Evaluation of functional heterogeneity in the potent responses of single CAR-T effector cells

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Abstract

Over the last decade, chimeric antigen receptor (CAR)-T cell immunotherapies have produced some miraculous cures in advanced stage, aggressive leukemias and lymphomas in both children and adults. However, many barriers continue to limit the availability and effectiveness of these therapies, including the lack of reliable potency assays for these cell-based "living" medicines, which by nature are highly heterogeneous. An engineered lot of cells inevitably contains individual cells with a variety of growth properties, cytolytic activity, gene expression patterns, and secretion profiles, and it is not clear which cell subpopulations are most correlated with positive clinical outcomes.

Here we report results of an evaluation of phenotypic heterogeneity in a population of anti-CD19 CAR-T cells co-cultured with target cells in an array of >300,000 microwells. Our approach uses high throughput time lapse imaging to continually monitor the cell-cell interactions between effector and target cells. We maintained cell viability in the microwells for more than 5 days, which gave us sufficient time to observe the full range of antigen-mediated cell killing phenotypes, and also evaluate growth rates of the potent effector cells. We tracked thousands of individual co-cultures through time lapse imaging, and cell behavior was evaluated and sorted by deep learning algorithms. We were able to quickly identify and retrieve clones that exhibited desirable phenotypes for full molecular characterization.

Our analysis shows that three fundamental subpopulations of effector cells can be clearly distinguished and quantified. These are 1) the effector cells that kill the targets and then die, 2) the effector cells that kill the target and then proliferate, and 3) the effector cells that do not kill the target. In future work, these populations will be further dissected by characterizing their secretion and transcriptomic profiles, to identify further subtype classifications.

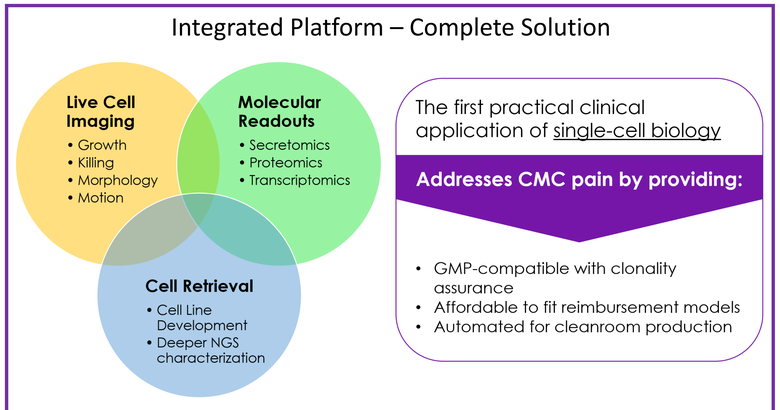

Fig. 1: Standard 96-well format plate, with each well containing thousands to tens of thousands of microwells. Simple loading by direct addition of cells, without microfluidics, cell stress, or clogging. Simple assembly of co-cultures, and no cell disturbance during washes or liquid exchanges. Automated imaging and recognition of desired phenotypes, with ability to select and pick out desired clones.

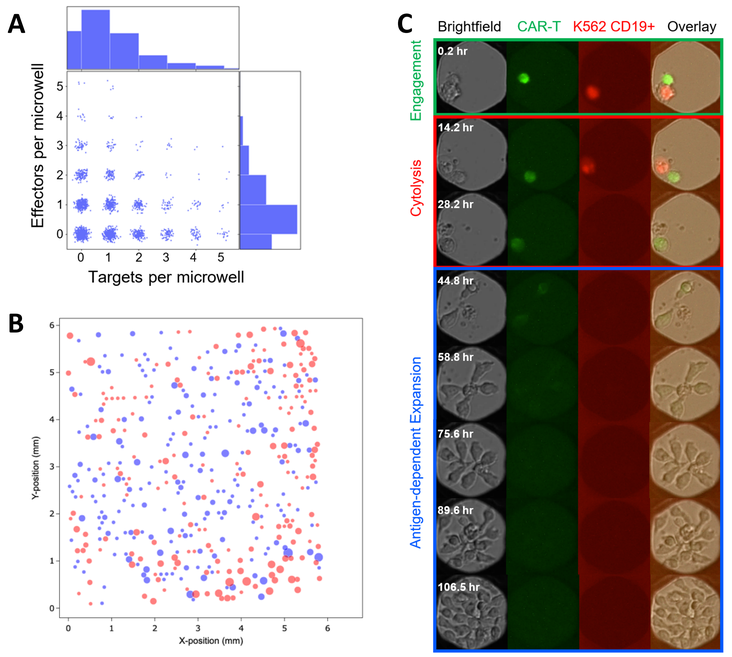

Fig. 2: (**A**) Cell trapping statistics for effector:target distribution in an average well. Each well naturally contains co-cultures along with monoculture controls. (**B**) Map of interactions within a well. Red dots indicate microwells in which effector T-cells engaged with their targets; blue dots indicate microwells without engagement. (**C**) Representative time lapse image series of co-cultures in brightfield and 2 fluorescent channels. Antigen-specific engagement, cytolysis, and post-killing expansion of the effector cell is observed.

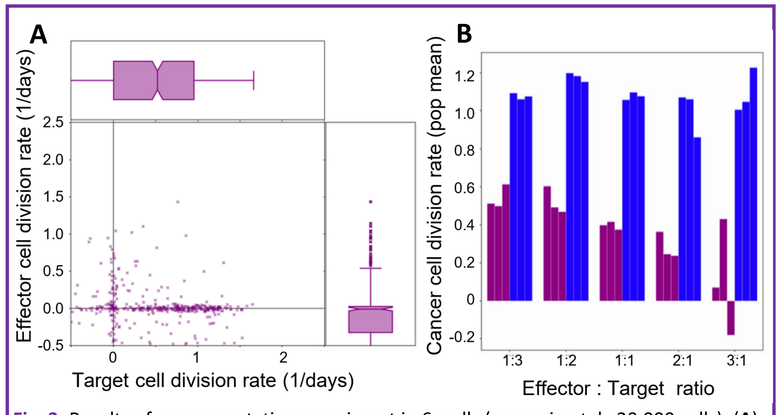

Fig. 3: Results of a representative experiment in 6 wells (approximately 20,000 cells). (**A**) The growth rate distributions of the target + effector cocultures for exactly 1:1 effector:target ratio microwells. Each dot represents one microwell. (**B**) Average target growth rate vs E:T ratio for all 6 wells. Blue bars represent triplicate of wells containing non-target (CD19-) and effectors, while the purple bars show triplicate of wells containing target (CD19+) and effectors. Division rate reduction reflects death of a subpopulation of target cells, and occurs only in microwells with target-expressing cells, while off-target cells show unhampered division.

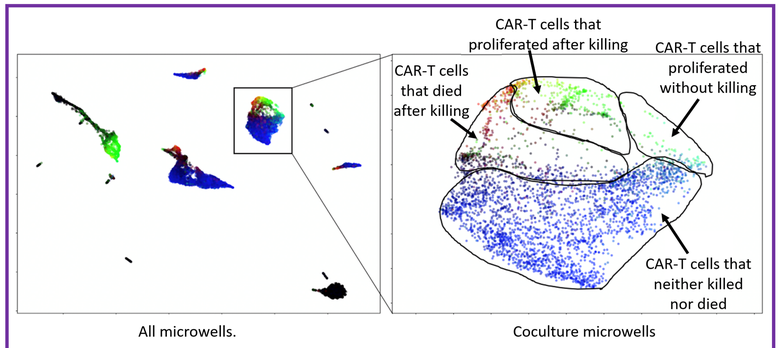

Fig. 4: UMAP analysis of phenotypic cell data. (Left) All microwells separated by UMAP; note that coculture wells cluster together. (Right) Zoom on the coculture cluster, showing separation between four populations of CAR-T cells. The largest population does not kill or replicate, while the remainder either kill the target then die, or kill it and start proliferating. A small number of CAR-Ts initiate replication without killing.

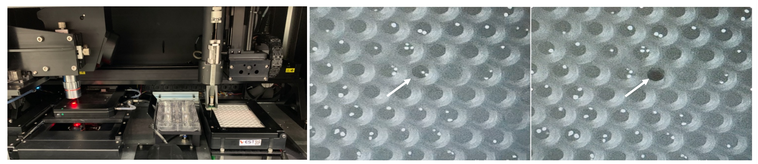

Fig. 5: Integrated automated cell picker hardware can remove and transfer selected cells into a separate 96-well plate for further assays and genomic/transcriptomic analysis.

Conclusions

Data generated by direct measurement of phenotypes and behaviors of single cells within bulk populations represents a fundamentally new way of measuring cytotoxic potency of effector cells, and their therapeutic potential. This resolution cannot be obtained from measurements in bulk cultures, where all individual cell behaviors are averaged over a heterogenous population. Populations can be screened for the percentages of potent, proliferative T-cells (such as the ones seen along the y-axis of Fig. 3A), and such cells can be further isolated for more detailed analysis. The commercial platform will include measurements of cytokine secretion and subtyping of effector cells.

The Celldom platform is an integrated solution for next-generation cell assays, allowing researchers to make precise connections between phenotype and genotype at the single cell level. Simplified workflows and easy-to-use consumables facilitate adoption, reduce costs, and eliminate sample losses caused by easily clogged microfluidics. Temperature, humidity, and atmosphere-controlled incubation chamber allows easy short- or long-term live imaging of cellular interactions, with automated phenotyping at high throughput.

Beyond CAR-T screening and potency testing, this technology is useful for extremely high throughput antibody screening (>1,000,000 B-cells per run) with affinity and specificity measurements, enabling efficient searches for antibodies against complex or difficult targets. The same high throughput, ease of use, and automated analysis enable previously unfeasible approaches to biologics discovery and cell line development. The platform also allows screening of primary cancer samples for drug resistance, identification of effective combination therapies, and measurements of dose-response effects on various aspects of cellular physiology and behavior.

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