

#3766. Radiation Enhances Anti-Tumor Activity of Checkpoint Blockade in Syngeneic Tumor Models

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Introduction and Background

- More than 50% of cancer patients receive some sort of radiation therapy during the course of their illness.
- While radiation treatment is a mainstay in clinical oncology, there is limited preclinical data in syngeneic models.
- The advent of image-guided small animal irradiators such as the Small Animal Radiation Research Platform (SARRP; Xstrahl) allow for use of targeted focal irradiation (RT) in a broad range of models.
- Here we evaluated the use of RT to broaden efficacy and response duration of immunomodulatory therapies. In the GL261-Luc glioblastoma model we also examined possible changes in lymphoid and myeloid immune cells by flow cytometry and looked at infiltration of CD4+ T cells by immunohistochemistry.

Materials and Methods

- Cells were implanted either subcutaneous (SC) on the flank (A20 and CT26), in the mammary fat pad (EMT-6, E0771, 4T1-Luc), or intracranially (GL261-Luc) into the appropriate syngeneic mouse host.
- Image-guided irradiation was performed under 1-2% isoflurane anesthesia on the Small Animal Radiation Research Platform (SARRP; Xstrahl Inc., Suwanee, GA). Treatment was delivered at 220 kV and 13.0 mA using an appropriately sized collimator to the total indicated dose (in Gray; Gy) in 2 equally weighted beams. Unless otherwise indicated, focal RT was given once. Antibodies were acquired from Bio X Cell and delivered through intraperitoneal injections.
- In the SC and mammary fat pad models, focal irradiation was delivered only to the tumor and tumor growth changes were tracked by caliper measurements. Mice were euthanized at a tumor volume of approximately 2,000 mm³.
- In the GL261-Luc model, mice were implanted intracranially with 10 µL total volume. Mice were injected with Carprofen at 5 mg/kg and anesthetized using 2% isoflurane and then secured in a stereotaxic frame (ASI Instruments, Inc.). Antibody treatments were initiated on the same day that radiation was given.
- In the IC model, focal irradiation was delivered to the brain and tumor burden was tracked by bioluminescence imaging (BLI) over time. BLI was performed using an IVIS Spectrum (Caliper Life Sciences, Hopkinton, MA).
- For immune cell profiling, whole brains were collected and digested to a cell suspension for flow cytometry (Miltenyi, Germany). MI-CompT™ panel (CD8+ T cells, CD4+ T cells and regulatory T cells [Tregs]) and MI-TAM™ panel (monocytic and granulocytic myeloid derived suppressor cells [M-MDSC & G-MDSC], M1 and M2 tumor associated macrophage [M1-TAM & M2 TAM]) were used on an Attune™ NxT Flow Cytometer (Thermo Fisher Scientific, Waltham, MA) and analyzed with FlowJo software (Tree Star, Inc., Ashland, OR).
- For immunohistochemistry, whole brains were harvested, fixed in 10% NBF and embedded in paraffin for sectioning. Tissue sections were then processed and labeled using direct methods with chromogen substrate on the Bond RXm (Leica Biosystems). Images were obtained on the Aperio VERSA (Leica Biosystems).

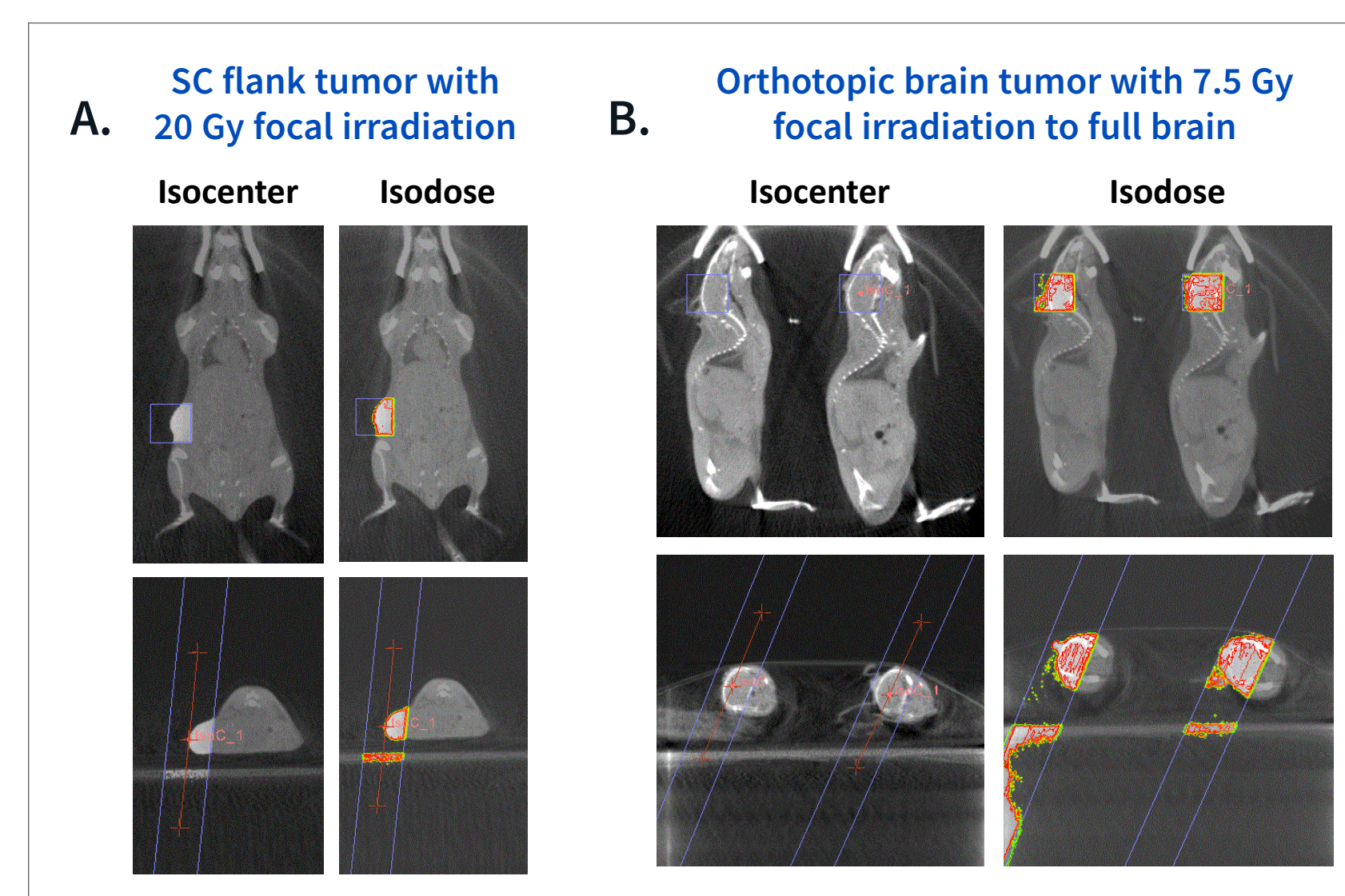


Figure 1. Small animal radiation research platform. Following placement onto the treatment bed, a CBCT is performed for treatment planning. The resultant CT is then loaded into the treatment planning software and a treatment plan is optimized for each target. In 1A and 1B treatments were delivered using a 10x10 collimator and the total indicated dose (in Gray; Gy) was administered in 2 equally weighted beams. The isodose figures shown on the right hand panels of 1A and 1B show homogeneity of the delivered dose with limited/no scatter.

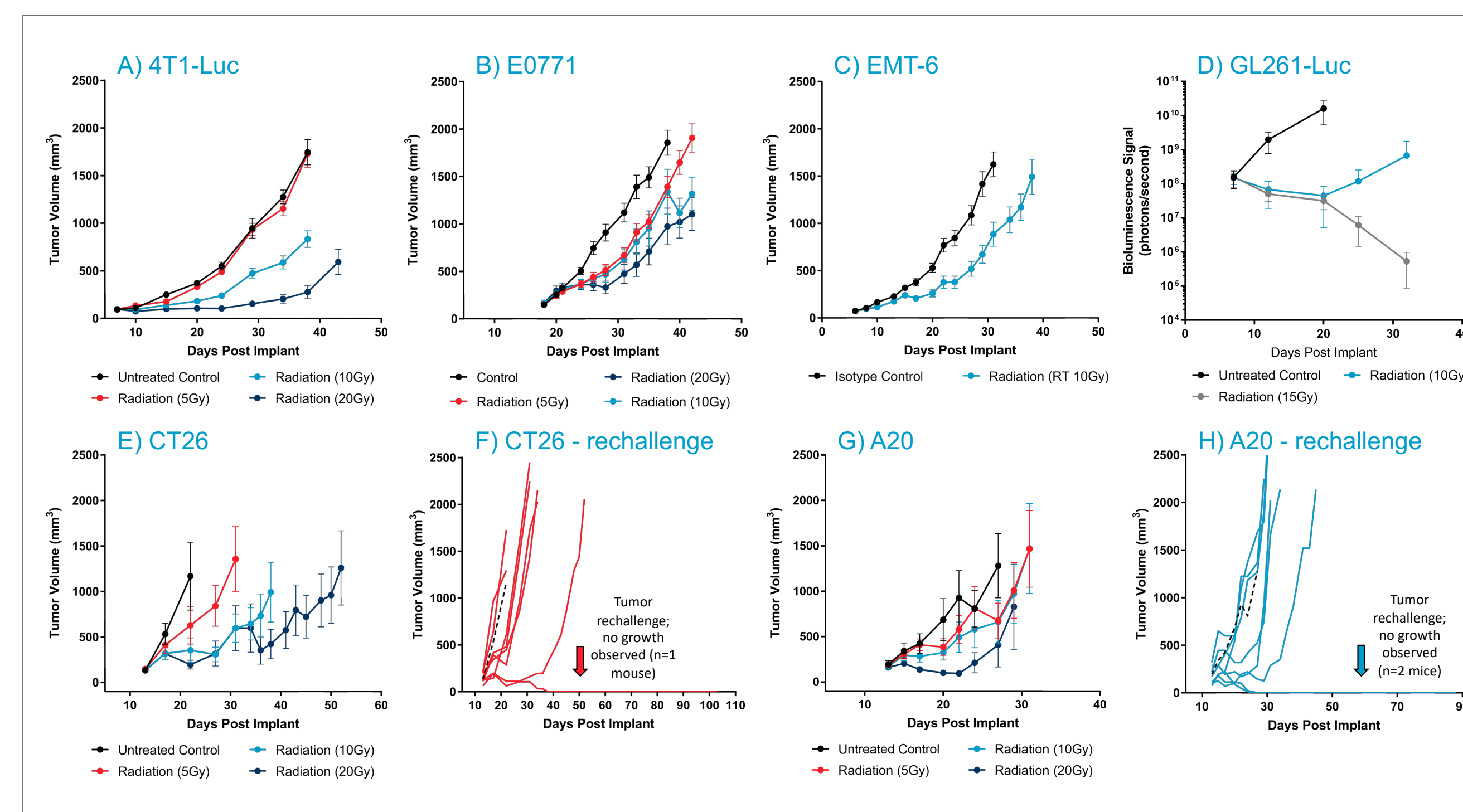


Figure 2. Sensitivity of murine tumor lines to focal radiation. Mice were implanted as described and treated with a single dose of radiation. In 2F and 2H, tumor-free mice were reimplanted on the contralateral flank with either CT26 or A20, respectively to evaluate presence of immunological memory.

Results and Conclusions

- Murine syngeneic tumor models have varying sensitivities to focal RT. In all models tested to date, we have been able to identify a dose of RT that provides moderate single agent activity suitable for testing drug combinations.
- RT combinations with immune modulatory agents can differ between models and between agents. Evaluation of both RT dose/schedule along with appropriate immunotherapies should be considered on a model-to-model basis.
- EMT-6, CT26 and GL261-Luc all demonstrated enhanced anti-tumor activity when RT was combined with an I/O agent.
- Further examination of the immune cell subsets in the GL261-Luc model demonstrated that RT alone and in combination with an anti-PD-1 antibody can result in modulation of some, but not all cell populations. Evaluation of these changes in other murine models is ongoing.
- We found no CD4+ T cells on the non-tumored hemisphere of the brain (not shown) in this study.
- Immunohistochemistry can provide additional detail on the spatial localization of immune cells in these models.

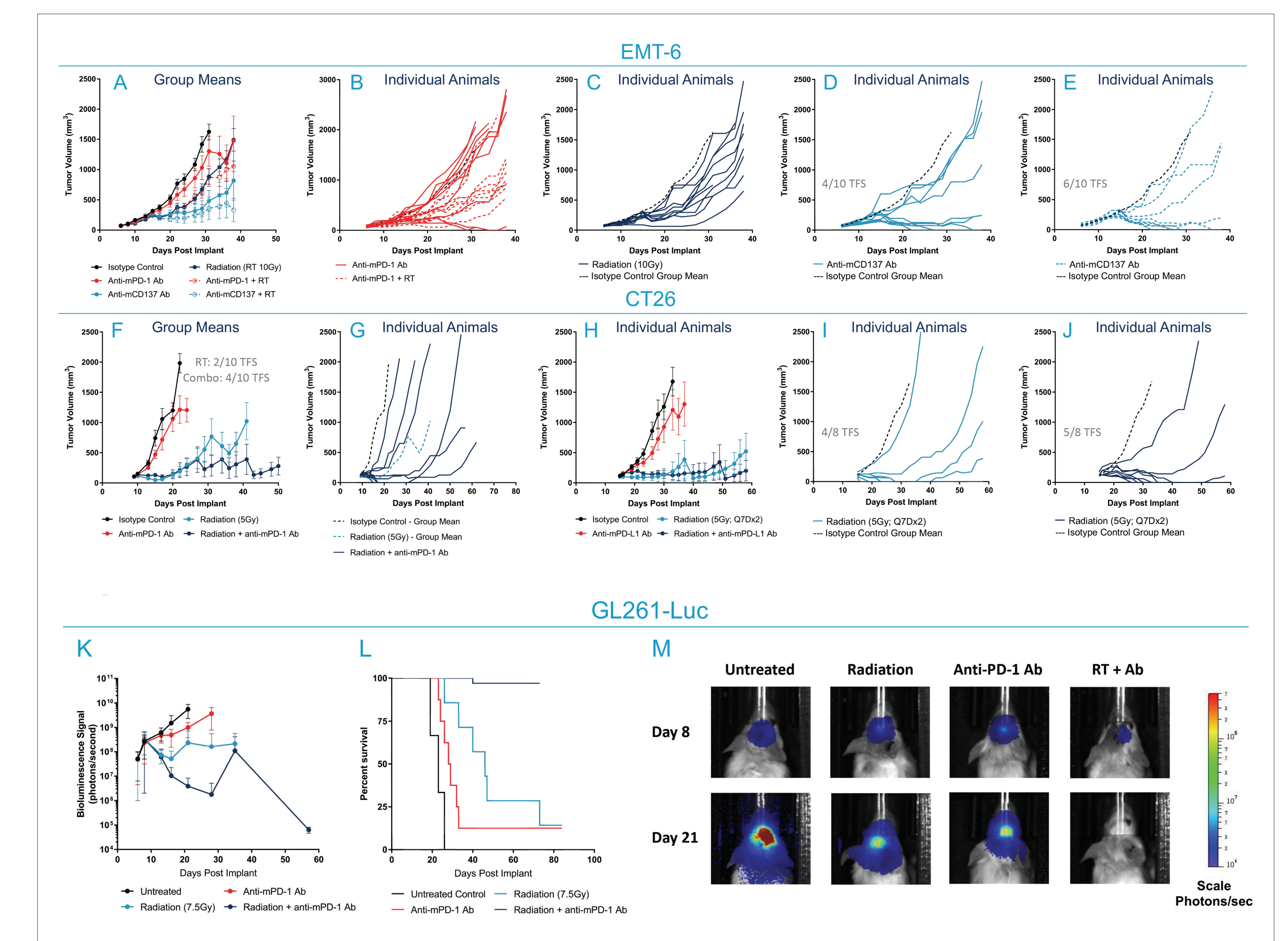


Figure 3. Immune modulators in combination with focal radiation. Mice were implanted as described. Once tumors were established mice were sorted into groups and treated with focal radiation +/- anti-mPD-1, +/- anti-mCD137 or anti-mPD-L1 as indicated. Tumor volumes were tracked by caliper measurements (A-J) or bioluminescence imaging (K, M).

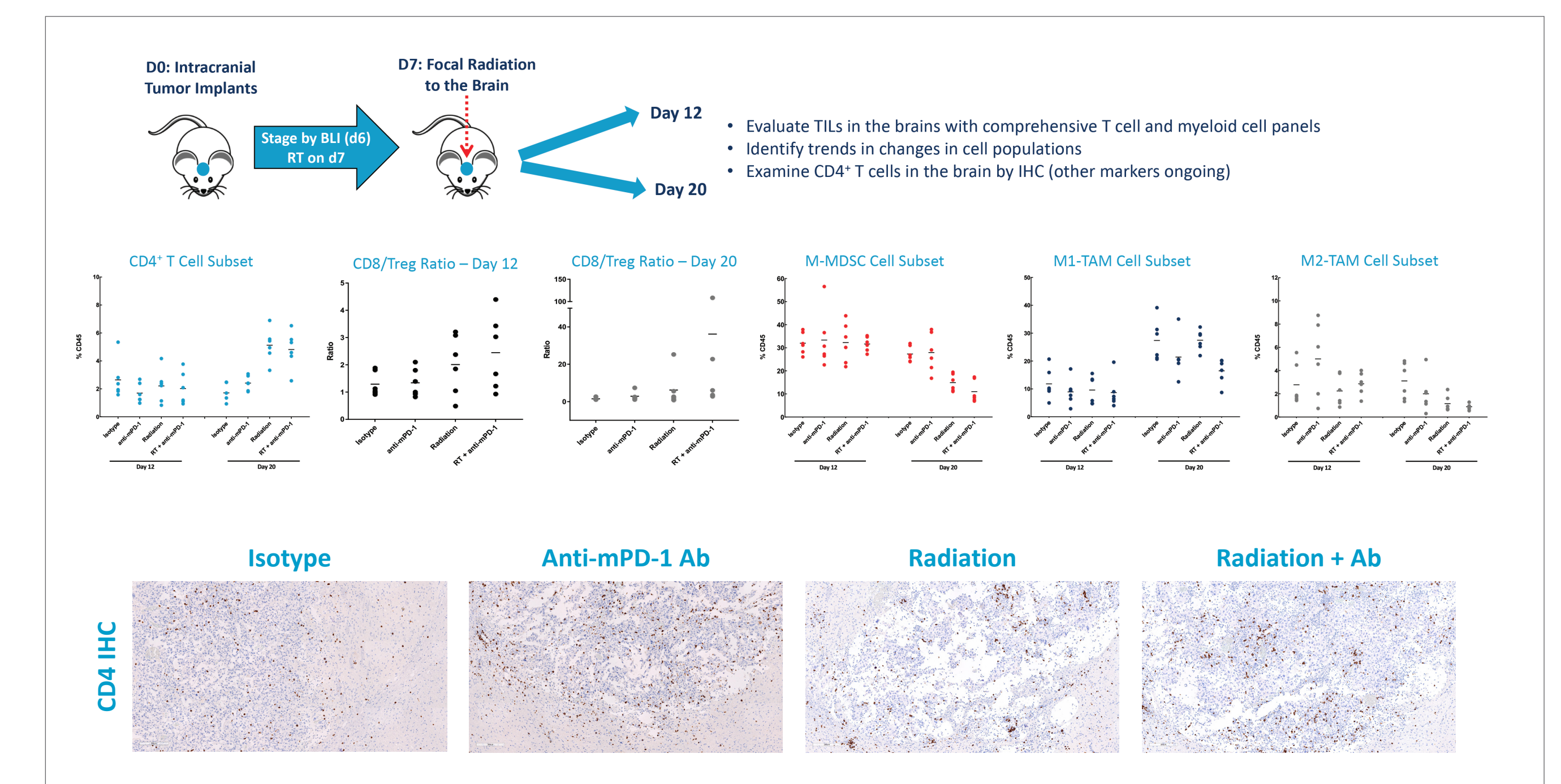


Figure 4. Immune cell modulation following focal radiation.