# Utilizing the zebrafish xenograft model to evaluate anticancer drug efficacy

## Overcoming therapeutic resistance with Bcl-2 functional converters.

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# Background

- **The zebrafish xenograft model is a powerful tool for assessing tumorigenic potential and drug efficacy.**
- **Model provides an *in-vivo*, high-throughput, low-cost platform for drug-screening and toxicity assessment.**
- **Provides a more robust, and representative model of cancer-drug response than cell culture.**
- **We leverage this model for evaluating anti-cancer action of Bcl-2 functional converter (BFC) compounds.**
- **Bcl-2 is upregulated in therapy-resistant cancers as a mechanism of acquired resistance.**
- **BFCs convert Bcl-2 from a pro-survival protein to a pro-apoptotic conformation, exposing the BH3-death domain.**
- **Palbociclib (Pd) is a recently approved (2015) clinical drug currently used for ER-positive/HER2-negative breast cancer treatment. Pd is a CDK4/6 inhibitor, preventing cell-cycle progression.**
- **Pd is initially effective, but acquired therapy-resistance still observed in the clinic.**
- **Urgent need still exists for targeting resistant cancers.**

# Methods

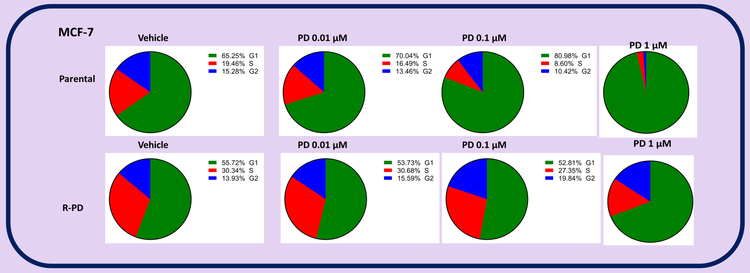
**<u>Zebrafish Xenograft Experiments:</u>** **Fish were acquired from Sinhuber Aquatic Research Laboratory and maintained in (.0003%) phenylthiourea in E3 media following 24 hpf to prevent melanization. Pd-Resistant MCF7 cells were previously generated by continuous culture in drug for 6-8 weeks with an increasing concentration regiment (.001-1 µM). The day of injection, MCF7 cells were stained with fluorescent CM-DiI and injected into the yolk sac of 48 hpf fish by air-driven micro-pressure injector via a borosilicate glass needle. Cell suspension was 1 x $10^7$ cells/mL, and approximately 200 cells were used per fish.**

**Fish were imaged 1 day and 4 days post-injection. For imaging, fish were anesthetized by 0.2 mg/mL Tricaine and embedded in 0.8% low melting point agarose in a 96-well plate. Imaging was performed with the High-content microscope, with Z-stacks captured on a 10X objective.**

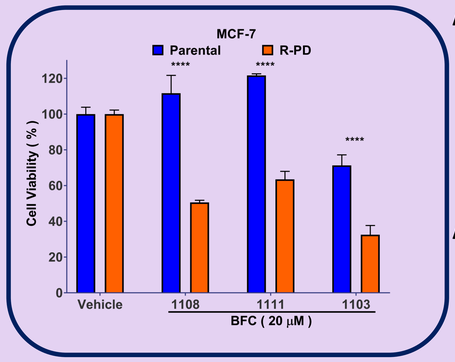
**Cancer growth was analyzed using Fiji software, by making a maximum projection image of the z-stack and applying a median filter. Area was calculated by creating a binary mask from thresholds with the Otsu algorithm and calculating total area of resulting segmented objects.**

# Results

## A) <u>Generation of a therapy-resistant breast cancer line – MCF7 cells become insensitive to Pd</u>



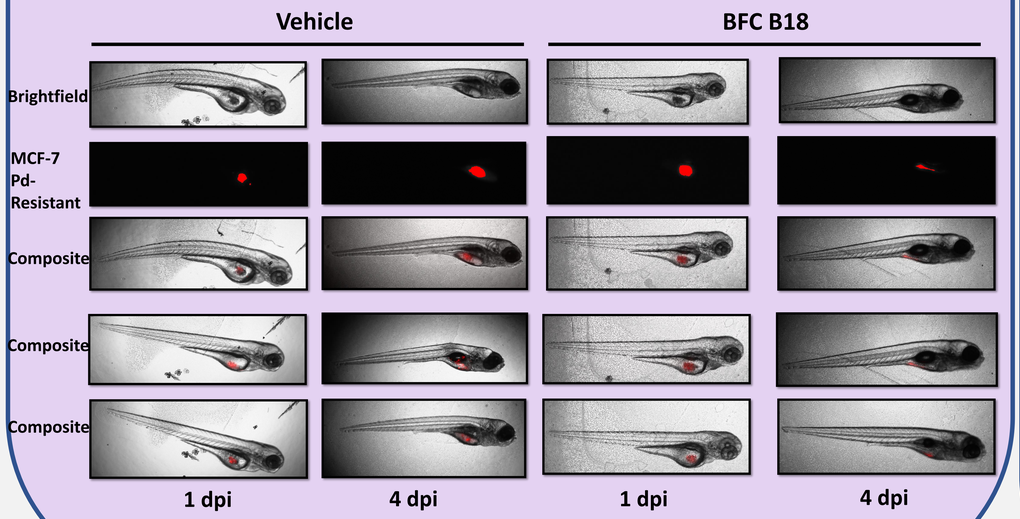

## B) <u>Pd-Resistant cells are more sensitive to B18 treatment than parental cells.</u>




A) <u>MCF-7 cells acquire resistance to Pd (Palbociclib)</u>: Cell cycle analysis by flow cytometry shows parental MCF7 cells are sensitive to PD, with increasing dosages promoting G1 arrest (green). Resistant (R-PD) cells are no longer sensitive to Pd treatment, and proceed through S/M phases. Resistant cells were generated by 6-8 weeks Pd-treatment (10nM-1 µM).

A) <u>Pd-Resistant cells are sensitive to BFC treatment:</u> Treatment with Bcl-2 functional converters shows R-Pd cells are more sensitive than parental cells. BFC-1108 was found to have greatest therapeutic difference between parental and resistant lines.

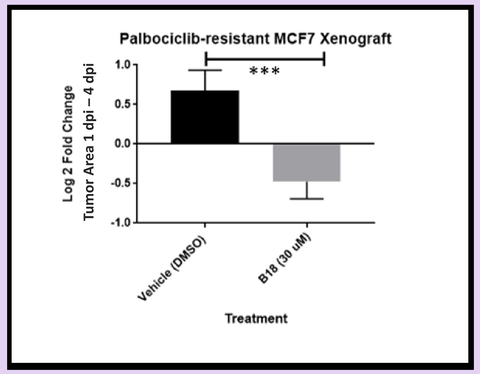
## C) <u>B18 reduces tumor burden in Pd-resistant xenografts</u>




C) Representative images, day 1 and day 4 post-injection, of fish treated with Vehicle or 30 µM B18.

# Results

## D) B18 suppresses tumor growth




D) <u>B18 suppresses therapy-resistant xenografts</u>: Tumor area was quantified 1 day and 4 days post-injection with Pd-resistant MCF-7 cells. Bars represent the average log-base 2 fold-change between day 1 and 4. (n=55, n=65 for Veh./Treat) Error bars are SEM. (p=.0003 Student's Unpaired T-test).

# Conclusions and Future Directions

- **Bcl-2 represents a logical molecular target for overcoming acquired resistance to therapy and is upregulated in numerous cancers, including ER-positive breast cancer.**
- **Bcl-2 functional conversion by B18 is capable of suppressing Pd-resistant MCF7 cells in the zebrafish xenograft model, and showed no signs of overt toxicity to fish.**
- **BFCs have further shown efficacy in breast cancer xenografts resistant to other therapeutics such as paclitaxel (data not shown here).**
- **<u>Future directions include:</u>**
  - **Evaluating B18 activity in xenografts with parental MCF7 to demonstrate differential sensitivity in this model.**
  - **Demonstrating Bcl-2 functional conversion as mechanism of suppression by staining for BH3 domain exposure in sectioned zebrafish.**