Institute of Molecular and Translational Medicine
Faculty of Medicine and Dentistry, Palacky University Olomouc

http://www.imtm.cz/

CZECH REPUBLIC

high-capacity screening site

OLOMOUC

an official partner site of eu openscreen

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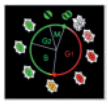
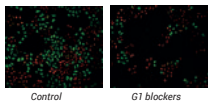
THE PROJECTS

Cell cycle analysis in HTS/HCA

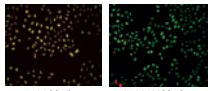
High content screening (HCS) is a method that uses automatic microscopy and image analysis techniques to extract multiple physiologically relevant measurements at cellular level. Our goal was to use HCS to develop robust, homogeneous and inexpensive assay for cell cycle analysis as alternative to low throughput FACS. In our endeavors we used HeLa cells stably expressing FUCCI (Fluorescent Ubiquitination-based Cell-Cycle Indicator) probe. The FUCCI probe was generated by fusing red and green fluorescent proteins with ubiquitination domains of Cdt1 and Geminin respectively. As a means of tracking cell cycle progression FUCCI exploits cyclical expression and degradation of the ubiquitination oscillators Cdt1 and Geminin to specifically mark cell cycle phases in living cells. Cell cycle perturbation can be evaluate d by measuring mean fluorescence intensity of Cdt1-Red and Geminin-Green in single cell and eventually based on fluorescence intensity range cells can be classified as one of G1, G1/S, S/G2/M and M cell cycle phase. The assay was designed for automatization of cell plating, addition of compounds, cytotoxicity measurement and high content microscopy on our triple arm robotic system.



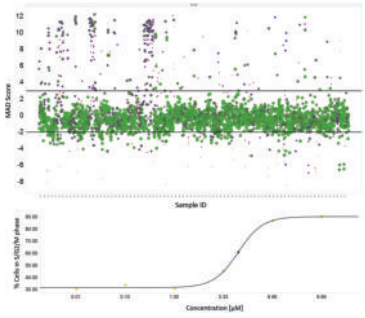
F u c c i Probe—Changes in probe fluorescence during cell cycle



Control G1 blockers



G1/ S blockers S/ G2/ M blockers



Results of Primary Screen for cell cycle Blockers. Compounds with MAD score >3 were considered as primary hits and used for active confirmation

Active confirmation of Primary screen hits. There dose dependent accumulation of cells I n S/G2/M phase

Cytotoxicity profiling of new chemical compounds

Mostly in large chemical library screens a primary consideration is whether the compounds are toxic. Therefore in vitro cytotoxicity testing has become an essential aspect of drug discovery; it is a convenient, cost-effective, fenotypic and predictive mean of characterizing the toxic potential of new chemical entities.
In our HTS laboratory the MTS assay as a cytotoxicity test was validated. Eight tumor cell lines covering various cancer phenotypes and 2 normal fibroblast cell lines were treated with with 90 selected representative compounds in 384- and 1536- well plates. The results were compared and validated (Fig.1). We have tested about 15.000 proprietary compounds and tests our entire library of 110.000 small molecules are in progress.

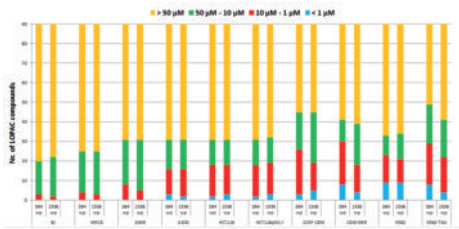
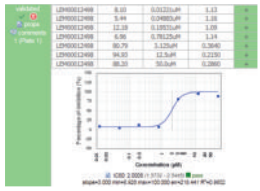


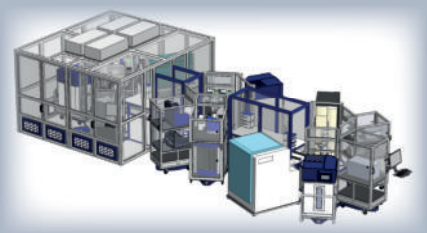
Fig.1: Distribution of reference compounds according their cytotoxicity evaluated in 384- and 1536-well plate format.

To quantify the suitability of cytotoxic assay in a HTS the Z-factor was determined for each plate and cell line.

Example of data evaluation in MTS assay



THE HARDWARE



Triple uStar Robotic Station (HighResBiosolution, USA) Chemical library with capacity about 4.000.000 compounds connected to one robotic arm system (HighResBiosolution, USA)

Equipment
- BioTek EL 406
- Multidrop Combi
- Multidrop Combi nl
- Echo Labcyte 550, 555
- Freedom EVO Tecan 150
- Mosquito
- MicroSpin
- Agilent Sealer
- Desealer (Brooks)
- Ambistores
- Automatic incubators (Steristore, HRB)

Readouts/ Screening technologies
- High Content Screening
- Yokogawa CV7000, Operetta automated microscopes
  AlphaLisa, Fluorescence Polarization, Luminescence, Absorbance, Time Resolved Fluorescence, qPCR
- FLIPR-TETRA, MicroBeta LumiJet, Envision, Enspire, ViewLux
- Automatic X-Ray irradiator
- LightCycler1536 (Roche)

Compound libraries
- Lopac Pfizer, Prestwick, Enzo - 4000 molecules
- Library of 110 000 small molecules
- Library of 1800 fluorescence compounds
- Proprietary Library of 15000 compounds.

THE SOFTWARE

Data Analysis Tools
- Tibco Spotfire
- Dotmatics
- Matlab
- Proprietary database and portal MedChemBio

Software tools
- Columbus
- ImageJ
- CellProfiler
- Acapella Studio

THE OUTPUT

1. ŠKROTT, Z., M. MISTRÍK, K. ANDERSEN, S. FRIIS, D. MAJERA, J. GURSKÝ, T. OŽDIAN, J. BARTKOVA, Z. TURI, P. MOUDRÝ, M. KRAUS, M. MED VEDÍKOVÁ, J. VÁCLAVKOVÁ, P. DŽUBÁK, I. VROBEL, P. POUCKOVA, J. SEDLACEK, A. MILOVICOVA, A. KUTT, J. MATTOVA, C. DRIESSEN, Q. DOU, J. OLSEN, M. HAJDÚCH, B. CVEK, R. DESHAIES a J. BÁRTEK. Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4. Nature. 2017, 552(7684), 194-199. IF: 40.137.
2. TICHÝ, M., S. SMOLEN, E. TLOUŠŤOVÁ, R. POHL, T. OŽDIAN, K. HEJTMÁNKOVÁ, B. LIŠKOVÁ, S. GURSKÁ, P. DŽUBÁK, M. HAJDÚCH a M. HOCEK. Synthesis and Cytostatic and Antiviral Profiling of Thieno-Fused 7-Deazapurine Ribonucleosides. Journal of Medicinal Chemistry. 2017, 60(6), 2411-2424. IF: 5.589.
3. DAS, V., T. FÜRST, S. GURSKÁ, P. DŽUBÁK a M. HAJDÚCH. Evaporation-reducing Culture Condition Increases the Reproducibility of Multicellular Spheroid Formation in Microtiter Plates. Journal of Visualized Experiments. 2017, 121, doi: 10.3791/55403. IF: 1.113.
4. BÜRGLOVÁ, K., G. RYLOVÁ, A. MARKOS, H. PŘICHYSTALOVÁ, M. SOURAL, M. PETRÁČEK, M. MEDVEDÍKOVÁ, G. TEJRAL, B. SOPKO, P. HRADIL, P. DŽUBÁK, M. HAJDÚCH a J. HLAVÁČ. Identification of Eukaryotic Translation Elongation Factor 1-α 1 Gamendazole-Binding Site for Binding of 3-Hydroxy-4(1 H)-quinolinones as Novel Ligands with Anticancer Activity. Journal of Medicinal Chemistry. 2018, 61(7), 3027-3036. IF: 6.259.

THE COLLABORATIONS

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