rhIL-7-hyFc (efineptakin-alfa, NT-I7) increases tumor-specific CD8⁺ T cells despite FOLFOX cytotoxicity effect

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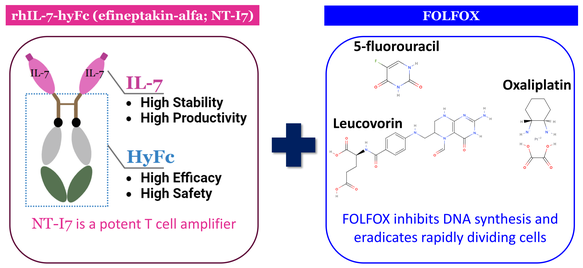
Abstract

rhIL-7-hyFc (efineptakin-alfa; NT-I7) is a potent T cell amplifier, comprised of two molecules of interleukin-7 (IL-7) fused to the hybrid Fc domain of IgD/IgG4 immunoglobulin. NT-I7 safely, significantly and persistently increases the absolute lymphocyte counts (ALC) in humans in a dose-proportional manner. Importantly, this increase is primarily due to an increase in T cells (1). Previous studies have shown that NT-I7 not only significantly increases the number of CD8⁺ T cells in peripheral blood but also dramatically boosts the presence of tumor-infiltrating CD8⁺ T cells, including both tumor-specific (p15E⁺PD-1⁺) and tumor non-specific (PD-1⁻) CD8⁺ T cells in mice (2). FOLFOX is a chemotherapy regimen composed of 5-fluorouracil, leucovorin, and oxaliplatin that inhibits DNA duplication and eradicates rapidly dividing cells (3). Although the mechanism of FOLFOX results in direct tumor killing, it also has the potential to kill newly dividing lymphocytes during NT-I7 treatment (4-6). Therefore, in this study, we aimed to evaluate the feasibility of combining FOLFOX with NT-I7 in a syngeneic MC38 mouse tumor model. Our results show that NT-I7 improves the efficacy of FOLFOX, irrespective of the timing of NT-I7 administration: simultaneous administration or delayed 3 hours, or 2 days after FOLFOX administration. This improvement persists despite a significant decrease in ALC for the first 6 days, which was significantly lower than the ALC observed with FOLFOX alone. FOLFOX decreases the absolute numbers of tumor non-specific CD8⁺ T cells. However, adding FOLFOX does not affect the NT-I7 driven increase of tumor-specific CD8⁺ T cells within the tumor. Taken together, our data demonstrate that FOLFOX does not compromise NT-I7's ability to increase tumor-specific T cells within the tumor and the combination enhances the anti-tumor response irrespective of the timing of NT-I7 administration.

Schematic Hypothesis



Considering that FOLFOX is a cytotoxic chemotherapy regimen that inhibits DNA synthesis and eradicates rapidly dividing cells, and that it also has the potential to kill newly dividing lymphocytes during NT-I7 treatment, is it possible to combine NT-I7 with the FOLFOX regimen, and would this increase efficacy?

NT-I7 improves the efficacy of FOLFOX, irrespective of the timing of NT-I7 administration

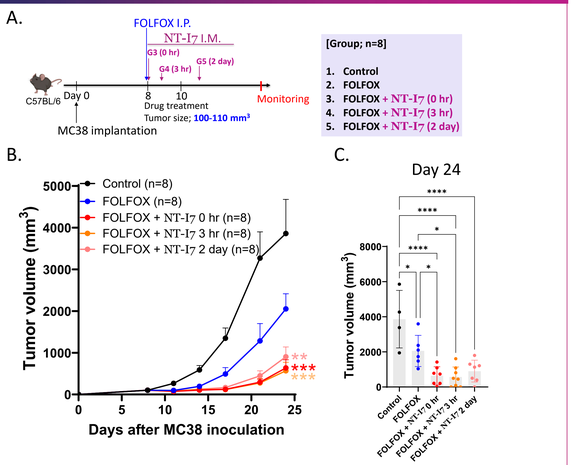


Figure 1. Comparison of anti-tumor efficacy between time-interval and simultaneous treatment in NT-I7 and FOLFOX combination therapy in MC38 syngeneic tumor model. C57BL/6 mice implanted with MC38 tumors (5 × 10^5 cells) were intraperitoneally dosed with FOLFOX (5-fluorouracil 50 mg/kg, Leucovorin 90 mg/kg, and Oxaliplatin 6 mg/kg) on day 8. NT-I7 (10 mg/kg) was then administered intramuscularly either simultaneously with FOLFOX, or 3 hours or 2 days post FOLFOX administration (n=8 per group). (A) Schematic overview of experimental design. (B) Tumor growth curves of subcutaneous MC38 tumors in syngeneic mice treated with the indicated therapies. (C) Tumor volumes on indicated day post implant. The data represent the Mean ± SEM (B) or ± SD (C). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. The *P value* in (B) represents comparison between the FOLFOX monotherapy group and NT-I7 combination groups. Two-way (B) or one-way ANOVA (C) with Tukey's test were done.

FOLFOX has transient effect on NT-I7-induced ALC increase regardless of NT-I7 timing

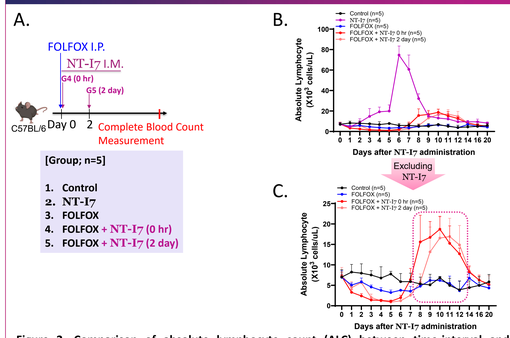


Figure 2. Comparison of absolute lymphocyte count (ALC) between time-interval and simultaneous treatment in NT-I7 and FOLFOX combination therapy in naïve mice. Naïve B6 mice were intraperitoneally dosed with FOLFOX (5-fluorouracil 50 mg/kg, Leucovorin 90 mg/kg, and Oxaliplatin 6 mg/kg) on day 0. NT-I7 (10 mg/kg) was then administered intramuscularly either simultaneously with FOLFOX, or 2 days post FOLFOX administration (n=5 per group). (A) Schematic overview of experimental design. (B) Kinetics of ALC in the blood at the indicated time points after treatment with the indicated therapies. (C) Kinetics of ALC in the blood excluding the NT-I7 monotherapy group, as shown in the (B), at the indicated time points after treatment with the indicated therapies. The data represent the Mean ± SD.

FOLFOX reduces peripheral blood PD-1⁻CD8⁺ T cells, but not tumor-specific (p15E⁺PD-1⁺) CD8⁺ T cells in the tumor microenvironment

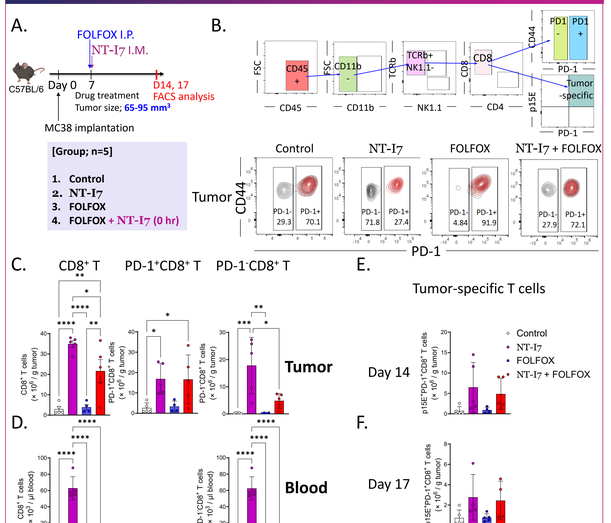


Figure 3. FOLFOX impact on the reduction of peripheral blood PD-1⁻CD8⁺ T cells, but not PD-1⁺CD8⁺ T cells in the tumor microenvironment. B6 mice implanted MC38 tumors (5 × 10^5 cells) were intraperitoneally dosed with FOLFOX (5-fluorouracil 50 mg/kg, Leucovorin 90 mg/kg, and Oxaliplatin 6 mg/kg) on day 7. NT-I7 (10 mg/kg) was then administered intramuscularly simultaneously with FOLFOX. (C, E, F) Tumor tissues or (D) peripheral blood was harvested on (C-E) day 14 or (F) day 17. (A) Schematic overview of experimental design. (B) Schematic overview of the gating strategy (upper) and representative plots (lower). (C) Absolute numbers of CD8⁺, PD-1⁺CD8⁺, and PD-1⁻CD8⁺ T cells in MC38 tumors. (D) Absolute numbers of CD8⁺ and PD-1⁻CD8⁺ T cells in peripheral blood. (E, F) Absolute numbers of p15E⁺PD-1⁺CD8⁺ T cells in MC38 tumors. The data represent the Mean ± SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. One-way ANOVA with Tukey's test was done.

NT-I7 improves anti-tumor efficacy when combined with repeated administration of FOLFOX

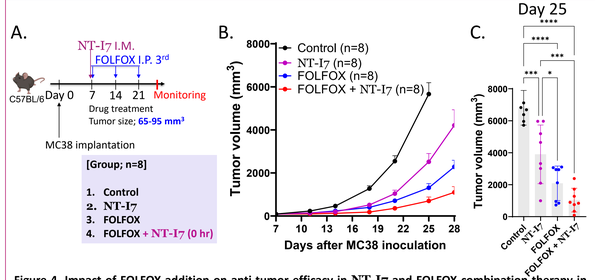
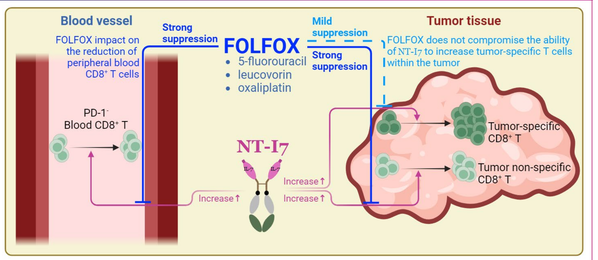


Figure 4. Impact of FOLFOX addition on anti-tumor efficacy in NT-I7 and FOLFOX combination therapy in the MC38 syngeneic tumor model. B6 mice implanted MC38 tumors (5 × 10^5 cells) received three intraperitoneal doses of FOLFOX (5-fluorouracil 50 mg/kg, Leucovorin 90 mg/kg, and Oxaliplatin 6 mg/kg) on days 7, 14, and 21. NT-I7 (10 mg/kg) was administered intramuscularly at the same time as the first dose of FOLFOX on day 7 (n=8 per group). (A) Schematic overview of experimental design. (B) Tumor growth curves of subcutaneous MC38 tumors in syngeneic mice treated with the indicated therapies. (C) Tumor volumes on indicated day post implant. The data represent the Mean ± SEM (B) or ± SD (C). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Two-way (B) or one-way ANOVA (C) with Tukey's test were done.

Conclusions

- **NT-I7 significantly improves the anti-tumor efficacy of FOLFOX in the MC38 syngeneic tumor model, regardless of administration timing.**
- **Enhanced efficacy stemming from NT-I7 persists, despite a significant decrease in ALC induced by FOLFOX.**
- **NT-I7-driven increase in tumor-specific CD8⁺ T cells is not affected by FOLFOX, despite the decrease in absolute numbers of peripheral blood CD8⁺ T cells and tumor non-specific CD8⁺ T cells.**
- **NT-I7 improves anti-tumor efficacy when combined with repeated administration of FOLFOX in the MC38 syngeneic tumor model.**

The proposed mechanism of FOLFOX and NT-I7 combination therapy



※ Take home message

Despite FOLFOX's impact on certain immune cells, NT-I7 significantly enhances the anti-tumor efficacy of FOLFOX by maintaining an increase in tumor-specific CD8⁺ T cells. In conclusion, our study provides compelling evidence supporting the potential of NT-I7 to be effectively combined with cytotoxic chemotherapy like FOLFOX.

References

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