

ChemoINTEL™: A high-throughput, multi-parametric compound screening platform for intelligent lead compound and therapeutic combination identification.

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ABSTRACT

Pierian Biosciences has developed the ChemoINTEL™ platform for high-throughput, semi-automated quantification of cellular responses to chemotherapeutics. ChemoINTEL™ relies on a microscopy-based process that captures and analyzes single cell behavior to reflect responses within a population. The platform is customizable to a variety of small molecule panels, either specific to certain tumor types or for titration screening of small molecules in development. Automated imaging of each well of a 384-well plate over time provides real-time kinetic response data under a variety of treatment conditions. Brightfield and fluorescent images are used to determine quantitative changes in morphology and molecular metrics reflecting induced apoptosis and cell death. Unique to ChemoINTEL™ as a drug screening platform is the quantification of intensity values of each fluorescent probe at each timepoint through a high-throughput analysis routine. These data are further processed through an internally developed algorithm to compare a treated population's response relative to an untreated control and reports a sensitivity score. By combining different treatment conditions, the platform provides intelligent design of single agent or combination treatment approaches. By capturing images of the population versus whole well population-based absorbance data, ChemoINTEL™ aids in better understanding response of tumor cell sub-populations to different chemotherapeutic agents. As a compound library screening tool or potential clinical diagnostic, Pierian Biosciences' quality-controlled processes ensure all of its equipment, reagents and processes follow ISO17025 guidelines to ensure the quality of all data generated. Each assay is internally controlled to include a standard cell line, whose response is monitored for accuracy of the reported results. In collaboration with several biorepositories and through the development of a standardized dissociation approach resulting in purification of viable primary tumor cells, the ChemoINTEL™ platform is also under development to assess chemotherapeutic response within patient populations. In addition to the ChemoINTEL™ platform, Pierian Biosciences has also developed a sophisticated multi-color flow cytometry platform (ImmunoINTEL™) that when used in conjunction with ChemoINTEL™ provides information on the purity of isolated primary tumor cells prior to plating, as well as information on the cell populations present within the tumor microenvironment. These two platforms can be further complexed to evaluate immunotherapies in combination with front-line therapies. In summary, Pierian Biosciences has developed a high-throughput, multi-parametric platform for both drug development and diagnostic applications.

Introduction and Objectives

- Development of a high-throughput compound screening platform to support biopharmaceutical initial target validation, clinical trial development, and commercial launch
 - Deliver high-throughput imaging system for assessment of apoptotic responses via fluorescent reporter activity
 - Provide services in a highly automated and regulated fashion under ISO 17025 certification
 - Combine with multi-parametric flow cytometry to further evaluate functional/molecular biomarkers
 - Provide ever increasing insight into biology as related to drug activity and disease characterization

Methods and Workflow

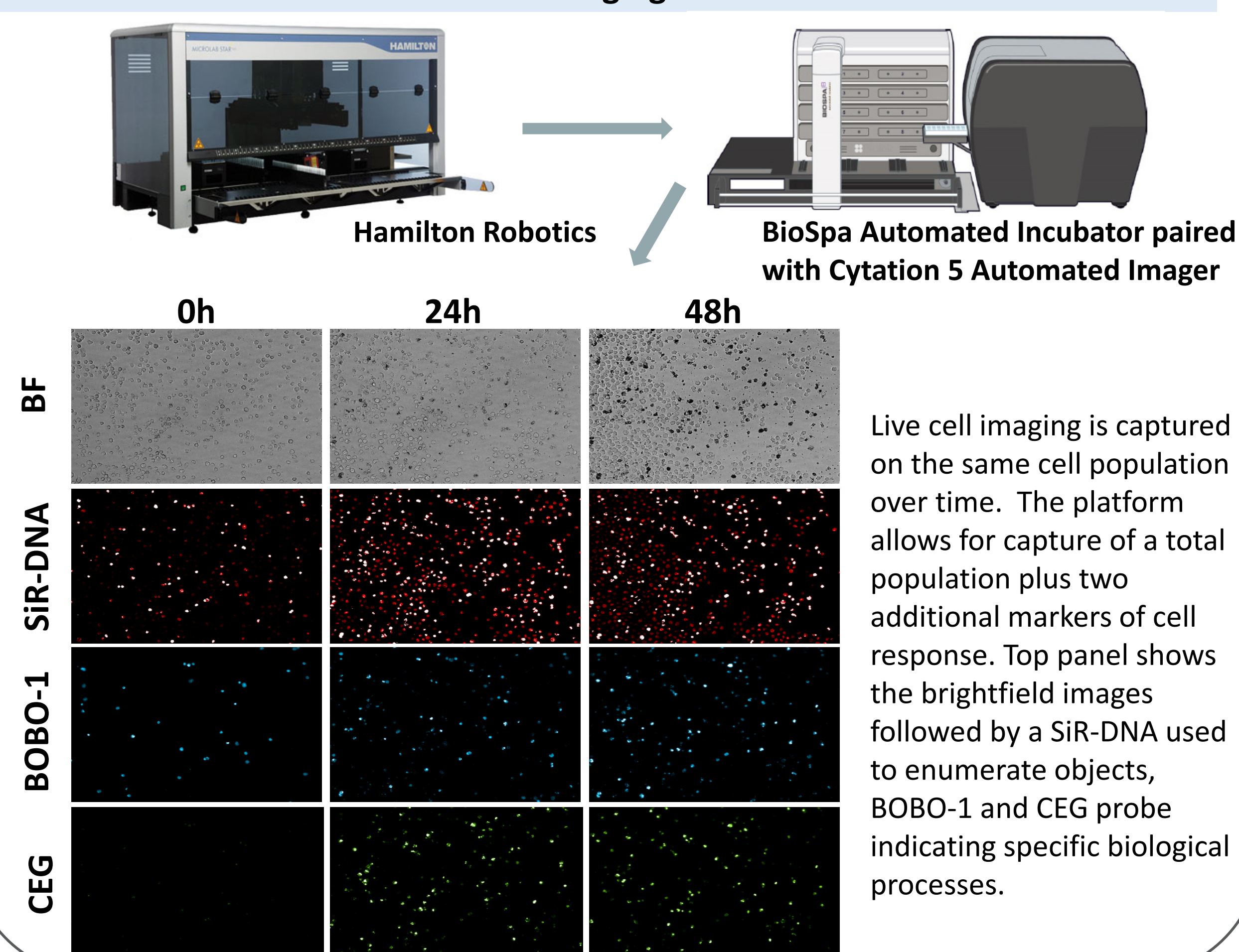
The ChemoINTEL™ platform utilizes a semi-automated process for sample preparation, plating, and imaging

- Pre-Analytical Steps**
- Sample collection
 - Sample shipment
 - Sample receipt and accessioning

- Sample Processing**
- Hamilton Liquid Handling (plating and drug treatment)
 - BioTek BiosPa 8 Automated Incubator
 - BioTek Cytation5 real-time kinetic imaging

- Analytical Steps**
- Sample Acquisition: automated imaging and Flow Cytometry
 - Automated gating

Figure 1. Pierian Biosciences ChemoINTEL™ platform utilizes kinetic live-cell imaging



RESULTS

Figure 2. ChemoINTEL™ semi-automated single cell analysis captures ability to define cellular responses to lead compounds and chemotherapy.

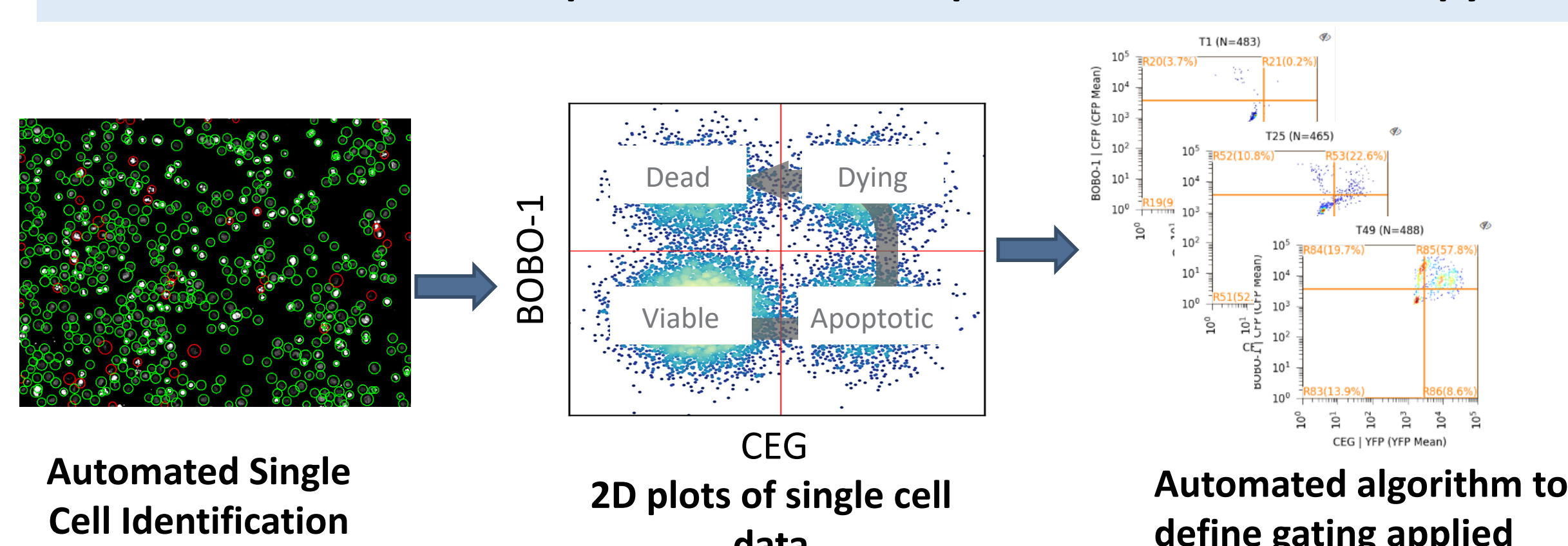
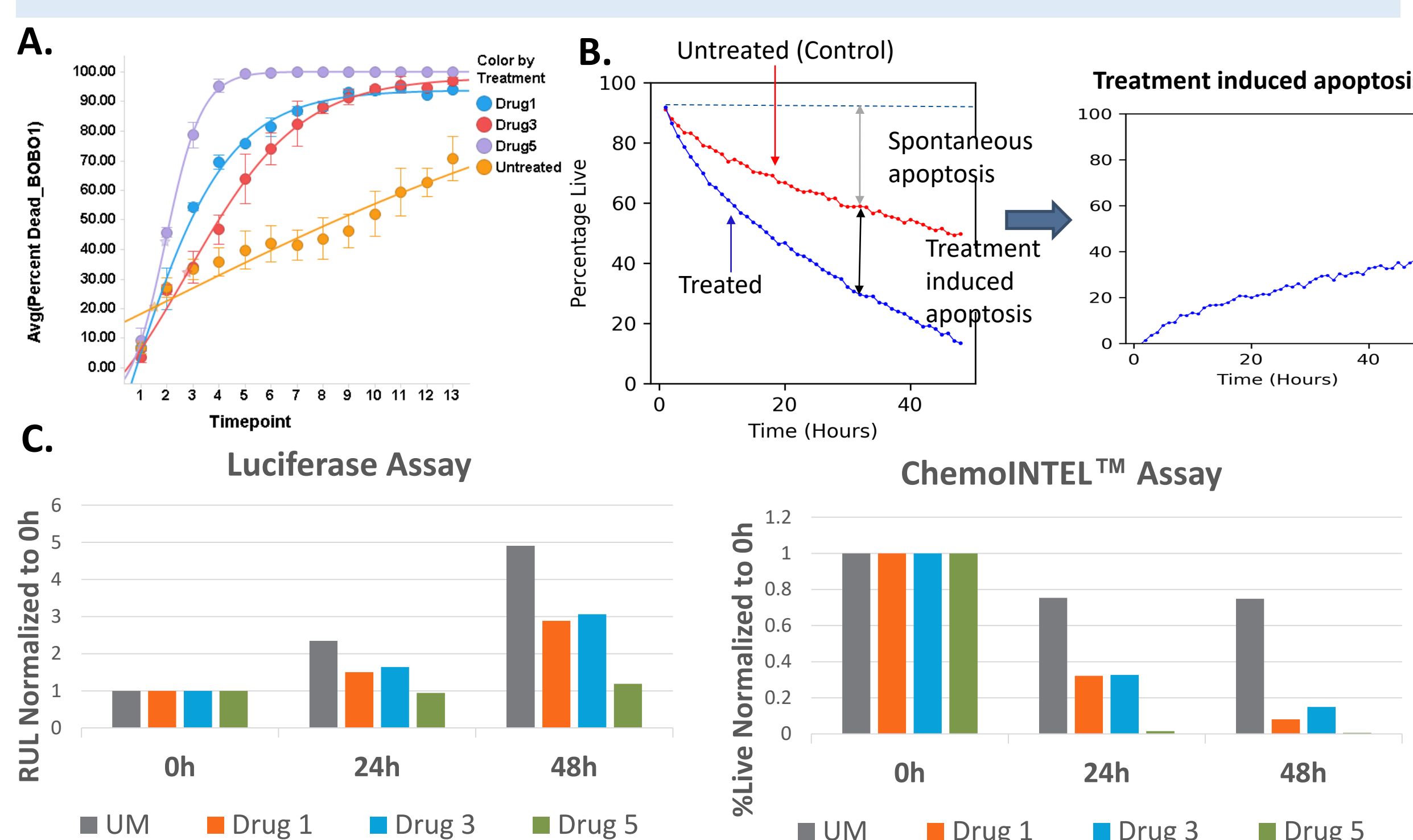
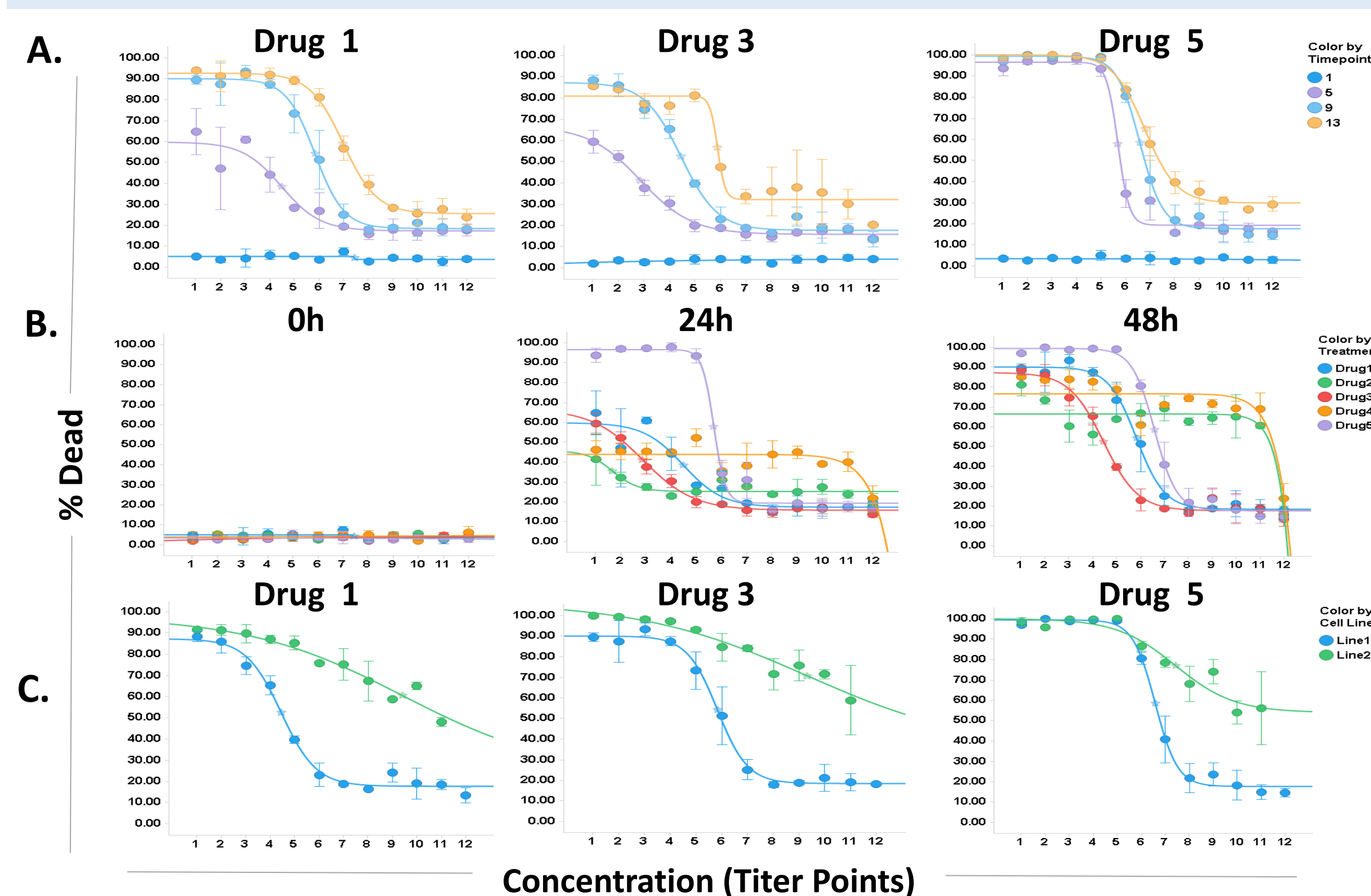


Figure 3. Representative single cell data demonstrates ability to characterize cellular response to chemotherapy.



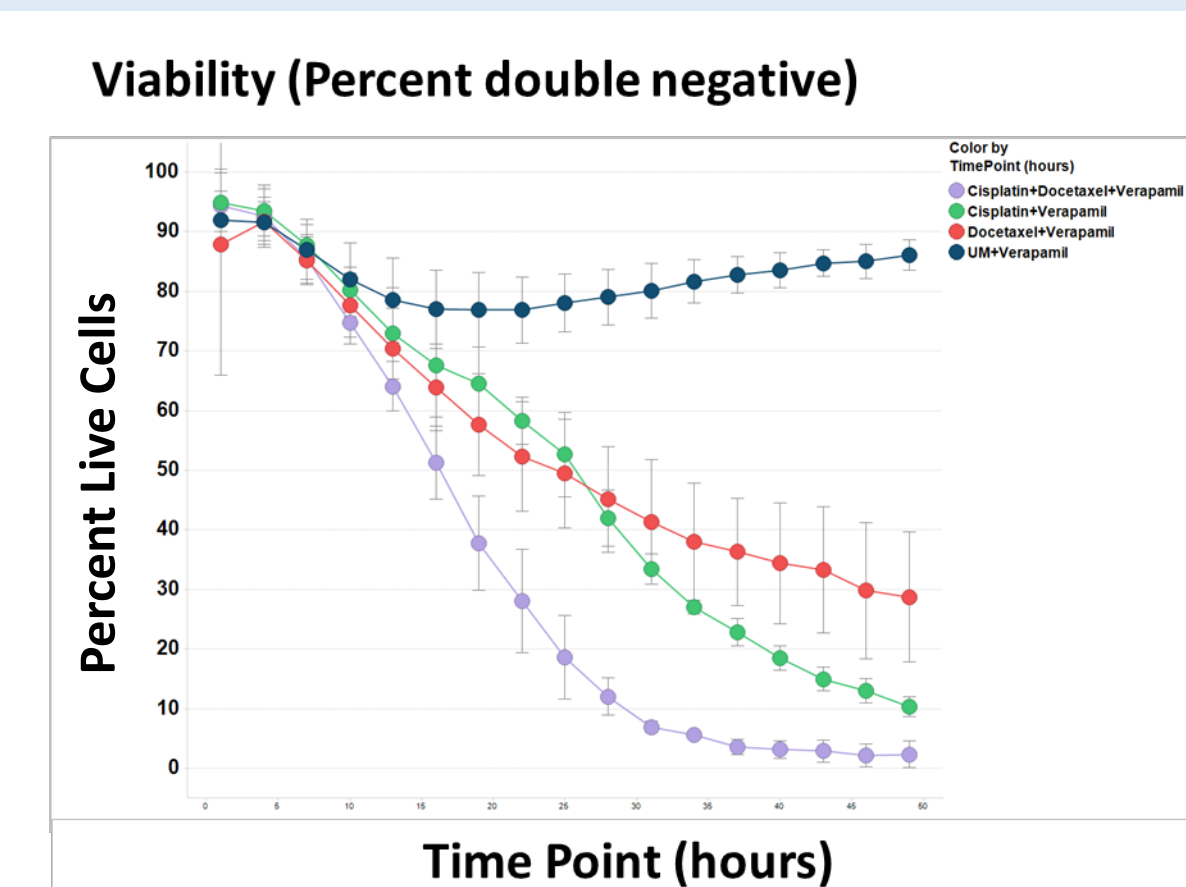
A. One-dimensional graphical representation of percent dead across a number of timepoints. B. Representative example of ChemoINTEL™ treatment induced apoptosis measurements. C. ChemoINTEL™ results were compared to a luciferase-based endpoint viability measurement demonstrating the accuracy and sensitivity of the ChemoINTEL™ platform.

Figure 4. Optimized drug concentrations & lead compound identification utilizing the ChemoINTEL™ platform



A. Determining the EC₅₀ value of different small molecule inhibitors at different time points through 12pt serial titrations. B. Comparison of the kinetics of the cellular response to multiple different drugs at different timepoint. C. Assessment of the effect of different drugs on cell viability in different cell populations as represented by the two cell lines.

Figure 5. Custom Plate Design Allows for Easy Drug Combination Testing

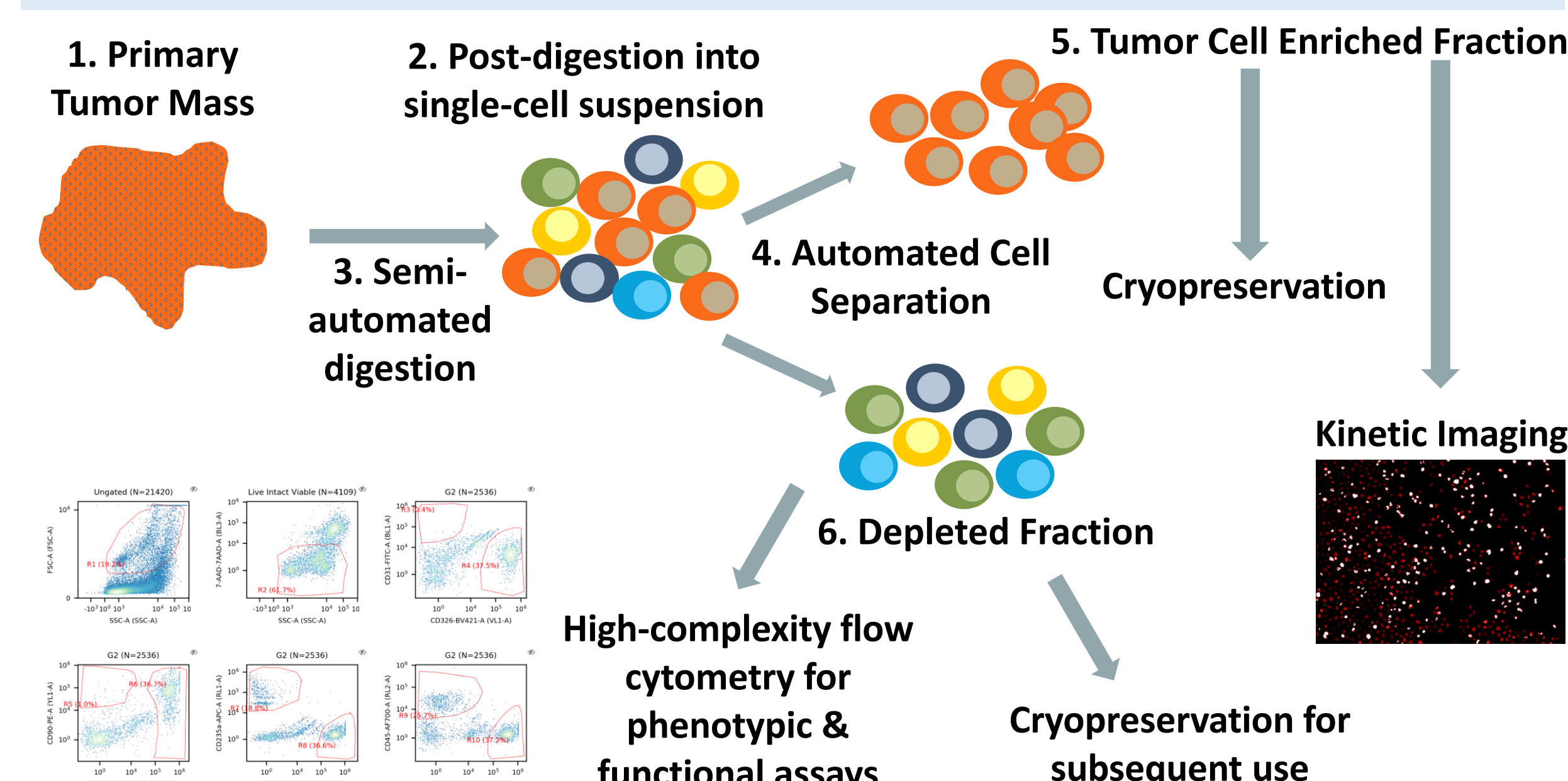


JURL-MK2 cells treated with chemotherapeutic agents and fluorescent probes for real-time imaging of cell viability and drug-induced apoptosis.

The percentage of non-apoptotic (live) cells plotted as a function of time. Live cell frequency calculated for each assay point as the double negative population (BOBO1-, CEG-)

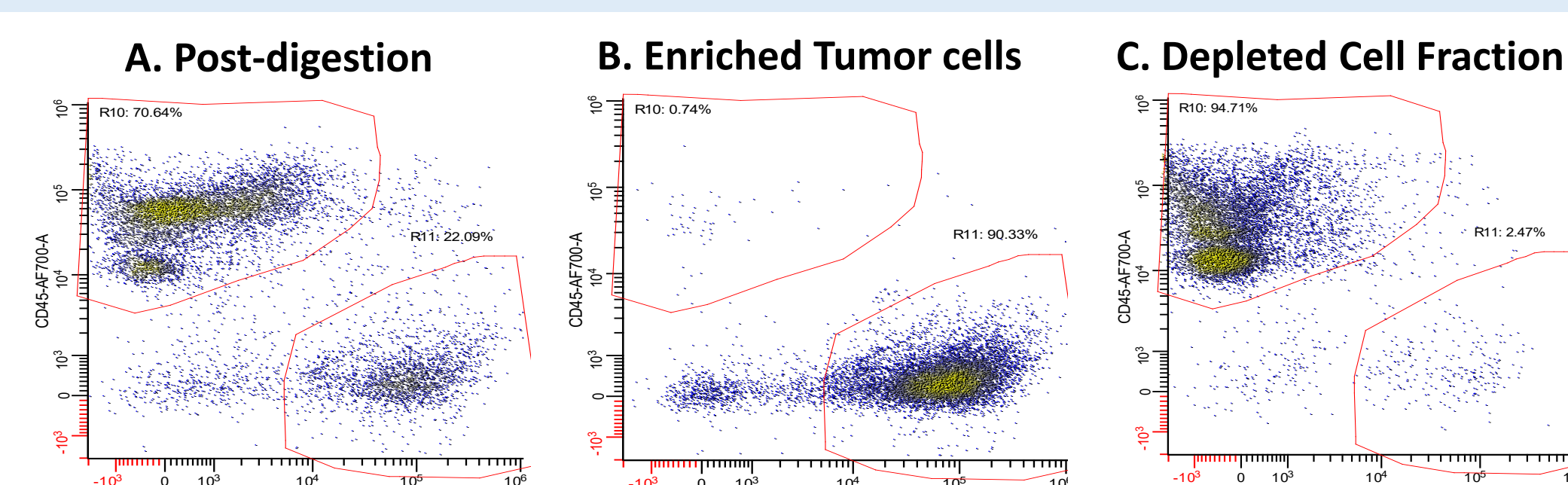
RESULTS

Figure 6. Pierian Biosciences ChemoINTEL™ technology can be applied to primary tumor processing & enrichment



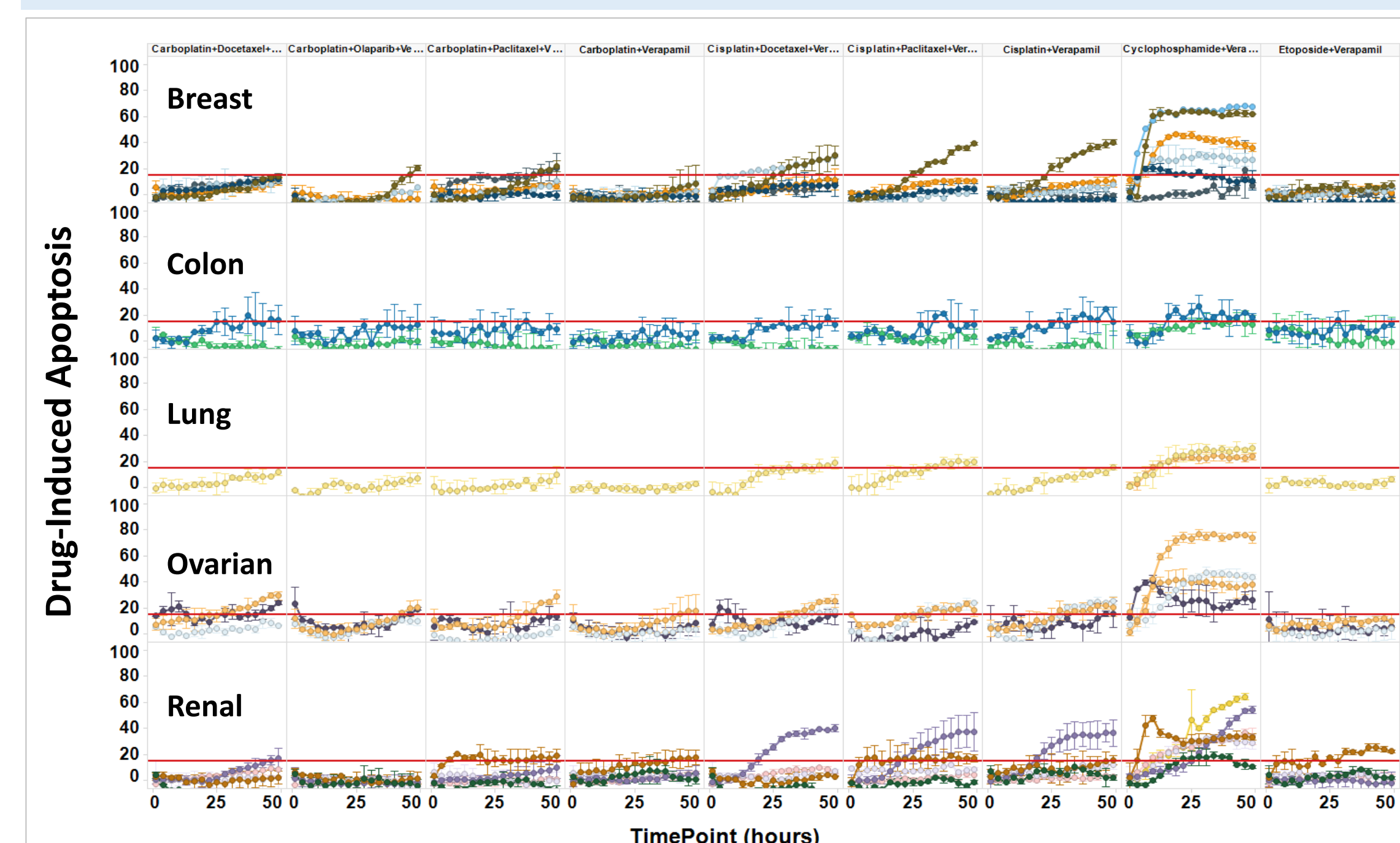
1) A solid tumor mass is converted to 3) a single cell suspension through a 2) semi-automated dissociation process involving chemical and mechanical digestion resulting in high viability of the heterogenous tumor. The post-digestion material is then further enriched through 4) an automated cell separation process to generate two populations of cells 5) an enriched viable epithelial tumor cells for plating and 6) a depleted fraction containing fibroblasts, immune cells and red blood cells

Figure 7. ChemoINTEL™ in combination with flow cytometry verifies purity of isolated tumor cell population (Abstract 1361 for more information).



A. Single cell suspensions from digested solid tumors are stained for epithelial cell markers (CD326+, shown), tumor-infiltrating lymphocytes (CD45+, shown) and other cell populations (not shown). B. A semi-automated processes enriches for viable epithelial tumor cells and C. the depleted fraction contains the TILs

Figure 8. ChemoINTEL™ is used to assay drug response in enriched human tumor cells.



Enriched tumor cells are treated with chemotherapeutic agents, both single and in combination, and fluorescent probes for real-time imaging of cell viability and drug-induced apoptosis. The amount of drug-induced apoptosis in treated cells is calculated and plotted as a function of time.

CONCLUSIONS

- Pierian Biosciences has developed a high-throughput, semi-automated, multi-parametric platform intended for use in both drug development and diagnostic applications
- The ChemoINTEL™ platform provides detailed information about effective concentrations and kinetic profiles
- The downstream software-based HTP analysis accelerates data analysis/extraction and increases the quality of data obtained from cell line based work or patient-derived specimens
- The ChemoINTEL™ platform can serve as a drug discovery/evaluation tool (a) in early stage drug development; (b) for patient stratification in clinical trials; (c) for identifying the most effective treatment regimen in the clinic

FOR MORE INFORMATION

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