# **Developing Cytotoxic Drugs that Target Estrogen Receptors in Breast Cancer Cells**



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### Abstract

Breast cancer is a growing problem in the United States that takes the lives of approximately 40,000 women a year. Clearly, this is a serious issue that must be solved. Current chemotherapy treatments often result in widespread cell death, including the killing of healthy cells, making it necessary to find alternative treatments that specifically target cancer cells. Many breast cancer cells over express estrogen receptors, which are vital to the rapid cell division and growth of tumors. Estrogen is a steroid hormone that enters the cell, binds to its receptor, translocates to the nucleus, and leads to gene expression. Previous work from our group has resulted in the development of a drug which targets estrogen receptor-positive breast cancer cells called Est-3-Melex. The drug contains a DNA methylating group (methyl sulfonate, Melex) conjugated to estrogen. The mechanism of action of the drug is by the binding of the estrogen portion of the molecule to its receptor that ultimately translocates to the nucleus. While in the nucleus, the Melex portion of the compound is brought in close proximity to the DNA and methylates the adenines, eventually resulting in cell death. Essentially, this is a receptor targeted cancer therapy.

In this study, we show that Est-3-Melex is more toxic to cancer cells that overexpressed the estrogen receptor compared to those that did not. Treating the estrogen receptor-positive breast cancer cells with excess amounts of estrogen inhibited Est-3-Melex-induced cell death. Fluorescence imaging was also utilized to visualize localization of the drug. The results showed that the drug localized to the nucleus and this localization was inhibited by estrogen and Fulvestrant. Our results suggest that Est-3-Melex is effective in specifically killing estrogen receptor positive breast cancer cells by binding to the estrogen receptor. Additional investigations are underway to identify the mechanism of cell death.

### **Breast Cancer**

- Breast cancer takes the lives of approximately 40,000 U.S. women each year, with about 1 in 12 women (8%) developing breast cancer during the course of their life
- Current treatments include surgery, chemotherapy, radiation therapy, hormone therapy, and targeted therapy
- Chemotherapy often results in widespread cell death, including the killing of healthy cells
- Many breast cancers overexpress estrogen receptors, which can be exploited for targeted therapy
- Estrogen binds to its receptor in the cytoplasm, the complex translocates to the nucleus, interacts with DNA, and results in gene expression and cancer proliferation
- Estrogen stimulates the proliferation of breast cancer cells and two major subtypes of breast cancer overexpress the estrogen receptor

### **Goal of Project**

To test the mechanism of entry of Est-3-Melex in estrogen receptor-positive breast cancer cells.



Figure 1. % Cell survival values in MCF-7 cells treated with increasing concentrations of *Est-3-Melex and Melex.* 





Figure 3. Fluorescence imaging of MCF-7 Cells treated with 5.63  $\mu$ M NBDPP-3 at 20x magnification



Figure 4. Fluorescence imaging of MCF-7 Cells treated with 21.36 µM NBDPP-3 and 0.5 mM Estradiol at 20x magnification

### MTT Cytotoxicity Assay

Day 1.) Plate MCF-7 or 293HEK cells in 96-well plate (~5000 cells/well) Day 2.) Treat plate with varying concentrations of Est-3-Melex, Melex, and/or Estradiol Day 3.) Treat cells with MTT and measure absorbance at 540 nm using a spectrophotometer (measuring cell survival)

Figure 2. % Cell survival values in MCF-7 cells treated with increasing concentrations of Estradiol and  $EC_{50}$ *Est-3-Melex* 



Figure 5. Fluorescence imaging of MCF-7 Cells treated with 21.36 µM *NBDPP-3 and 1* µM *Fulvestrant at 40x* magnification



Figure 6. Fluorescence imaging of 293HEK Cells treated with 21.36 µM NBDPP-3 at 40x magnification

Cell Death

cancer cells 293HEK cells

Methods

### **Fluorescent Imaging**

Day 1.) Plate ~10,000 cells on HCl-pretreated coverslips in a six-well plate and add medium Day 2.) Treat wells with varying concentrations of NBDPP-3, Estradiol, and/or Fulvestrant Day 3.) Wash coverslips with PBS and Paraformaldehyde. Prepare slides Day 4.) Look at slides using fluorescence microscope

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### Conclusions

- Est-3-Melex is more effective than Melex at killing MCF-7 breast
- Estradiol inhibits the cytotoxicity of Est-3-Melex
- Molecules that bind to the estrogen receptor, Estradiol and Fulvestrant, inhibit Est-3-Melex nuclear localization
- Est-3-Melex does not enter the nucleus in Estrogen receptor negative

### Acknowledgements



