368 TIME-LAPSE ANALYSIS OF TCR-T CELLS HIGHLIGHTS HETEROGENEITY AND KINETICS OF RESPONSES UPON ANTIGEN STIMULATION

[1]Amanda S Montoya*, [1]Peixin Jiang, [2]Yulia Shulga, [2]Sarah Forward, [2]Emane Rose Assita, [1]Emily G Bontekoe, [1]Minying Zhang, [3]Jared K Slone, [1]Ludovica La Posta, [1]Eleonora Dondossola, [3]Lydia Kavraki, [1]Jianjun J Zhang, [2]Trevor Brown, [1]John V Heymach, [2]Sheldon Kwok, [1]Alexandre Reuben. *[1]The University of Texas MD Anderson Cancer Center, Houston, TX, USA*; *[2]LASE Innovation, Waltham, MA, USA*; *[3]Rice University, Houston, TX, USA*

Background Oncogenic mutations are prevalent in non-small cell lung cancer (NSCLC) and associated with lack of response to checkpoint blockade immunotherapy. Cell therapies using tumor-infiltrating lymphocytes (TILs) have been deployed and resulted in impressive responses; however, most patients do not derive durable benefit, potentially due to prevalence of bystander TILs, lack of functional potential, or limited tumor specificity. Accordingly, T cell receptor (TCR) engineering targeting critical tumor antigens has emerged as an alternative that could overcome many limitations. Unfortunately, heterogeneity in TCR-transduced populations, particularly their changes in phenotype and function over time and in response to stimuli, has not been investigated.

Methods We evaluated a library of validated TCRs recognizing EGFR, KRAS, and HER2 mutations, which recognize different classes of tumor antigens including tumor-associated antigens, single nucleotide variants, duplications, and insertions and deletions, presented on several of the 10 most prevalent HLA alleles in the USA. TCR-T cells were tested for sensitivity and specificity of binding to cognate antigens by peptide dose-response and alanine scan. TCR-T cells also underwent Time-Lapse flow™ cytometry, where cells were barcoded with laser particles (LP) and measured over time following antigen exposure (baseline, 12 hours, 36 hours) to evaluate phenotypic and functional changes in individual TCR-T cells at the single cell level. Activation (CD8, CD25, CD69, PD-1, CD154, CD137), memory (CD45RA, CCR7), and checkpoint expression were evaluated (PD-1, TIM-3, LAG-3, CD137).

Results TCR-T cells exhibited specificity for mutant epitope and lack of reactivity against wildtype counterpart as measured by IFN-g ELISpot, MIP-1b secretion, and cytotoxicity via LDH or chromium release assays. CD69, CD25, and PD-1 expression of individual cells over time revealed unique signatures for TCR-T cells in the presence of cognate antigen. Preliminary data revealed TCR-T cells could be grouped into 9 well-separated clusters based on marker expression trajectories, with one prominent cluster only observed with cognate antigen and consisting of cells with largely de novo expression of activation markers. Analysis of exhaustion markers showed LAG-3 was always co-expressed with TIM-3, but not vice versa. IFNγ-secreting-cells tended to either sustain or induce CD69 at 36 hours following antigen exposure. Overall, TCR-T cells were strikingly phenotypically and kinetically heterogeneous despite harboring the same TCR, which could provide insights into clinical responses and lack thereof.

Conclusions Our studies highlight the phenotypic and functional heterogeneity of TCR-T cells via time-lapse flow cytometry. Analyses are underway to assess the impact of repeated antigen exposure on TCR-T.

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