

# Improved Efficacy of Radiation in Combination with Systemic Immunomodulatory Therapy in a Syngeneic Mouse Model of Colorectal Cancer

Kristina H. Young, MD, PhD<sup>1</sup>, Marka Crittenden, MD, PhD<sup>2</sup>, Talicia Savage<sup>2</sup>, Benjamin Cottam<sup>2</sup>, Michael J. Gough, PhD<sup>2</sup>

<sup>1</sup>Oregon Health & Science University, Portland, OR, <sup>2</sup>Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, OR

## Purpose/Objectives

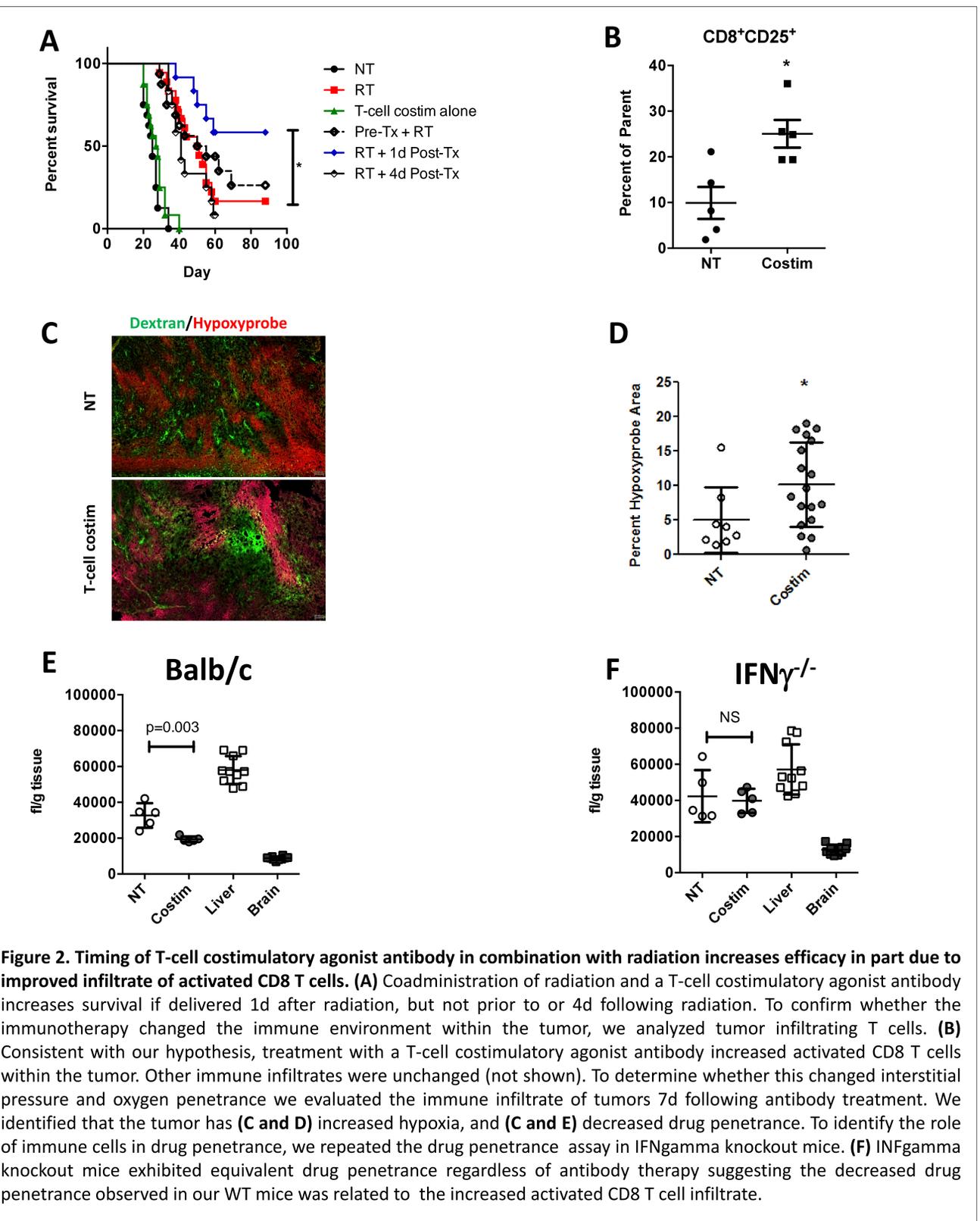
Forty thousand cases of rectal cancer are diagnosed annually in the United States and along with colon cancer, is the third leading cause of cancer death. Important recent data indicates that disease-free survival and overall survival in colorectal cancer patients correlates more closely with the immune environment in tumor samples, than TNM staging. While patients with high levels of tumor-infiltrating T cells have a better prognosis than patients with low levels of tumor-infiltrating T cells, it is not known whether elevating T cell tumor infiltration is sufficient to improve prognosis, and what the mechanism might be. We hypothesize that increasing T cell tumor infiltrates by immunotherapy will improve the outcome of radiation therapy. We test this hypothesis using murine models of colorectal cancer treated with T cell costimulatory antibodies.

## Materials/Methods

We established CT26 colorectal or 4T1 mammary carcinomas subcutaneously in the hind limb of immunocompetent BALB/c mice. Radiation was delivered using a clinical linear accelerator to doses of 20Gy utilizing a half-beam block to minimize doses to radiation sensitive regions of the torso and draining lymph nodes. Immunotherapy included agonist antibodies to a T cell costimulatory molecule. Tumor kinetics, lung metastases, and survival were measured for all groups. Small molecule penetrance and hypoxia were measured using quantitative fluorimetry for FITC-dextran and immunohistochemistry for hypoxyprobe adducts, respectively. Immune infiltrate was measured using flow cytometry and immunohistochemistry.

## Results

CT26 cells demonstrated equal radiosensitivity to 4T1 cells *in vitro*, but were markedly more sensitive to radiation doses *in vivo*, suggesting that differences in their *in vivo* environment affects their response to radiation (Figure 1). Immunotherapy, which was not efficacious alone, significantly improved control of CT26 tumors by radiation therapy. Importantly, the ability of immunotherapy to synergize with radiation therapy was dependent on the timing of administration (Figure 2). Drug penetrance was significantly impaired in tumors compared to other organs, and immunotherapy significantly increased T cell infiltration into tumors (Figure 2).



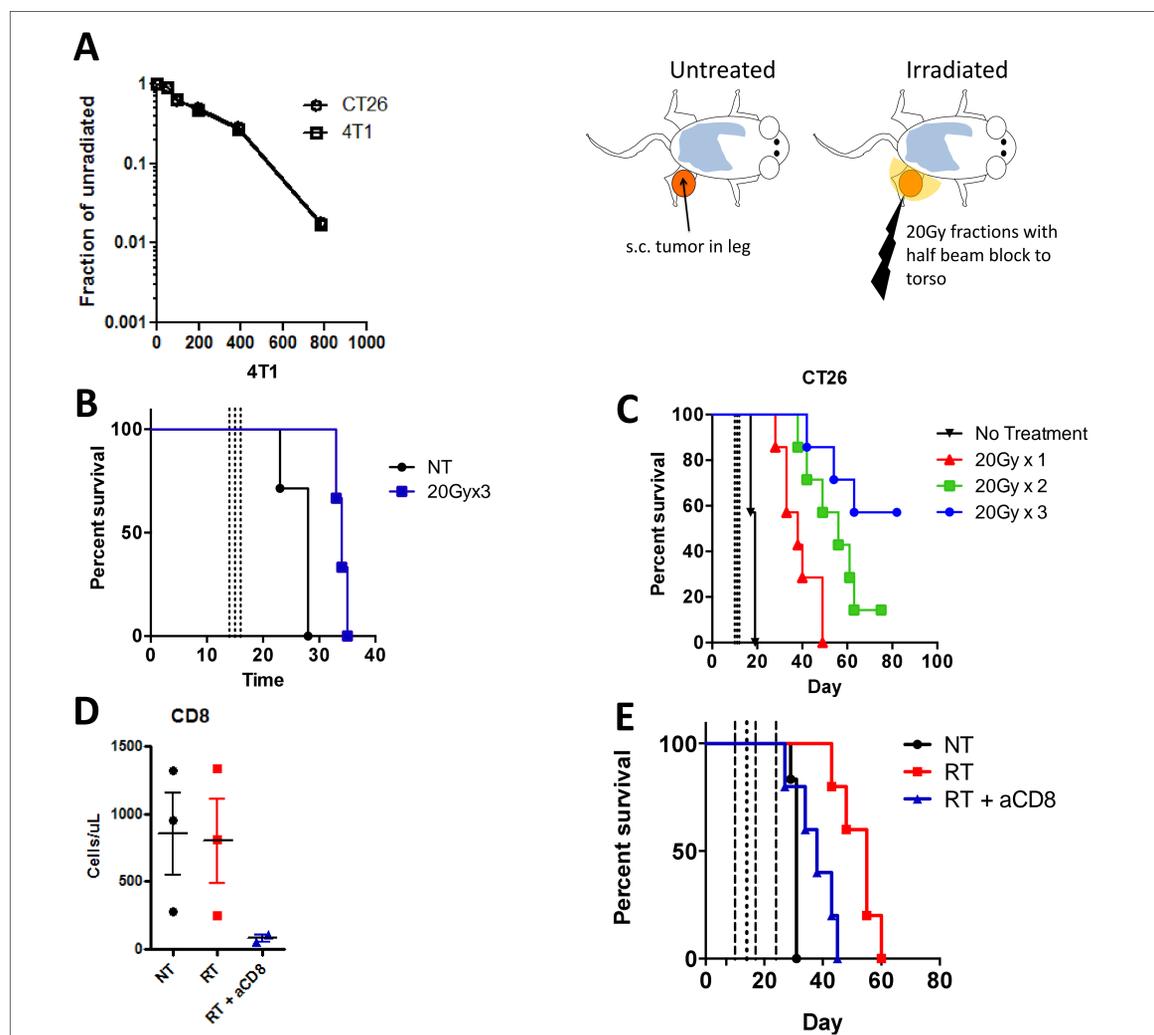
**Figure 2. Timing of T-cell costimulatory agonist antibody in combination with radiation increases efficacy in part due to improved infiltrate of activated CD8 T cells.** (A) Coadministration of radiation and a T-cell costimulatory agonist antibody increases survival if delivered 1d after radiation, but not prior to or 4d following radiation. To confirm whether the immunotherapy changed the immune environment within the tumor, we analyzed tumor infiltrating T cells. (B) Consistent with our hypothesis, treatment with a T-cell costimulatory agonist antibody increased activated CD8 T cells within the tumor. Other immune infiltrates were unchanged (not shown). To determine whether this changed interstitial pressure and oxygen penetrance we evaluated the immune infiltrate of tumors 7d following antibody treatment. We identified that the tumor has (C and D) increased hypoxia, and (C and E) decreased drug penetrance. To identify the role of immune cells in drug penetrance, we repeated the drug penetrance assay in IFN $\gamma$  knockout mice. (F) IFN $\gamma$  knockout mice exhibited equivalent drug penetrance regardless of antibody therapy suggesting the decreased drug penetrance observed in our WT mice was related to the increased activated CD8 T cell infiltrate.

## Conclusions

This study investigated a novel mechanism to explain the limitations of chemoradiotherapy in colorectal cancer and evaluated an approach to overcome this limitation *in vivo*. Immunomodulatory therapy, in combination with radiation therapy, has the potential to improve therapeutic response rates. Further experiments are ongoing, and investigation into the mechanism and long-term outcome of immunoradiotherapy is warranted.

## Acknowledgements

RSNA R&E Foundation Research Resident Grant  
ABR B. Leonard Holman Research Pathway  
OHSU Rubinstein Research Scholar program



**Figure 1. Increased radiosensitivity of CT26 tumors *in vivo* abrogated by CD8 depletion.** (A) *In vitro* clonogenic assay of CT26 and 4T1 tumor cells were indistinguishable. We established subcutaneous tumor in immunocompetent Balb/c mice, and treated with radiation beginning on day 14. (B) Radiation of subcutaneous 4T1 tumors with 60Gy in 3 fractions resulted in ~10d improvement in survival (MS 28d to 34d). (C) Radiation of subcutaneous CT26 tumors with 20Gy resulted ~25d improvement in survival (MS 19d to 38d), while 60Gy in 3 fractions resulted in tumor resolution in 57% of inoculated mice. Given the vastly different *in vivo* radiosensitivity of CT26, we investigated whether the immune microenvironment was responsible. We depleted CD8 cells with a once weekly injection of anti-CD8 antibody, resulting in loss of CD8 cells as detected in the peripheral blood (D). (E) CD8-depleted mice had significantly decreased survival following radiation suggesting CD8 T cells are necessary for the improved efficacy of radiation in CT26 tumors.