#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 supraspinal 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 orthotopic 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 primary treatment 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 site 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 stereotactic 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 DH12 mutational status, which results in an accumulation of the oncometabolite 2-hydroxyglutarate (2HG) 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 (mortality 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; Xatrax Inc., Swannee, 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 (Muranip, Xatrax Life Sciences) and a treatment plan applied and optimized for each target.
- For U87MG-Luc: Radiolab RAD source cabinet irradiator was used. Following calibration of the instrument with the ACCuDose meter, mice were placed in Plexiglas jigs and lead shielding was placed over the head 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.)

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 diffuse throughout the brain. SARRP treatment produced a dose-dependent response with 10Gy 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.