#1928. Radiation Response in Preclinical Orthotopic Models of Brain Cancer

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Introduction and Background

- Glioblastoma multiforme (GBM) is a fast-growing, aggressive type of central nervous system tumor that forms on the supportive tissue of the brain. It is the most common primary brain tumor.
- The American Cancer Society estimates that in 2019 there will be >23,000 new cases diagnosed and >17,000 people will die from their brain cancers.
- Preclinical GBM models are essential in evaluating the unique environment where these tumors grow. The brain offers a nutrient-rich location protected by the blood brain barrier. With optical and translational imaging, assessing orthoptic tumor growth and response to therapy over time allows for more quantitative analysis of data.
- Primary treatment options for patients with GBM have changed little over the years, with the first two options being surgery and radiation treatment (RT). Radiation therapy has proven to be curative in a number of types of cancer when localized to the specific tissue of interest.

Materials and Methods

U87MG-Luc, GL261-Luc2 and BT142 Cell Lines

All three cell lines were implanted intracranially into female mice of the indicated strains via stereotaxic frame. Tumors were allowed to become established prior to initiation of radiation treatment.

- U87MG-Luc (nu/nu mice) is a human glioblastoma cell line that was developed from an astrocytoma.
- GL261-Luc2 (Albino C57Bl/6) is a murine glioblastoma model that grows aggressively in the mouse brain.
- BT142 (NOD SCID) is a human anaplastic oligoastrocytoma that is relatively unique due to its DH1/2 mutational status, which results in an accumulation of the oncometabolite 2-hydroxyglugarate (2-HG) in the tumor.

Tumor Monitoring

- For the U87MG-Luc and GL261-Luc2 models: tumor burden was tracked with bioluminescence imaging (BLI) over time. BLI was performed using an IVIS Spectrum (Perkin Elmer, Waltham, MA).
- For the BT142 model: tumor burden was tracked by MRI. MRI was performed using a 7 Tesla magnet to assess anatomical tumor volume (BioSpec 70/20 7T, Bruker, Bellerica, MA).
- All studies were coupled with traditional survival endpoints (morbidity and mortality) to assess biological impact of radiation therapy.

Radiation Treatment

- For GL261-Luc2 and BT142: Image-guided irradiation was performed on the Small Animal Radiation Research Platform (SARRP; Xstrahl Inc., Suwanee, GA). Following placement on the treatment bed, animals were imaged with an open field at 60 kV and 0.5 mA for a planning CBCT. The resultant CT was then loaded into the treatment planning software (Muriplan, Xstrahl Life Sciences) and a treatment plan applied and optimized for each target.
- For U87MG-Luc: Radcal RAD source cabinet irradiator was used. Following calibration of the instrument with the ACCuDose meter, mice were placed in plexiglass jigs and lead shielding was placed over the body leaving only the head exposed to radiation treatment.

Flow Cytometry

- For immune profiling, brains with GBM tumors were collected and dissociated into single cell suspensions (gentleMACS™, Miltenyi), samples were labeled with a comprehensive leukocyte panel and analyzed by an Attune™ NxT flow cytometer (Thermo Fisher Scientific). Immune subsets were delineated using FlowJo (FlowJo, LLC, Ashland, OR.)

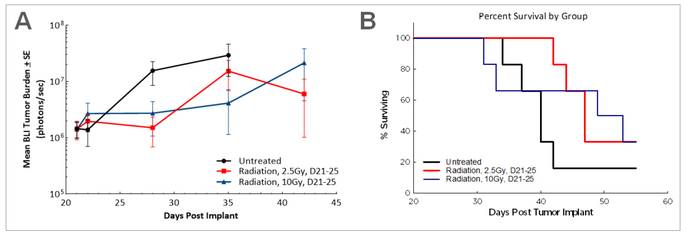

Figure 1. Cabinet RT reduces U87MG-Luc tumor burden and increases life spans. Athymic nude mice bearing orthotopic U87MG-Luc tumors treated with cabinet radiation to the head only. Mean (A) BLI of primary tumor response and (B) overall survival were tracked over time.

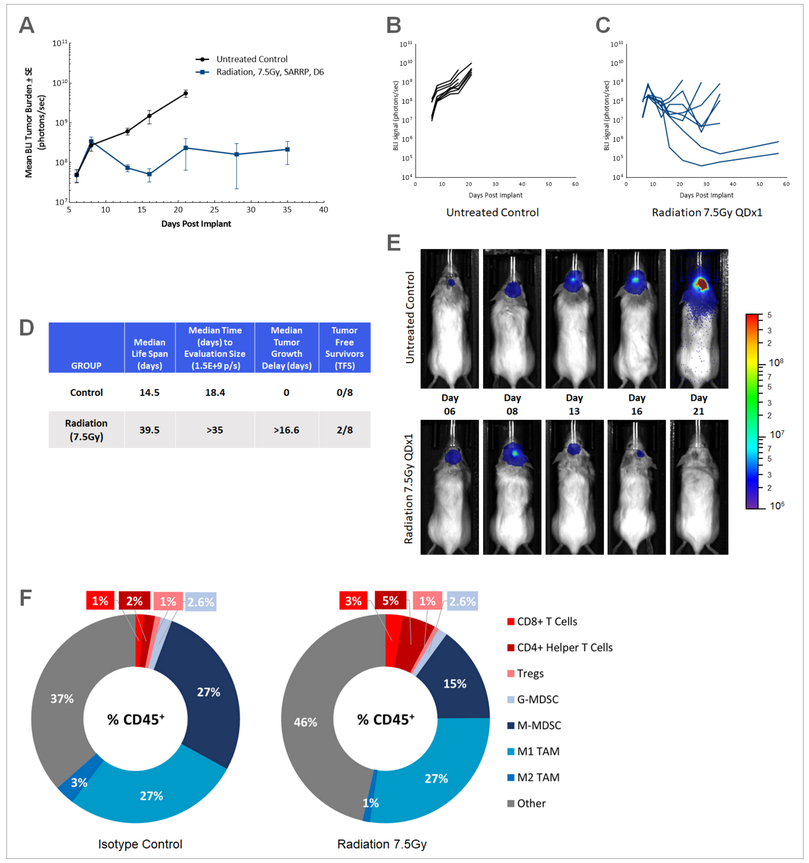

Figure 2. Focal RT reduces primary GL261-Luc2 tumor burden. Albino C57BL/6 mice bearing orthotopic GL261-Luc2 tumors treated with focal beam radiation (SARRP). Longitudinal assessment of mean (A) and individual (B-C) tumor growth and treatment response by BLI. (D) Summary data table comparing untreated to focal radiation treatment. (E) Representative images of control and treated mice over time. (F) Baseline and Day 20 (15 days post RT) immunophenotype in orthotopic GL261-Luc2 tumors. Data represents percentages of subsets among total CD45+ cells. Whole brains were collected and dissociated for flow cytometry analysis of tumor infiltrating cells. Analysis determined that a relatively small but measurable immune compartment (<5% of cells were CD45+) was observed. Slight decrease in the M-MDSC cells population and slight increase in the CD8+ and CD4+T cells population which results in an increased CD8:Treg ratio.

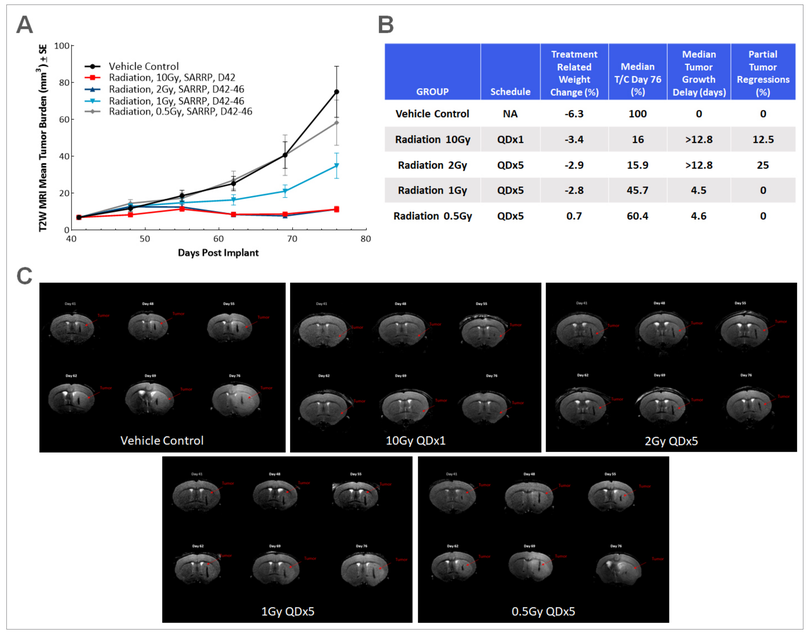

Figure 3. Focal RT reduces primary BT142 tumor burden. NOD SCID mice bearing orthotopic BT142 tumors treated with focal beam radiation (SARRP). (A) Mean longitudinal assessment of tumor growth and treatment response with MRI. (B) Summary data table comparing untreated to focal radiation treatment. (C) Representative images of control and treated mice over time.

Results and Conclusions

- In the human U87MG-Luc model, tumor growth is reliable and reproducible. Cabinet radiation was well tolerated and effective, producing a 48% (2.5 Gy) and 86% (10 Gy) Day 35 tumor growth inhibition (TGI). This inhibition of tumor growth resulted in an increase in life span of 8 and 12 days, respectively.
- In the syngeneic GL261-Luc2 model, tumor growth is very aggressive, which allows for a narrow treatment window. Focal beam radiation (SARRP) treatment is curative at a single 15 Gy dose (data not shown) but response is more moderate at a single 7.5 Gy dose, producing 30% tumor regressions. Thus, there is ample room for improvement with combination therapy. Minimal immune profile changes following treatment were seen by flow. IHC demonstrated that all immune cells were localized to the hemisphere with the tumor (not shown).
- In the human BT142 model, tumor growth is slow and diffuses throughout the brain. SARRP treatment produced a dose dependent response with 10 Gy single dose and 2 Gy daily for 5 treatments (10 Gy total dose) being relatively equivalent. Lower dosage levels given on the fractionated schedule are not as effective.
- In these models, radiation therapy is an effective monotherapy. However, there is a significant window for improvement with combination treatments. The utilization of SARRP reduces the incidence of commonly seen adverse side effects typically reported with radiation therapy.
- These data support the use of these preclinical models and approaches for work in GBM drug discovery and development.

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