Preclinical evaluation of the anti-tumor activity of Fc-fused interleukin-7 in both monotherapy and combination therapy

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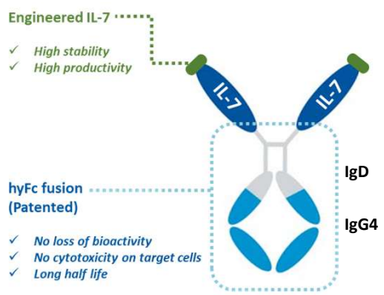
Abstract

A remarkable progress of cancer immunotherapy in a recent decade, including immune checkpoint blockades (ICB), has shed a new light on the medical treatment of cancer patients. These successes of immunotherapies affirm the notion that modulation of immune-related environment, although not directly targeting a tumor cell, might lead to a better efficacy for cancer treatment. Interleukine-7 (IL-7), a member of the common γ chain family cytokine, plays important roles in the development and homeostasis of lymphocytes in both mouse and human, in particular T lymphocytes. Positive effects of recombinant IL-7 on anti-tumor activity in preclinical models have placed IL-7 as a strong candidate for a novel immunotherapeutic agent in clinics; however, a short half-life of recombinant protein has remained a challenge. Here, we investigated anti-tumor effects in mice of NT-I7, the long-acting form of recombinant human IL-7 fused with hybrid Fc (IL-7-hyFc) in syngeneic tumor models. A dramatic inhibition of tumor growth was achieved when NT-I7 is given in a single subcutaneous injection. NT-I7 administration significantly enhanced both absolute number and frequency of $CD8^+$ T cells in a dose-dependent manner. The frequency of $CD8^+$ tumor-infiltrating lymphocytes (TILs) was highly increased after NT-I7 treatment in several different syngeneic tumor models as well. Of interest, the fraction of PD-1^+CD8^+ TILs was decreased by NT-I7 treatment. Therefore, NT-I7 is able to expand tumor antigen specific $CD8^+$ effector T cells, resulting in the enhanced infiltration and functional recuperation of TILs. To increase the therapeutic efficacy of NT-I7, we combined single injection of the conventional chemotherapeutics cyclophosphamide (CPA) with a moderate dose in which CPA confers immunogenic tumor cell death without severely depleting immune compartment. The combinatorial treatment of NT-I7 with CPA increased T cells in periphery, especially $CD8^+$ T subpopulations. The combination therapy of NT-I7 with CPA augmented the infiltration of $CD8^+$ TILs and the ratio of $CD8^+$ to Treg, leading to an enhanced antitumor efficacy in the advanced tumor model. In sum, NT-I7 confers the effective anti-tumor responses through reconstructing $CD8^+$ T lymphocytes; this activity was highly enhanced in combination with the chemotherapeutics. Thus, these results imply that NT-I7 can be applied to various cancer immunotherapy regimens as monotherapy or a combination partner with conventional and other immunotherapy, such as ICB.

Schematic Diagram of NT-I7 (long acting IL-7 fused with non-cytolytic Fc)

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



Acknowledgements

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Dose-dependent Tumor Suppression by NT-I7 Monotherapy

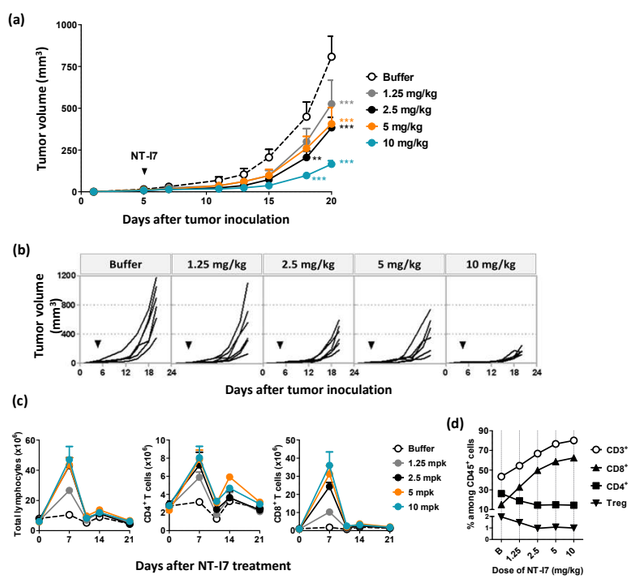

Figure 1. C57BL/6 mice with MC-38 tumors were treated with different doses of NT-I7 subcutaneously after 5 days of tumor inoculation. **(a)** Tumor growth curves of all groups. **(b)** Tumor growth curves of individual mice. **(c)** Changes in absolute numbers of total lymphocytes, CD4 single-positive(SP) and CD8 SP T cells in blood from treated mice. Percentages of CD4 or CD8 SP cells were calculated based on the total lymphocytes counts obtained per volume (mL) of blood by CBC counter. **(d)** Percentages of T lymphocytes compartments among $CD45^+$ cells from blood at 7 days post treatment. **p<0.01, ***p<0.001 versus Control by two-way ANOVA with Bonferroni post-tests for tumor volumes. Data are mean±SEM.

NT-I7 Monotherapy Inhibits Tumor Growth by Increasing $CD8^+$ TILs

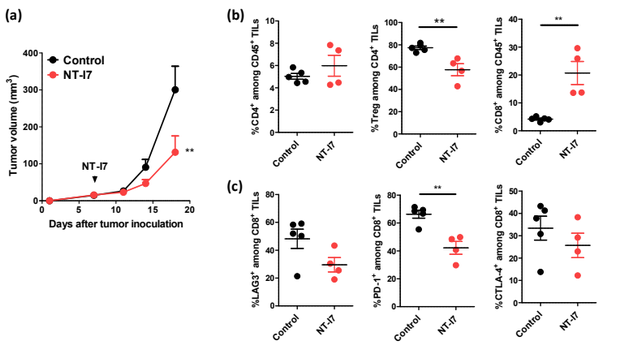

Figure 2. C57BL/6 mice bearing MC-38 tumors were treated with 1.25 mg/kg of NT-I7 subcutaneously after 7 days of tumor inoculation. **(a)** Tumor growth curves. Analysis of tumor-infiltrating lymphocytes(TILs) was done after 7 days of NT-I7 treatment. **(b)** Different T lymphocytes compartments among $CD45^+$ or CD4 SP TILs. **(c)** Percentages of $LAG3^+$, PD-1^+ or CTLA-4^+ cells among CD8 SP TILs. **p<0.01 versus Control by two-way ANOVA with Bonferroni post-tests for tumor volumes or t-test for other graphs. Data are mean±SEM.

NT-I7 Increases $CD8^+$ TILs in Several Tumor Models

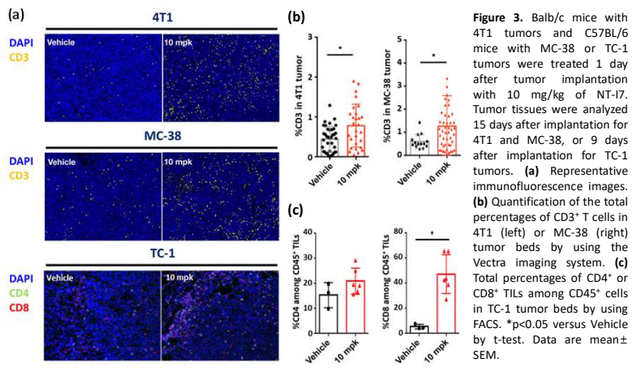

Figure 3. Balb/c mice with 4T1 tumors and C57BL/6 mice with MC-38 or TC-1 tumors were treated 1 day after tumor implantation with 10 mg/kg of NT-I7. Tumor tissues were analyzed 15 days after implantation for 4T1 and MC-38, or 9 days after implantation for TC-1 tumors. **(a)** Representative immunofluorescence images. **(b)** Quantification of the total percentages of $CD3^+$ T cells in 4T1 (left) or MC-38 (right) tumor beds by using the Vectra imaging system. **(c)** Total percentages of $CD4^+$ or $CD8^+$ TILs among $CD45^+$ cells in TC-1 tumor beds by using FACS. *p<0.05 versus Vehicle by t-test. Data are mean± SEM.

CPA Treatment Enhances the T-cell Increasing Activity of NT-I7

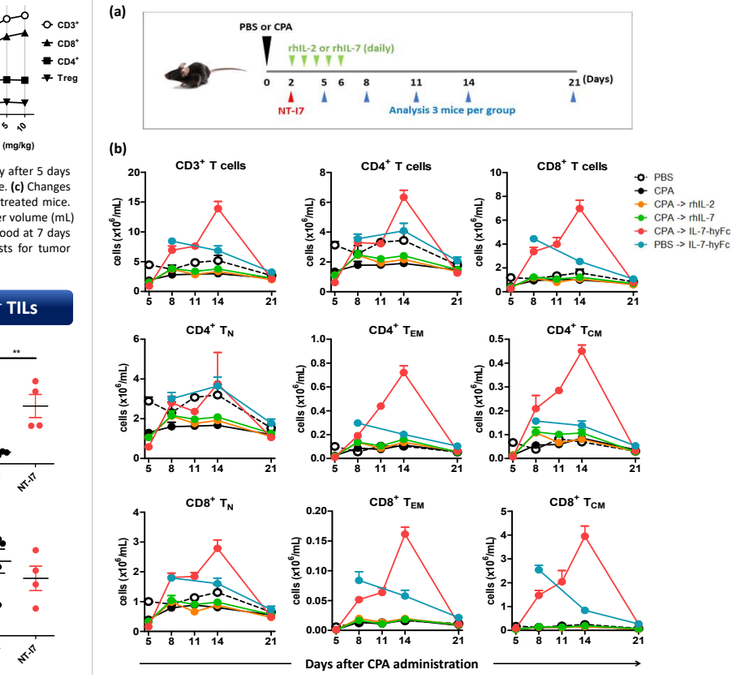

Figure 4. C57BL/6 mice were treated with PBS or 150 mg/kg of CPA intraperitoneally. Some groups of mice were subcutaneously treated with 1 ug of recombinant cytokines daily for 5 times or 25 ug of NT-I7 which is composed with 5 ug of hIL-7(by ELISA). The absolute numbers of lymphocytes compartments were analyzed after 5, 8, 11, 14 and 21 days of CPA treatment from PBMCs by CBC and FACS. **(a)** Experimental scheme. **(b)** Kinetics of lymphocytes counts. CD44 and CD62L were used as markers for gating of T lymphocytes subsets; naïve (T_N;$CD44^-CD62L^+$), effector-memory (T_{EM};$CD44^+CD62L^-$), and central-memory (T_{CM};$CD44^+CD62L^+$) phenotypes. Data are mean±SEM.

Titration of CPA Dose for Suppressing Tumor Growth

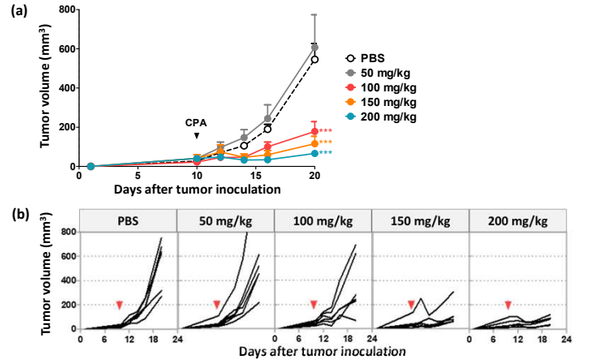

Figure 5. C57BL/6 mice with advanced MC-38 tumors were treated with different doses of CPA after 10 days of tumor inoculation. **(a)** Tumor growth curves of all groups. **(b)** Tumor growth curves of individual mice. Arrows mean treatment time point. ***p<0.001 versus PBS by two-way ANOVA with Bonferroni post-tests. Data are mean±SEM.

Combined Therapy with NT-I7 and CPA Enhances Antitumor Efficacy in the Advanced Tumor Model

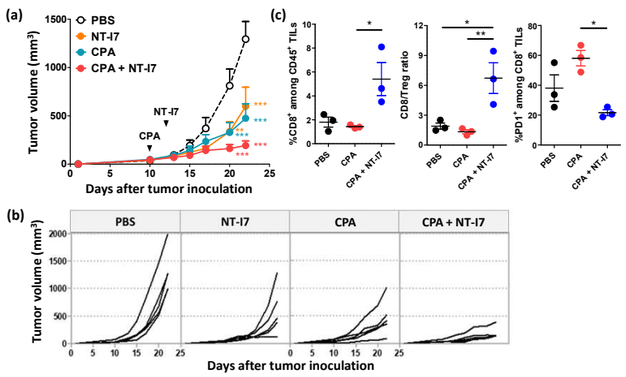

Figure 6. (a-b) C57BL/6 mice bearing MC-38 advanced tumors were injected with 100 mg/kg of CPA, 10 mg/kg of NT-I7, or combined treatment. CPA was administered intraperitoneally on day 10 after tumor inoculation, NT-I7 was administered subcutaneously on day 12 after tumor inoculation. **(a)** Tumor growth curves of all groups. **(b)** Tumor growth curves of individual mice. Arrows mean treatment time points of each treatment. **p<0.01; ***p<0.001 versus PBS by two-way ANOVA with Bonferroni post-tests. **(c)** C57BL/6 mice with advanced MC-38 tumors were injected with 150 mg/kg of CPA on day 14 after tumor inoculation, 1.25 mg/kg of NT-I7 on day 16 after tumor inoculation. TILs were analyzed after 7 days of NT-I7 treatment. *p<0.05, **p<0.01 versus Control by one-way ANOVA with Tukey's multiple comparison test. Data are mean±SEM.

Summary

- ✓ Single systemic administration of NT-I7 inhibits the growth of tumors in a dose-dependent manner.
- ✓ The antitumor effect of NT-I7 is achieved by elevation of tumor-specific $CD8^+$ T cells that infiltrate into tumor.
- ✓ Combined therapy with NT-I7 and CPA increases T cells, which leads to the dramatic antitumor effect in mice bearing advanced tumors, compared to monotherapy.
- ✓ Our data suggest that NT-I7, the long-acting form of human IL-7, might be a suitable combination partner for other cancer therapeutic agents, like chemo-reagents or immune checkpoint blockade.