DISSECTING INTRATUMORAL IMMUNE ORGANIZATION: DEFINING THE COMPARATIVE CELLULAR COMPOSITION OF TERTIARY LYMPHOID STRUCTURES T-CELL SUPPORTIVE ANTIGEN PRESENTING NICHES IN RENAL TUMORS

Caroline Jansen*, Ewelina Sobierajska, Rachel Greenwald, Jennifer Carlisle, Patrick Mullane, Nataliya Prokhnevskaya, Maria Cardenas, Mehmet Bilen, Adeboye Osunkoya, Viraj Master, Haydn Kissick. *Emory University, Decatur, GA, USA*

Background Tumor infiltrating T-cells have a prognostic benefit in many tumor types,^{1–8} and we recently sought to determine whether the level of T-cell infiltration into renal tumors predicts clinical outcomes. In our recent publication,⁹ we showed that patients with high of CD8 T-cell infiltration have improved progression free survival (PFS). Further, we found that this T-cell response is supported by TCF1+ stem-like CD8 T-cells, which reside within dense regions of closely clustered antigen presenting cells within the tumor. Interestingly, aggregations of immune cells have also been described in other tumor types and termed ‘tertiary lymphoid structures’ (TLS), which are typically defined as B-cell-dominant aggregates, containing high endothelial venules and reactive germinal centers.^{10–12} Together, these findings raise several important questions, which we explore here—(1) what additional cell types comprise these niches?⁹ and (2) how are these niches similar to or different from TLS?

Methods Tumor tissue was collected from patients with renal tumors undergoing surgery at Emory University Hospital. Intraoperative tumor samples were analyzed by flow cytometry, RNA sequencing, immunofluorescence, and immunohistochemistry. Immunofluorescence data was analyzed using our custom quantitative analysis pipelines, which allows for delineation of cell type and location, cell-cell distance, and density of cellular aggregation.

Results The proportion of CD8 T-cells infiltration human renal tumors varied widely, consistent with our previous reports.⁹ TCF1+ stem-like CD8 T-cells were identifiable by both flow cytometry and immunofluorescence and resided in dense antigen presenting niches. Quantitative immunofluorescence revealed the location of aSMA+ fibroblasts within tumor tissue, in relation to antigen presenting niches, and in tumors with many infiltrating T-cells. Pathologist scored hematoxylin and eosin-stained slides were delineated TLS+ or TLS-. Quantitative immunofluorescence imaging analysis revealed the detailed composition of tumor infiltrating immune cell populations and the contrasting cellular organization in TLS as compared to in antigen presenting niches.

Conclusions As we have shown CD8 T-cell infiltration to predict PFS in renal tumors and that antigen presenting niches containing stem-like cells maintain the anti-tumor T-cell response,⁹ it is critical to understand the additional cell types present in these niches and to understand how these niches relate to previously described phenomena of immune organization, such as TLS.^{10–12} This mechanistic understanding of the anti-tumor immune response represents an opportunity to inform development of enhanced prognostic tools and innovative therapeutic possibilities.

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Ethics Approval Samples are collected under an approved IRB protocol (The Urological Satellite Specimen Bank at Emory University, IRB00055316). All patients provided informed consent.

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