Identification and profiling of cell cycle-interfering compounds in phenotypic assays using the iQue Screener

Authors: Daniela Brodbeck[1], Serge Parel[1], Kim Luu[2], Tom Duensing[2], Stephan Fasler[1]

[1]Exquiron Biotech AG, Reinach, Switzerland, and [2]IntelliCyt Corporation, Albuquerque, NM, USA

Abstract

We have used IntelliCyt's novel iQue Screener system to profile selected compounds in phenotypic assays employing the MultiCyt™ Cell Cycle and 4-Plex Apoptosis Screening kits.

Using a panel of five known cell cycle inhibitors as templates, we used our proprietary SAR expansion approach to select a virtual subset of compounds. From these, 160 were cherry-picked and tested for induction of cell cycle arrest and apoptosis markers in Jurkat cells. We identified seven compounds resulting in a perturbation of the cell cycle at different phases, with some of these compounds resulting in concomitant induction of apoptosis markers. Further characterization of these compounds is required to establish time course and dose-dependence profiles.

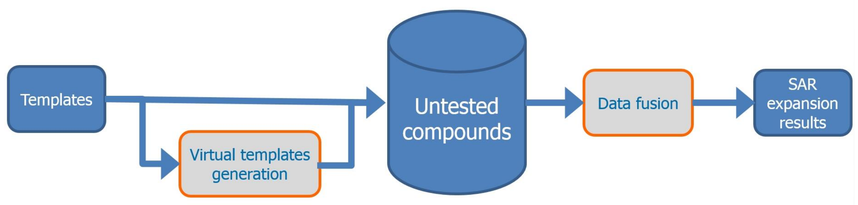
This study demonstrates the rapid identification and characterization of cell-cycle interfering compounds by gathering phenotypic data in high throughput mode, with small sample volumes, fast turnaround times and powerful data analysis tools, for the profiling of candidate compounds emanating from hit finding campaigns.

iQue Screener Technology

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.

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

Canonical cell cycle inhibitors block the cell cycle and induce apoptosis markers

As part of the validation experiments, Jurkat cells were treated for 24h 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 (Fig. 3), and parallel induction of apoptosis markers (Fig. 4).

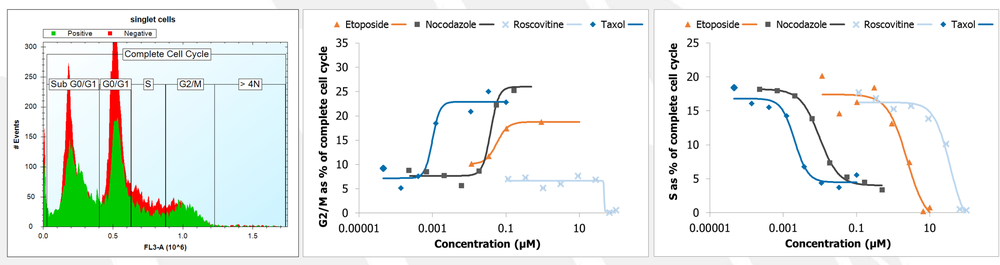


Fig. 3: The distribution of cells in different stages of the cell cycle, and the gates applied, are illustrated for the negative control population treated with DMSO (red histogram), and the positive population treated with the cell cycle inhibitor Nocodazole (green histogram, left panel). Taxol, Nocodazole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

Compounds causing cell cycle perturbation

Jurkat cells were treated with the test set of 160 compounds, at a single timepoint of 24h in independent experiments, and were stained using the MultiCyt™ cell cycle kit. Seven compounds were identified which showed perturbation of normal cell cycle progression (Fig. 1).

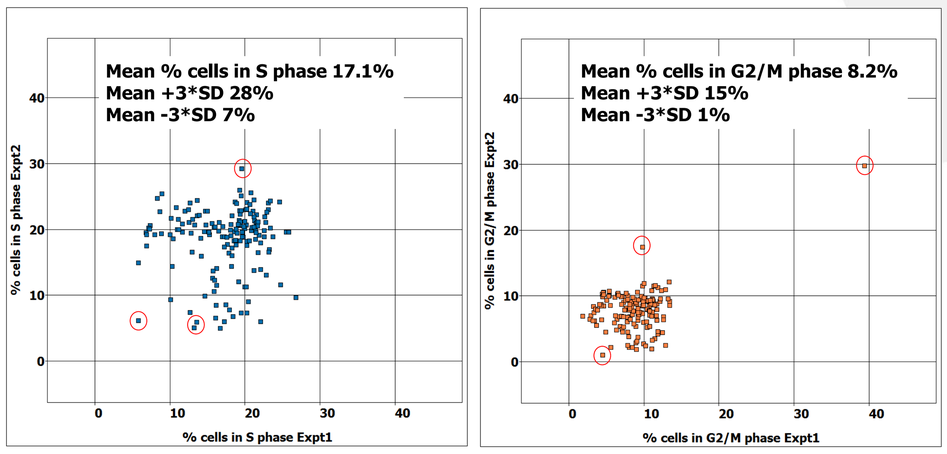


Fig. 1: Scatter plots of two independent determinations at 20 µM. Plotted are % of cells in S phase (left panel) or G2/M phase (right panel). Seven representative compounds with a difference >3*SD from the mean (values indicated) are circled.

Cells from the same treatment were further profiled in a four parameter apoptosis assay, and a score value derived for cell viability, and for several apoptosis markers (Fig. 2).

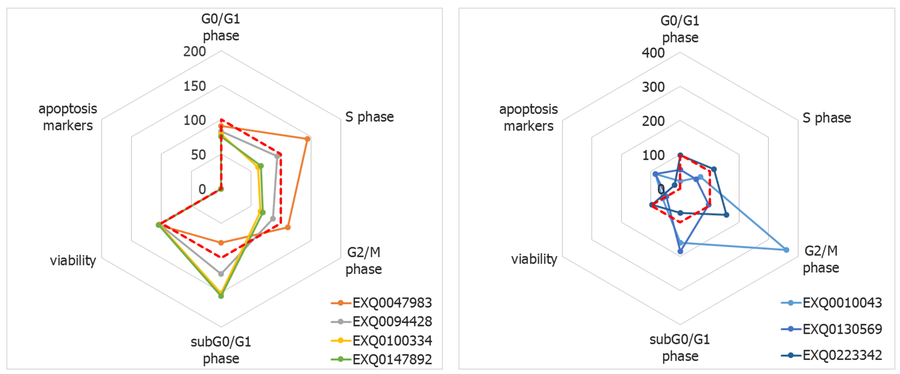


Fig. 2: Radar plots displaying % of cells in different phases of the cell cycle and apoptosis marker data, relative to DMSO treated cells (red dashed line). Left panel shows compounds affecting percentage of cells in S phase without induction of apoptosis markers. Compounds resulting in G2/M phase arrest also show concomitant induction of apoptosis markers (right panel), effects which are also found for Nocodazole and Taxol.

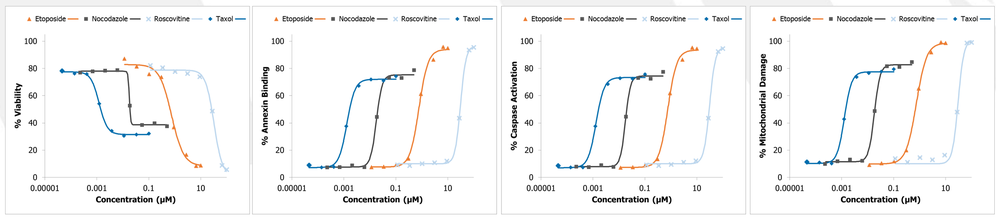


Fig. 4: Analysis with the four parameter apoptosis kit after 24h treatment revealed for all cell cycle inhibitors tested a dose-dependent decrease in viability (outer left panel), and an increase in several apoptosis markers (annexin V binding, Caspase 3 activation, and mitochondrial damage, center left to right panels), concomitant with the cell cycle blocking effects.

Results

- We used IntelliCyt's novel iQue Screener to efficiently generate phenotypic assay data by rapid screening of cells in solution
- Exquiron's SAR expansion approach was employed to select compounds which may affect normal cell cycle progression, and a subset of 160 compounds chosen for this study
- The subset was tested on Jurkat cells, with a single treatment timepoint. Seven compounds resulted in a perturbation of the cell cycle
- Parallel analysis in a four parameter apoptosis assay provided additional valuable information, further aiding in the selection of compounds for characterization in time-course and dose response experiments
- Compounds resulting in G2/M arrest showed profiles of cell cycle distribution and apoptosis markers similar to those elicited by the canonical cell cycle inhibitors Taxol, Nocodazole and Etoposide
- IntelliCyt's iQue Screener has proven highly efficient for the profiling of compounds in multiparameter phenotypic assays, with simple to handle assay protocols providing high quality data with fast turnaround times

Exquiron Biotech AG
Kägenstrasse 17
4153 Reinach, Switzerland
www.exquiron.com

IntelliCyt Corporation
9620 San Mateo Blvd. NE
Albuquerque, NM 87113
www.intellicyt.com