1051 RADIATION DOSE-RELATED TEMPORAL CHANGES IN STING-ASSOCIATED IMMUNE GENES IN MURINE CD8 T CELLS

Ana Torres*, Amanda Shea, Wonjong Jin, Caroline Kerr, Kaleb Schroeder, Paul Clark, Zachary Morris. *University of Wisconsin-Madison, Madison, WI, USA*

Background Systemic lymphopenia from radiation results in poor outcomes for patients. Clinical interventions can reduce lymphopenia; however, more efficient treatments could drastically improve patient outcomes, particularly when receiving immunotherapeutic treatments. Interactions of T cells, radiation, and the tumor microenvironment remain largely unknown, and in this study, we examined modulation of CD8 T cell transcriptional activity after radiation at various doses and time points.

Methods Five spleens of C57BL/6 mice were disaggregated into single cell suspensions and positively sorted with a CD8 T cell isolation kit. The $CD8^+$ cell suspensions were expanded *in vitro* for 9 days with CD3/CD28 plate-bound stimulation and then left unirradiated (0Gy control) or administered 2Gy, 8Gy, 12Gy, or 20Gy. At 24-, 48-, and 72-hours post-irradiation, the cells were collected for RNA, followed by expression analysis using qPCR. Fold-change (with relation to 0Gy) expression data were compared among multiple cohorts using a two-way ANOVA with a post hoc Tukey test.

Results We identified several immune genetic changes in irradiated CD8 T cells compared to unirradiated controls. *Ifn*-??, a direct stimulator of type 1 IFN response, expression was highest at 72 hours post radiation, with all doses exhibiting >10-fold increases ($p<0.006$). *Irf3*, a transcription factor of type 1 interferons, was increased in 8Gy, 12Gy, and 20Gy conditions, and expression levels were highest 48 hours post radiation in the 8Gy condition ($p=0.0009$).*Trex1*, a potential negative regulator of STING-response, increased in a dose dependent manner with the highest expression being a 14-fold increase at 48 hours post radiation (p-values for all conditions <0.0001, except 2Gy ns). Interestingly, *Ifn*-??, a Type 2 interferon response and activated T cell interferon, was also increased ($p<0.0001$) at 8Gy 48 hours post radiation. *Pd-L1* and *Mhc-1* expression both increased at all radiation doses with highest expression at 48 hrs (*Mhc-1*: 2-10 fold increase; *Pd-L1*: 2-7 fold increase).

Conclusions Radiation dose-related and temporal changes in transcription of various immune genes (*Ifn*-??, *Irf3*, *Trex1*, *Ifn*-??, *Pd-L1*, *Mhc-1*) suggests CD8 T cells play a role in activation, maintenance, and regulation of immune response, at least partially via the STING pathway. Changes included actionable targets such as *Pd-L1* and *Mhc-1* that could inform timing of immunotherapies to patients such as immune checkpoint inhibitors and CAR-T cells.

Ethics Approval All animal experiments were performed with the approval of UW Madison Institutional Animal Care and Use Committee (IACUC). PI Zachary Morris M005670. No human materials were used in the experiment described. All experiments were conducted under BSL-1 conditions with the approval of the UW Institutional Safety Board (B00000510).

http://dx.doi.org/10.1136/jitc-2022-SITC2022.1051