Scintillant BIOSCIENCE

Advancing Cancer Therapeutics Through Targeted PARG Inhibition: A Case Study

The Challenge

Dysfunctional DNA repair processes are a common feature of various cancer types. Mistakes in DNA repair can lead to the accumulation of genetic mutations in tumor suppressor genes and proto-oncogenes, driving malignant transformation and enabling cancer cells to evade cell death. However, too much DNA damage will conversely result in cancer cell death. Consequently, there is a balance between the acquisition of mutations that drive oncogenesis and the mutational burden that promotes cell lethality. Thus, DNA repair mechanisms are critical for maintaining the growth of both healthy and malignant cells.

Among the crucial players in maintaining DNA integrity is the process of PARylation, mediated by the enzyme Poly(ADP-ribose) polymerase (PARP). PARylation, involving the addition of poly(ADP-ribose) (PAR) chains to proteins, acts as a signaling mechanism in response to DNA damage. This modification recruits repair factors to damaged DNA sites, facilitating the repair process. However, it is imperative to maintain a dynamic balance between PARylation and the removal of PAR chains for cellular homeostasis.

Poly(ADP-ribose) glycohydrolase (PARG) is an enzyme responsible for the timely degradation of PAR chains, effectively reversing the PARylation process and ensuring the completion of DNA repair. Depletion or inhibition of PARG in cancer cells increases sensitivity to DNA-damaging agents, underscoring its role in preserving cell survival.

The Solution

Targeting PARG with specific inhibitors has the potential to disrupt the ability of cancer cells to repair DNA damage, rendering them more susceptible to cell death, especially in conjunction with chemotherapeutic agents. Consequently, developing targeted PARG inhibitors represents a promising strategy in the quest for more effective cancer therapeutics and therapeutic combinations.

To address this challenge, we undertook contract research on behalf of a mid-sized pharmaceutical company. Our goal was to develop an assay capable of ranking PARG inhibitors by their potency of inhibition. We began by adopting a previously published high-content screening assay (James DI et al., "An assay to measure poly(ADP ribose) glycohydrolase (PARG) activity in cells," F1000Research 2016, 5:736), which we subsequently modified, validated, and implemented to suit our client's needs.

The assay was conducted in a 384-well format over multiple medicinal chemistry cycles. For each cycle, we received batches of 30 to 60 compounds. Each compound was subjected to a 10-point dose-response analysis, with four technical replicate wells per compound concentration. To ensure reliability, each dose-response curve for every compound was generated twice in two independent experiments.

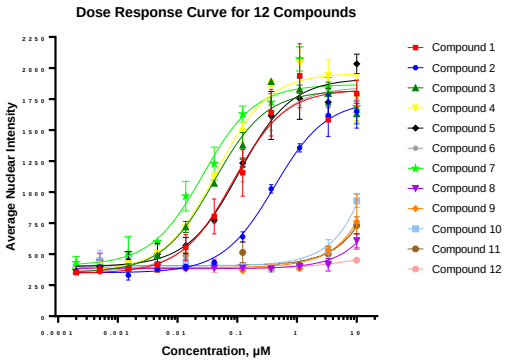
The Results

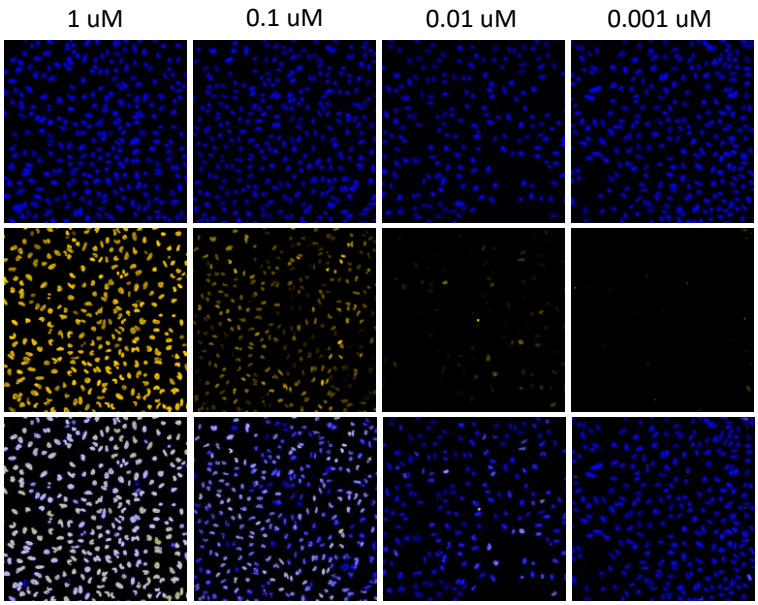
Our efforts yielded high-quality and highly reproducible data for our client. The assay effectively aided the tracking of structure-activity relationships (SAR), allowing them to identify increasingly potent compounds with each successive medicinal chemistry cycle. Our client expressed great satisfaction with our development and implementation of the assay, as it empowered them to optimize candidates for prospective advancement into human clinical trials.

See examples of experimental data, including images and dose-response curves, on the following two pages:

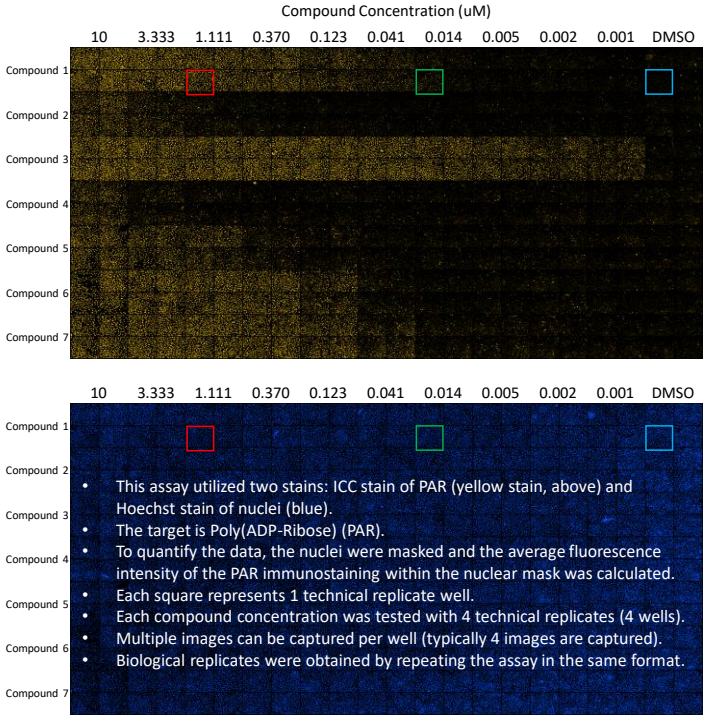
ICC for PAR Polymer (PARG Inhibition)

Precise and accurate dose-response curves can be generated for compounds against a target of interest using fluorescence imaging. With our high-content imaging system, many compounds may be tested simultaneously and rank-ordered by potency with several technical and biological replicates per condition to ensure confidence in the experimental results.



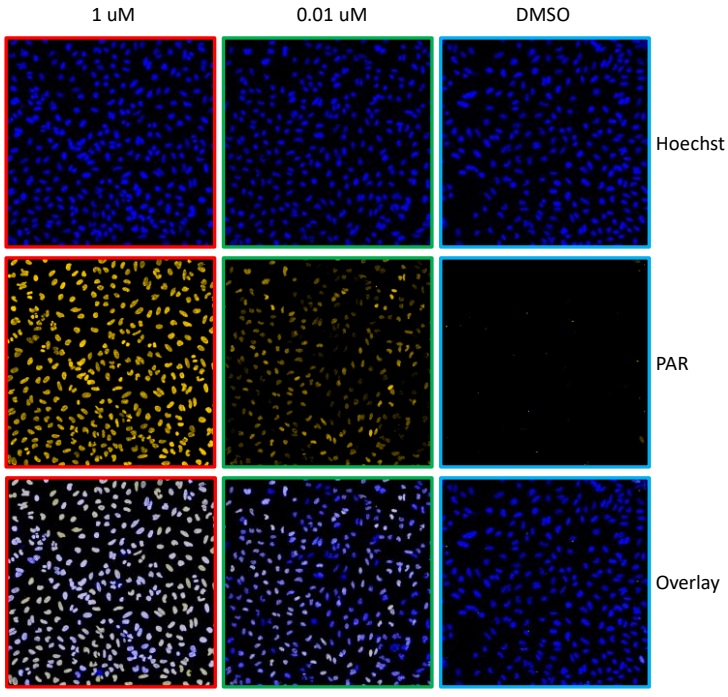


Thumbnail images of all wells in a 384-well plate (edge wells excluded)



- This assay utilized two stains: ICC stain of PAR (yellow stain, above) and Hoechst stain of nuclei (blue).
- The target is Poly(ADP-Ribose) (PAR).
- To quantify the data, the nuclei were masked and the average fluorescence intensity of the PAR immunostaining within the nuclear mask was calculated.
- Each square represents 1 technical replicate well.
- Each compound concentration was tested with 4 technical replicates (4 wells).
- Multiple images can be captured per well (typically 4 images are captured).
- Biological replicates were obtained by repeating the assay in the same format.

Larger images of selected wells (color-coded in thumbnail images)



Assay Reproducibility

Biological Replicate 1

These thumbnail images are from two 384-well plates run on separate days with the same experimental conditions, demonstrating the reproducibility of the assay. From this data, 10-point dose response curves were generated with high confidence to determine IC50 values

Biological Replicate 2