Dissecting Treatment Response in Heterogeneous Cell Populations by Linking Live-Cell Dynamics to Transcriptomics

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INTRODUCTION

Innovative therapeutics have had tremendous patient impact, strategically targeting different MoAs with compatible treatment modalities. However, heterogeneity in patient response, treatment durability, and adverse events remain a universal challenge. Studying tumor responses against these treatments have revealed significant cellular heterogeneity due to cell type composition and multiple states or subtypes within the ame cell types. Thus, it is probable that heterogeneity in patient responses is in part, driven by heterogeneity in cellular responses to treatments, including persistence and outgrowth of drug-resistant clones.

Single cell transcriptomics has enabled the field to discern cellular heterogeneity and identify novel subpopulations of tumor cells, some of which are postulated to be linked to treatment resistance. If these populations do not have sortable markers, researchers generate a series of scRNA-seq datasets to evaluate their role in treatment responses by observing their loss or emergence. This is costly, especially for longitudinal studies evaluating treatment durability, does not directly differentiate death from cell-state transitions, and fails to capture mechanistic nuances (e.g., cell cycle arrest vs. death vs. apoptosis). An ideal approach for identifying and characterizing drug resistant or responsive clones would be to longitudinally evaluate cellular phenotypes and link them to their transcriptomics. To develop such an assay, we leveraged Cellanome's novel platform technology. Unlike classic imaging-based platforms, which are limited to static analysis of single-cells, Cellanome enables attribution of temporal dynamic cell behavior (e.g., morphological changes, proliferation) to a single clone, because tens of thousands of individual cells are encapsulated within semi-permeable hydrogel compartments called CellCage™ enclosures (CCEs) and cultured. Single cells are kept in place within the CCEs, and their behavior tracked via longitudinal imaging. Sequencing data is collected from the same, single cells to generate a linked, multi-modal dataset across functional, phenotypic, and genomic data.

CELLANOME TECHNOLOGY

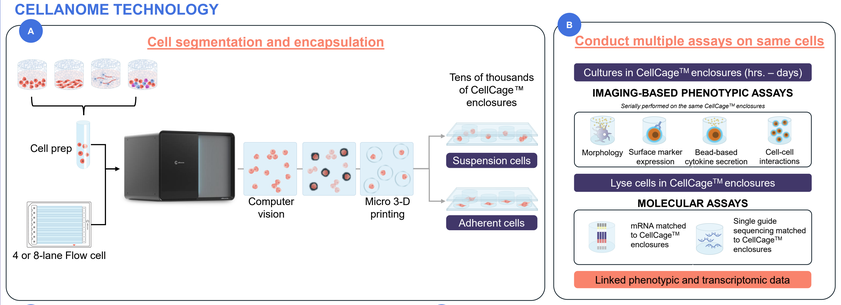
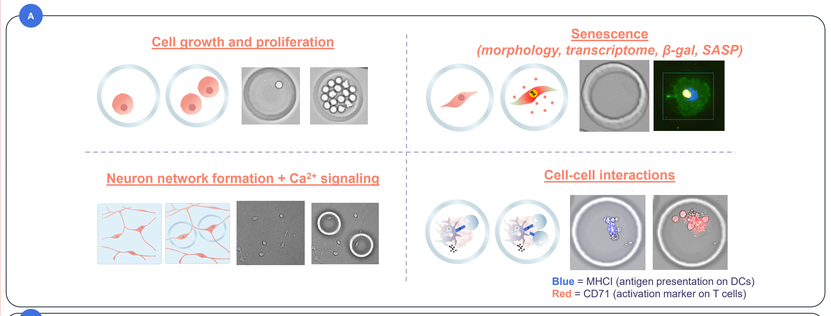


Fig 1. Cellanome's Technology enables the measurement of multiple phenotypic and functional assays from the same cells

(A) Tens of thousands of cells are mixed with hydrogel precursor and loaded on an 8-lane flow cell. Cell positions are identified and CellCage™ enclosures (CCEs) automatically generated around cells with light-guided polymerization. Bio-compatible CCEs can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine beads). CCEs 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 are lysed within CCEs to generate cDNA for downstream library prep and sequencing off the instrument. (B) Imaging-based phenotypic analysis enabled for single CCEs across flow cell lane. Cells within the same CCE can be processed for sequencing readouts and linked to imaging data. (C) Reagents can be diffused through semi-permeable CCE walls. This feature can be used to deliver nutrients, small molecules, and antibodies to all CCEs at once, at any point of the experiment. (D) Cells within CCEs are serially imaged for longitudinal, multi-functional analysis, before being processed within the CCEs for transcriptomic analysis. This enables the generation of highly parallel, integrated single-CCE datasets.

ADAPTING SINGLE-CELL AND CO-CULTURE ASSAYS FOR DRUG DISCOVERY AND DEVELOPMENT



CONFIGURING CORE CELLANOME ASSAY TO IDENTIFY DRUG RESISTANT AND RESPONSIVE CLONES WITH TRANSCRIPTOMIC DATA FOR PATHWAY ANALYSIS

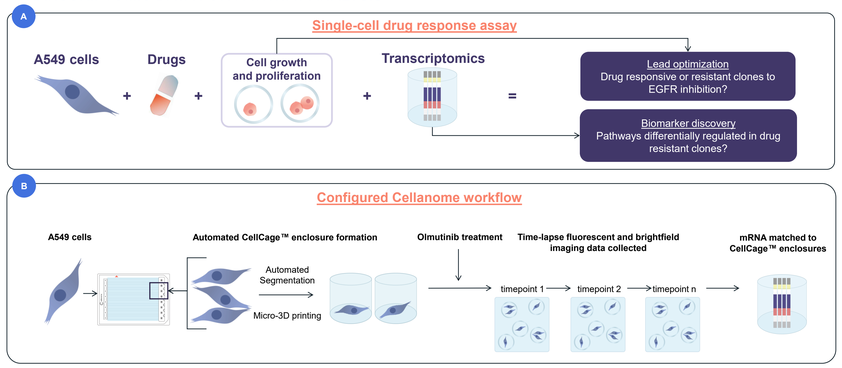


Fig 3. Developing a novel model for longitudinal evaluation of response to EGFR inhibitors by lung cancer cells

(A) Schematic illustrating how a single-cell proliferation assay can be configured to identify drug-resistant clones and generate data from those same clones for biomarker discovery. (B) A549 cells are loaded and encapsulated in CCEs as described in Figure 1a. Single cells are held in place within CCEs, treated with olmutinib, and longitudinally imaged, before the cells are lysed within the same CCEs for RNA-sequencing off the instrument. (C) A549 cells proliferated in no drug treatment control lane, but A549 cells showed heterogeneous phenotypes in olmutinib treated lane (D) Quantification of A549 death in response to treatment over time. Each individual dot represents a single CCE. Blue Mean Sub represents Annexin V signal. Increase in Blue Mean Sub reflects increase in apoptotic cells from 0 to ~30 hours.

LINKING CELLULAR PHENOTYPES TO TRANSCRIPTOMICS

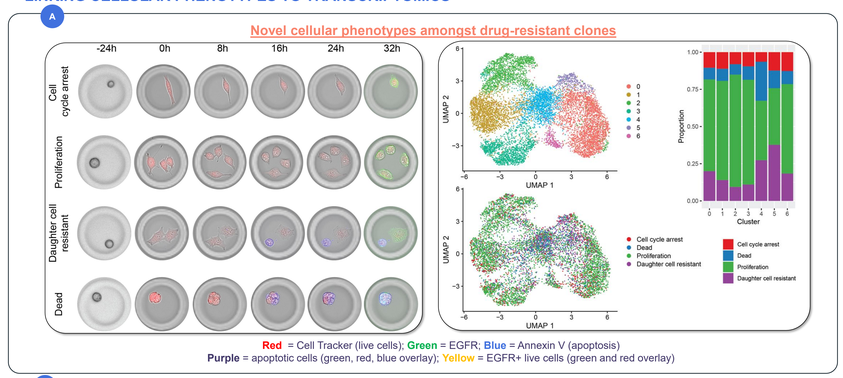


Fig 4. Cellanome's technology can discover novel cellular phenotypes within drug-resistant clones

(A) Representative images of diversity of phenotypes observed in Olmunitib treated condition. UMAP visualization and unsupervised clustering of the transcriptomic data (top), colored by the phenotypic classification (bottom). Each transcriptomic cluster contains cells spanning all four of the imaging-based phenotypes, revealing limited concordance between the two classification types. (B) The combination of transcriptomic clustering with imaging classification identified 15 unique pathways not found in either single-modality strategy. Gene networks that are positive (left) or negative (right) predictors of the Daughter Cell Resistant class. The Daughter Cell Resistant class is over-represented in cluster 5, which includes p53 transcriptional targets associated with a quiescent/slow-cycling drug resistant state

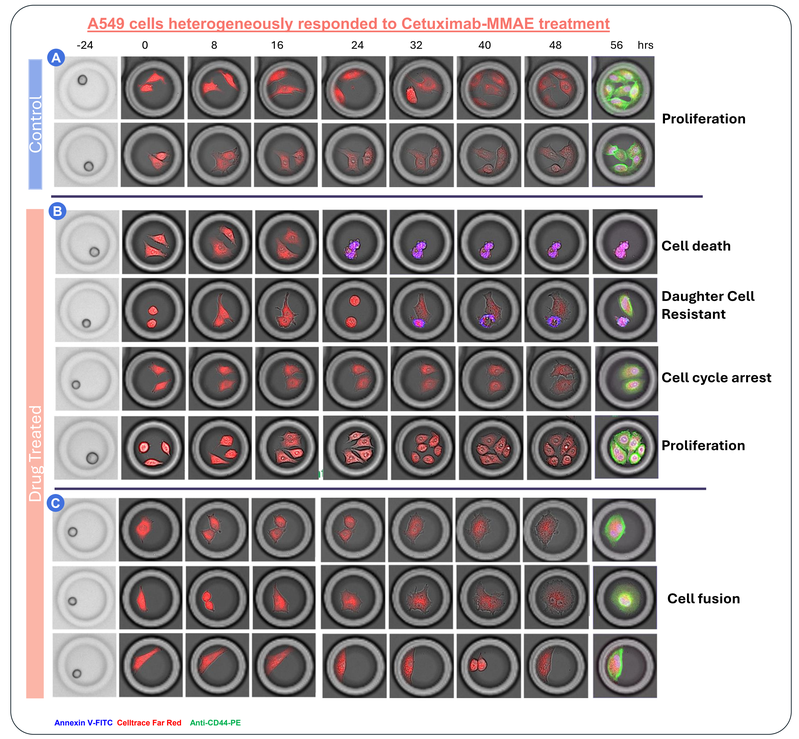
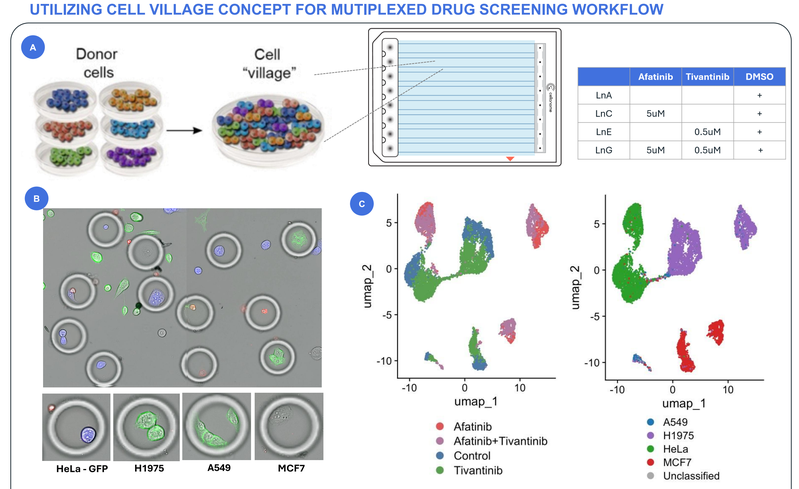


Fig 5. Establishing compatibility of Cellanome's R3200 with antibody-drug conjugate (cetuximab-MMAE) on identifies cellular phenotypes shared and distinct form Olmutinib, consistent with distinct MoA. A549 cells were treated with antibody drug conjugates at 50nM for 56 hours, cells were imaged every 8 hours to monitor drug response. (A) Representative longitudinal images of un-treated A549 cells, cultured in CCEs and imaged every 8 hours to monitor drug response. (B) Similar cellular phenotypes between Cetuximab - MMAE and Olmutinib were identified. (C) Distinct cellular phenotypes between Cetuximab - MMAE and Olmutinib were identified. Individual clones were able to proliferate, divide, and then fuse. This phenotype is challenging to identify with other platforms, supporting that Cellanome's platform can unearth biologically and therapeutically relevant cellular phenomenon.

UTILIZING CELL VILLAGE CONCEPT FOR MUTIPLEXED DRUG SCREENING WORKFLOW



	Afatinib	Tivantinib	DMSO
LnA			+
LnC	5uM		+
LnE		0.5uM	+
LnG	5uM	0.5uM	+

Fig 6. Cell Village concept allows testing multiple cell types against a drug within a single lane. (A) Cell village concept utilizes mixture of 4 cancel cell lines mixed together before caging inside flowcell lane and lanes are treated with 4 different conditions (B) Representative image of region of control lane at the end of 48 hour treatment duration. Shown below are 4 cell types that are enclosed within the CCEs. (C) UMAP clustering of the 4 lanes showing clusters corresponding to various conditions. The UMAP on the right shows overlaid identities of cells, as established by the fluorescence measurement at the endpoint. The impact of drug or combination is measured on proliferation and gene expression for each of the 4 cancel cells

DISCUSSION

These results demonstrate that Cellanome's platform, in a single assay, can identify drug resistant clones and discover mechanistic insights by characterizing dynamic cellular phenotypes, linked to the transcriptomics of single clones. Next, we aim to 1) mine the transcriptomics to identify potential biomarkers for treatment response, 2) design assays that leverage these new phenotypes as readouts for evaluating treatment dosage and / or combinations suitable for eliminating drug resistant clones, and 3) evaluate which clones can regain proliferative capacity and potentially contribute towards minimum residual disease. The integrated imaging and sequencing datasets can serve as ideal data inputs for foundation models designed to predict cellular and molecular features that correspond to oncology therapies with enhanced safety and durability.