HIGH-DIMENSIONAL ANALYSES OF INTRATUMORAL MYELOID CELLS HIGHLIGHTS PRESENCE OF DISTINCT MYELOID CELL PHENOTYPES IN IMMUNE CHECKPOINT-SENSITIVE AND RESISTANT TUMORS

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Background Immune checkpoint therapy (ICT) has revolutionized cancer treatment, however, has produced around 20% durable clinical response rates. 1 Multiple studies demonstrate myeloid cell abundance and function in the tumor microenvironment (TME) correlates with poor outcome in ICT resistant cancers. 2-3 Despite the wealth of knowledge regarding the biology of these cells, our ability to distinguish immune-suppressive versus immune-stimulatory myeloid cells remains a major challenge and, thus, targeting myeloid cells has had limited clinical success. Our objective is to better understand the phenotype of these immunosuppressive myeloid cells to identify potential combination strategies to improve response to ICT.

Methods Comparative analyses of intratumoral myeloid cell subsets was performed in orthotopic murine models of ICT sensitive (B16F10 melanoma) and resistant (MT4 pancreatic) tumors at baseline and post ICT treatment (anti-PD-1 and/or anti-CTLA-4 antibodies) using single cell RNA sequencing (scRNAseq). To determine myeloid cell evolution with tumor progression, longitudinal scRNAseq was performed on MT4 tumors.

Results We observed two-fold higher abundance of macrophages and neutrophils in baseline MT4 tumors as compared to B16F10 tumors from our scRNAseq analysis (figure 1A-C). Overall, we identified 4 distinct macrophage subsets and 1 neutrophil subset (figure 1B). Mac_1 and mac_2 (MT4 macrophages) were the dominant macrophage clusters in MT4 tumors and while mac_1 expressed mmp14, axl and mafb; mac_2 had high expression of vegfa, ang1, ccl24 and fn1 (figure 1D). In contrast, mac_3 (B16F10 macrophages), the most abundant macrophage cluster in B16F10 tumors, expresses antigen presenting genes (cd72) and interferon-induced genes (cxcl10, isg15) (figure 1D). MT4 macrophages were significantly enriched TGF beta signaling, angiogenesis, hypoxia, glycolysis and B16F10 macrophages which enriched in oxidative phosphorylation and interferon gamma and alpha response pathways (figure 1E). The neutrophil subset was present specifically in MT4 tumors and expressed cd24a, cxcl2, il1b and cd274 (figure 1D); consistent with the suppressive tumor-associated neutrophil (TAN) phenotype. Overall, at baseline, MT4 myeloid cells possess characteristics associated with T cell inhibition whereas B16F10 myeloid cells show T cell activating phenotypes. Post ICT treatment, macrophage subsets decreased moderately, however, MT4 TAN abundance increased, indicating possible compensatory resistance mechanisms (figure 1F). Longitudinal analyses indicated that neutrophil and macrophage subsets were abundant in early stage tumors and persisted during MT4 tumor progression (figure 1G).

Conclusions Distinct myeloid subsets are present in ICT sensitive and ICT resistant tumors. Myeloid targeting prior ICT treatment might be necessary to generate an effective immune response in resistant tumors. This study provides the foundation to identify novel myeloid specific targets in resistant solid tumors.

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REFERENCES

Ethics Approval All mice were housed in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and NIH standards. Experiments were conducted according to protocol 00000893-RN02 and approved by the University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee.

Abstract 505 Figure 1 (A) Representation UMAP plot of intratumoral immune cell landscape in B16F10 and MT4 tumors. 3 tumors in each were pooled for internal control. All major immune cell subsets were identified. (B) Cluster frequency plots of each immune subset in B16F10 and MT4 tumors. (C) Characterization of immune subsets identified in figure 1A based on their marker gene expressions. (D) Heatmap of functional markers for the individual myeloid subsets providing phenotypic information. Expression levels are scaled between minimum and maximum expression for each gene across all clusters. (E) GSEA analysis of hallmark pathways enriched in mac_1 and mac_2 versus mac_3. F) Cluster frequency plots of indicated myeloid subsets after treatment with ICT antibodies obtained from scRNAseq analyses. (G) Representative UMAP plots of intratumoral immune subsets collected from Day 5, 7, 10 and 15 of MT4 tumors. Highlighted regions in blue and brown indicate macrophage and neutrophil subsets, respectively.