by Two-way ANOVA with Dunnett post hoc test.

NT-I7 and SLC-3010 increase PD-1$^+$ CD8$^+$ T cells in the tumor

Precursor exhausted, transitory and exhausted CD8$^+$ T cells in Tumor

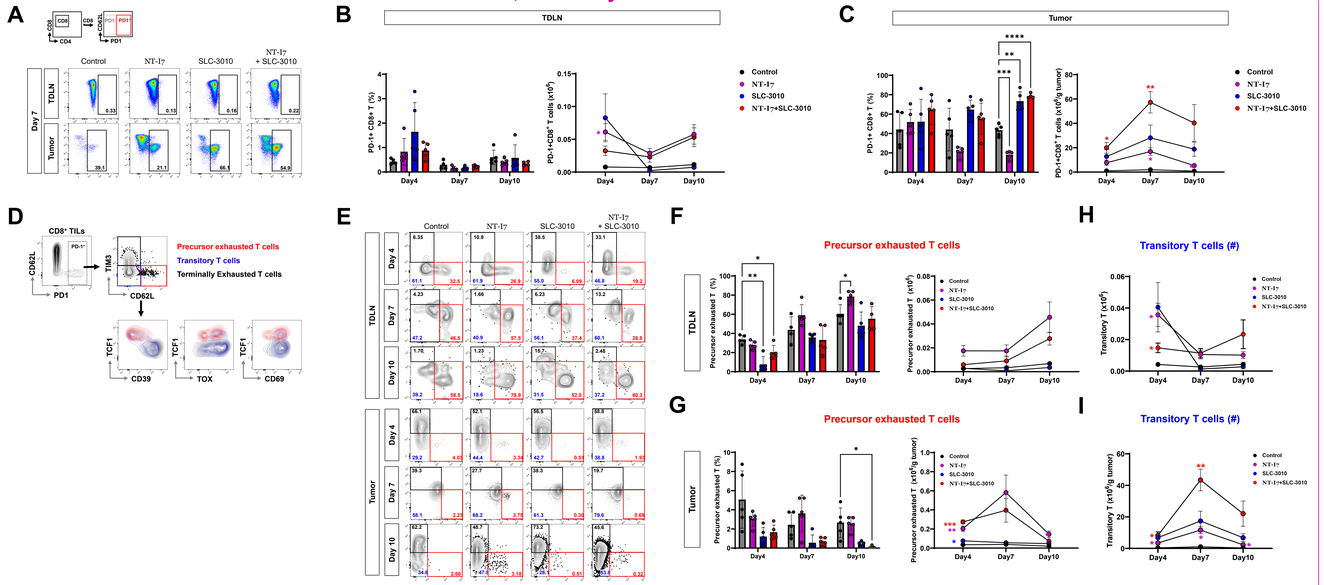
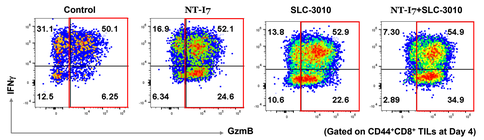


Figure 3. Frequency and number of precursor exhausted, transitory and exhausted CD8$^+$ T cells in the tumor and TDLN. (A) FACS analysis strategy (upper) and representative plots (lower) for the frequency of PD1$^+$CD8$^+$ T cells at day7 in TDLN and tumor. (B) Frequency (left) and cell numbers(right) of PD1$^+$CD8$^+$ T cells in TDLN. (C) Frequency (left) and cell numbers (right) of PD1$^+$CD8$^+$ T cells in tumor. (D) Gating strategy for defining the distinct subset of PD1$^+$CD8$^+$ T cells. (E) Representative FACS plots for the frequency of CD62L$^+$TIM3$^-$(precursor exhausted), CD62L$^-$TIM3$^-$(transitory) and CD62L$^-$TIM3$^+$(terminally exhausted) T cells. (F) Frequency (left) and numbers (right) of CD62L$^+$TIM3$^-$ (precursor exhausted) T cells in TDLN. (G) Frequency (left) and numbers (right) of CD62L$^+$TIM3$^-$ (precursor exhausted) T cells in tumor. (H and I) Numbers of transitory T cells in TDLN (H) and tumor (I). Data are Mean±SD (frequency) and Mean±SEM (cell number) and representative of 2 or 3 independent experiments (n = 3~5 per group per experiment) *(*p≤0.05; **p ≤ 0.001; ***p ≤0.0001; ****p ≤0.00001)*. Statistical analysis was performed by Two-way ANOVA with Dunnett post hoc test.

NT-I7 and SLC-3010 enhance cytotoxicity

Cytokine expression in CD8$^+$ T cells



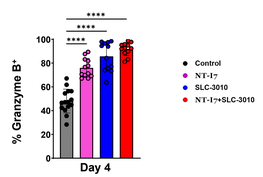


Figure 4. Increased frequency of Granzyme B (GzmB) in tumor-infiltrating cells. (A-B) Representative FACS plots (A) and frequency (B) of GzmB$^+$ cells in CD8$^+$ CD44$^+$ TILs after PMA/ionomycin stimulation. Data are Mean±SD and representative of 2 or 3 independent experiments (n = 12~15 per group)*(*p≤0.05; **p ≤ 0.001; ***p ≤0.0001; ****p ≤0.00001)*. Statistical analysis was performed by One-way ANOVA with Dunnett post hoc test.

Conclusion

NT-I7 can be applied in combination with other immunotherapies such as IL-2/TCB2 (SLC-3010) to enhance the anti-tumor response

NT-I7 (rhIL-7-hyFc) increases total CD8$^+$ TILs and increases % of less differentiated CD8$^+$ T cells in the tumor and TDLN

- **Increased % T_{CM} (most of which is newly infiltrated T cells)**
- **Increased CD8$^+$/Treg ratio in tumor**
- **Increased % T_{pex} in TDLN and Increased # T_{pex} in tumor**
- **Increased # T_{trans} in TDLN and tumor**

SLC-3010 (IL-2/TCB2) increases % of more differentiated CD8$^+$ T cells in the tumor and TDLN

- **Increased % T_{EM} in tumor**
- **Increased CD8$^+$/Treg ratio in tumor; did not significantly affect Treg levels in tumor**
- **Decreased % T_{pex} in TDLN**

For more information, please contact science@neoimmunetech.com