954 FLUORESCENCE TRACKING DEMONSTRATES A RAPID CYCLE OF TREG RECIRCULATION TO TUMOR DRAINING LYMPH NODES AND BACK TO TUMORS THAT IS INTERRUPTED BY RADIATION THERAPY

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Background Tumor infiltration with immune cells is linked to outcome following treatment with immunotherapies and conventional cancer therapies. For T cells this is a dynamic process, with some populations remaining resident, but with the majority of T cells recirculating via lymph nodes back to the peripheral blood. We aimed to understand the movement of T regulatory cells (Treg) to and from tumors and the impact of radiation therapy on Treg recirculation dynamics and tumor repopulation following treatment.

Methods Tumors were implanted in Kaede mice and once established tumors, TdLN, or NdLN were selectively photoconverted with UV light. In select experiments, tumors were then randomized to receive 12Gy radiation therapy using CT guidance. Tumors, TdLN, or NdLN were harvested 1–3 days later to identify photoconverted cells by flow cytometry.

Results Following photoconversion of the tumor photoconverted T cells are detectable in the TdLN and at later time points in NdLN. T regulatory cells in the TdLN contain a significantly higher proportion of photoconverted cells than non-Treg CD4 or CD8 T cells in mice bearing MC38, Moc1, and Moc2 tumors, indicating that Treg are particularly active in movement to the TdLN. To confirm active exit from the tumor, mice were treated with FTY720, resulting in a decrease in photoconverted Treg and non-Treg movement to the TdLN. Photoconvertion of the TdLN and analysis of the tumor and NdLN demonstrated that few TdLN Treg moved to the NdLN, and most moved to the tumor. Photoconversion of the TdLN or NdLN and analysis of the tumor demonstrated that significantly more Treg and non-Treg CD4 T cells were recruited from the TdLN than the NdLN, Radiation therapy of the tumor results in a decrease in the proportion of Treg and non-Treg CD4 T cells in the TdLN that are photoconverted, consistent with cytoxicity in the upstream irradiated tumor. Following radiation therapy the tumor continues to recruit Treg resulting in a rapid repopulation of the tumor and reactivation of the cycle of Treg recirculation.

Conclusions These data demonstrate that there is a selective and rapid cycle of Treg recirculation between the tumor, the TdLN, and back to the tumor, with limited involvement of distant lymph nodes. These data may inform immunotherapies intended to manipulate the tumor or TdLN environment, and the impact of tumor Treg targeting on local versus distant immunity.

Ethics Approval Animal protocols were approved by the Earle A. Chiles Research Institute (EACRI) Institutional Animal Care and Use Committee (Animal Welfare Assurance No. D16–00526).

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