NT-I7, a novel long-acting interleukin-7, improves engraftment of patient immune cells and efficacy of anti-PD-1 therapy in a preclinical humanized melanoma model

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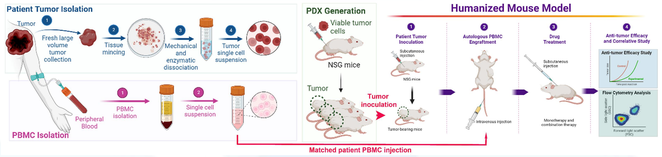
BACKGROUND

Although immune checkpoint inhibitors (ICIs) have led to significant improvements in melanoma survival, half of these patients fail to benefit, necessitating the discovery of novel strategies. There is a pivotal need for the development of preclinical models to evaluate 'next-generation' immunotherapies prior to clinical investigation.

In this study, we developed and optimized our all-autologous humanized melanoma mouse model for the study of NT-I7 (efineptakin alfa), a long-acting human IL-7. Because NT-I7 has been shown to enhance T cell proliferation and survival in both humans and mice, we hypothesized NT-I7 would both improve the engraftment of patient immune cells and the efficacy of anti-PD-1 therapy in our humanized mouse model.

METHODS

Both tumor cells and peripheral blood lymphocytes (PBLs) were collected from melanoma patients to establish our autologous humanized melanoma mouse model. Tumor-bearing NSG mice were infused with matched melanoma PBLs prior to receiving 10 mg/kg NT-I7. Optimization of the model was performed and evaluated by measuring engraftment of the patient immune cells via flow cytometry and comparing tumor growth. The optimized autologous melanoma model was then used to test the efficacy of NT-I7 combined with anti-PD-1 therapy.



CONCLUSIONS

- Our all-autologous humanized melanoma mouse model allows us to evaluate novel immunotherapies in the context of matched patient immune and tumor cells.
- NT-I7 is well tolerated and dramatically improves engraftment of patient PBLs with CD4+ T cell enrichment in both the periphery and tumor.
- NT-I7 and anti-PD-1 combination therapy significantly enhanced the anti-tumor response.

In sum, we have developed a platform to feasibly design NT-I7 combination therapies to identify optimal strategies that can be translated into clinical investigations.

Implications: NT-I7-induced CD4+ T cells could play a key role in regulating tumor progression. Of note, tumor-specific CD4+ T cells in human melanoma have been shown to correlate with CD8 T cell, B cell and macrophage function, including survival and activation[1-3].

FUNDING

NeoImmuneTech

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REFERENCES

[1] Veatch et al., Apr 2022, Cancer Cell 40*(4)*, 393-409.
[2] Alspach et al., Oct 2019, Nature 574*(7780)*, 696-701.
[3] Tran et al., May 2014, Science 344*(6184)*, 641-645.

RESULTS

Figure 1. NT-I7 promotes PBL engraftment in the periphery and tumor

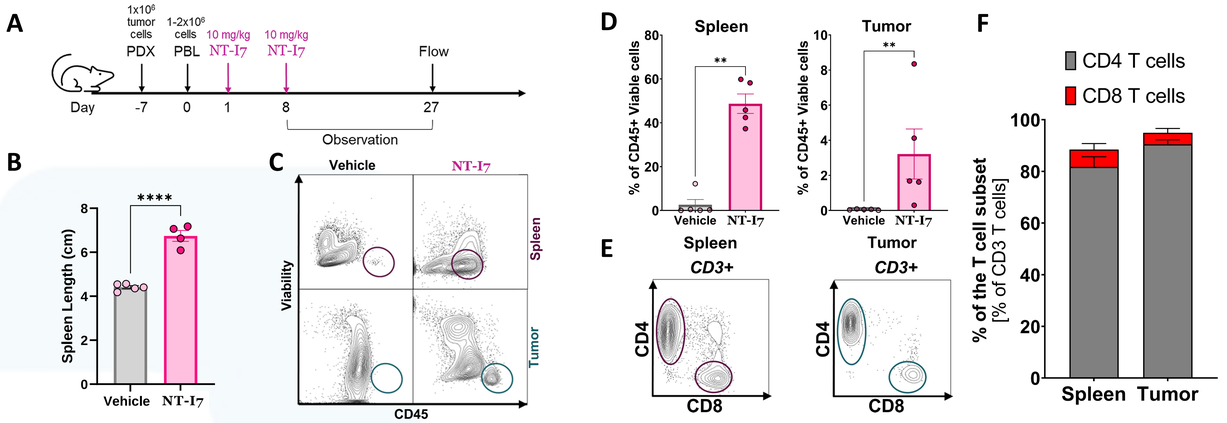

Fig 1. (A) Model optimization required transferring un-expanded human peripheral blood lymphocytes (PBLs) into tumor-bearing NSG mice, followed by subcutaneous administration of 10 mg/kg NT-I7. (B) Measurement of spleen length at day 27 post-PBL transfer. Mice treated with NT-I7 have larger spleens. Data graphed as mean ± SEM; **** p<0.0001; unpaired t test. (C) Flow analysis of spleen and tumor at day 27 post-PBL transfer. A representative dot plot showing enhancement of live CD45+ cells in the spleen and tumor after NT-I7 treatment is shown. (D) Quantification of viable CD45+ cells in the spleen and tumor, demonstrating a significant increase after NT-I7 treatment. Data graphed as mean ± SEM; ** p<0.01; Mann Whitney test. (E) Representative dot plot of CD4+ and CD8+ T cells from the spleen and tumor of NT-I7 treated mice. (F) Quantification of CD4+ and CD8+ frequency within the spleen and tumor, showing a majority of T cells are CD4+ after NT-I7 treatment.

Figure 2. NT-I7 improves anti-tumor response

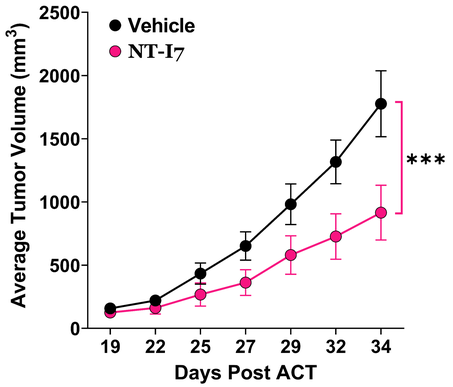

Fig 2. Tumor growth curves of NSG tumor-bearing mice treated with NT-I7 or vehicle control as shown in Figure 1A. NT-I7 treatment significantly improves tumor control. All error bars represent mean ± SEM;*** p-value < 0.001; 2-way ANOVA.

Figure 3. NT-I7 and anti-PD-1 combination therapy enhances CD45+ engraftment

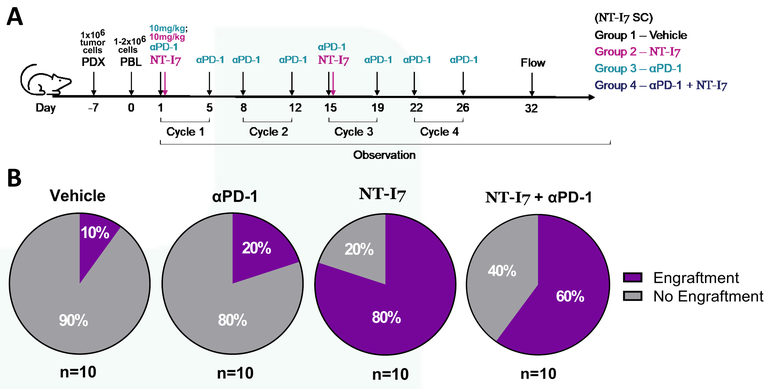

Fig 3. (A) Schematic illustration of experimental design for combination treatment. (B) On day 32 post-PBL transfer, spleens were collected and flow analysis was used to compare successful PBL engraftment. Engraftment was defined as having at least 500 viable CD45+ cells. Mice treated with NT-I7 and combination NT-I7 + anti-PD-1 show better PBL engraftment compared to either anti-PD-1-treated mice or vehicle control. (C) Further flow analysis was performed on samples with successful engraftment. Mice treated with NT-I7 (n=8) or NT-I7 + anti-PD-1 (n=6) consistently show most T cells are CD4+. All data graphed as mean ± SEM.

Figure 4. NT-I7 + anti-PD-1 enhances anti-tumor efficacy

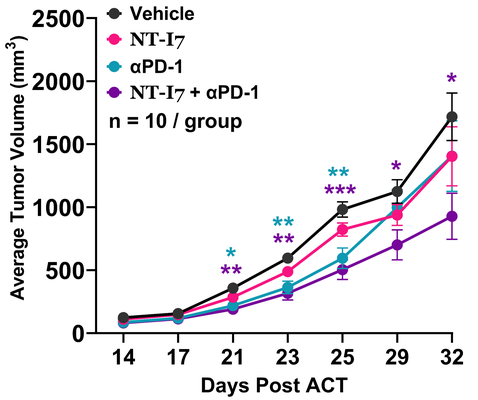

Fig 4. Tumor growth curves of NSG tumor-bearing mice treated as shown in Figure 3A. While NT-I7 and anti-PD-1 monotherapy had a slight effect on tumor growth, combination treatment significantly perturbs tumor growth. All error bars represent mean ± SEM; * p < 0.05; ** p < 0.01; *** p < 0.001; 2-way ANOVA, Dunnett's multiple comparisons test. purple*, vehicle vs NT-I7 + αPD-1; green*, vehicle vs αPD-1.