Rapid Profiling of Compounds for Cell Cycle and Apoptosis: Using Single Micro-Scale Compound Treatments for Multiple Multiplexed Assays

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Abstract

Compound profiling is an important component of drug discovery and emerging assay technologies are enabling the implementation of profiling earlier in the process. Information gathered from multiplexed phenotypic screens enables the classification and characterization of compounds based on cellular efficacy, mechanism of action, and cytotoxicity. This information can be key to improving the success of potential chemical series as they move through the discovery process. Here we demonstrate the ability of the iQue™ Screener to serve as a robust, high-throughput profiling platform to generate information on five different cellular endpoints using a single micro-scale compound treatment.

IntelliCyt's iQue Screener is a suspension assay technology that has very low sample volume requirements. Using this technology enabled us to generate data for cell cycle modulation and 4 different apoptosis endpoints from a single compound treatment plate. For this study, we used a compound library generated by Exquiron Biotech using their proprietary structure-activity relationship expansion protocol. Starting with a set of templates derived from 5 known cell cycle inhibitors, Exquiron computationally expanded the set into a virtual collection of ~13,000 compounds with structural features similar to cell cycle modulators. A subset of 160 compounds was chosen from Exquiron's library for a profiling feasibility experiment. After treating Jurkat cells with the compounds in 50 µL volumes within 384 well plates, aliquots of the treated cells were transferred to 2 separate assay plates; one for cell cycle analysis and one for 4-plex apoptosis analysis.

From this study we were able to observe two apparent profiles for the compounds tested: 1) potential G2/M-phase cell cycle modulators that also induced apoptosis and 2) potential S-phase cell cycle modulators that did not induce apoptosis. 7 of the 160 compounds (4.4%) demonstrated perturbation of either the S-phase or G2/M phase of the cell cycle. Of these 7 compounds, 3 demonstrated concomitant induction of all 4 apoptosis-specific markers. The remaining 4 compounds demonstrated cell cycle perturbation without evidence of apoptosis induction.

Taking advantage of low assay volume requirements and multiplexed assays, we demonstrated a phenotypic screening technology that can be used as a powerful compound profiling platform. By aliquoting small volumes into multiple assay plates, an extensive phenotypic profile can be generated from a single compound treatment plate.

Compound Profiling with iQue Screener Technology

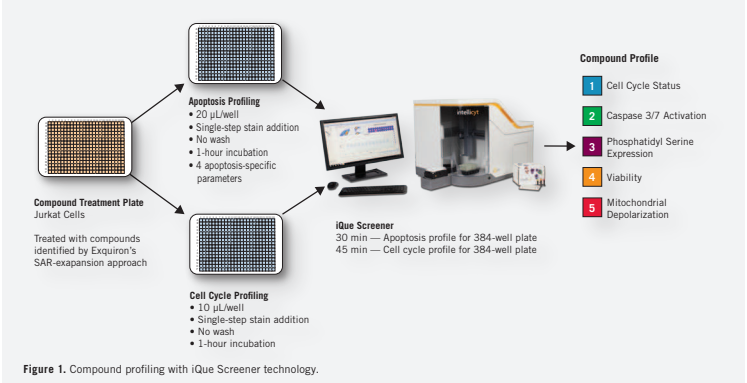

Figure 1. Compound profiling with iQue Screener technology.

Preparation of compound treatment plates is often the most time-consuming and variable component of an assay. To save time, reduce variability, and improve the ability to cross-correlate results between multiple assays, we used a single 384-well compound treatment of Jurkat cells to generate a 5-endpoint profile for each compound. Small aliquots from the compound treatment plate were used for staining with IntelliCyt's MultiCyt™ Cell Cycle and Apoptosis Kits. Both plates were analyzed using the iQue Screener. The resulting compound profile contained: cell cycle status, caspase 3/7 activation, phosphatidyl serine surface expression, viability, and mitochondrial depolarization.

IntelliCyt's iQue™ Screener integrates our patented technology for rapid sample delivery and high content single cell-level analysis into a bench-top system ideal for the screening environment. Researchers can rapidly profile compound effects on non-adherent cell lines, primary immune system cells and determine molecular interactions using cell or bead-based assays. The iQue Screener platform operates on ForeCyt® screening software and features low-volume, high throughput, multiplexed endpoints in an automation-friendly system. Screening relevant metrics in terms of hits or dose responses can be identified directly from the software and accelerate the time to discovery.

Exquiron's SAR Expansion Approach

Using five known cell cycle inhibitors as templates, a SAR expansion protocol[1] was run against Exquiron's 260,000 compound collection, resulting in a pool of 13,661 compounds before data fusion.

To assemble a test set of 160 diverse molecules for this study, of this pool of compounds 60 were selected which were found by >3 methods, and a further 40 by starting from at least 2 different templates; these were complemented with 60 compounds from the remaining pool.

[1] A. Bergner, S. P. Parel, *J. Chem. Inf. Model.* 2013, 53, 1057.

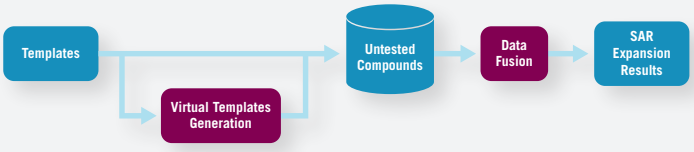

Figure 2. SAR expansion protocol.

Canonical Cell Cycle Inhibitors Block the Cell Cycle and Induce Apoptosis Markers

As part of the validation experiments, Jurkat cells were treated for 24 hours with canonical cell cycle inhibitors, and effects on the cell cycle and on induction of apoptosis were monitored. The cell cycle inhibitors showed dose-dependent block in G2/M or G0/G1 phase, with a decreased frequency of cells in S phase, and parallel induction of apoptosis markers.

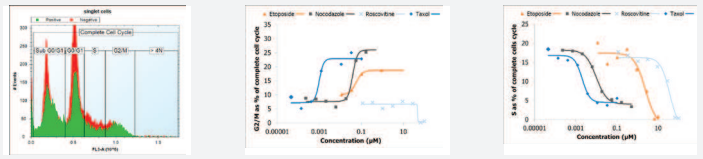

Figure 3. The distribution of cells in different stages of the cell cycle, and the gates applied, are illustrated for the negative control population treated with DMSO, and the positive population treated with the cell cycle inhibitor Nocodazole (left). Taxol, Nocodazole and Etoposide resulted in a dose-dependent cell cycle arrest in the G2/M phase, manifest by an increased percentage of cells in this phase (middle), and decreased population in S phase (right).

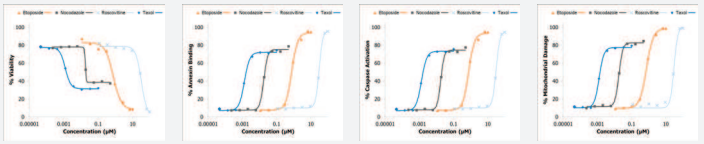

Figure 4. Analysis with the multi-parameter apoptosis kit after 24 hours treatment revealed for all cell cycle inhibitors tested a dose-dependent decrease in viability, and an increase in several parameters indicative of apoptosis (Annexin V binding, Caspase 3 activation, and mitochondrial damage) concomitant with the cell cycle blocking effects.

Compounds causing cell cycle perturbation

The 160 compounds were tested at a single timepoint of 24 hours, in independent experiments, using the MultiCyt™ cell cycle kit. Seven compounds were identified which showed perturbation of normal cell cycle progression (**Figure 5**). The entire test set was further profiled in a four parameter apoptosis assay, a score value derived for cell viability, and for several apoptosis markers (**Figure 6**).

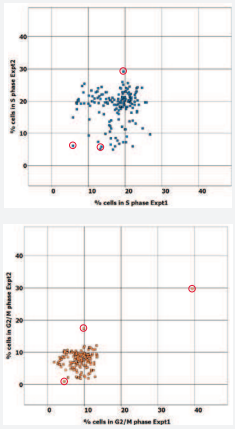

Figure 5. Correlation plots of two independent determinations at 20 µM. Plotted are % of cells in S phase (blue, upper panel) or G2/M phase (orange, lower panel). Seven representative compounds with a difference >3SD from the mean (values indicated) are circled.

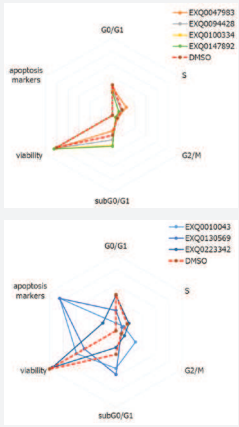

Figure 6. Radar plots show that the seven identified compounds can be grouped into two categories which differ from mean distributions, as exemplified by cells treated with DMSO (red). Those altering % cells in S phase do not show induction of apoptosis at the 24h timepoint (upper panel). Compounds resulting in an arrest in G2/M phase after 24h induce apoptosis (lower panel), similar to the canonical cell cycle inhibitors Nocodazole and Taxol.

Results

- The low volume requirements of IntelliCyt's iQue™ Screener allowed us to save time and enhance the ability to cross-correlate results by using a single compound treatment to profile compounds with multiple assays.
- Compounds were profiled using IntelliCyt's MultiCyt™ Cell Cycle and Apoptosis Kits, allowing us to assess cell cycle status, caspase 3/7 activation, phosphatidyl serine surface expression, viability, and mitochondrial depolarization — all in no-wash, mix-and-read assay formats with just 1-hour staining times.
- Using Exquiron's SAR expansion approach, a targeted selection for compounds with potential effects on cell cycle control was made.
- 160 compounds were tested at a single timepoint in this mini-SAR expansion, among these, seven were identified which showed a perturbation of the cell cycle.
- Parallel analysis in a multiparameter apoptosis assay resulted in additional valuable information, aiding in the classification of those compounds selected for further profiling in time-course and dose response assays.
- The use of IntelliCyt's iQue Screener for profiling compounds in multiparameter phenotypic assays has proven highly efficient, with simple to handle assay protocols providing high quality data with fast turnaround times.

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