ChemoINTEL™: A high-throughput, multi-parameter compound screening platform to personalize cancer treatment by guiding therapeutic selection

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ABSTRACT

Background: Cancer is the leading cause of death worldwide 9.7 million deaths in 2022. There were 20 million new diagnosed cases of cancer in 2022 and an estimated increase to 35 million by 2050. Most patients undergo cytotoxic chemotherapy as the "backbone" of their cancer treatment driven by evidence-based guidelines. However, each patient tumour is unique and requires personalized therapeutic guidance. Pierian Biosciences has developed the ChemoINTEL™ platform to quantify patient tumour cell responses to chemotherapeutics and guide physicians in the selection of the most appropriate chemotherapy.

Methods: ChemoINTEL™ platform utilizes automated imaging of single tumour cells providing real-time kinetics of induced apoptosis and cell death to panel of treatment conditions and predicts patient response using internally developed algorithms. By combining different treatment conditions, the platform provides intelligent design of single agent or combination treatment approaches. As a compound library screening tool or potential clinical diagnostic, Pierian Biosciences' quality-controlled processes ensure all its equipment, reagents and processes follow ISO17025 guidelines to ensure the quality of all data generated.

Results: Drug induced apoptosis have been measured for a wide range of tumour types where patient specimens were treated with chemotherapeutic agents as single drugs or in combination. Synergistic as well as antagonistic effect of combination therapy is also evaluated.

Conclusions: ChemoINTEL™ platform can be utilized for both, drug development and clinical diagnostic, predicting the most effective treatment regimen for cancer treatment.

INTRODUCTION AND OBJECTIVES

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

- The selection of cytotoxic drugs used for cancer treatment continues to be guideline driven and based on:
  1) clinical evidence from treatment protocols developed from large, population-based, phase 3, prospective, randomized, multi-centre studies
  2) patient cohort treatment outcomes (progression free survival [PFS] and overall survival [OS])
- This approach was necessitated by the stark reality that no "predictive" or "treatment-directing" diagnostic technologies were available, a circumstance that, to an overwhelming degree, remains unaltered at this time. However, every patient is unique, and their clinical response is varied.
- ChemoINTEL™ assay predicts tumour responses to cytotoxic treatment in individual patients and identifies which chemotherapeutic agent a patient is most likely to respond to.
- Identifying which specific drugs is going to be the most effective for a patient is needed and would lead to significant benefits including:
  - Improved patient outcomes to 1st Line Treatment
  - Reduced toxicity-associated side effects through decreased use of less effective agents
  - Reduced cost and improved quality of life
- The currently undergoing ChemoINTEL™ Algorithm Development Study aims to develop a predictive algorithm by investigating the correlation between *in vitro* cytotoxic drug-induced apoptosis and clinical response to administered chemotherapy in epithelial ovarian cancer patients.

METHODS AND WORKFLOW

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

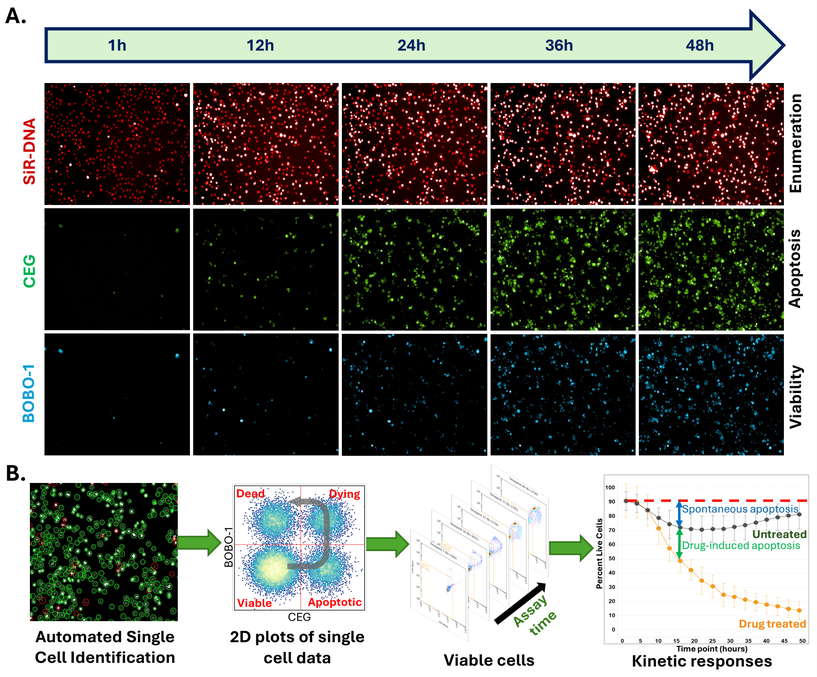
- Tumour specimens are shipped to Pierian Biosciences under temperature-controlled conditions for processing within 24 hours of surgical resection

Pre-Analytical Steps
- Sample collection and shipment
- Sample receipt and processing within 24 hours

Sample Processing
- Semi-automated dissociation of tissues into single-cell suspensions using benchtop gentleMACS™ Dissociator
- Automated enrichment of tumour cells using benchtop autoMACS™ ProSeparator
- Hamilton Liquid Handling robot (master drug plate preparation, sample plating and drug stamping)
- BioTek BioSpa 8 Incubator and BioTek Cytation 10 (for real-time kinetic imaging system)

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

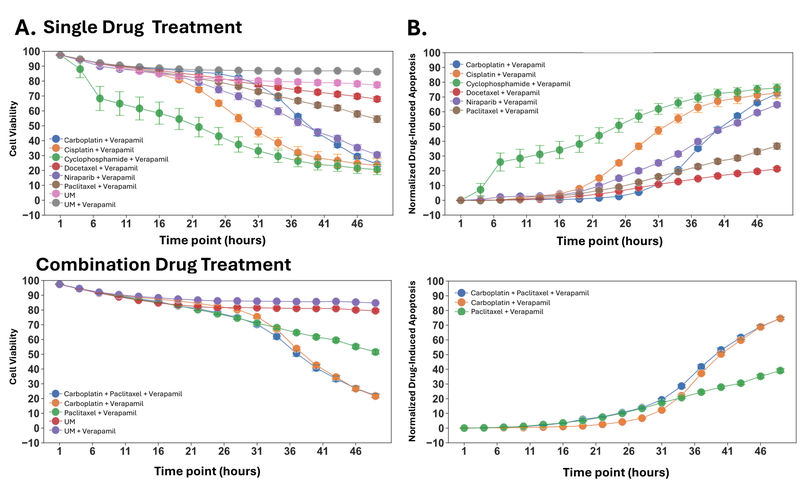
Figure 1. Pierian Biosciences ChemoINTEL™ platform captures drug-induced cellular responses



A. Representative single cell fluorescent images illustrating drug response in the ChemoINTEL™ assay. Cells are enumerated with the SiR-DNA nuclear stain (**top panel**) while CellEventGreen (CEG) (**middle panel**) and BOBO-1 (**bottom panel**) label apoptotic and dead cells, respectively.
B. Representative ChemoINTEL™ analysis showing raw data fluorescent image, 2D plots of single cell responses, kinetic gating plots, and **drug treated** versus **untreated** kinetic curves displayed as a percentage of live cells (double BOBO-1$^-$ and CEG$^-$ negative population).
Drug-induced apoptosis is measured as a difference in Percentage Live Cells between untreated and treated conditions. Additional normalization is applied to account for differences in the rate of spontaneous apoptosis between samples.

RESULTS

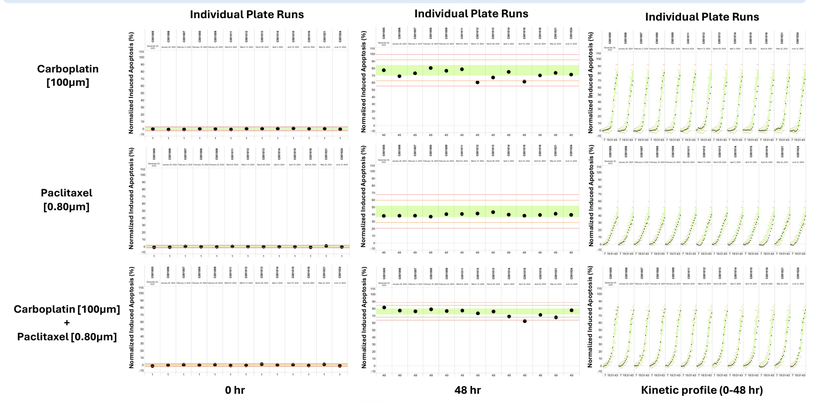
Figure 2. On-plate Cell Line Assay Quality Control



JURL-MK2 cells treated with chemotherapeutic agents and fluorescent probes for real-time imaging of cell viability and drug-induced apoptosis
- Carboplatin, Cisplatin, Cyclophosphamide, Docetaxel, Niraparib and Paclitaxel
- Single drug treatments (**top panels**) and combination treatments (**bottom panels**)

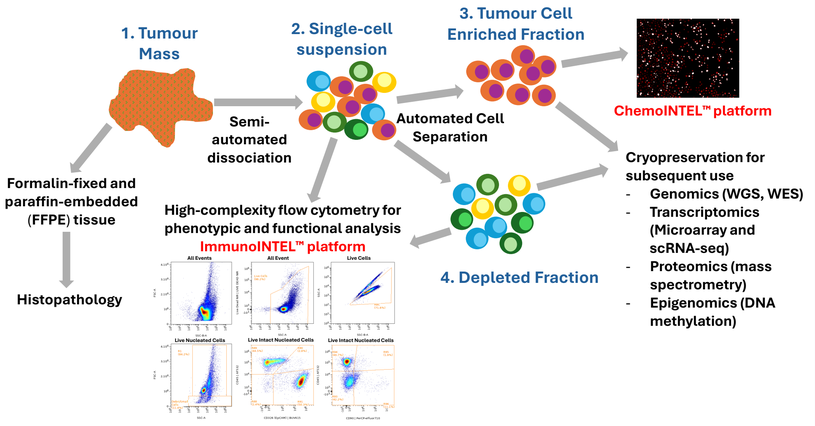
A. The percentage of non-apoptotic (live) cells plotted as a function of time (**left panel**)
B. The amount of drug-induced apoptosis in treated cells calculated and plotted as a function of time (**right panel**)

Figure 3. ChemoINTEL™ assay performance monitoring and longitudinal tracking



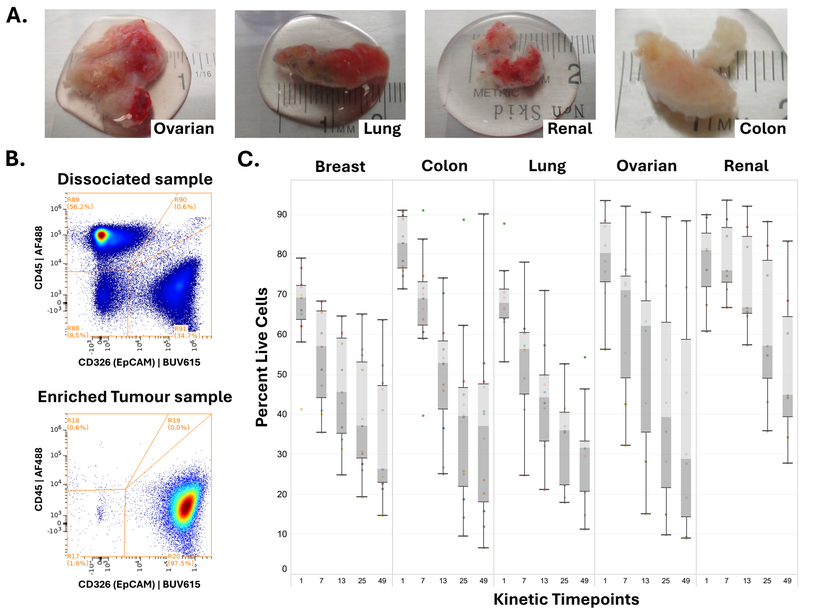
- JURL-MK2 cells assayed as ChemoINTEL™ control sample (representative plots showing data over 6-month period).
- Normalized Induced Apoptosis plotted in response to 100µm Carboplatin, 0.80µm Paclitaxel and both in combination.
- Performance of 17 chemotherapeutic agents at two concentrations and 6 two drug combinations are evaluated at each run and monitored overtime.

Figure 4. Pierian Biosciences ChemoINTEL™ technology can be applied to primary tumour processing and enrichment



1) A solid tumour is converted to **2)** a single cell suspension (SCS) through a semi-automated dissociation process. The post-digestion fraction is then processed to generate two populations of cells **3)** an enriched epithelial tumour cells for **ChemoINTEL™ platform** and **4)** a depleted fraction containing fibroblasts, immune cells and red blood cells. All fractions are analysed by flow cytometry with the SCS fraction being subjected to a 37-colour **ImmunoINTEL™ platform**.

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



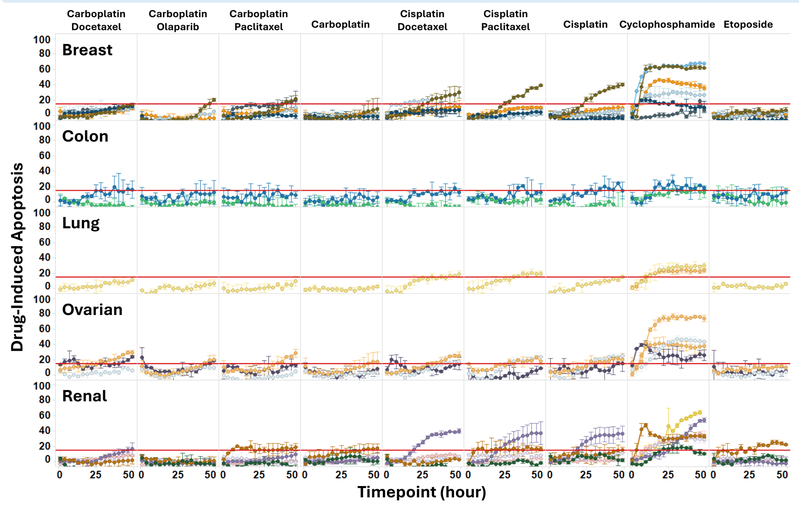
A. Representative images of surgically removed primary tumours shipped under controlled temperature to Pierian Biosciences
- Tumour sizes averaged between 1.0 - 2.0cm^2

B. Flow cytometric analysis of disassociated tumours to determine purity of enrichment
- Top panel showing CD45+ leukocytes (56.2%) and CD326+ tumour cells (34.7%) in the post- dissociation sample
- Bottom panel showing CD45+ leukocytes (0.6%) and CD326+ tumour cells (97.5%) in the enriched tumour sample

C. Box and whisker plots of viability data plotted for different tumour types over time
- Percent Live Cells plotted at 1, 6, 12, 24, and 48 hours after plating

RESULTS

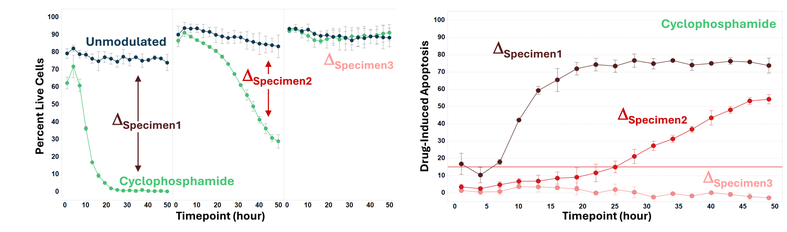
Figure 6. Drug-induced apoptosis is readily observed in tumour cells isolated from patient specimens treated with chemotherapeutic agents as single drugs or in combination



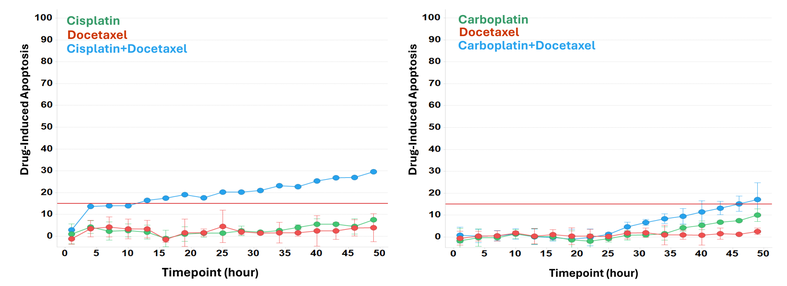
Enriched tumour cells treated with chemotherapeutic agents and fluorescent probes for real-time imaging of cell viability and drug-induced apoptosis for different tumour types
- Cisplatin, Carboplatin, Docetaxel, Paclitaxel, Olaparib, Etoposide and Cyclophosphamide
- Single drug treatments and combination treatments

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

A. Range of tumour cell responses with Cyclophosphamide



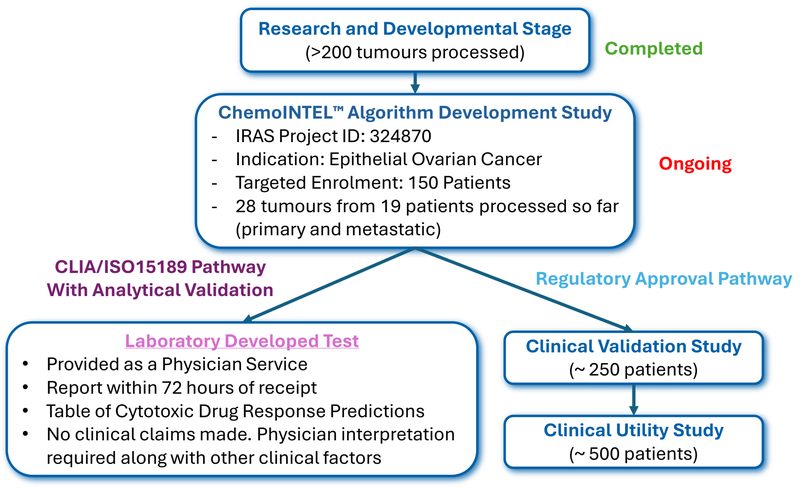
B. Combination drug responses in patient tumours



A. Detection of differential response to Cyclophosphamide in dissociated primary tumours exhibiting low levels of spontaneous cell death
- Potential for patient response stratification

B. Identification of synergistic treatment regiments utilizing drug combinations
- Potential for identifying beneficial combinatorial treatment strategies

Figure 8. Ongoing algorithm development study



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 drug concentrations and kinetic drug profiles
- The downstream software-based HTP analysis accelerates data analysis/extraction and increases the quality of data obtained from the control JURL-MK2 cell line and enriched tumour cells from 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
- The ChemoINTEL™ Algorithm Development Study is ongoing to model and develop a prediction algorithm for patient tumour cell sensitivity to chemotherapy drugs which will lay the foundation for Clinical Validation and Utility studies.

FOR MORE INFORMATION

Please see poster P-082 for more information about Pierian Biosciences ImmunoINTEL™ flow cytometry platform to support biopharmaceutical assay and drug development

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