ChemoINTEL™: A multi-parametric assay of chemotherapeutic responses in isolated primary tumor cells

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ABSTRACT

The efficacy of chemotherapeutic cancer treatment is limited by the absence of "personalized" therapies that pair individual patients with therapeutic agents optimized for their specific disease. The ChemoINTEL™ platform utilizes kinetic measurements of apoptotic and cell death responses in primary tumor cells treated with a panel of cytotoxic agents to score the in vitro response to each drug as a potential measure of drug-induced apoptosis of these malignant cells in vivo. The ChemoINTEL™ platform provides a semi-automated system for brightfield and fluorescence-based assessments of cell responses utilizing a combination of automated equipment for: tumor dissociation and enrichment, sample and drug delivery to 384-well imaging plates, and kinetic brightfield and fluorescent imaging. The platform is fully supported through integrated systems of data analytics including sample management (SMS), information management (LIMS), and data analysis (integration of multiple software systems), as well as reagent formulation and a quality and document management program under ISO regulatory guidelines. Assay development studies completed with multiple cell lines demonstrate fluorescent measurements of apoptosis and cell death (delta values between treated and untreated) and provide endpoint responses equivalent or better than established drug testing methods by determining both kinetics and magnitude of response. In proof-of-concept studies utilizing primary carcinomas, enriched tumor cells isolated from 62 patient solid-tumor samples were screened through a panel of 6 cytotoxic agents and one PARP inhibitor (either as single treatment agents or in combination) to assess cytotoxic responses. Tumor viability varied significantly across patient samples and tumor types (ovarian, breast, lung, renal, gastric, colon, and pancreatic) with initial median viabilities of 75% (Interquartile Range (IQR) 19%) dropping to a median of 39% (IQR 29%) at 24 hours and a median of 34% (IQR 27%) at 48 hours. Using pre-defined inclusion criteria, 23 of 87 samples from multiple tumor types exhibited drug responses to the limited drug panel tested. Most tumors responded to multiple drugs, including Cyclophosphamide, Cisplatin, and Etoposide as single agents, and Carboplatin in combination with Docetaxel or Paclitaxel. Applications of this technology to research and development may facilitate screening of early stage therapeutic reagents in primary tumor samples, and clinical applications have the potential to improve patient outcomes and minimize toxicities by limiting the use of cytotoxic agents not suited to the patient's disease.

INTRODUCTION AND OBJECTIVES

Defining chemotherapeutic treatments for cancer that are specific to the patient through an in vitro assessment of drug responses in enriched tumor cells

- **The response of tumor cells to cytotoxic agents varies by patient, leading to heterogeneous responses in clinical settings across drug types, tumor types, and treatment regimens**
  - Chemotherapeutic treatments tailored to the patient
- **The ability to pair patients with the specific drug most effective in treating their specific disease would lead to significant benefits including:**
  - Greatest cytotoxicity in patient tumor cells and improved patient outcomes
  - Reduced toxicity-associated side effects through decreased use of less effective agents

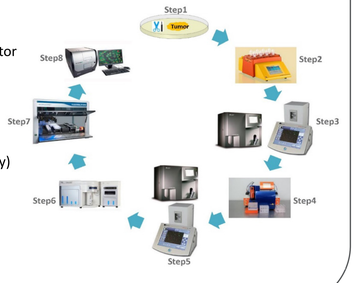
METHODS AND WORKFLOW

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

- **Tumor specimens are shipped to Pierian Biosciences under controlled conditions**
  - Temperature controlled overnight shipment of tumor samples
  - Isolation and enrichment of tumor cells for in vitro assessment of chemosensitivity
- **A semi-automated process provides a reliable in vitro platform for assessing drug-induced apoptosis without extended culturing of cells**
  - Viable tumor cells isolated from specimens using enzymatic/mechanical dissociation and enriched through labeling with magnetic beads in a negative-selection strategy
  - Enriched tumor cells are transferred to 384-well imaging plates and treated with fluorescent imaging probes and cytotoxic agents using a Hamilton Liquid Handling System
  - Real-time kinetic imaging of drug-induced cell apoptosis and death is achieved with the Biotek Cytation5 Imaging System

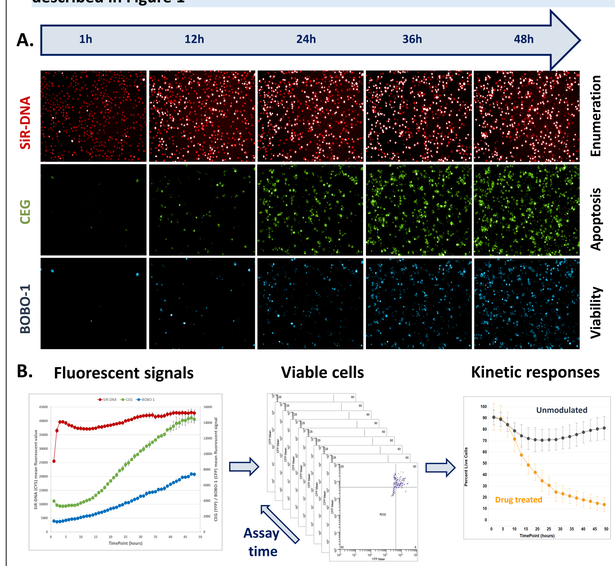
Figure 1. Tumor cell isolation, enrichment, plating, and imaging

- **Step 1 –** Tumor dissociation to 2mm
- pieces
- **Step 2 –** Miltenyi gentleMACs dissociator (single cell suspension)
- **Step 3 –** Cell viability and counting
- **Step 4 –** Miltenyi autoMACS Pro Separator (enrichment)
- **Step 5 –** Cell viability and counting
- **Step 6 –** Sample purity (flow cytometry)
- **Step 7 –** Hamilton Liquid Handling (plating and treatment)
- **Step 8 –** BioTek Cytation5 real-time kinetic imaging



METHODS AND WORKFLOW

Figure 2. Pierian Biosciences ChemoINTEL™ representative assay results as described in Figure 1



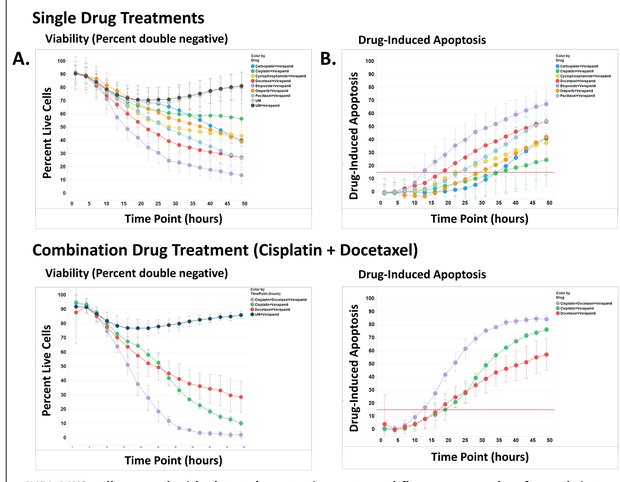
A) Representative single cell fluorescent images illustrating kinetic drug response in the ChemoINTEL™ assay

- Cells are enumerated with the SiR-DNA nuclear stain (**top panel**) while CellEventGreen (**middle panel**) and BOBO-1 (**bottom panel**) label apoptotic and dead cells, respectively

B) Representative ChemoINTEL™ analysis showing raw data fluorescent values (left panel), kinetic gating plots (middle panel), and treated versus untreated kinetic curves (right panel) displayed as a percentage of live cells

RESULTS

Figure 3. Cell viability and drug-induced apoptosis in the JURL-MK2 cell line reveals the range of responses and the potential for measuring synergy in combination treatments



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

- Carboplatin, docetaxel, cisplatin, paclitaxel, etoposide, cyclophosphamide, and olaparib
- Single drug treatments (**top panels**) and combination treatments (**bottom panels**)

A) The percentage of non-apoptotic (live) cells plotted as a function of time (left panels)

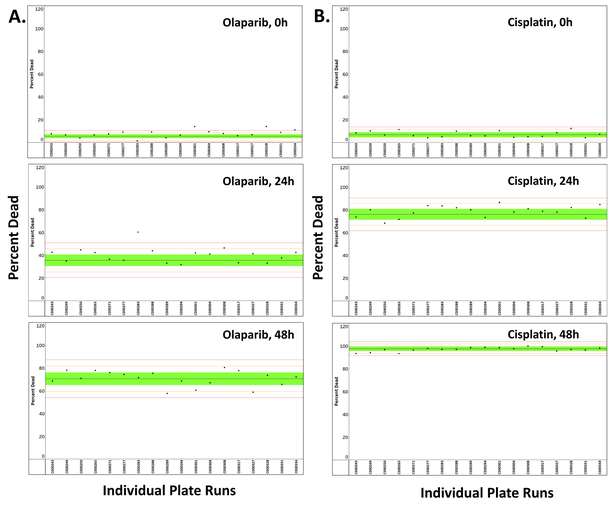
- Live cell frequency calculated for each assay point as the double negative population (BOBO1-, CEG-)

B) The amount of drug-induced apoptosis in treated cells calculated and plotted as a function of time (right panels)

- Drug-Induced Apoptosis = Percent Live Cells (untreated) – Percent Live Cells (treated)

RESULTS

Figure 4. ChemoINTEL™ assay performance monitoring; Longitudinal tracking of the assay control cell line JURL-MK2



JURL-MK2 cells assayed as ChemoINTEL™ control sample

- Representative plots showing data over a 5 month period

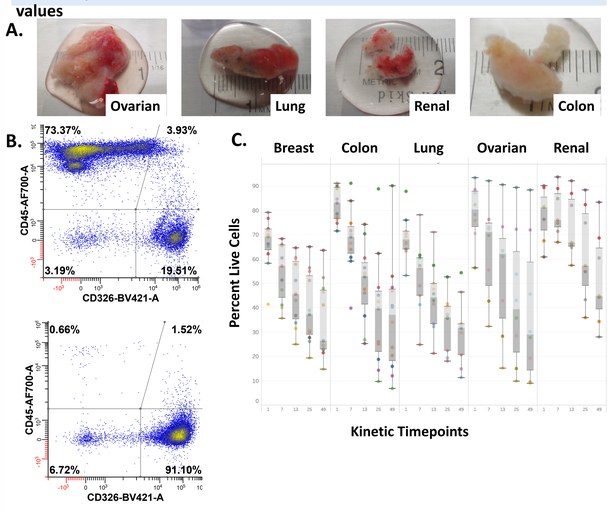
A) The percentage of dead cells plotted in response to 10μM Olaparib (PARP inhibitor) treatment

- Percent dead calculated for each assay point (0h, 24h, and 48h) as the double positive population (BOBO1+, CEG+)
- Average values plotted with ±1StdDev, ±2StdDev, and ±3StdDev

B) The percentage of dead cells plotted in response to 2.5μM Olaparib (PARP inhibitor) treatment

- Percent dead calculated for each assay point (0h, 24h, and 48h) as the double positive population (BOBO1+, CEG+)
- Average values plotted with ±1StdDev, ±2StdDev, and ±3StdDev

Figure 5. A wide range of tumor cell viabilities are observed over the course of the ChemoINTEL™ assay, establishing limits for statistically significant response values



A) Representative images of surgically removed primary tumors shipped overnight in controlled temperature shippers to Pierian Biosciences

- Tumor sizes averaged between 1.0-2.0cm^2

B) Flow cytometric analysis of disassociated tumors to determine purity of enrichment

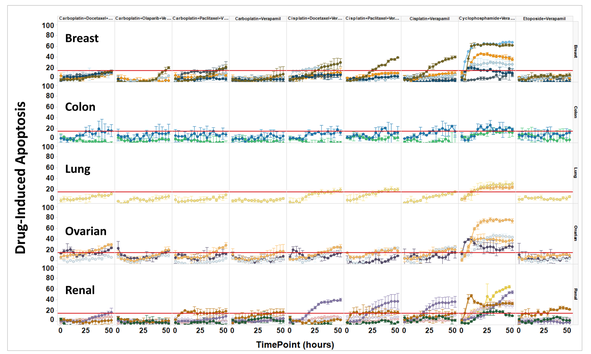
- Top panel showing CD45+ leukocytes and CD326+ tumor cells post dissociation
- Bottom panel showing CD45+ leukocytes and CD326+ tumor cells post enrichment

C) Box and whisker plot of compiled viability data plotted as a function of tumor type over time

- Percent Live Cells plotted at 1, 6, 12, 24, and 48 hours after plating

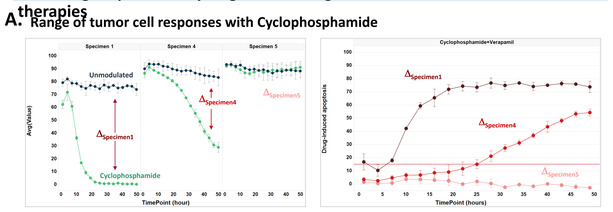
RESULTS

Figure 6. Drug-induced apoptosis is readily observed in tumor cells isolated from patient specimens treated with chemotherapeutic agents as single drugs or in combination



- **Enriched tumor cells treated with chemotherapeutic agents and fluorescent probes for real-time imaging of cell viability and drug-induced apoptosis**
  - Carboplatin, docetaxel, cisplatin, paclitaxel, etoposide, cyclophosphamide, and olaparib
  - Single drug treatments and combination treatments
- **The amount of drug-induced apoptosis in treated cells calculated and plotted as a function of time**
  - Drug-Induced Apoptosis = Percent Live Cells (untreated) – Percent Live Cells (treated)

Figure 7. ChemoINTEL™ can detect a wide range of cellular response as well as informing on potential synergistic or antagonistic effects of combination therapies



A) Detection of differential response to cycolophosphamide in dissociated primary tumors exhibiting low levels of spontaneous cell death; Potential for patient response stratification
B) Identification of synergistic (left panel) and antagonistic (right panel) treatment regiments utilizing chemotherapeutics and/or small molecule inhibitor combinations; Potential for identifying beneficial and ineffective or even detrimental combinatorial treatment strategies

CONCLUSIONS

- **Pierian Biosciences has developed a high-throughput, semi-automated, multi-parametric platform intended for providing patient specific treatment direction recommendations**
- **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 and efficiencyof data obtained from cell line based work or patient-derived specimens**
- **The ChemoINTEL™ platform can potentially identify the best mono- or combination-therapy for patient treatment**
- **ChemoINTEL™ can potentially inform on synergistic or antagonistic chemotherapy regiments**

FOR MORE INFORMATION

- **Please see Poster 294 for more information on using ChemoINTEL to support biopharmaceutical assay and drug development**

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