# Effects of Daminozide on Triple Negative Breast Cancer Cells

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## Introduction

Breast cancer, as the world's most prevalent cancer, continues to pose significant global health challenges with 2.3 million women diagnosed and 685,000 deaths reported in 2020 alone. One of the most aggressive and treatment-refractory subtypes is triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER) and progesterone receptor (PR), as well as lack of human epidermal growth factor receptor 2 (HER2) overexpression.

**Frequency of Breast Cancer Subtypes**
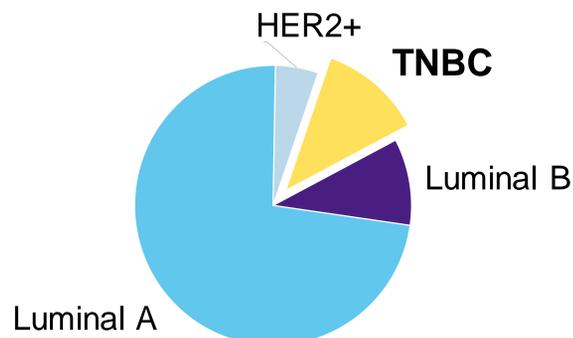


**Targetable Receptors in Breast Cancer**



Hormone Positive | HER2 Enriched | Triple Negative
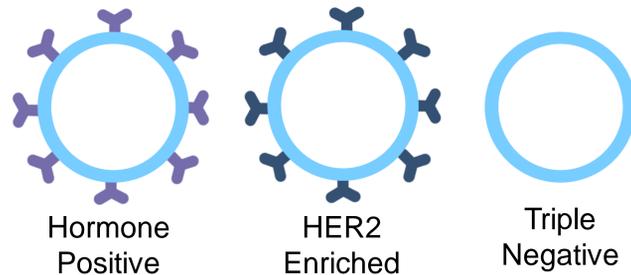
The epigenetic regulator, Plant Homeodomain Finger protein 8 (PHF8), offers promise as a potential target for TNBC. PHF8 modulates histone methylation, a fundamental cellular process that contributes significantly to gene regulation and cancer development. Daminozide (DAM), a plant growth regulator, is the only available selective PHF8 inhibitor. The current study aims to investigate the therapeutic implications of inhibiting PHF8 with daminozide in TNBC cell lines.
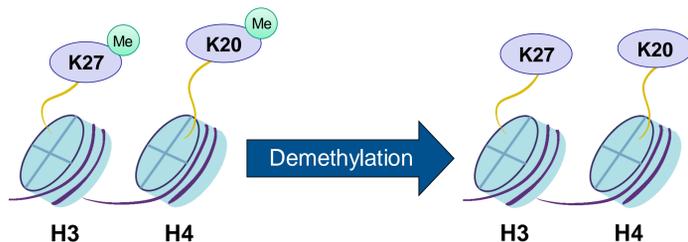


## Relapse Free Survival Rate
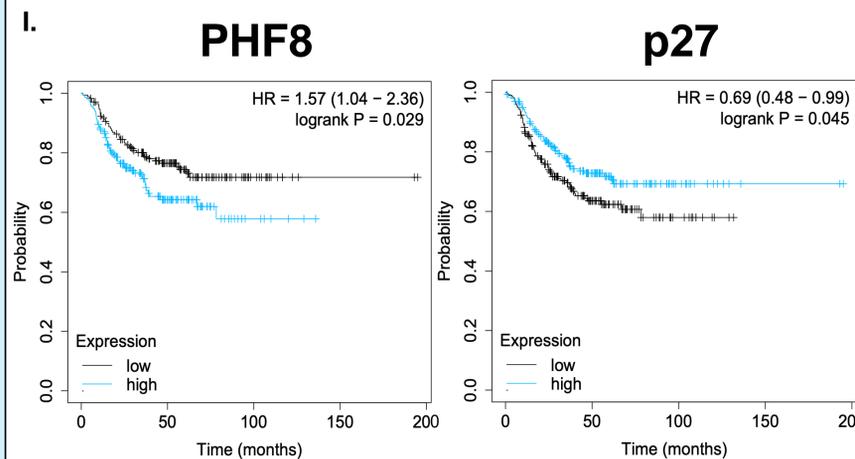


Figure 1. Kaplan-Meier curves obtained with the 215065_at (PHF8) and 203114_at (p27) signature. This set has relapse free survival information and genome-wide expression profiles obtained with RNA-seq & included 392 triple negative breast tumor samples

## Molecular Docking
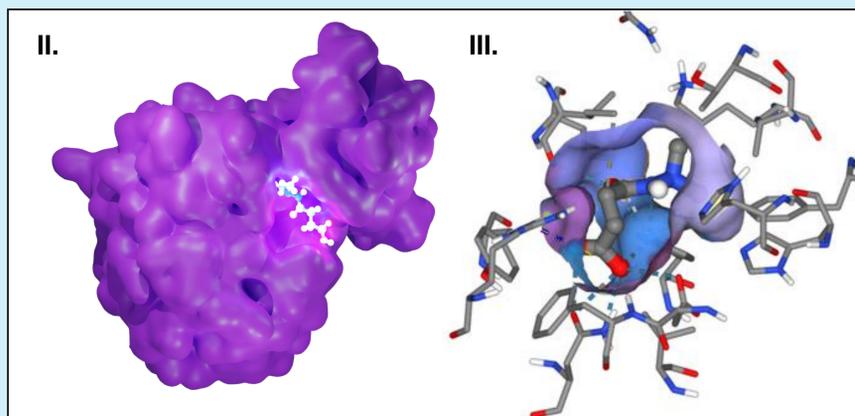


Figure 2. Computational molecular docking 3D model of PHF8 and daminozide using RSCB Protein Data Bank
Figure 3. Active-site structure of PHF8 showing the hydrogen-bonding network with daminozide docked

## Methods

 ❖ **MTS** assay was used to determine cell viability.

 ❖ **RT-qPCR** was performed to detect mRNA expression.

 ❖ **Western Blots** were performed to assess the expression and activation of proteins.

 ❖ **Flow cytometry assays** were performed to detect cell cycle arrest.

## Results
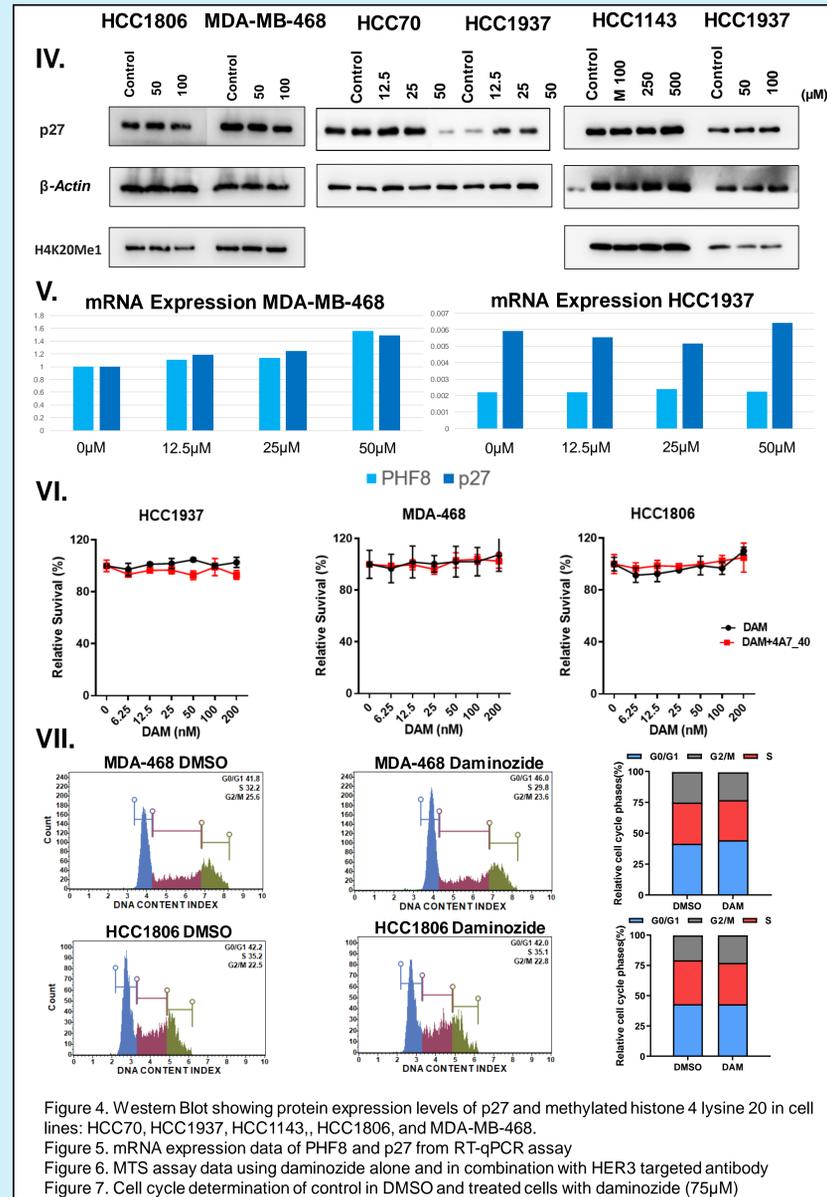


Figure 4. Western Blot showing protein expression levels of p27 and methylated histone 4 lysine 20 in cell lines: HCC70, HCC1937, HCC1143,, HCC1806, and MDA-MB-468.
Figure 5. mRNA expression data of PHF8 and p27 from RT-qPCR assay
Figure 6. MTS assay data using daminozide alone and in combination with HER3 targeted antibody
Figure 7. Cell cycle determination of control in DMSO and treated cells with daminozide (75µM)

## Conclusion

Daminozide has not demonstrated to be an effective inhibitor for PHF8 in the tested triple-negative breast cancer cell lines. Moving forward, more research is needed to identify novel inhibitors with the potential to target PHF8, with the goal of improving patient outcomes in this challenging subtype of breast cancer.



Daminozide | Derivatives (Potential Inhibitors)