The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

High Throughput Screening of KPC-Familial Pancreatic Cancer for Selective Inhibitors of BRCA2 Deficient Cells

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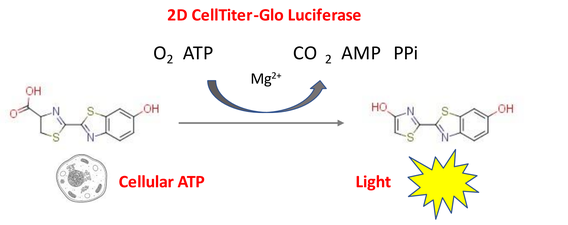
Abstract

Pancreatic cancer is one of the most common malignant and progressive types of cancer. Current statistics show that only 11 out of 100 diagnosed patients survive more than 5 years after being diagnosed. About 10% of pancreatic cancer cases are related to familial genes such as BRCA1/2, ATM and PALB2. The most common genes in familial pancreatic cancer (FPC) are BRCA2 and CDKN2A. Patients with FPC may be able to undergo more gene specific treatments to increase their chance at survival. To address this unmet need, isogenic murine pancreatic cancer (KPC) cell lines were mutated by CRISPR/Cas9 to model FPC patient genetic profiles. High-throughput drug screening was conducted on both BRCA2 KO and ROSA26 cell-lines, in parallel, for a direct hit comparison using ROSA26 as a control. The screening consisted of more than 3,500 known drugs in 1536 well plate format. One drug, called JQ1 was found to be a selective inhibitor, and further research was done to show that BRCA2 deficient cells are more sensitive to bromodomain and extraterminal (BET) inhibitors. Overall, the data suggests BET inhibition may be used as a novel therapeutic strategy for FPC patients presenting with BRCA2 deficiencies.

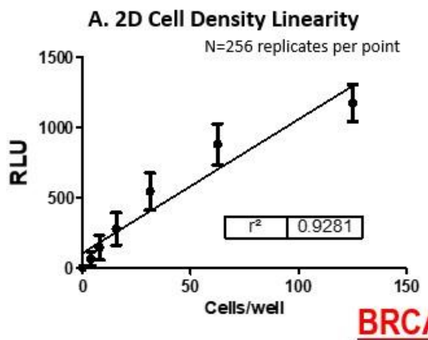
Assay Principle



Scripps proposed using the 2D CellTiter-Glo (CTG) Luciferase assay to determine cell viability after the cells had been treated with the specified compounds for 3 days. The luminescence in RLU will provide us with relative viability for each compound per cell line.

QR CODE

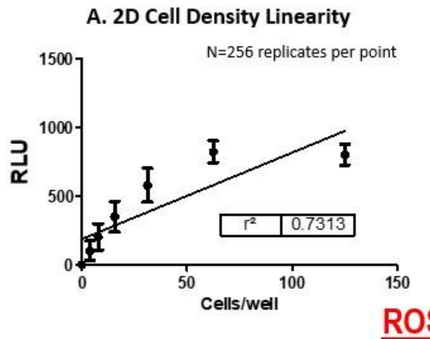
Experimental Design



B. Assay Statistics vs. [Cells]

Cells/Well	Z'	S/B
3.91	-1.61	17.03
7.81	-0.88	37.28
15.63	-0.28	70.84
31.25	0.25	138.43
62.50	0.49	223.68
125.00	0.66	298.07

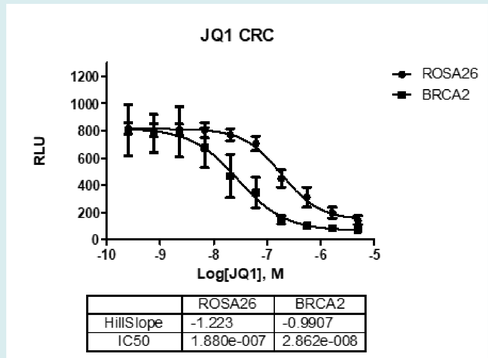
BRCA2KO



B. Assay Statistics vs. [Cells]

Cells/Well	Z'	S/B
3.91	-1.18	32.25
7.81	-0.44	63.46
15.63	0.02	108.54
31.25	0.35	179.05
62.50	0.70	254.47
125.00	0.71	248.27

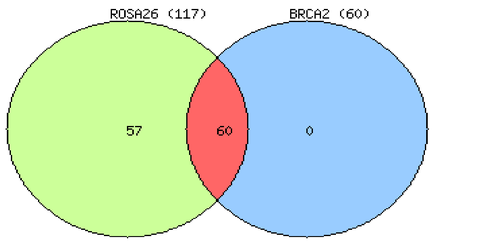
ROSA26



	ROSA26	BRCA2
HillSlope	-1.223	-0.9907
IC50	1.880e-007	2.862e-008

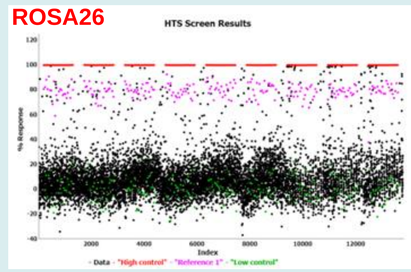
After optimizing the cell density, the team determined compound response using the JQ1 compound in a dose dilution. Previous studies by the Hwang lab showed promising results using this JQ1 compound. The figure above shows it can be used as a positive control, and the experiment works at that cell density with the incubation periods specified.

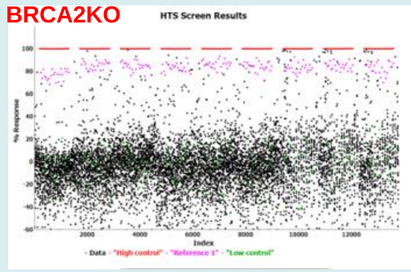
ROSA26 vs BRCA2KO



mKPC Run Statistics ROSA26 (n=9 plates)
- 3,295 compounds tested at ~2uM in triplicate.
- Ave Z' = 0.74 ± 0.04
- Ave S:B = 123.67 ± 3.97
- Hit Cutoff= 28% (AVG + 3*SD of LC)
- Hit Rate: 3.55%
- 117 Hits

mKPC Run Statistics BRCA2 (n=9 plates)
- 3,295 compounds tested at ~2uM in triplicate.
- Ave Z' = 0.53 ± 0.11
- Ave S:B = 109.36 ± 4.05
- Hit Cutoff= 47% (AVG + 3*SD of LC)
- Hit Rate: 1.82%
- 60 Hits



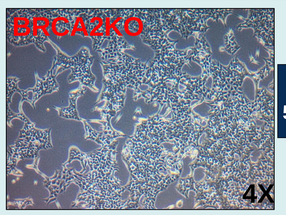


HTS Screening

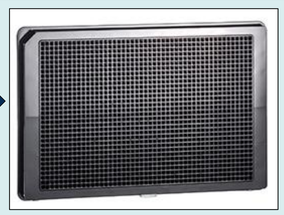
1536wp Assay

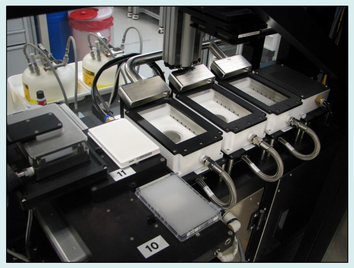
- Seed cells in 5 µL culture medium at 62.5 cells/well into 1536 Greiner black clear treated plates
- Incubate cells for 24 hrs | 37°C 5%CO_2 95% RH
- Pintool transfer 10nL of compounds
- Incubate the cells + compounds for an additional 3 days at 37°C 5%CO_2 95% RH
- Dispense 5 µL CellTiter-Glo 2D reagent, Incubate for 10min at RT in the dark
- Read plate(s) luminescence on the ViewLux

Final volume = 10 µL



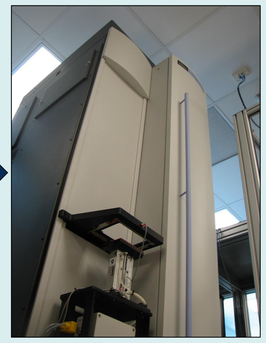
62.5cells/well
5ul media/well



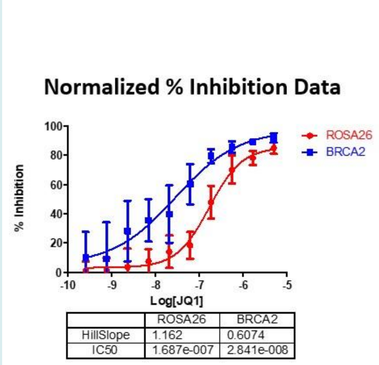


10nL Kalypsys Pintool Transfer

Incubate & Read

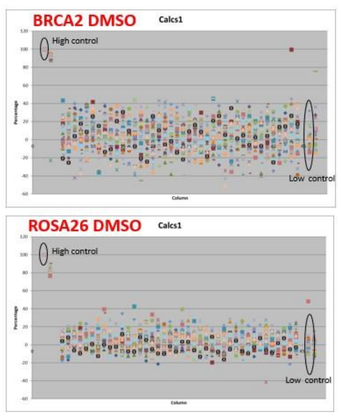


ViewLux



	ROSA26	BRCA2
HillSlope	1.162	0.6074
IC50	1.687e-007	2.841e-008

% Response = 100 × (Test Well – Median Low Control Wells) / (Median High Control – Median Low Control Wells)



Plates at 96hrs time post seeding, 72hrs post pinning

High control - MEDIA+DMSO, Low control - Cells + DMSO

mKPC Run Statistics BRCA2 (n=3 plates)
- 133 compounds tested starting at ~5uM in 10-point 2-fold dilution in triplicate.
- Ave Z' = 0.55 ± 0.14
- Ave S:B = 118.11 ± 8.67

mKPC Run Statistics ROSA26 (n=3 plates)
- 133 compounds tested starting at ~5uM in 10-point 2-fold dilution in triplicate.
- Ave Z' = 0.62 ± 0.17
- Ave S:B = 123.09 ± 12.06

Conclusions

Assay performance was great and had an average of Z' of 0.66±0.08 for BRCA2KO and Z' of 0.75±0.03 for ROSA26 the triplicate data was reproducible. The average signal-to-background ratio (S: B) was 130.76 ±5.61 for BRCA2KO and 137.39 ±5.61 for ROSA26 (n=3 plates). There are 38 active hits with an IC50<1uM for a 35% hit rate on ROSA26 (BRCA2KO had 36 active hits). The compound JQ1 obtained by the assay provider performed upon the best of the dose compounds.

Future Implications

In the future, the assay provider will move forward with determining the best compound and concentration for specific BRCA2 deficient cell inhibition. Additional studies have been completed by Dr. Hwang's team at UC Davis, and "Selective vulnerability to BET inhibition due to enhanced autophagy in BRCA2 deficient pancreatic cancer" is a manuscript currently under review at the Cancer Research Journal. Manuscript full author list: Chang-Il Hwang, EunJung Lee, Keely Ji, Suyakarn Archasappawat, Jocelyn Pena, Verneliz Fernandez-Vega, Ritika Gangaraju, Nitin Beesabathuni, Martin Kim, Qi Tian, Priya Shah, Louis Scampavia, and Timothy Spicer.