# Leveraging a novel, multi-modal platform to identify drug resistant clones that exhibit distinct cellular phenotypes in response to EGFR inhibitors

Yunmin Li[1], Shan Sabri[1], Teresa Ai[1], Pier Federico Gherardini[1], Annarita Scaramozza[1], Gary Schroth[1], Mostafa Ronaghi[1], Tarun Khurana[1]
[1]*Cellanome, Foster City*

## 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 same 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

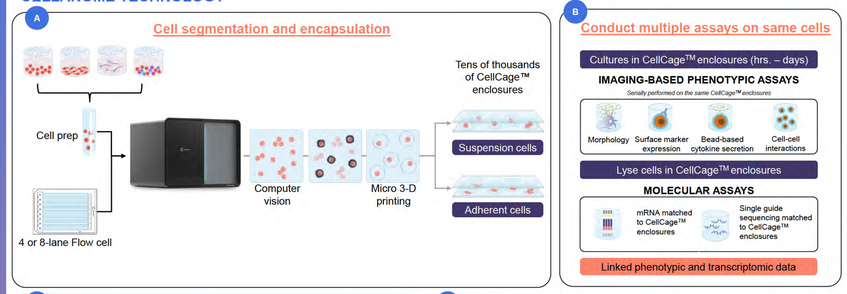
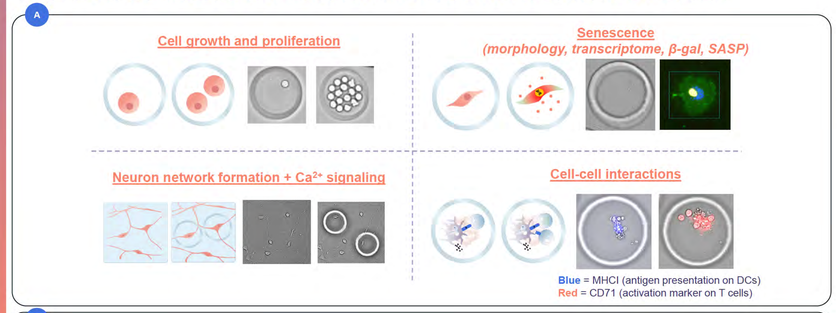



**Fig 1. Cellanome's Technology enables the measurement of multiple phenotypic and functional assays from the same cells**

(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



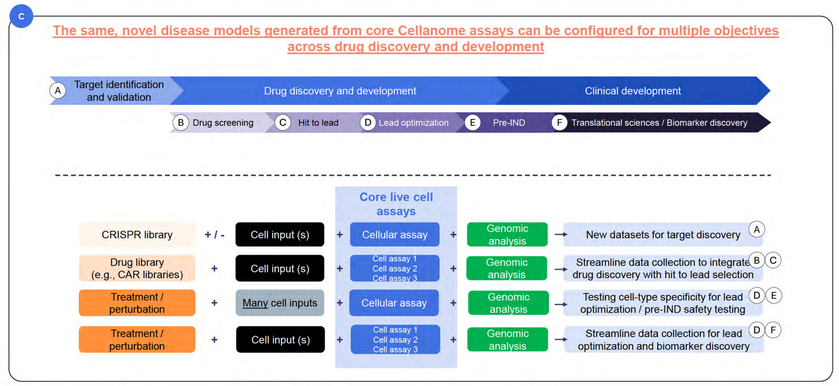
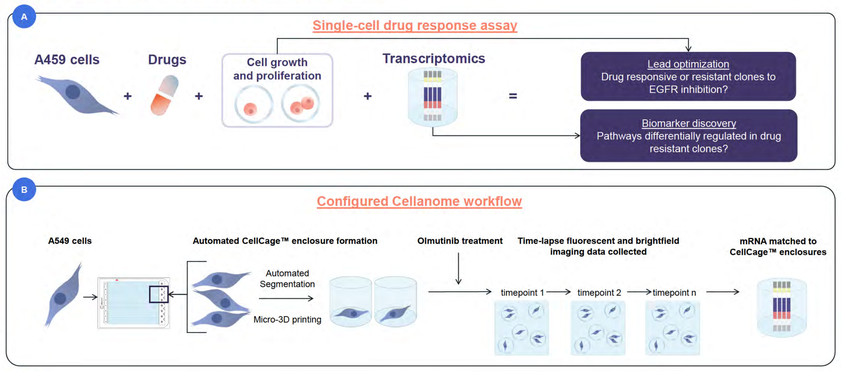
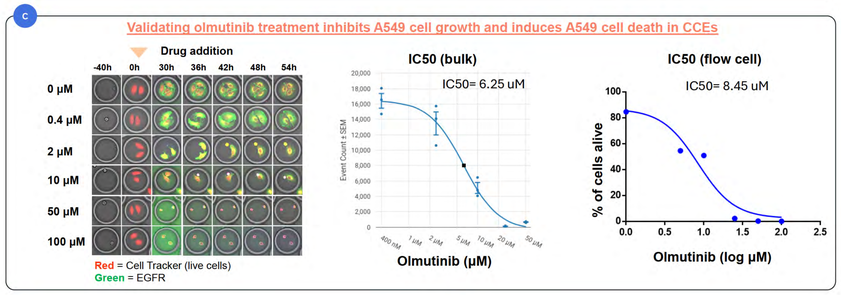




**Fig 2. Novel Cellanome assays are building blocks for novel *in vitro* disease models**

(A) Established single-cell or co-culture assays within CCEs on Cellanome's platform technology. (B) Disease models established by configuring cell inputs for Cellanome assays. (C) Schematic illustrating the flexibility enabled by Cellanome's platform technology. Compatibility with many cell types, perturbation methods (e.g., small molecules, CRISPR, antibodies), and linkage to sequencing enables end-to-end discovery from target to drug with a single, core Cellanome assay.

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



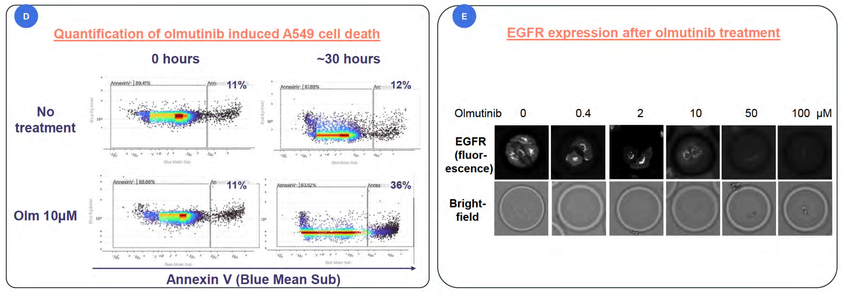
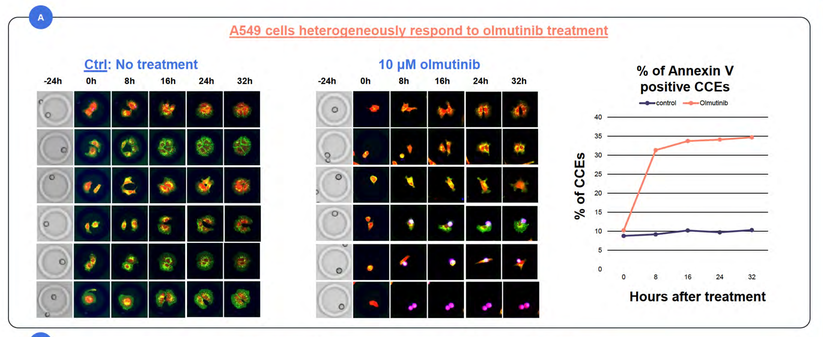




**Fig 3. Developing a novel model for longitudinal evaluation of response to EGFR inhibitors by lung cancer cells**

(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) A459 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) Olmutinib titrations validate A549 cells behave as expected, with higher drug concentration correlating with more cell death over time in both bulk and Cellanome's flow cells. (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. (E) Imaging analysis enables validation of single-cell EGFR expression in conjunction with cell morphology and proliferation. EGFR expression declines with increased concentration of olmutinib treatment within the CCEs at 42 hours, as expected



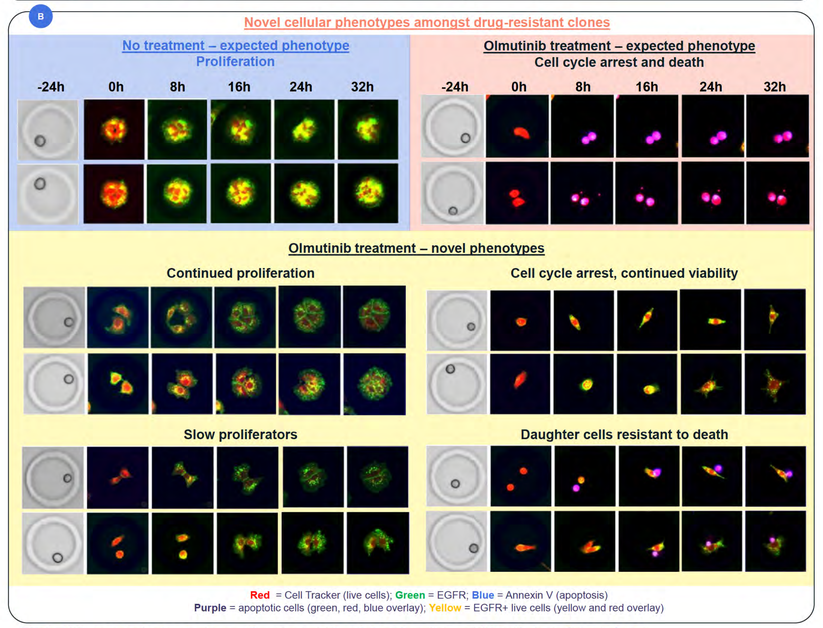




**Fig 4. Cellanome's technology can discover novel cellular phenotypes within drug-resistant clones**

(A) Representative images of treated and non-treated A549 cells (10 μm olmutinib), with quantification of Annexin V signal across all CCEs. (B) Representative images of non-treated controls and distinct cellular phenotypes within drug-resistant clones.

## LINKING CELLULAR PHENOTYPES TO TRANSCRIPTOMICS

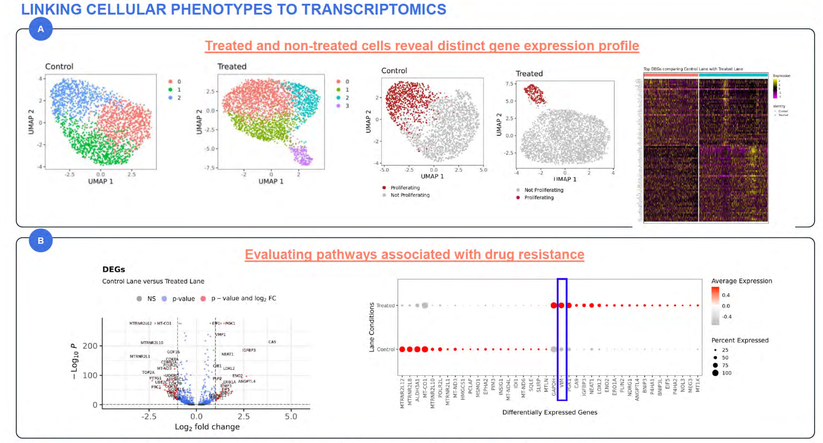



**Fig 5. Transcriptomics of drug-resistant clones validate expression of drug-resistant pathways**

(A) scRNAseq generated from single clones imaged within individual CCEs. Treated cells include a population of cells that continue to proliferate with drug treatment. (B) Differential gene expression analysis comparing treated and un-treated cells. Pathways associated with drug resistance observed.

## 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.