16 MULTI-DIMENSIONAL ANALYSIS OF CD19-CAR T CELLS AT SINGLE CELL RESOLUTION ON THE BERKELEY LIGHTS PLATFORM ENABLE UNIQUE INSIGHTS INTO RELATIONSHIPS BETWEEN CYTOTOXICITY KINETICS AND CYTOKINE SECRETION

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Background CD-19 CARs have tremendous efficacy in the clinic and complete clinical responses persisting for >10 years. However, outcome of clinical response cannot be predicted. Traditional analytical methods are complex, labor/cost intensive, with high sample requirements, thus generating deep characterization datasets to define critical quality attributes is an unmet need. In addition, linking parameters at single-cell resolution has not been possible. We show here the ability to rapidly measure and link analytes at single-cell resolution for thousands of cells in parallel.

Methods T cells from a healthy donor were activated with CD3/CD28 beads and transduced with lentivirus encoding CD-19 CAR. Single control or CD-19 CAR T cells were loaded on OptoSelect® chips and placed in co-culture with Raji CD19+ or Raji CD19-KO single cells and a bead conjugated to anti-IFN-y antibody. Co-cultures were under perfusion media culture containing Caspase-3 detection reagent over 22-hour timelapse with 30-minute imaging intervals. Time to detection of Caspase-3 signal and mean fluorescent intensity of anti-IFN-y-PE was recorded.

Results Hundreds to thousands of single cell co-cultures of the populations described above were obtained. Detectable IFN-y signal was observed in 45% of the co-culture events in the CAR-T+CD-19 Raji group while all others were below 1%. Cytotoxicity was observed in 25% of the co-culture events in the CAR-T+CD-19 Raji group, 10% of the co-culture events in the CAR-T+CD-19-KO Raji group and <=1% in the other groups. Rapid cytotoxicity (<= 7 hours) was enriched in the CAR-T+CD-19 Raji group >6 fold compared to the CD-19-KO control. Slow cytotoxicity (>7 hours) showed no significant difference between the CD-19+ and KO groups. Co-cultures double positive for IFN-y and cytotoxicity comprised 13% of the CAR-T + CD-19 Raji population and not present in any others. Significant clustering of rapid killers with low IFN-y secretion was observed.

Conclusions We demonstrate the ability to characterize T cells in co-culture with antigen-presenting cells (APCs) at single-cell resolution. Classic functions of antigen-dependent interactions between CAR-T cells and APCs such as IFN-y secretion and cytotoxicity are enriched when both CAR and antigen are present but largely absent in mismatches as expected. Evaluation of cytotoxicity kinetics and semi-quantitative measurements of cytokines enable identification of potential multimodal mechanisms of action and insights into potency of cell products. This deep characterization dataset can be generated in two days with limited numbers of cells which will enable definition of critical quality attributes to drive next-generation cell therapies.

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