Anti-GITR Antibody and Stereotactic Radiation Induces Immune-mediated Prolongation of Survival in Murine Intracranial Glioma

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Introduction

The most prevalent brain tumor, GBM is associated with a median overall survival of 1-2 years and a 5-year survival rate of <10% [1-3]. Temozolomide, surgical resection and radiation comprise the current standard of care for GBM, but even standard therapy has only modestly improved overall survival [4]. Current immunotherapies may have the potential to work synergistically with the standard of care for GBM, providing promise for improvements in overall survival beyond the current standard. Immunotherapeutic agents have begun to alter the landscape of anti-tumor therapy for a number of cancer types, and GBM is no exception. **Glucocorticoid-induced tumor necrosis factor receptor (GITR) is an immune checkpoint up-regulated on antigen-experienced CD4+ and CD8+ T-cells and constitutively expressed on regulatory T-cells (Tregs)** [5-6]. Ligation of the GITR receptor augments CD4+ and CD8+ lymphocyte effector function and destabilizes Treg suppressive function through loss of Foxp3 [6]. Anti-GITR monoclonal antibody (mAb) therapy has induced tumor regression in flank tumor models, but the effect of anti-GITR in intracranial glioma is unknown [7-8]. **Stereotactic radiosurgery (SRS) is focal radiation therapy for the treatment of brain cancer.** Radiation has been known to create a pro-inflammatory tumor microenvironment by inducing the up-regulation of immunogenic ligands and release of soluble antigens [9]. The combination of SRS with immunotherapy has proven successful in intracranial glioma [10-11]. **We report that the combination of an anti-GITR agonist mAb (anti-GITR(1)) alongside adjuvant SRS promotes immune-mediated tumor regression in murine intracranial GL261 glioma, primarily mediated by CD4+ Th1 cells.**

Methods

Our model was a GL261 mouse glioma cell line transfected with luciferase. Mice were implanted with GL261 and began SRS and anti-GITR isotype IgG1 (anti-GITR(1)) treatment after 10 days. Mice were randomized to four treatment groups: (1) control; (2) SRS only; (3) anti-GITR(1) only; (4) anti-GITR(1)+SRS. SRS was delivered to the tumor in one fraction of 10 Gy, and mice were treated with mAb thrice i.p. Mice were euthanized on day 21 to analyze the immunologic profile of brain tumor, spleen, and tumor draining lymph nodes.

Results

FIGURE 1: Eradication of intracranial GL261 tumors with anti-GITR(1) mAb plus SRS combination therapy. (A) Kaplan-Meier survival curve, n>10 mice per group. P<.05 between anti-GITR(1)+SRS treatment arm and all other treatment arms. (B) Bioluminescent imaging of 4 representative mice per treatment arm. (C/D) Tumor infiltrating CD4 and CD8 T cells (gated on CD3+ cells) and Tregs (gated on CD4+ cells) by treatment group; **** P<.0001.

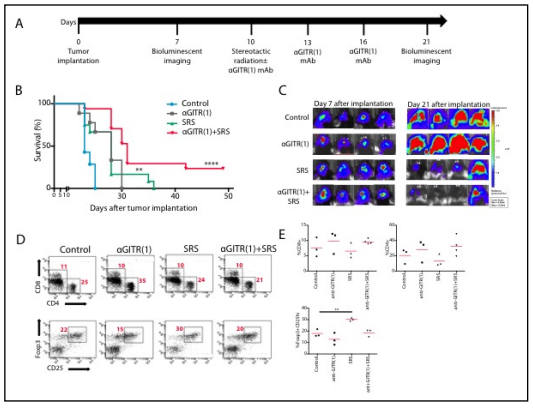


FIGURE 2: Survival benefit conferred by anti-GITR(1)/SRS treatment of murine glioma requires CD4+ T cells. (A) Depletion of CD4+ T cells abrogated the anti-GITR(1)/ SRS treatment effect as reflected by significantly decreased survival compared to non-depleted mice. (B) Depletion of CD8+ cells did not significantly diminish anti-GITR(1)/SRS treatment effect. Survival difference between control mice and mice depleted of CD8+ T cells was significant, but was not significant in the absence of CD4+ cells; n>7 per group. *P<.05; **P<.01. (C/D) Depletion of Tregs does not alter treatment effect.

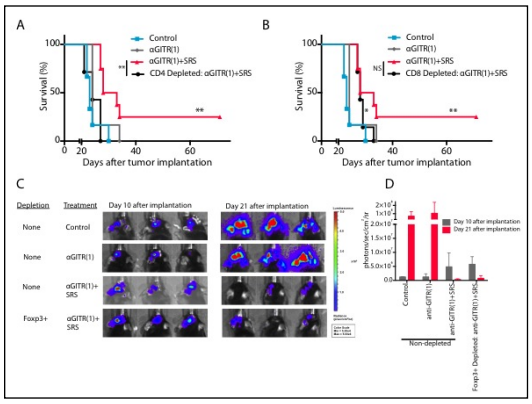


FIGURE 3: Tumor infiltrating lymphocytes (TIL) in the anti-GITR(1)/SRS group have a Th1 immunophenotype and elevated effector to Treg ratio in the anti-GITR(1)/SRS group. (A) CD8+ TIL produced significantly elevated IFNg and TNFa. (B) CD4+ TIL produced significantly elevated IFNg and IL-2. (C) There was a significantly elevated CD4+IFNg+/Treg ratio in the anti-GITR(1)/SRS group. *P<.05.

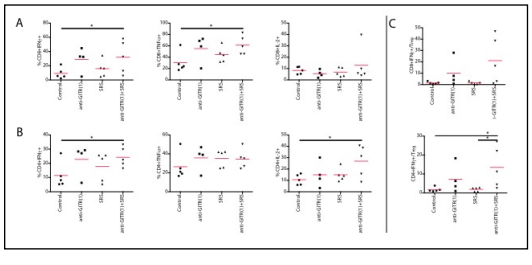
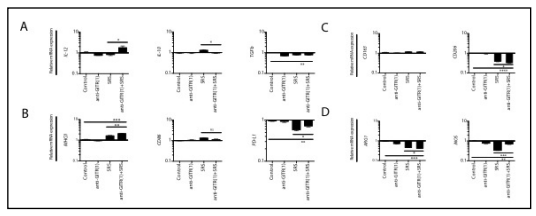


FIGURE 4: Anti-GITR(1)/SRS yields intratumoral myeloid cells with overall lower expression of M2 and higher expression of M1 markers. mRNA expression in intratumoral CD11b+CD45+ mononuclear cells relative to the control group of (A) cytokines, (B) cell surface molecules, (C) cell surface receptors, and (D) cellular enzymes.



Conclusions

Anti-GITR mAb and SRS significantly prolongs survival in murine orthotopic glioma. These findings provide preclinical evidence for the use of anti-GITR non-depleting antibodies alongside SRS in human GBM.

References

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