178 LEVERAGING A NOVEL, MULTI-MODAL PLATFORM TO IDENTIFY DRUG RESISTANT CLONES WITH DISTINCT MECHANISMS OF RESISTANCE TO EGFR INHIBITORS

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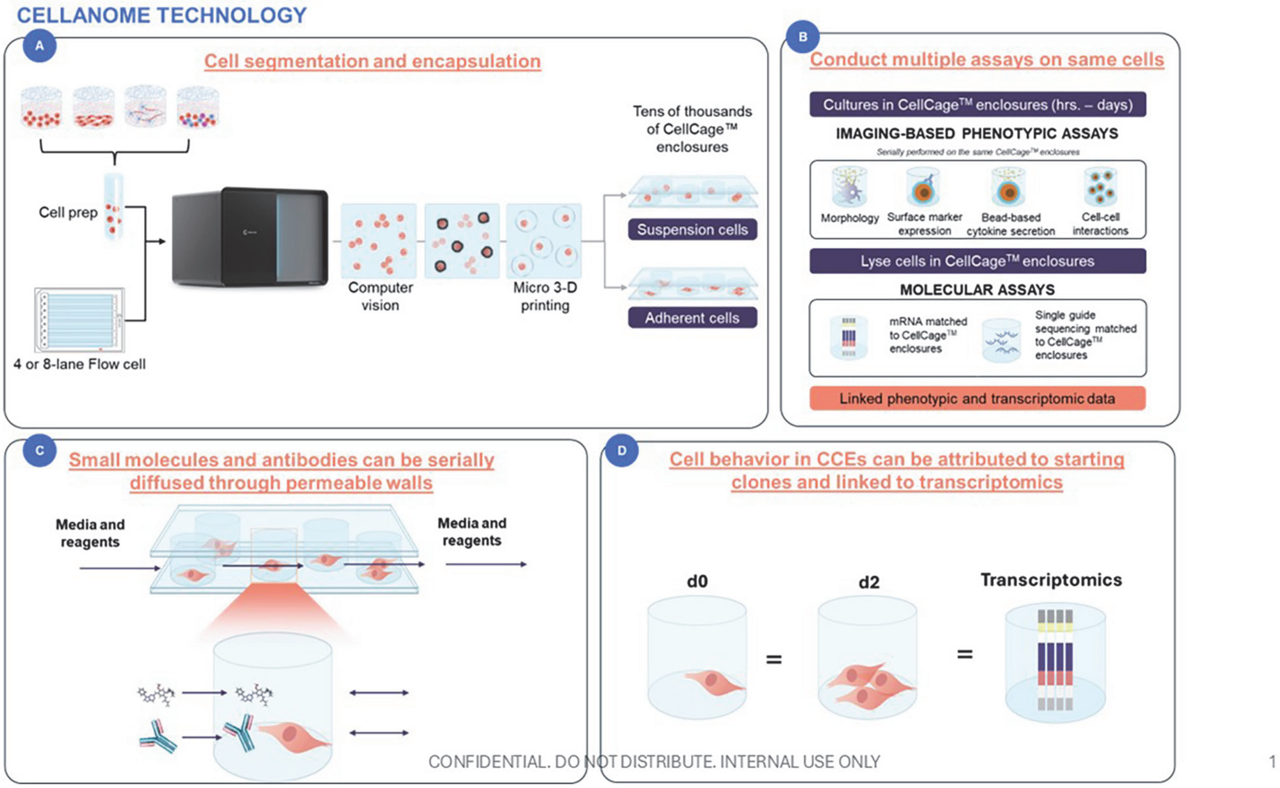
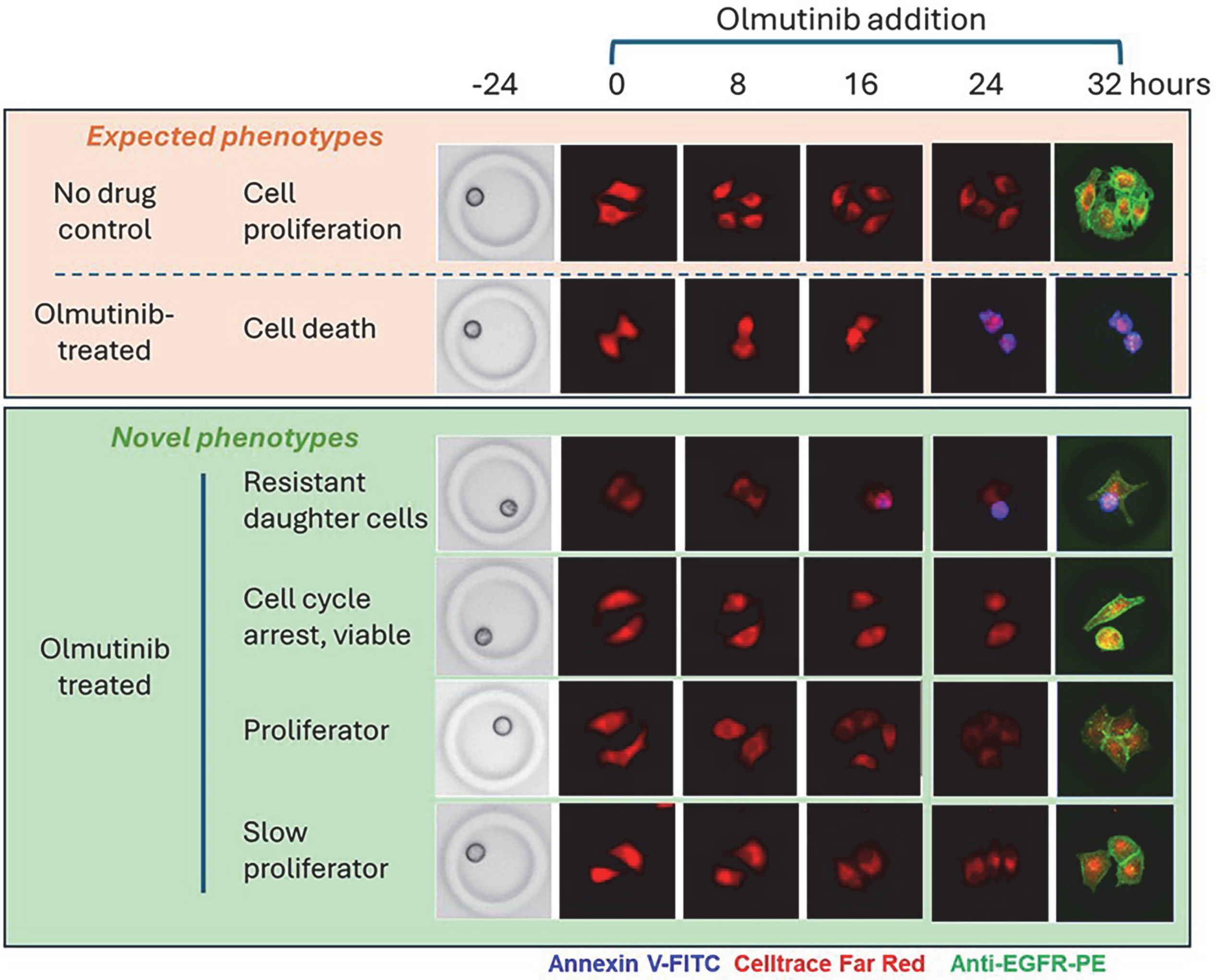
Background Heterogeneity in the tumor microenvironment reflects heterogeneous patient responses to therapeutics, including treatment failure, residual disease, and/or tumor recurrence. Single cell transcriptomics has enabled the field to discern cellular heterogeneity and identify novel subpopulations of tumor cells, some of which are treatment resistant. However, scRNA-seq faces – it is costly, does not directly evaluate cell-state transitions, and fails to capture mechanistic nuances only discernible by observing live cell behavior. An ideal approach for identifying and characterizing drug resistant or responsive clones would.

Methods We leverage Cellanome's R3200 platform, a high-throughput, multi-modal platform that can culture thousands of single cells, longitudinally track their phenotypes and function via imaging, and link the imaging data to underlying transcriptomics. Tens of thousands of single lung cancer A549 cells were within semi-permeable hydrogel compartments called CellCage™ Enclosures (CCEs) and treated with EGFR inhibitors (Olmutinib) or antibody drug conjugate (Cetuximab-MMAE) (figure 1). Containment within individual CCEs enables attribution of temporal dynamic cell behavior to a single starting clone. Proliferation, morphological changes, and apoptosis via Annexin V were evaluated for 3 days, after which they were lysed within CCEs to generate barcoded libraries for mRNA sequencing. The resulting linked, multi-modal dataset were analyzed to identify cell populations with novel cellular phenotypes and their underlying molecular pathways.

Results Most treated cells exhibited cell cycle arrest and eventual death after 32 – 48 hours, but a population of cells emerged as treatment-resistant. Interestingly, within these resistant cells, we observe multiple phenotypes, suggesting distinct mechanisms of drug resistance. This includes 1) cells that were arrested, but remained viable (56%), 2) strong proliferators (4%), 3) weak proliferators which eventually underwent apoptosis (22%), and 4) arrested clones with daughter cells that remain viable (12%). Preliminary analysis of mRNA-seq data across these populations identified upregulation of MAPK signaling pathways and canonical drug resistance genes (ABCC1, ABCC2, ABCC3, and ABCC4) (figure 2). These shared pathways amongst cells with distinct cellular phenotypes suggest there may be a foundational program governing drug-resistance that works in cooperation with additional



Abstract 178 Figure 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 CellCageTM 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.



Abstract 178 Figure 2 Novel cellular phenotypes were identified within drug-resistant clones using Cellanome's technology. Representative images of non-treated controls and distinct cellular phenotypes within drug-resistant clones

mechanisms that influence whether these cells eventually succumb to treatment or persist long-term.

Conclusions These results demonstrate that Cellanome's platform can identify drug resistant clones and discover novel mechanistic insights that can advance efforts to 1) identify biomarkers for treatment response or 2) design combination treatments that maximize patient outcomes.

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