INTRAVITAL MULTIPHOTON MICROSCOPY OF INFILTRATING T CELL AND TUMOR CELL METABOLISM IN A MURINE MELANOMA MODEL
1Alexa Heaton*, 2Tiffany Heaster, 2Anna Hoefges, 2Alexander Rakhmilevich, 2Amy Erbe, 2Paul Sondel, 1Melissa Skala. 1University of Wisconsin and Morgridge Institute for Research, Madison, WI, USA; 2University of Wisconsin, Madison, WI, USA

Background Intravital multiphoton microscopy (IMM) provides single-cell imaging within intact living systems. IMM of the autofluorescent metabolic co-enzymes NAD(P)H and FAD, or optical metabolic imaging (OMI), provides in vivo label-free imaging of metabolic changes. The metabolism of tumor cells and immune cells is closely associated with cancer progression, 1-3 so we aim to study metabolic trends before and after administration of an established, effective, triple-combination immunotherapy within murine melanoma tumors. 4 This therapy includes 12 Gy external beam radiation, intratumoral administration of a hu14.18-IL2 immunocytokine (anti-GD2 mAb fused to IL2), and intraperitoneal administration of anti-CTLA-4 leading to in situ vaccination and cure of GD2+ murine tumors. 5 Previous work has shown that a T cell response is critical to the efficacy of this therapy, 4, 5 so we created mCherry-labeled T cell mouse models to study T cell response. Here, IMM was used to image concurrent tumor cell and T cell metabolic trends, T cell infiltration, and tumor microenvironment composition.

Methods We created mCherry-labeled T cell mouse models through CRISPR/Cas9 knock-in and Cre-lox genetic modifications. We then implanted syngeneic B78 (GD2+) melanoma cells intradermally into the flanks of C57BL/6 mice to induce measurable tumors. Mice were anesthetized, skin flap surgery performed, and tumors imaged at varying time points. IMM was performed using 750-1040 nm to excite NAD(P)H, FAD, and mCherry through a 40X (1.15 NA) objective. Fluorescence lifetime data was collected using time correlated single photon counting electronics. Murine tissues were later harvested and analyzed via flow cytometry and immunohistochemistry to confirm mCherry expression in mouse models and IMM findings.

Results Here we demonstrate the feasibility of our IMM platform to perform single-cell resolution imaging in vivo. We established that our genetically engineered mouse models enable clear identification and tracking of mCherry T cell populations. In addition, we show that label-free OMI provides metabolic trends and structural information in vivo (figure 1). Overall, we demonstrate concurrent imaging of intravitral tumor cell and T cell populations within the tumor microenvironment.

Conclusions Our preliminary results suggest that the combination of IMM and our mCherry mouse models with OMI allows for concurrent imaging of T cell infiltration and metabolic trends. With continued work, this imaging platform has the potential to provide dynamic, metabolic information on tumor cell and immune cell populations to inform further immunotherapy development.

Acknowledgements This work is supported by the Morgridge Institute for Research (Interdisciplinary Fellowship awarded to A.R.H.) and the NIH (R01 CA205101 and R35 CA197078). The authors thank the University of Wisconsin Carbone Cancer Center (UWCCC) Support Grant P30 CA014520, the UWCCC Translational Research Initiatives in Pathology laboratory - supported by the UW Department of Pathology and Laboratory Medicine and the Office of The Director NIH (S10OD023526), the UWCCC Flow Cytometry Laboratory, and the Genome Editing and Animal Models Laboratory for core services.

Ethics Approval All animal work was approved by the University of Wisconsin Institutional Animal Care and Use Committees.

REFERENCES

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0042