Establishing a multi-modal, cell therapy characterization workflow to identify cell subsets with heightened cytotoxicity

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QUESTIONS?

ABSTRACT

Cell therapies have been groundbreaking for B-cell lymphomas but optimizing efficacy for solid tumors remains challenging. Lack of consensus on potency measures, unknowns about novel engineering strategies, and product heterogeneity drive extensive characterization to discover product specific mechanisms of action (MoAs). This process is costly and limited by variable results from simple molecular assays and need to infer relationships between data from disparate technologies. Thus, we created a multi-modal technology with which to establish a multi-functional, longitudinal cellular characterization workflow. With a single sample input, this workflow evaluates multiple live phenotypes from the same individual cells across tens of thousands of cells at once. We leveraged TALL-104 cells before validating compatibility with clinical-stage cell therapies with AIC100 - a Phase 1 CAR-T product targeting thyroid cancer (NCT04420754). Cellanome's technology enables multi-modal evaluation of live, cellular behavior at the resolution of single cells or single-cell interactions. Within an 8-lane flow cell, tens of thousands of single TALL-104 cells were enclosed with K562 target cells in bio-compatible hydrogel compartments called CellCage™ enclosures (CCEs). These CCEs enable longitudinal monitoring of single TALL-104 cells interacting with K562 cells for hours to days with time-lapse imaging. During this period, imaging-based evaluation of surface markers, cytokine secretion, and cytotoxicity are integrated from the same single T-cells. Our analysis pipeline links target cell death to single T cells in CCEs, enabling cell killing to be studied with unprecedented resolution. Similar to flow cytometry via CD107 as a surrogate for killing, with Cellanome's technology, we could identify cytotoxic T cells (~85%). Uniquely, we can directly evaluate killing by single TALL-104 cells and further segment them into groups. Group 1 consists of strong killers (killing 100% of targets), Group 2 of medium killers (50-99%), and Group 3 of weaker killers (0-49%). We next characterized single AIC100 cells enclosed with HeLa as targets and observed that, unlike TALL-104 cells where Group 2 was the largest, for AIC100, Group 3 was the largest, suggesting distinct killing mechanisms. Thus, we asked whether AIC100's heterogeneous composition of CD4 / 8 cells impacts product efficacy. We configured this workflow to test killing by ~100 combinations of CD4:CD8 AIC100 cells in a single experiment. Preliminarily, more killing occurred in CCEs containing a mixture of CD4 and CD8 T cells relative to CCEs containing only CD4 or CD8 cells. We are continuing to identify the optimal CD4:CD8 compositions for greater potency. We envision multi-modal characterization of clinical products with our platform, pre- and post-patient infusion, will inform efforts to advance cell therapies for solid tumors.

CELLANOME TECHNOLOGY

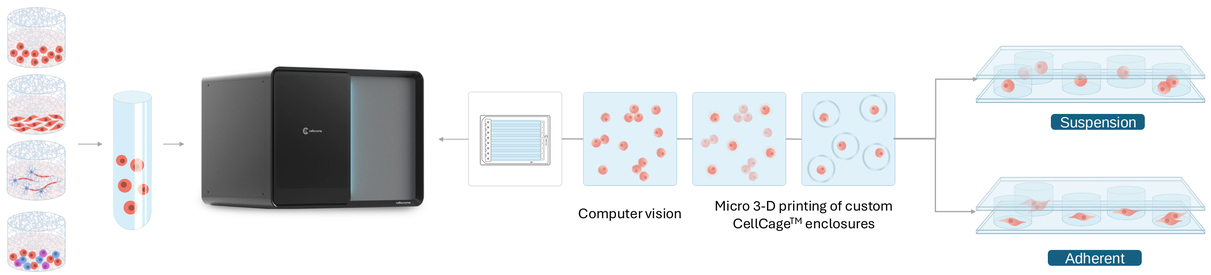


Fig 1. Schematic of workflow for novel Cellanome technology enabling the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. Tens of thousands of cells in suspension are mixed with hydrogel precursor and loaded on an 8-lane flow cell. Positions of cells are identified and CellCage™ enclosures are generated around cells with light-guided polymerization in an automated fashion. CellCage™ enclosures are permeable to reagents enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells (e.g., small molecules, immunofluorescent antibodies). Cells can be lysed within CellCage™ enclosures to generate barcoded cDNA for downstream library prep and sequencing off the instrument.

PROFILING THE KILLING ACTIVITY OF CLINICAL GRADE CAR-T CELLS PRODUCTS

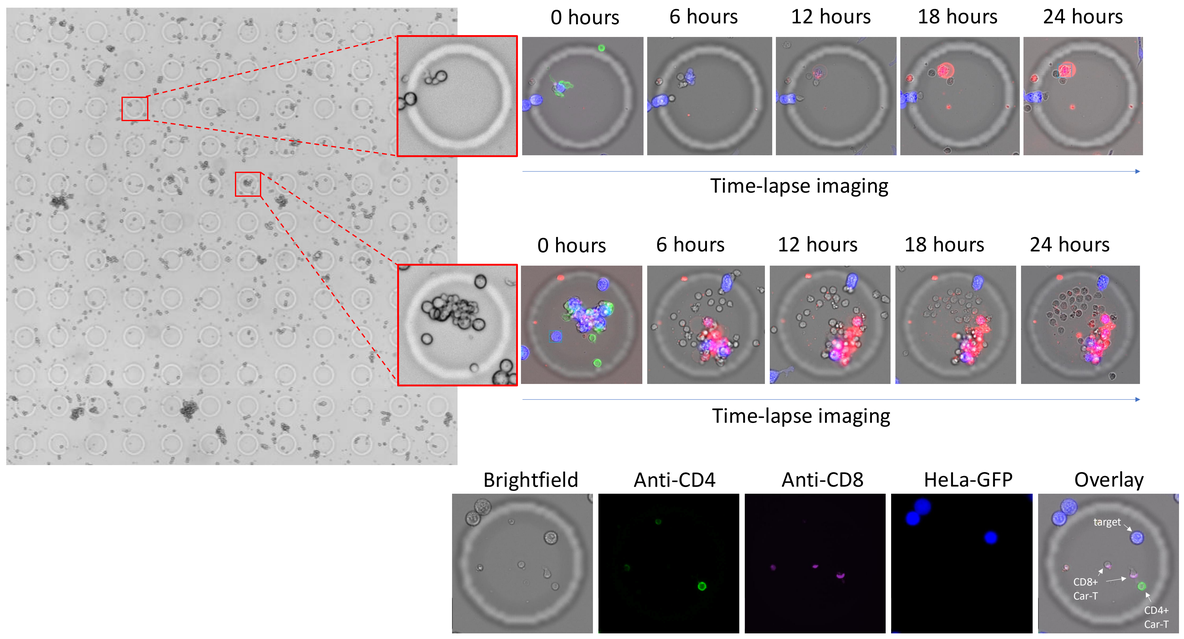


Fig 2. Identification of T-cell types and longitudinal monitoring cell killing activity using AIC100 CAR T-cells. AIC100 cells, labeled with anti-CD4 (green) and anti-CD8 (violet) antibodies were co-encapsulated in CellCage™ enclosures with HeLa-GFP target cells (blue). Cell killing, as indicated by Annexin V staining (red), was monitored for each CellCage™ enclosures via time-lapse imaging over 24 hours.

A NEW APPROACH TO THE ANALYSIS OF CELL KILLING DATA

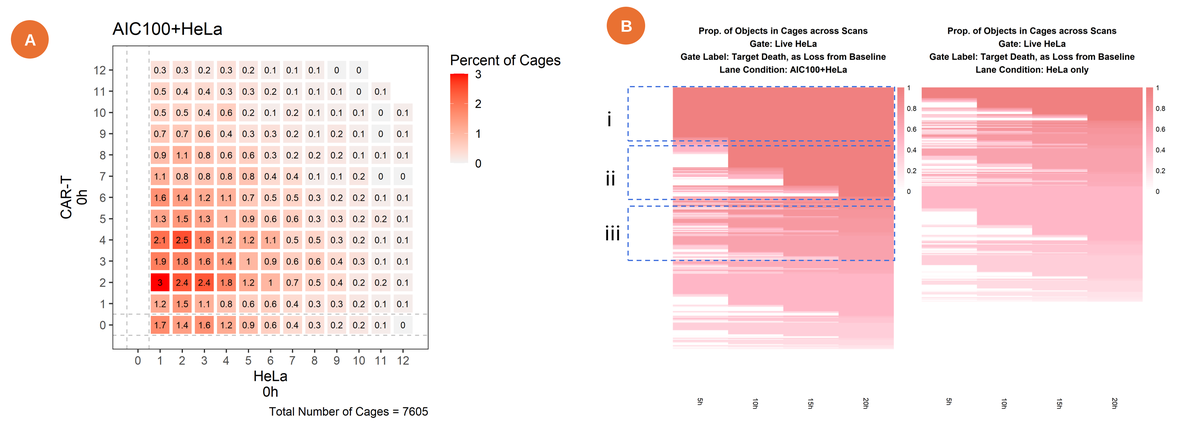
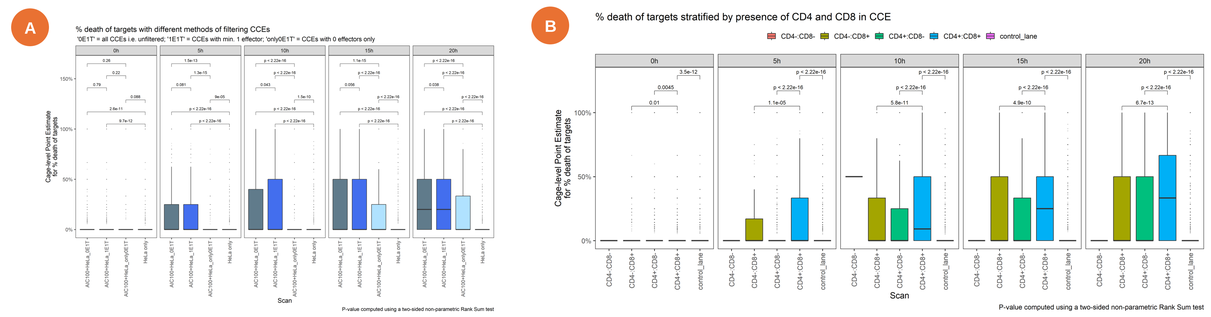


Fig 3. (A) The distribution of cell combinations encapsulated in CCEs across the co-cultured lane. The shading indicates the percentage of CCEs containing *x* HeLa-GFP cells and *y* AIC100 cells, each of which occurs in a Poisson distribution. (B) The rate and proportion of target cytotoxicity is tracked for each individual CCE (y-axis) across time-lapse imaging scans (x-axis), when HeLa-GFP targets were co-encapsulated with AIC100 cells (left) or without AIC100 cells (right). Boxed dotted lines indicate CCEs with (i) fast and efficient killing, (ii) slow and efficient killing, or (iii) slow and less efficient killing. Cytotoxicity is the proportion of live HeLa-GFP cells in each CCE that die or disintegrate at each timepoint.

DETERMINATION OF CONDITIONS THAT MEDIATE EFFECTIVE CELL KILLING



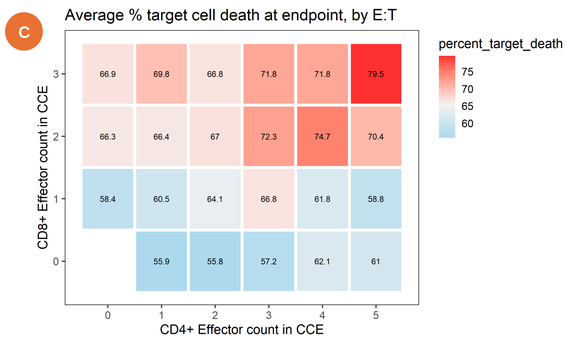


Fig 4. Correlation of CCEs with high and low cell killing with AIC100 cell type and number elucidates conditions needed for effective killing. (A) The distribution of target cytotoxicity among CCEs with different filtering conditions within the same lane. These distributions show that the presence of effectors in CCEs leads to a statistically significant increase in target cytotoxicity, (B) The distribution of target cytotoxicity is dependent on the composition of the effectors encapsulated in the same CCE. In later timepoints, we observe a statistically significant difference in target cytotoxicity among CCEs in the presence of CD4+ and/or CD8+ AIC100 effectors. CCEs that contain both CD8+ and CD4+ cells are associated with the highest average target cytotoxicity, compared to CCEs with only CD4+ or only CD8+ AIC100 cells. This suggests that CD8+ and CD4+ T-cells can effect greater cytotoxicity when encapsulated together in close proximity.

C) Average target cytotoxicity varies by the specific numbers of different effector cell profiles, defined by surface positivity for CD4 and CD8. CCEs have increasing target cytotoxicity with increasing numbers of both CD4+ effectors and CD8+ effectors, independently and together. This dependence is stronger with CD8+ effector count in the CCE, and aligns with our observation in (B).

DISCUSSION AND FUTURE DIRECTIONS

In this study we established an experimental workflow that can be leveraged by cell therapy developers / researchers to deeply understand the products and engineering strategies they are developing. By creating high-resolution, multi-modal data that can maximize information yielded from product or patient samples, researchers and developers can conserve precious samples and extend discovery efforts. Cellanome's technology provides data on cell behavior with multi-modal measurements across surface receptors, cytokine secretions, and cytotoxic capabilities from single cells and multi-cell interactions. Because they are generated from the same cell, this platform yields matched datasets, removing the need to infer relationships across molecular properties from cells collected across different samples, experiments, and technologies. The observation that a combination of CD4+ and CD8+ AIC100 CAR T-cells increased cytotoxicity suggests that individual CAR T effectors are not sufficient for optimal activity. Effectors and targets were analyzed based on stochastic distribution, enabling the survey of a wide range of E:T ratios within a single lane. A greater proportion of HeLa-GFP cell death was observed in the lane with AIC100 CAR T effectors compared to the HeLa-GFP only negative control lane. CCEs that encapsulated both CD8+ and CD4+ effectors had the highest proportion and rate of target cell death. Further analysis of these CCEs showed that the increasing the number of CD8+ effectors alone increased target cell death while increasing CD4+ effectors alone did not, suggesting that CD8+ CAR T-cells played a more direct role in target cell cytotoxicity. Interestingly, increasing the number of both CD8+ and CD4+ led to the greatest amount of target cell death, suggesting that the coordinate activities of both T-cell types were required for efficient cell killing. Being able to make these observations underscores the capabilities of the Cellanome platform in not only executing cytotoxicity assays but also enabling the examination of conditions required for effective cell killing. These results underscore the value to the CAR-T field of being continuously vigilant in comprehensively characterizing CAR-T products both pre- and post-patient infusion across modalities, coupled with patient response data. Our next steps are to leverage Cellanome's platform to comprehensively profile AIC100 cells from patient blood, evaluate what cellular and molecular predictors correlate with better or worse patient responses, and compare them to the properties of functional subsets identified in vitro.