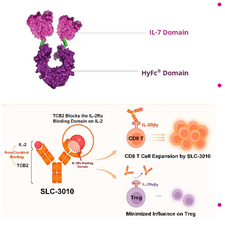
Abstract # ABST-000187

The combination of NT-I7 and hIL-2/TCB2c promotes the development of an immune-stimulatory tumor microenvironment that enhances the anti-tumor efficacy in combination with checkpoint inhibitors

Minji Lee[1], Sun-Kyoung IM[1], Seungtae Baek[1], Mankyu Ji[1], Miyoung Kim[1], Eun Ju Lee[1], Seung Taek Ji[1], Sara Ferrando-Martinez[2], Alexandra Wolfarth[2], Jun-Young Lee[3], Daeun Kim[3], Donghoon Choi[1]

[1]Research Institute of NeoImmuneTech, Co., Ltd., Pohang, 37666, Republic of Korea
[2]NeoImmuneTech, Inc., Rockville, MD, USA
[3]Selecxine, Pohang, 37666, Republic of Korea

BACKGROUND



- NT-I7 (efineptakin alfa), a long-acting IL-7, is a potent T cell amplifier that increases systemic stemness as a monotherapy or in combination with checkpoint inhibitors (CPIs). NT-I7 combination has been shown to boost immunotherapy antitumor efficacy in both preclinical and clinical settings.
- The IL-2/anti-human IL-2 mAb complex, IL-2/TCB2c (SLC-3010), is an IL-2 complexed with a novel anti-human IL-2 antibody clone, TCB2, which binds to the central part of the CD25-binding interface of IL-2. IL-2/TCB2c preferentially stimulates CD122^{high}CD8+ T cells and NK cells over regulatory T (Treg) cells and has been shown to exhibit strong anti-tumor activity in various mouse tumor models.
- Here, we aim to explore the anti-tumor efficacy and the underlying mechanisms of a combined therapy involving NT-I7 and IL-2/TCB2c.

STUDY DESIGN

- MC38 colon carcinoma cells were inoculated subcutaneously in the flank of syngeneic C57BL/6 background female mice. At 7-10 days after MC38 cell injection, mice were randomized into treatment groups.
- Treatment was administered as follows:
  - NT-I7; a single intramuscular injection of 10 mg/kg
  - IL-2/TCB2c; a single intravenous injection of 0.9 mg/kg,
  - Anti-PD-1; intraperitoneal injection of 5 mg/kg every three days; total of three.
- To compare the composition of immune cells between each monotherapy and the combination therapy, we analyzed tumors, tumor-draining lymph nodes (inguinal), and peripheral blood by flow cytometry at 4- , 7-, and 10-days after first injection.

RESULTS

The combination of NT-I7 and hIL-2/TCB2c enhances anti-tumor efficacy

- Both NT-I7 and hIL-2/TCB2c monotherapies each resulted in significant delays in tumor growth compared to the untreated group.
- Combination of NT-I7 and hIL-2/TCB2c significantly inhibited the growth of MC38 tumors compared to the non-treated control or either monotherapy.

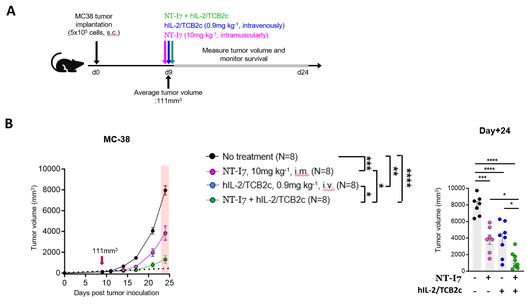


Figure 1. NT-I7 and hIL-2/TCB2c combination therapy significantly improves anti-tumor activity compared with single agents. (A) Schematic overview of experimental design. MC38 tumor cells were injected subcutaneously at day 0 (d0). 10 mg/kg NT-I7 or 0.9 mg/kg hIL-2/TCB2c was administered intramuscularly and intravenously on d9. Tumor growth of MC38 tumors treated with the indicated therapies (n = 8 per group) is shown as (B) average tumor size per treatment group; red arrow indicates treatment start; tumor volumes d24 post-implant are highlighted in a bar graph.

Comprehensive profiling of immune infiltrates following treatments

- NT-I7 treatment decreases the proportions of CD11b+ cells, leading to higher ratio of CD8+ to CD11b+ cells
- The significant and persistent expansion of CD8 T cells in tumor and tumor-draining lymph node is mainly mediated by NT-I7.

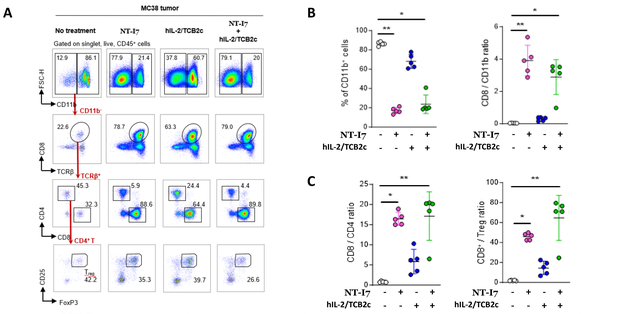


Figure 2. NT-I7 treatment results in increased CD8+/CD11b+ ratio and CD8+/Treg ratio in the tumor. (A) Representative flow cytometry plots showing the frequency of CD11b+, CD8+, CD4+, CD4+CD25+FoxP3+ regulatory T cells isolated from MC38 tumors 7 days after treatment and (B) Summary graphs showing the percentage of CD11b+ cells (left) and CD8/CD11b ratio (right) at 7 days post-treatment. (C) Pooled data showing CD8/CD4 ratios (left) and CD8/Treg ratios (right) in tumor.

NT-I7 reduces the proportion of the more immunosuppressive TIM-3+CD39+ Treg cells

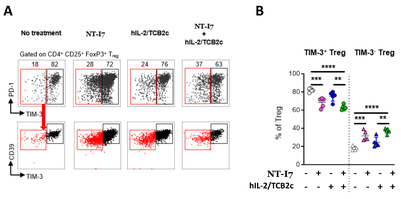


Figure 3. NT-I7 significantly reduces the frequency of TIM-3+CD39+ Treg cells within the tumor. (A) Representative flow plots showing the percentage of two subpopulations of intratumoral Treg cells based on the expression of TIM-3. Red dots represent the TIM-3- Tregs. (B) Pooled data of the frequency of TIM-3+ and TIM-3- Treg cells is shown in the right panel. *P<0.05; **P<0.01; ***P<0.001, ****p<0.0001; summary data shown as mean ± SD.

NT-I7 drives the treatment-related expansion and persistence of CD8+ T cells

- The CD8 T cell count in the tumor significantly increases after 4 days and reached its peak at 7 days following NT-I7 or hIL-2/TCB2c administration.
- The significant and persistent expansion of CD8 T cells in the tumor and the peripheral organs (tumor-draining lymph node and peripheral blood) is driven by NT-I7.

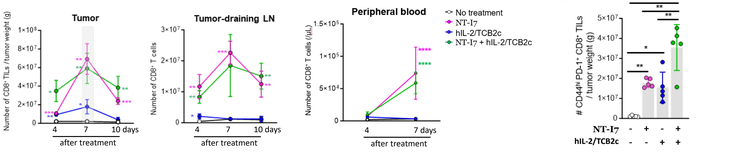


Figure 4. Expansion kinetics of CD8+ T cells from tumor-bearing mice following treatment with individual monotherapies or combination therapy. (A) Absolute counts of total CD8+ T cells in tumor (left), tumor-draining lymph node (middle), and peripheral blood (right) from MC38 tumor-bearing mice at 4-, 7- and 10 days post-treatment (B) Absolute numbers of CD44^{high}PD-1+CD8+ T cells in MC38 tumors. *P<0.05; **P<0.01; ***P<0.001, ****p<0.0001; summary data shown as mean ± SD.

NT-I7 and hIL-2/TCB2c greatly increases both tumor non-specific and -specific CD8+ T cells within tumors

- NT-I7 monotherapy increases the absolute numbers of both tumor-reactive and tumor non-reactive CD8+ T cells, with a stronger and significant effect observed in the non-reactive compartment
- The combination of NT-I7 plus hIL-2/TCB2c significantly increases the absolute number and frequency of polyclonal tumor-reactive and tumor-specific/reactive p15E-specific CD8+ T cells.

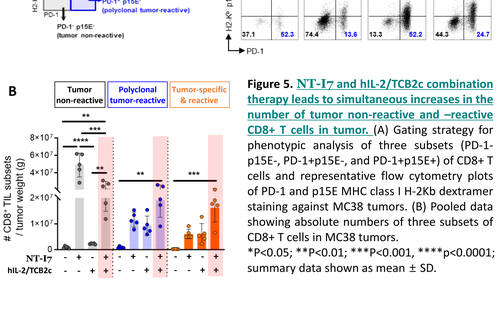


Figure 5. NT-I7 and hIL-2/TCB2c combination therapy leads to simultaneous increases in the number of tumor non-reactive and –reactive CD8+ T cells in tumor. (A) Gating strategy for phenotypic analysis of three subsets (PD-1-p15E-, PD-1+p15E-, and PD-1+p15E+) of CD8+ T cells and representative flow cytometry plots of PD-1 and p15E MHC class I H-2Kb dextramer staining against MC38 tumors. (B) Pooled data showing absolute numbers of three subsets of CD8+ T cells in MC38 tumors.
*P<0.05; **P<0.01; ***P<0.001, ****p<0.0001; summary data shown as mean ± SD.

NT-I7 selectively increases CD62L+Ly108+ early progenitor of exhausted CD8+ T cells

- NT-I7 significantly increases the percentage and absolute number of CD62L+Ly108+ early progenitor of exhausted CD8+ T cells (TCF1+TIM-3-; T_{PEX}). NT-I7 enables T_{PEX} cells to retain a less-differentiated state.
- After hIL-2/TCB2c treatment, the proportion of terminally exhausted CD8+ T cells (TCF1-TIM-3+; $T_{EX\ term}$) is significantly increased, followed by the drastic reduction in the frequency of T_{PEX} cells.
- The combination strategy can prevent severe CD8+ T cell exhaustion, by increasing in the number of T_{PEX}.

Figure 6. NT-I7 can expand and maintain the progenitor pool of exhausted CD8+ T cells, whereas hIL-2/TCB2c induces terminal differentiation into PD-1+TIM-3+CD8+ T cells. (A) Gating strategy for the phenotypic heterogeneity of exhausted CD8+ T cells in the tumor: early T_{PEX} (PD-1+CD44+TCF1+TIM-3-CD62L+Ly108+), T_{PEX} (PD-1+CD44+TCF1+TIM-3-), $T_{EX\ int}$ (PD-1+CD44+TCF1-TIM-3-), and $T_{EX\ term}$ (PD-1+CD44+TCF1-TIM-3+) cells. (B) Representative flow cytometry plots of CD62L+Ly108+ early T_{PEX} cells among PD-1+CD44^{high} CD8+ TILs (C) Percentage (left) and absolute counts (right) of CD62L+Ly108+ early T_{PEX} cells. (D) Representative plots showing frequencies and (E) absolute numbers of T_{PEX}, $T_{EX\ int}$, $T_{EX\ term}$. (F) Pooled data shows the percentage of CD101- and CD101+ on CD8+ $T_{EX\ term}$.
*P<0.05; **P<0.01; ***P<0.001, ****p<0.0001; summary data shown as mean ± SD.
T_{PEX}, progenitor exhausted T cell. $T_{EX\ int}$, intermediate exhausted T cell. $T_{EX\ term}$, terminally exhausted T cell.

NT-I7 promotes proliferation of CD8+T_{PEX} cells within tumor-draining lymph nodes

Figure 7. NT-I7 significantly increases the proliferation rate of CD8+ T_{PEX} cells. (A) Representative contour plots showing Ki-67+ T_{PEX} cells in tumor-draining lymph node (tdLN) (B) Frequencies of Ki-67+ T_{PEX} among total CD8+ T_{PEX} cells in tdLN.

NT-I7 and hIL-2/TCB2c improves the antitumor efficacy of PD-1 blockade therapy

Figure 8. NT-I7 and hIL-2/TCB2c combination synergizes with PD-1 blockade. (A) Average growth curves of MC38 tumors (B) Pooled data showing the percentage of tumor-free mice.

CONCLUSIONS

- NT-I7 can expand and maintain the progenitor pool of exhausted CD8+ T cells, whereas hIL-2/TCB2c promotes their differentiation into $T_{EX\ term}$.
- NT-I7 and hIL-2/TCB2c can overcome the limited therapeutic effectiveness of PD-1 blockade, culminating in the complete regression of tumors.
- NT-I7 could be a mainstay modality for expanding tumor-infiltrating T_{PEX} cells, thereby improving efficacy of both IL-2 and anti-PD-(L)1 cancer immunotherapy.

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For more information, please contact Dr. Donghoon Choi (dhchoi@neoimmunetech.com)