Long-acting recombinant interleukin-7, NT-I7, improves survival following oncolytic Zika virus treatment in the SB28 immunosuppressive and treatment-resistant murine glioma model

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Introduction

- Glioblastoma (GBM) does not respond to immunotherapy for several reasons, including limited cancer-specific neoantigens and poor T cell infiltration in the tumor microenvironment. The SB28 murine glioma model recapitulates these aspects of human GBM.
- A long-acting recombinant human IL-7, NT-I7 (NeoImmuneTech, Inc.), increases systemic T cell abundance and improves tumor infiltration. The addition of NT-I7 to temozolomide and radiation resulted in a modest survival benefit in mouse studies.[1]
- Intratumoral Zika virus (ZIKV) treatment for GBM works through multiple mechanisms, including direct tumor killing and induction of a CD8+ T cell anti-tumor response. However, ZIKV is less effective against the immunosuppressive and treatment-resistant SB28 tumors.[2]

Hypothesis

We hypothesized that combining NT-I7 with oncolytic ZIKV therapy would enhance the anti-tumor immune response and improve survival in mice bearing SB28 syngeneic tumors.

Methods

- Mouse model: 1×10³ SB28 tumor cells were intracranially implanted in C57BL/6J mice. Tumor engraftment was confirmed with bioluminescence imaging on day 6. Mice were treated with NT-I7 (10mg/kg, subcutaneous) on post tumor implantation day 7 and 10, followed by intratumoral ZIKV on day 14 to coincide with the peak in peripheral T cell expansion. Anti-PD1 (10mg/kg, intraperitoneal) treatment was given on days 15, 17, 19, and 21. Mice were monitored for survival. After 120 days, cured mice were rechallenged with a contralaterally implanted tumor.
- Immune profiling: Mice were bled weekly to assess the systemic T cell response following NT-I7 treatment. Immunoprofiling of brain, draining lymph node, and peripheral blood via flow cytometry was done on day 17 and 21.
- Statistical analysis: Statistical analyses were performed using GraphPad Prism7 (GraphPad Software). Expression of various markers between groups were compared using the two-sided Student's t-test or ANOVA. P-values <0.05 were considered statistically significant.

NT-I7 + intratumoral ZIKV significantly increases survival in SB28 models

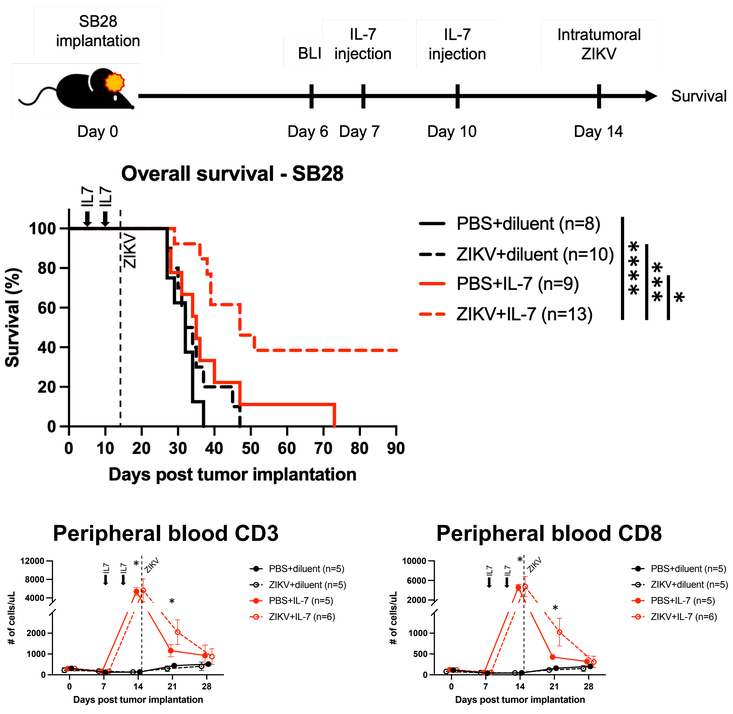

Figure 1: NT-I7 expands peripheral T cell abundance and NT-I7 + ZIKV significantly increases survival in SB28 mouse glioma model. The combination of NT-I7 and ZIKV treatment resulted in significant improvement in survival compared to NT-I7 or ZIKV treatment alone (p<0.05). Subcutaneous injection of NT-I7 significantly boosted systemic T cell abundance compared to control (5,623 cells/uL vs. 136 cells/uL, p<0.05), particularly cytotoxic CD8+ T cells (4,776 cells/uL vs. 47 cells/uL, p<0.05).

NT-I7 + ZIKV treatment increases infiltration of T cells into the tumor microenvironment

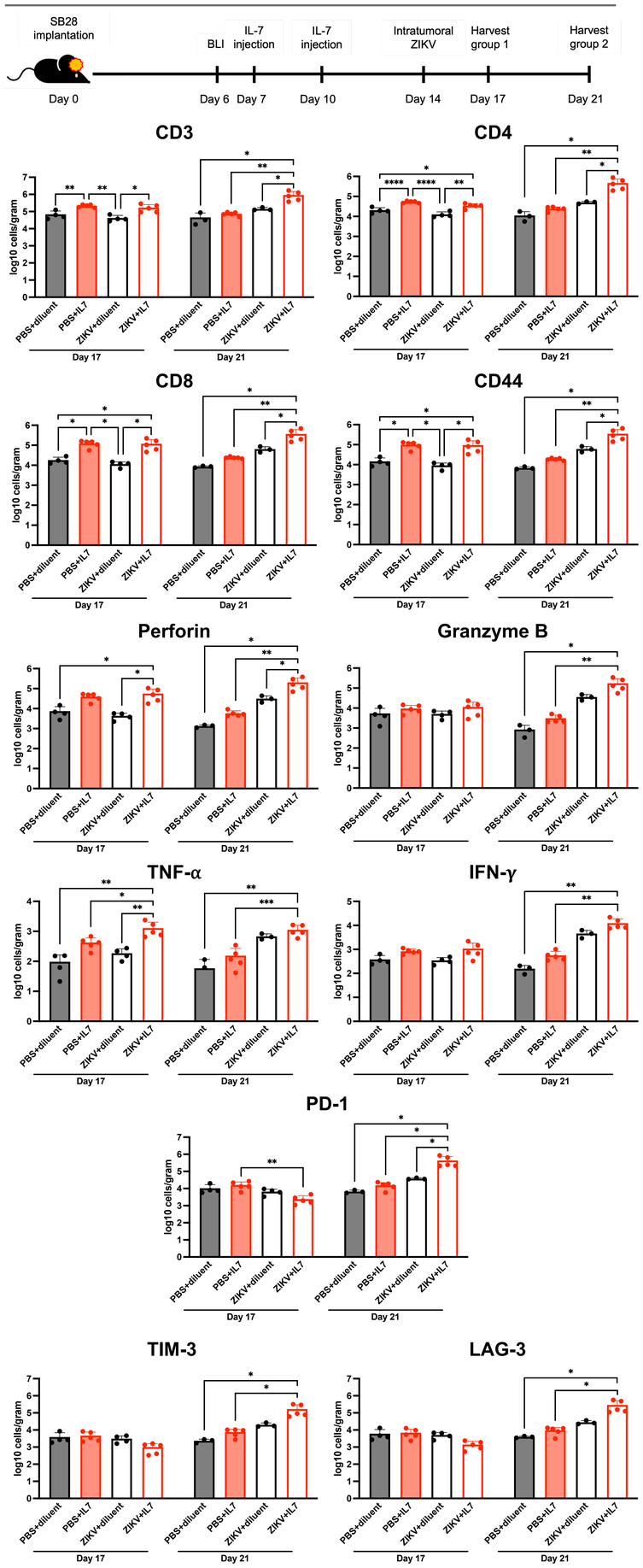

Figure 2: Immunoprofiling of brain tumor microenvironment via flow cytometry. NT-I7 + ZIKV treatment resulted in increased CD8+ T cell infiltration (369,814 cells/g vs. 23,947 cells/g and 63,961 cells/g, respectively) as well as increased perforin expression compared to either monotherapy (p<0.05). There was also increased T cell expression of granzyme B, TNF-α, and IFN-γ, compared to NT-I7 alone (p<0.05). On Day 21, but not Day 17, there were increased T cell exhaustion markers (PD-1, TIM-3 and LAG-3) in the NT-I7 + ZIKV treatment group.

Addition of immune checkpoint blockade further improves efficacy of NT-I7 + ZIKV treatment

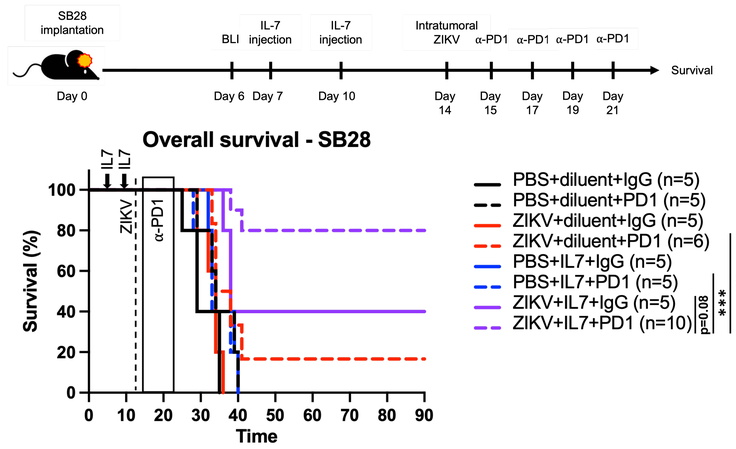

Figure 3: Addition of anti-PD1 checkpoint blockade further improves efficacy of NT-I7 and ZIKV treatment. Given the increased expression of T cell exhaustion markers seen at Day 21, we tested the hypothesis that adding an anti-PD1 checkpoint inhibitor with further improve survival. Overall, there was a trend towards improved overall survival (p=0.08) in the ZIKV + NT-I7 + anti-PD1 group compared to ZIKV + NT-I7 + IgG control.

Long-term survivors after NT-I7 + ZIKV treatment are protected against secondary syngeneic tumors

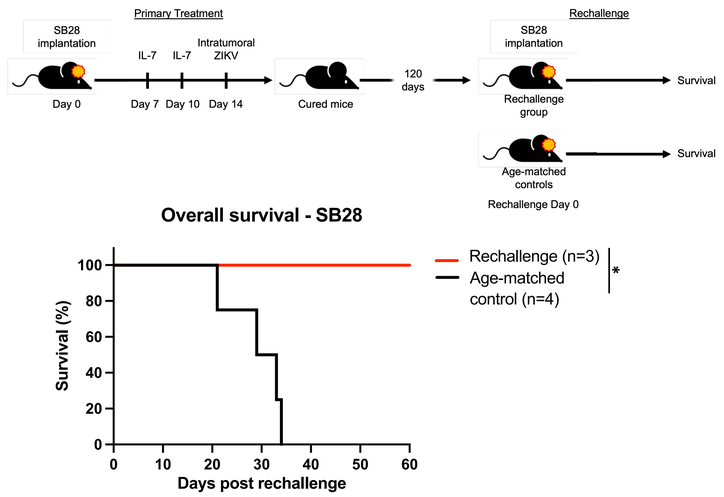

Figure 4: Long-term survivors after NT-I7 and ZIKV treatment are protected against tumor rechallenge. After 120 days following initial treatment, long-term survivors were rechallenged with left-sided implantation of a SB28 tumor. All long-term survivors were able to clear their tumor without additional treatment compared to 0/4 age-matched controls (p<0.05).

Conclusions

- Timing the oncolytic ZIKV injection with the peak in peripheral CD8+ T cells greatly increased tumor infiltration of cytotoxic T cells and improved survival in the immunotherapy resistant SB28 glioma model.
- Long-term survivors after NT-I7 + ZIKV treatment were protected against tumor rechallenge.
- Our work suggests a role for a new "prime and pull" approach for the treatment of highly immunosuppressive tumors such as GBM: priming the systemic immune system with NT-I7, followed by an oncolytic stimulus to draw them into the tumor microenvironment to engage and clear tumor cells.

Acknowledgements

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References

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2. Nair S, Mazzoccoli L, Jash A, et al. Zika virus oncolytic activity requires CD8+ T cells and is boosted by immune checkpoint blockade. *JCI Insight*. 2021;6(1):e144619.