The Combination of anti-TIM-3 and anti-PD-1 Checkpoint Inhibitors with Focused Radiation Resulted in a Synergistic Anti-Tumor Immune Response in a Preclinical Glioma Model

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Introduction

Checkpoint molecules like programmed death-1 (PD -1) and T cell immunoglobulin mucin-3 (TIM-3) act as negative regulators of the immune system and can be up-regulated in the setting of glioblastoma multiforme (GBM). Combined PD-1 blockade with a blocking antibody (Ab) and stereotactic radiosurgery (SRS) has been shown to improve antitumor immunity and produces long-term survivors in a murine glioma model [1]. However, tumor infiltrating lymphocytes (TILs) can express multiple checkpoints, and expression of =2 checkpoints (i.e. PD-1+TIM-3+) corresponds to a more exhausted T cell phenotype [2]. We hypothesized that adding a second checkpoint-blocking Ab could achieve additive or synergistic antitumor effects.

Objectives

- Determine the survival benefits of triple therapy with anti-PD-1 Ab, anti-TIM-3 Ab, and SRS in an orthotopic murine glioma model
- Demonstrate the effect of each treatment modality on the tumor immune microenvironment

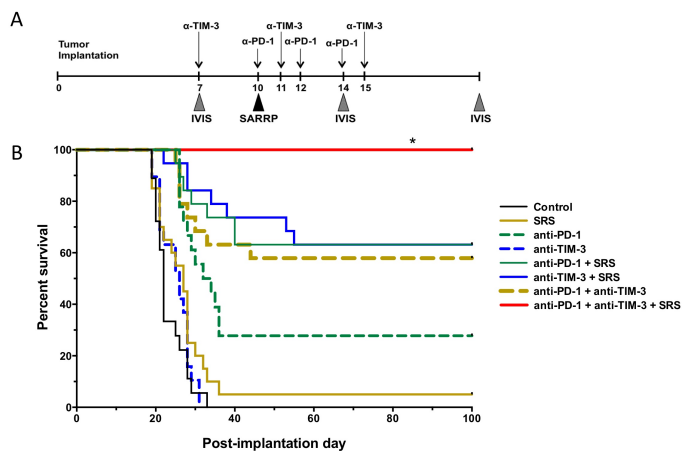
Methods

C57BL/6 mice were implanted with mouse glioma cell line GL261 transfected with luciferase and randomized into 8 treatment arms: (1) control, (2) SRS, (3) anti-PD-1 antibody, (4) anti-TIM-3 antibody, (5) anti-PD-1+SRS, (6) anti-TIM-3+SRS, (7) anti-PD-1+anti-TIM-3, and (8) anti-PD-1+anti-TIM-3+SRS. Bioluminescent imaging (IVIS) was used to track tumor progression, and overall survival (OS) was measured. Brains were harvested on day 21 to assess immune activation. Long-term survivors underwent flank rechallenge with 1,000,000 GL261 cells on day 100.

References

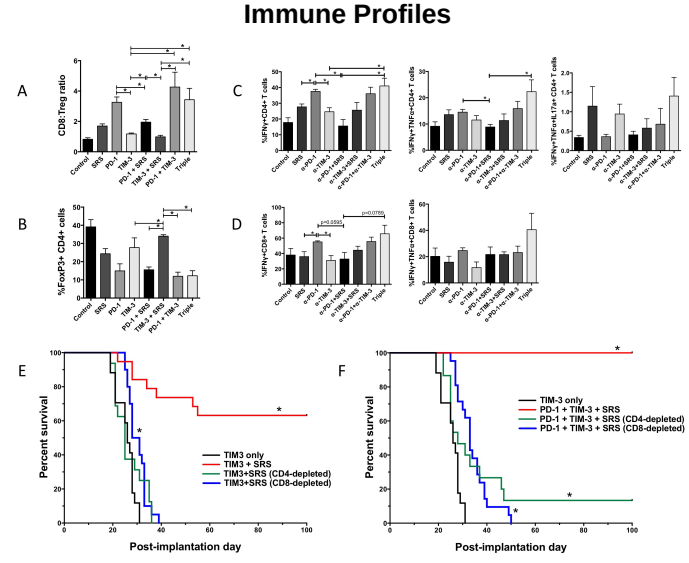
1. Zeng J, See AP, Phallen J, Jackson CM, et al. Anti-PD-1 Blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Rad Onc Bio 2013;86:343–349.
2. Sakuishi K, Apetoh L, Sullivan JM, et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 2010;207:2187-2194.

Overall Survival



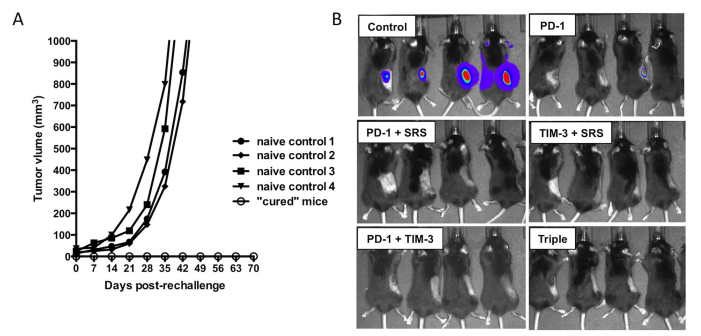
(A) Treatment scheme. (B) Kaplan-Meier analysis.

Immune Profiles



(A) CD8 to regulatory T cell (Treg) ratio. (B) Percent of total CD4+ TILs that express FoxP3+, a Treg marker. (C)-(D) Mono- and poly-clonal cytokine production by CD4 (C) and CD8 (D) T cells. (E)-(F) Effects of CD4+ or CD8+ T cell depletion

Immune Memory



(A)-(B) Flank tumor growth in long-term survivors. None of the “cured” mice grew tumors by day 30, compared to naïve control animals.

Results

Survival benefits were observed with combined anti-TIM-3 antibody+SRS compared to anti-TIM-3 antibody alone with a median survival (MS) of 69 vs. 25 days and OS of 50% vs. 0%, respectively ($p<0.001$ by log-rank Mantel-Cox). Dual blockade with anti-TIM-3+anti-PD-1 antibody also improved survival compared to TIM-3 blockade alone, (MS of 100 vs. 25 days, OS 60% vs. 0%, respectively, $p<0.05$). Notably, OS in the triple therapy group (anti-PD-1+anti-TIM-3+SRS) was 100% by day 100 ($p<0.05$), a significant improvement compared to all other treatment arms. Compared to the dual therapy groups, triple therapy increased tumor infiltration by interferon-gamma+ (IFNy) and tumor necrosis factor -alpha+ (TNFa)-producing CD4+ T cells, as well as IFNy+ CD8+ lymphocytes. CD4 depletion abrogated treatment response as reflected by a statistically significant decreased survival compared to non-depleted mice. Finally, long-term survivors were shown to have durable immune memory.

Conclusions

Combining anti-TIM-3 with anti-PD-1 and radiation improved TIL immunophenotype, and conferred a significant survival benefit.