1050 SINGLE CELL TRANSCRIPTOME AND EPIGENOME PROFILING REVEALS THE DIVERSITY OF T CELL STATES IN *EX VIVO* GROWN TUMOR-INFILTRATING LYMPHOCYTES FROM MALIGNANT PLEURAL MESOTHELIOMA

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Background Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer associated with exposure to asbestos that lacks effective treatment options.[1] Immunotherapy approaches remain challenging with a current paucity of knowledge on the tumor-infiltrating lymphocyte (TIL) landscape.[2] We aimed to generate a reference transcriptomic and epigenomic atlas of MPM T cell subpopulations that could inform on cellular features and states of propagated *ex vivo* cells to allow new immunotherapy design.

Methods TIL were expanded utilizing the MDACC 'TIL 3.0' method from surgically managed MPM patients (n=8, 6/8 cases received chemotherapy with treatment ending an average of 82 days prior to surgery).[3] Cells were processed for 10x Genomics 5' single cell (sc) RNA- and scATAC-seq profiling. Analysis was performed using 10x Genomics cell ranger pipelines. Downstream analysis was performed in R utilizing Seurat and Signac packages, following Monocle3 trajectory analysis.[4–6] The significant expression of key defining genes was used to label cell states. Wilcoxon Sum Ranking test was applied for determination of statistical significance of genes (adjusted p-value significance: 0≤*** <0.001≤** <0.01).

Results Profiling revealed 18 clusters: one CD4+ T cell cluster (CD4-CD40LG), twelve clusters representing different CD8+ states (CD8-CD27, CD8-MIF, CD8-ZNF683, two MAIT, IL9R-Tcells, five CD8-MKI67 and CD8-TOX), four gamma-delta T cell clusters (?d-TRDC), and one unique cluster (MALAT1). scATAC-seq analysis of the MPM TIL paired with their transcriptomic clusters validated the presence of existing cell states with trajectory analysis confirming the separation of the distinct cell states. Activation and inhibitory markers showed heterogenous pattern. Upregulation of activation markers OX40 (*TNFRSF4****) and ICOS*** was present in CD4-CD40LG and OX40 (*TNFRSF4****) marker in IL9R-Tcells. Moreover, CD4-CD40LG showed high upregulation of CTLA4*** and GITR (*TNFRSF18****), whereas, among CD8+ subsets, GITR *** expression was observed only in γδ-TRDC and IL9R-Tcells. γδ-TRDC also displayed heterogenous upregulation of other inhibitory markers as TIGIT, LAG3 and TOX. Progenitor exhausted state transcription factor TCF7*** was only observed in CD8-CD27 and across CD8-MK67 populations. Transcription factors *PRDM1****, *MAF**** promoting T cell exhaustion were present within MALAT1 cluster.

Conclusions As expected, CD8+ TIL states predominated the grown TIL products. The expression of signature genes suggested presence of several activated and proliferating CD8+ states, tissue resident memory CD8+, an effector CD4+ state and a γδ+ T-cell state. The presence of inhibitory receptors is heterogenous and informs the dysfunctional states of cells, which may be of use for design of novel immunotherapy strategies.

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Ethics Approval This study was written and conducted in accordance with the principles from the Declaration of Helsinki. Written informed consent was provided by all study participants or their legal representatives. The study (LAB08-0380) was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board.

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