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rhIL-7-hyFc (efineptakin alfa; NT-I7) enhances the anti-tumor response when combined with anti-TIGIT and anti-VEGF

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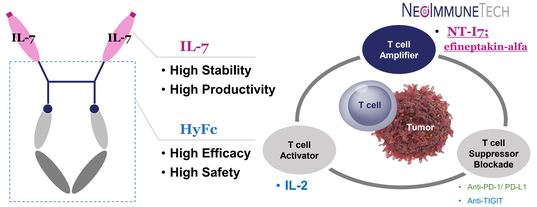
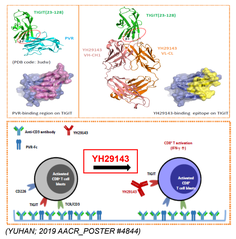
Abstract

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 two different T cell suppressor blockades; anti-TIGIT (YH29143) and anti-VEGF (Aflibercept) in MC38 tumor-bearing mice. NT-I7 was administered by intramuscular injection with the first dose of either YH29143 or Aflibercept. YH29143 or Aflibercept was administered every 3 days for 3 total doses, via intraperitoneal or intravenous route, respectively. The combination of NT-I7 with either T cell suppressor blockade enhanced the anti-tumor response. Surprisingly, NT-I7 combined with YH29143 increased the frequency of PD-1$^+$TCF-1$^+$TOX$^-$CD39$^-$ stem-like CD8$^+$ T cells in the draining lymph node. In addition, Aflibercept reduced the expression of TOX in PD-1$^+$CD8$^+$ T cells in the tumor. Our data suggests that NT-I7 can be applied in combination with other immunotherapies such as anti-TIGIT or anti-VEGF to enhance the anti-tumor response.

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

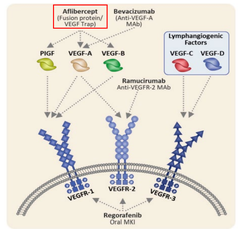


Anti-TIGIT (YH29143) ; T cell suppressor blockade 1



(YUHAN; 2019 AACR_POSTER #4844)

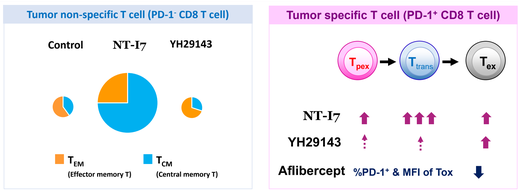
Anti-VEGF (Afilbercept) ; T cell suppressor blockade 2



(Cancers (Basel). 2020 Mar 31;12(4):844)

* *YH29143 (anti-TIGIT) was provided by Yuhan, South Korea.*

Schematic Hypothesis



Synergistic anti-tumor effect when NT-I7 is combined with YH29143 or Aflibercept

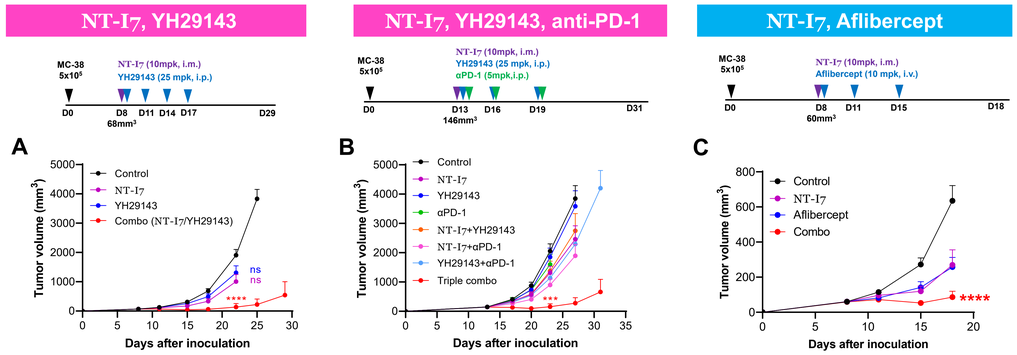


Figure 1. NT-I7 treated with YH29143, Aflibercept and/or anti-PD-1 inhibits the tumor growth in MC38-bearing mice. (A-C) Mean tumor growth curves (n=7~10 per group). (A) Tumor growth after treatment of small tumors with NT-I7 combined with YH29143. (B) Tumor growth after treatment of larger tumors with NT-I7 combined with YH29143 and/or anti-PD-1. (C) Triple combo effects with anti-VEGF blockade, Aflibercept, in MC-38-bearing mice. NT-I7 was administered by intramuscular injection with the first dose of either YH29143 or Aflibercept. YH29143 or Aflibercept was administered every 3 days for 3 total doses, via intraperitoneal or intravenous route, respectively. Data are Mean±SD and representative of 2 or 3 independent experiments (n = 7~10 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 increases CD8 T cells in TDLN and Tumor

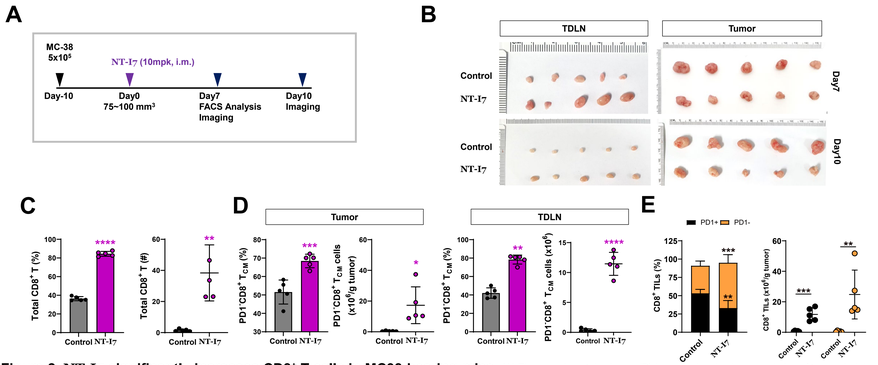


Figure 2. NT-I7 significantly increases CD8$^+$ T cells in MC38-bearing mice. (A) The experimental scheme (B) Images showing increased size of tumor-draining lymph node (TDLN) and decreased size of tumor after NT-I7 treatment. (C) Increased frequency (left) and numbers (right) of tumor-infiltrating CD8$^+$ T cells (TILs) in the NT-I7 group. (D) Increased frequency (left) and numbers (right) of CD8$^+$CD62L$^+$CD44$^+$ central memory T cells (T$_{CM}$) in tumor and tumor-draining lymph node (TDLN). (E) Reduced frequency (left) of PD1$^+$CD8$^+$ T cells and increased cell numbers (right) of PD1$^+$CD8$^+$ and PD1$^-$CD8$^+$ TILs at day 7 after NT-I7 administration . Data are Mean±SD and representative of 2 or 3 independent experiments (n = 5 per group per experiment). *(*p≤0.05; **p ≤ 0.001; ***p ≤0.0001; ****p ≤0.00001)*. Statistical analysis was performed by unpaired t-test (C,E) or Mann Whitney test (D).

NT-I7 and YH29143 increase PD-1$^-$ CD8$^+$ T cells in the tumor

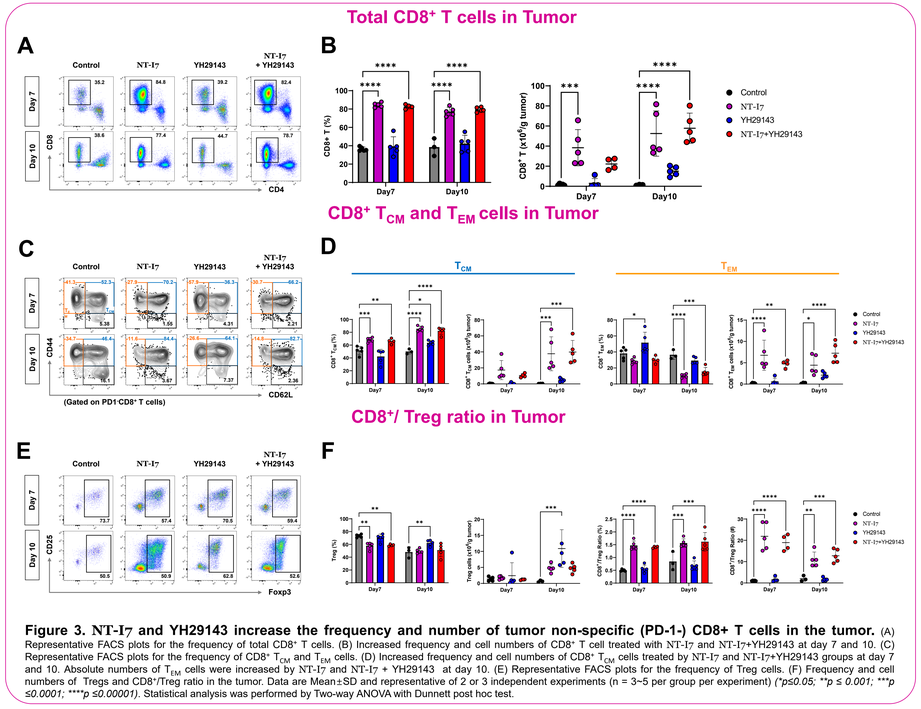


Figure 3. NT-I7 and YH29143 increase the frequency and number of tumor non-specific (PD-1-) CD8+ T cells in the tumor. (A) Representative FACS plots for the frequency of total CD8$^+$ T cells. (B) Increased frequency and cell numbers of CD8$^+$ T cell treated with NT-I7 and NT-I7+YH29143 at day 7 and 10. (C) Representative FACS plots for the frequency of CD8$^+$ T$_{CM}$ and T$_{EM}$ cells. (D) Increased frequency and cell numbers of CD8$^+$ T$_{CM}$ cells treated by NT-I7 and NT-I7+YH29143 groups at day 7 and 10. Absolute numbers of T$_{EM}$ cells were increased by NT-I7 and NT-I7 + YH29143 at day 10. (E) Representative FACS plots for the frequency of Treg cells. (F) Frequency and cell numbers of Tregs and CD8$^+$/Treg ratio in the tumor. Data are Mean±SD 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 YH29143 increase PD-1$^+$ CD8$^+$ T cells in the tumor

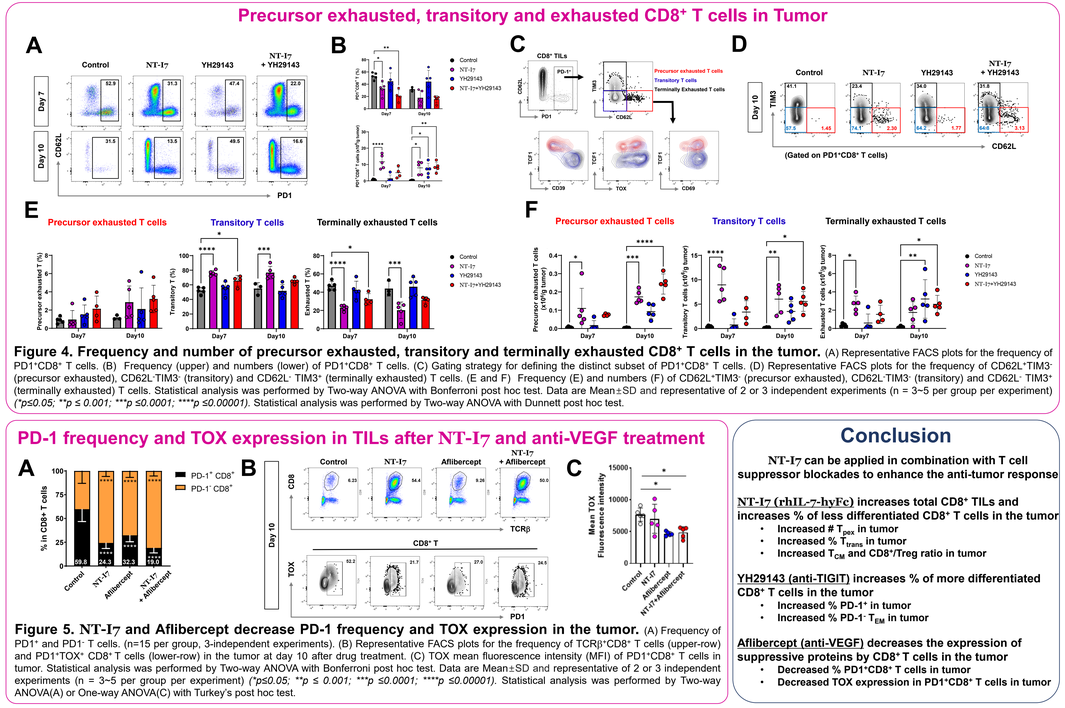


Figure 4. Frequency and number of precursor exhausted, transitory and terminally exhausted CD8$^+$ T cells in the tumor. (A) Representative FACS plots for the frequency of PD1$^+$CD8$^+$ T cells. (B) Frequency (upper) and numbers (lower) of PD1$^+$CD8$^+$ T cells. (C) Gating strategy for defining the distinct subset of PD1$^+$CD8$^+$ T cells. (D) Representative FACS plots for the frequency of CD62L$^+$TIM3$^-$ (precursor exhausted), CD62L$^-$TIM3$^-$ (transitory) and CD62L$^-$ TIM3$^+$ (terminally exhausted) T cells. (E and F) Frequency (E) and numbers (F) of CD62L$^+$TIM3$^-$ (precursor exhausted), CD62L$^-$TIM3$^-$ (transitory) and CD62L$^-$ TIM3$^+$ (terminally exhausted) T cells. Statistical analysis was performed by Two-way ANOVA with Bonferroni post hoc test. Data are Mean±SD 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.

PD-1 frequency and TOX expression in TILs after NT-I7 and anti-VEGF treatment

Figure 5. NT-I7 and Aflibercept decrease PD-1 frequency and TOX expression in the tumor. (A) Frequency of PD1$^+$ and PD1$^-$ T cells. (n=15 per group, 3-independent experiments). (B) Representative FACS plots for the frequency of TCRβ$^+$CD8$^+$ T cells (upper-row) and PD1$^+$TOX$^+$ CD8$^+$ T cells (lower-row) in the tumor at day 10 after drug treatment. (C) TOX mean fluorescence intensity (MFI) of PD1$^+$CD8$^+$ T cells in tumor. Statistical analysis was performed by Two-way ANOVA with Bonferroni post hoc test. Data are Mean±SD 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(A) or One-way ANOVA(C) with Turkey's post hoc test.

Conclusion

NT-I7 can be applied in combination with T cell suppressor blockades 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
- Increased # T$_{pex}$ in tumor
- Increased % T$_{trans}$ in tumor
- Increased T$_{CM}$ and CD8$^+$/Treg ratio in tumor

YH29143 (anti-TIGIT) increases % of more differentiated CD8$^+$ T cells in the tumor
- Increased % PD-1$^+$ in tumor
- Increased % PD-1$^-$ T$_{EM}$ in tumor

Aflibercept (anti-VEGF) decreases the expression of suppressive proteins by CD8$^+$ T cells in the tumor
- Decreased % PD1$^+$CD8$^+$ T cells in tumor
- Decreased TOX expression in PD1$^+$CD8$^+$ T cells in tumor

For more information, please contact science@neoimmunetech.com