MULTIPLEX MICROSCOPY REVEALS UNIQUE SPATIOTEMPORAL EFFECTS OF CANCER IMMUNOTHERAPIES

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Background Pancreatic ductal adenocarcinoma (PDA) is an aggressive and insidious cancer because it often remains undetectable until later stages and is typically refractory to current treatments. Additionally, the tumor microenvironment (TME) of PDA tumors is immunologically heterogeneous, which complicates therapies like checkpoint blockade. Many mouse models fail to recapitulate the tumor heterogeneity seen in human cancer, something that has severely constrained our understanding of the mechanistic response to therapy. To address this issue, we are using a library of tumor clones derived from the mouse model of PDA that elicit a spectrum of reproducibly unique immune cell infiltration profiles upon implantation into C57Bl/6 mice. Clones with high numbers of infiltrating T cells (T cell high-TCH) are responsive to a combination of checkpoint blockade and CD40 therapies, while clones with low numbers of infiltrating T cells (T cell low-TCL) are not [1]. However, the analysis of the TME in these divergent tumors is quite limited, and emerging high content imaging methods able to reveal the phenotype, activity, and spatial organization of the many cellular populations within these tumors remain to be employed.

Methods 1) Histocytometry: quantitative analysis of microscopy images that combines the spatial information of microscopy with the quantitative immune cell information of flow cytometry. (2) IBEX: iterative staining that facilitates high content tissue imaging (HCT) of immunofluorescent antibody (IF)-stained tissues [2]. (3) Two-photon intravital microscopy (2P-IVM): dynamically image tissues in real time

Results My initial goal was to utilize multiplex imaging methods to profile the immune landscapes of representative TCH and TCL clones at early days post tumor implantation; this would provide me with a ground truth for comparison to therapeutic intervention. As early as day 5 post tumor injection, I found striking differences between the TCH and TCL tumors: T cells are predominantly excluded to the outer rim of the TCL tumors, and there are well-defined myeloid cell clusters scattered throughout the TME (Figure 1). In contrast, TCH tumors have substantial T cell infiltration throughout the tumor proper and lack these distinctive myeloid cell clusters. Therapy also uniquely alters regulatory T cells in the TCH TME, severely depleting, reprogramming, and restricting the remaining cells to the tumor periphery.

IBEX was used to illustrate the complexity of myeloid cells within the tumor microenvironment

Conclusions These data raise important questions regarding the spatial positioning of cellular subsets, and the implications of these positions on therapeutic efficacy. This work was supported in part by the Intramural program of NIAID, NIH.

Abstract 574 Figure 1 Cold tumor myeloid cluster

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Ethics Approval This study involved the use of mice. This study (LISB 4E) obtained ethics approval via NIAID ACUC (Animal Care and Use Committee) and AAALAC.

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