INTRODUCTION

The key challenges to developing T cell-based therapies center on the fact that T cell-mediated tumor death relies on complicated cell-cell interactions and several complex mechanisms. These therapies have also been associated with significant side effects related to cytokine release syndrome (CRS) and neurotoxicity, placing importance on understanding T cell anti-tumor functions like cytokine release and killing kinetics. Ideally, T cell therapies would be tailored to mediate the rapid destruction of multiple tumor cells while reducing these side effects.

Various techniques are currently used to assess T cell function, including flow cytometry, live-cell imaging, and chromium release to assess target cell killing; and destruction of multiple tumor cells while reducing these side effects.

THE OPTO CELL THERAPY DEVELOPMENT 1.0 WORKFLOW

The Opto Cell Therapy Development 1.0 Workflow is a collection of software capabilities, reagents, and protocols that allow scientists to selectively measure cytokine secretion, visualize killing behavior, and sequence TCRs from individual cells in parallel. Here, we demonstrate its use for CAR and cytokine functional screening as well as the discovery of TCRs associated with specific T cell behaviors.

MATERIALS AND METHODS

Cytokine release and tumor killing assays with anti-CD19 CAR T cells

To assess CAR T function, we loaded IFNγ capture beads into OptiSelect chips in the Lightning system. T cells and CAR cells were reconstituted into T cell media containing our proprietary Loading Reagent. We assembled individual T cell target cell interactions by selectively placing single T cells and E-CD19+ or CD19- or CD19-CAR cells into NanoPen chambers containing cytokine capture beads. 75% of NanoPen chambers were loaded with CD19- T cells, while the remaining pens were loaded with CD19+ CAR T cells. We previously tracked T cell and target cell phenotype and killing by co-culturing cells and beads with constant perfusion of T cell media containing 5 μM Nuclease 40S Caspase-3 Substrate (Biotium) and imaging pens every 30 minutes in all fluorescence channels. After overnight incubation, we directly measured secreted IFNγ captured by IFNγ capture beads by incubating chips with a FITC-conjugated antibody against IFNγ (Biotium) and imaging in all fluorescence channels.

RESULTS

The cumulative percentage of pens with tumor cell caspase-3 activity increased over time in pens loaded with CD19+ tumors, peaking at 20% at 72 hours of incubation (A). This is in contrast to only 10% of pens displaying tumor cell death in control pens loaded with CD19- tumor cells, control pens also exhibited slower killing kinetics.

CONCLUSIONS

The Opto Cell Therapy Development 1.0 Workflow on Berkeley Lights systems enables researchers to:

- Correlate cytokine secretion to target cell killing behavior in CAR-mediated antigen recognition
- Discern CAR T cell subsets based on kinetics of target cell killing
- Link cytokine secretion and target cell killing behavior to TCR sequence in CAR-mediated antigen recognition