Abstracts

Regular Abstracts – Part 2

Biomarkers, Immune Monitoring and Novel Technologies

222-A THE BEACON® OPTOFLUIDIC SYSTEM ENABLES MULTIFUNCTIONAL CHARACTERIZATION OF T CELLS AND SEQUENTIAL RECOVERY OF SINGLE CELLS FROM CO-CULTURE FOR DOWNSTREAM TRANSCRIPTOMIC ANALYSIS

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Background T cells are heterogeneous in both phenotype and function and have variable responses to stimulation. Existing methods to assess T cell function are typically applied individually to separate cell populations such that relationships between functional parameters such as cytotoxicity and gene expression can only be inferred. We previously demonstrated the ability to evaluate rapidly cytokine secretion and cytotoxicity at single-cell resolution for thousands of cells in parallel. Transcriptomic analysis was performed on T cells that were activated with antigen-presenting beads and the results were correlated to T cell polyfunctionality. When antigen-presenting cells are present in the co-culture, downstream processing has been limited to T cell-specific readouts due to contaminating RNA transcripts. Here, we describe a high-throughput, automated workflow to isolate single cells of interest sequentially from a transient co-culture for downstream single-cell analysis. This workflow was demonstrated on proliferating T cells and may be applied toward assays requiring co-culture of multiple cell types.

Methods T cells from a healthy donor were activated with CD3/CD28 beads and labeled with CellTrace™ Far Red. Single T cells were isolated into NanoPen® chambers on OptoSelect® chips using optoelectronic positioning (OEP®). Single anti-CD3/CD28 activation beads were added to each NanoPen chamber and the cells were cultured with timelapse fluorescence imaging in 30-minute intervals to identify cell division events. Once sufficient cell division was observed, 192 T cells were isolated sequentially from 96 T cell pairs and recovered for downstream processing. Fluorescent images acquired during the cell recovery were used to map cells exported from specific NanoPen chambers to their respective well plate locations.

Results Over 3,000 single T cells were isolated for overnight culture. Detectable cell division was observed in 26% of Nanopen chambers after 7 hours of culture. T cells were sequentially isolated from 94 of the 96 T cell pairs attempted and the fluorescence intensity of each cell was recorded.

Conclusions The Beacon® Optofluidic System enables multidimensional characterization of T cell potency at single-cell resolution. Here, we expand on these capabilities and demonstrate the ability to isolate individual T cells sequentially from multicell cultures for downstream single-cell processing. Fluorescent images captured during single-cell isolation allow for tracing of specific cells or cell types, such as T cells and tumor cells in a co-culture, to sequencing results. This capability can be applied toward correlation of on-chip phenotypic assay data, including cytokine secretion, cytotoxicity, or surface expression to single-cell RNA sequencing that was previously not feasible.