T CELL PHENOTYPE DRIVES RESTRUCTURING OF TUMOR MICROENVIRONMENT TO BALANCE T CELL LONGEVITY AND TUMOR CONTROL: INSIGHTS FROM MULTIPLEXED IMAGING AND MULTI-SCALE AGENT BASED MODELING

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Background Immune cell therapies continue to have success in treatment of cancers yet face challenges of complexity, cost, toxicity, and low solid-tumor efficacy. Much work has focused on the phenotype characterization and control of ex vivo expanded cells; however, little is known about its relationship to changes in the tumor microenvironment in vivo. Thus, we imaged tumors treated with different phenotype tumor-specific CD8+ T cells with CODEX multiplexed imaging¹⁻⁴ that is able to visualize 42 antibodies at the same tissue in the tissue (figure 1A). To further probe this data in a systems immunology approach we created a multiscale agent-based model including critical circuits from the T cell-tumor microenvironment interactions (figure 1B).

Methods We initialized our agent-based models various percentages of either PD1+, PD1-, PDL1+, or PDL1- phenotypes and ran simulations for 72 hours. We also treated PMEL CD8+ T cells with or without 2 hydroxycitrate as a metabolic inhibitor during activation to achieve different input phenotypes of CD8+ T cells for therapeutic adoptive transfer on day 10 following B16-F10 tumors had been established. We performed neighborhood analysis on CODEX multiplexed imaging data by clustering neighboring cell types using a sliding window for neighborhood analysis.

Results Interestingly, the agent-based modeling indicated that the tumor phenotype switch to decrease proliferation was more effective than direct T cell killing. We observed spatially restricted inflammatory immune fronts when simulating with different initial percentages of PD1+ T cells and also from our CODEX multiplexed imaging. Quantitatively we observe that there is a drastic increase in the PDL1+, MHC1+, Ki67-tumor phenotype that increases with metabolically inhibited T cells. Neighborhood analysis indicated that metabolically treated T cells were able to create distinct immune cell environments that supported productive T cell-tumor interactions and also helped maintain T cell phenotype.

Conclusions This indicates there is a balance for therapeutic T cell to mitigate chronic tumor exposure while controlling tumor growth through killing and by changing tumor phenotype. We observe T cells create distinct tumor microenvironments that differs significantly based on the starting T cell phenotype. Controlling T cell phenotype to promote productive immune-tumor structures will be critical to maintain T cell functionality and efficacy. In the future we will investigate T cell recruitment of immune structures by similar systems biology technologies.

Acknowledgements J.W.H. is funded by an ACS Postdoctoral Fellowship (PF-20-032-01-CSM).

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Ethics Approval All studies involving mice were approved under Stanford's APLAC protocol 33502.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.180>