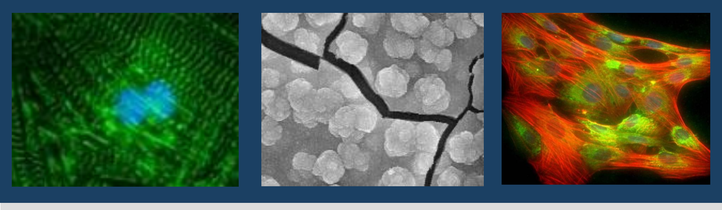

National Centre for Biomedical Engineering Science

Repurposing Drugs For The Treatment of Multi-Drug Resistant Breast Cancer

David Monaghan[1,2], Rachel Griffin[1], Amie Regan[1,2], Dr. Enda O'Connell[1], Dr. Howard Fearnhead[1,2]

[1] National Centre for Biomedical Engineering Science, NUI Galway, Ireland. [2] Department of Pharmacology and Therapeutics, NUI Galway, Ireland.

Introduction

Triple negative breast cancers lacking Estrogen, Progesterone and HER2 receptor expression, constitute approximately 15% of breast cancers. There are currently no targeted therapies, unlike Estrogen receptor positive (Tamoxifen) and HER2 positive (Herceptin) cancers, and acquisition of multi-drug resistance (e.g. increased MDR1 p-glycoprotein ABC transporter levels) limits the effect of cytotoxic chemotherapeutics.

Identifying targeted therapies for triple negative cancers is vital, but conventional drug discovery is expensive and time-consuming. Repurposing existing drugs is an attractive alternative, as preclinical and clinical data is widely available, greatly reducing the time and resources required to bring a candidate drug to clinical trial[1,2].

In this study, the Johns Hopkins Clinical Compound Library (JHCCL), containing approximately 1,500 FDA and foreign-approved clinical compounds, was used to screen a multi-drug resistant, triple negative breast cancer cell line, MDA16, for drug sensitivity.

Methods

MDA16 and MDA468 cells were provided by Dr. TW Gant (MRC Tox unit). Hs578t, HCC1937 and BT20 cells were provided by Dr. L O'Driscoll (TCD). Other cell lines were purchased from the ATCC. Cell viability was measured using the Alamar blue assay. Apoptosis was measured by Caspase activity assay, YO-PRO assay and Caspase and Lamin B processing via Western Blotting.

Primary and secondary screening protocols (i.e. drug dilution, cell seeding, drug treatment, Alamar blue addition and fluorescence detection) were performed using a Janus Automated Workstation and Victor X5 multilabel reader (Perkin Elmer), housed in a Class II Biosafety cabinet (BigNeat).

Results

1. Assay Optimisation

Before screening was performed, MDA16 cells were characterised alongside the parental cell line MDA468, to confirm a multi-drug resistance (MDR) phenotype (**Fig 1**).

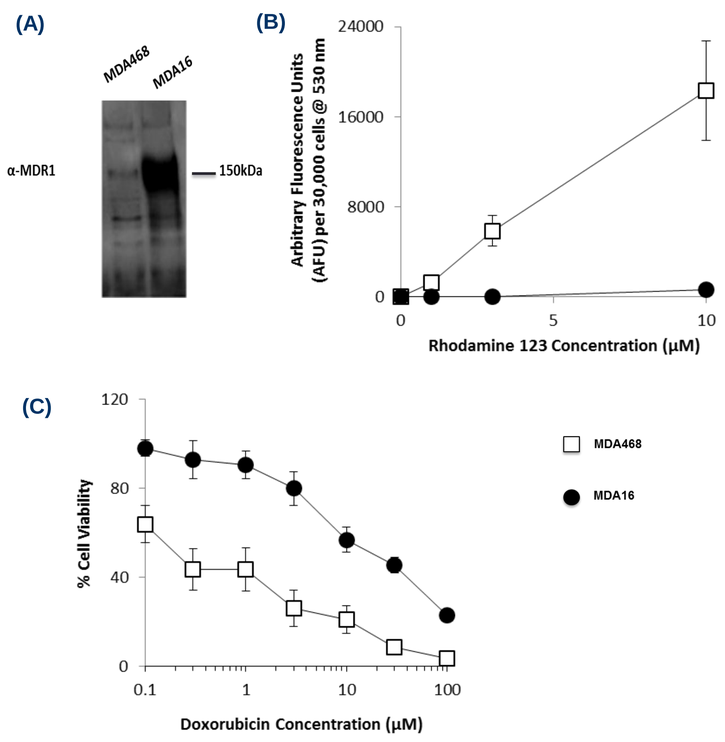

Fig 1: MDA16 cells possess a multi-drug resistant phenotype. MDA16 cells have increased MDR1 protein levels relative to MDA468 cells (**A**), resulting in a reduced cellular accumulation of the MDR1 pump substrate Rhodamine 123 (**B**). Accordingly, MDA16 cells exhibit increased resistance to Doxorubicin following 48 hour incubation (**C**).

To evaluate the robustness of the Alamar blue cell viability assay, a simple statistical parameter, the Z-factor, was determined[3]. To maximise the assay Z-factor, optimal screening conditions were established, including: cell seeding density; drug treatment time; Alamar blue treatment time; and automated liquid handling procedures (e.g. aspiration height, dispense height and mixing frequency) (**Fig 2**).

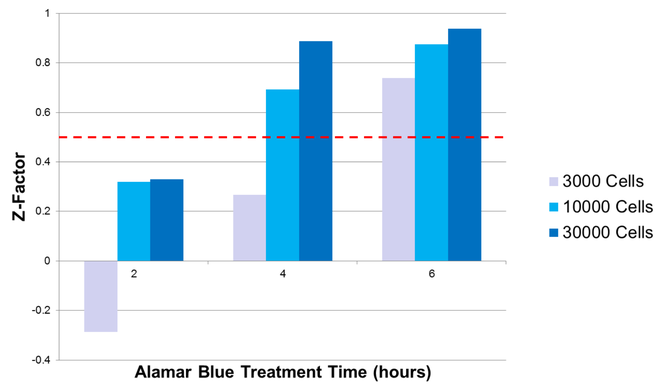

Fig 2: Alamar blue assay optimisation for high throughput screening. Optimisation of cell seeding density and Alamar blue treatment time parameters. A Z-factor of 1.0 indicates an ideal assay, while 1.0 > Z ≥ 0.5 indicates an excellent assay and 0.5 > Z > 0 indicates a weak assay.

2. Primary JHCCL MDA16 Screen

MDA16 cells were seeded at 30,000 cells/well, treated 24 hours later with 10 μM drug (1% DMSO) for 48 hours and treated with Alamar blue for 6 hours before fluorescence was detected (automated assay Z-factor = 0.75). Thirty bioactive compounds in fifteen different compound classes were identified (**Fig 3**).

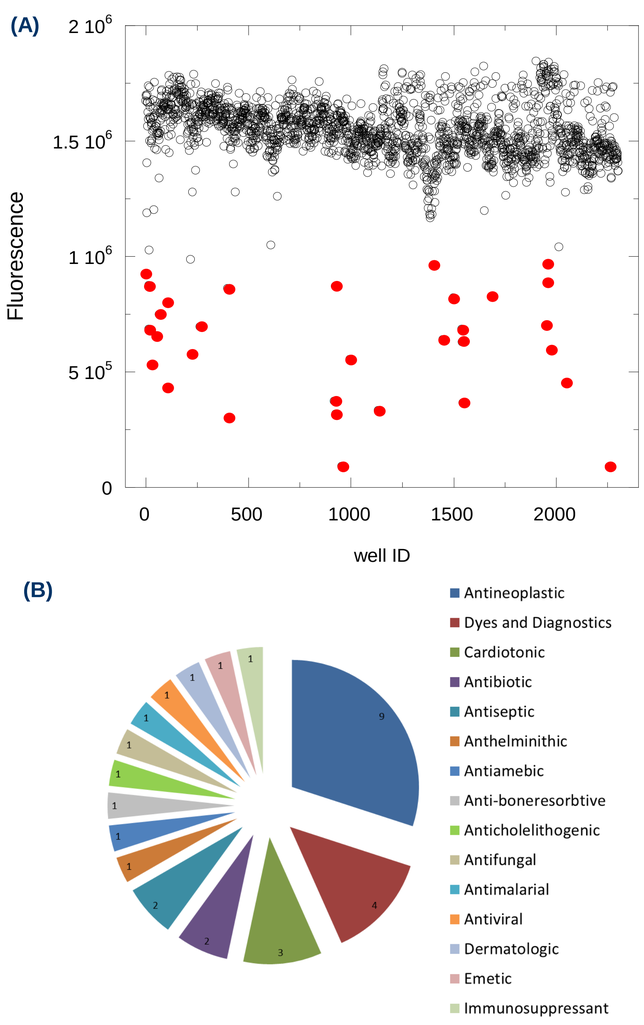

Fig 3: Identification of drugs with bioactivity against MDA16 cells. Using the definition of a "hit" as a compound that reduced MDA16 cell viability by ≥ 3.0 standard deviations from the mean, 30 bioactive JHCCL compounds were identified (**A**) in 15 different compound classes (**B**).

3. Secondary Validation Screen

Based on suitability and commercial availability from an alternative supplier (Sigma), 17 of the 30 'hit' compounds were selected for a secondary validation screen of both MDA16 cells and the parental cell line MDA468. Screening conditions from the primary screen were replicated. Bioactivity against MDA16 cells was confirmed in 12 of the 17 (70%) compounds and identified in 6 of the 17 (35%) compounds when MDA468 cells were treated (**Fig 4**).

Fig 4: Validation of "hit" compounds from primary JHCCL screen. The sensitivity of MDA16 and MDA468 triple negative breast cancer cells to 17 compounds was determined. Bioactivity defined as > 50% cell death.

4. Further Characterisation

A number of compounds, including Ciclopirox, a broad spectrum antifungal agent typically used for dermal infections, were selected for further characterisation.

Apoptosis was induced by Ciclopirox in MDA16 multi-drug resistant, triple negative breast cancer cells (**Fig 5**).

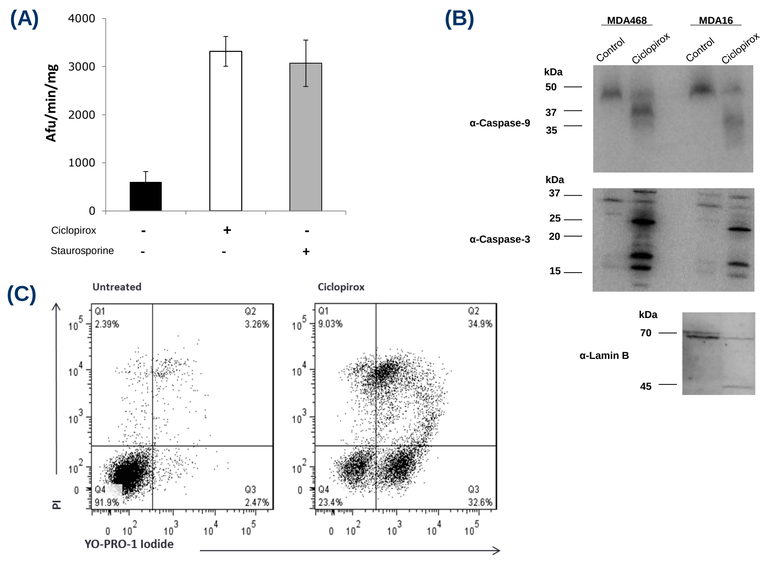

Fig 5: Ciclopirox induces apoptosis in MDA16 breast cancer cells. Ciclopirox stimulated similar levels of Caspase 3 activity as the positive control compound Staurosporine (**A**). In Ciclopirox treated cells, Caspase 9 and Caspase 3 were processed to their catalytically active fragments, while the cellular caspase substrate Lamin B was cleaved (**B**). Treated cells demonstrated increased fluorescence using the apoptotic dye YO-PRO as detected by flow cytometry (**C**).

The sensitivity of triple negative and non-triple negative breast cancer cell lines, and colorectal and prostate cancer cell lines to Ciclopirox was examined (**Fig 6**).

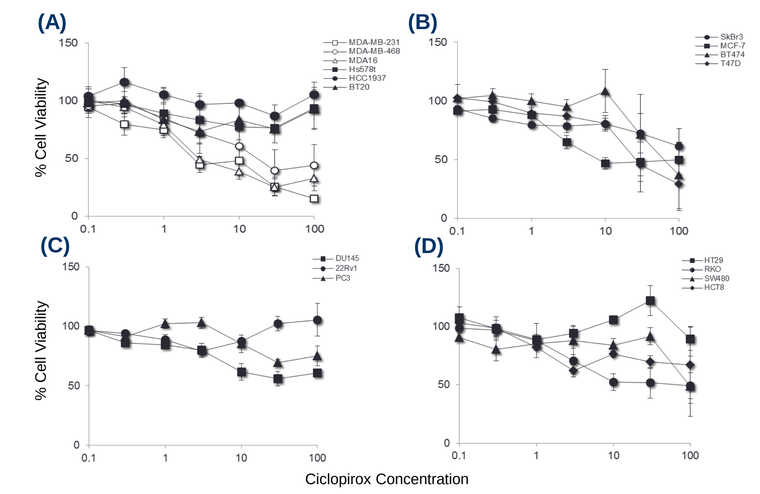

Fig 6: Sensitivity of 17 cancer cell lines to Ciclopirox treatment. The sensitivity of triple negative breast cancer cells (**A**), non-triple negative breast cancer cells (**B**), prostate cancer cells (**C**) and colon cancer cells (**D**) to Ciclopirox was examined. A subset of triple negative breast cancer cells were most sensitive to 48 hour treatment with Ciclopirox.

Discussion

To date, more than 100 drugs have shown activity *in vitro* against diseases other than those for which they were originally approved, e.g. the anti-amoebic Fumagillin has been shown to prevent angiogenesis and suppress cancer in mice[4]. This study identified 30 clinical compounds that reduced the cell viability of multi-drug resistant triple negative breast cancer cell line MDA16.

One compound, Ciclopirox, has recently been shown to possess anti-cancer activities against a number of haematological malignancies, as well as a breast cancer xenograft model[5,6]. In this study, Ciclopirox induced caspase activation and processing, implicating apoptosis as the method of cell death. Interestingly, Ciclopirox was most effective against a subset of triple negative breast cancers, suggesting a selective toxicity.

Assay optimisation is essential in High Throughput Screening to increase sensitivity, reproducibility and accuracy, while not compromising speed and efficiency. The Z-factor is a useful metric to evaluate assay optimisation, and in this study was increased to 0.75, which correlated with a hit confirmation rate of 70%.

1. Chong CR, Sullivan DJ. **New uses for old drugs**. Nature. 2007 Aug 9;448(7154):645-6.
2. Ekins S, Williams AJ. **Finding Promiscuous Old Drugs for New Uses**. Pharm Res.2011 May 24. [Epub ahead of print].
3. Zhang JH et al. **A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays**. J Biomol Screen. 1999;4(2):67-73.
4. Hou L et al. **Fumagillin inhibits colorectal cancer growth and metastasis in mice: in vivo and in vitro study of anti-angiogenesis**. Pathol Int. 2009 Jul;59(7):448-61.
5. Eberhard Y et al. **Chelation of intracellular iron with the antifungal agent ciclopirox olamine induces cell death in leukemia and myeloma cells**. Blood. 2009 Oct 1;114(14):3064-73.
6. Zhou H et al. **The antitumor activity of the fungicide ciclopirox**. Int J Cancer. 2010 Nov 15;127(10):2467-77.

HEA Higher Education Authority An tÚdarás um Ard-Oideachas
sfi science foundation ireland fondúireacht eolaíochta éireann
NUI Galway OÉ Gaillimh
NDP NATIONAL DEVELOPMENT PLAN