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# A novel approach for ER+ breast cancer treatment: A new compound that modulates aromatase and ER

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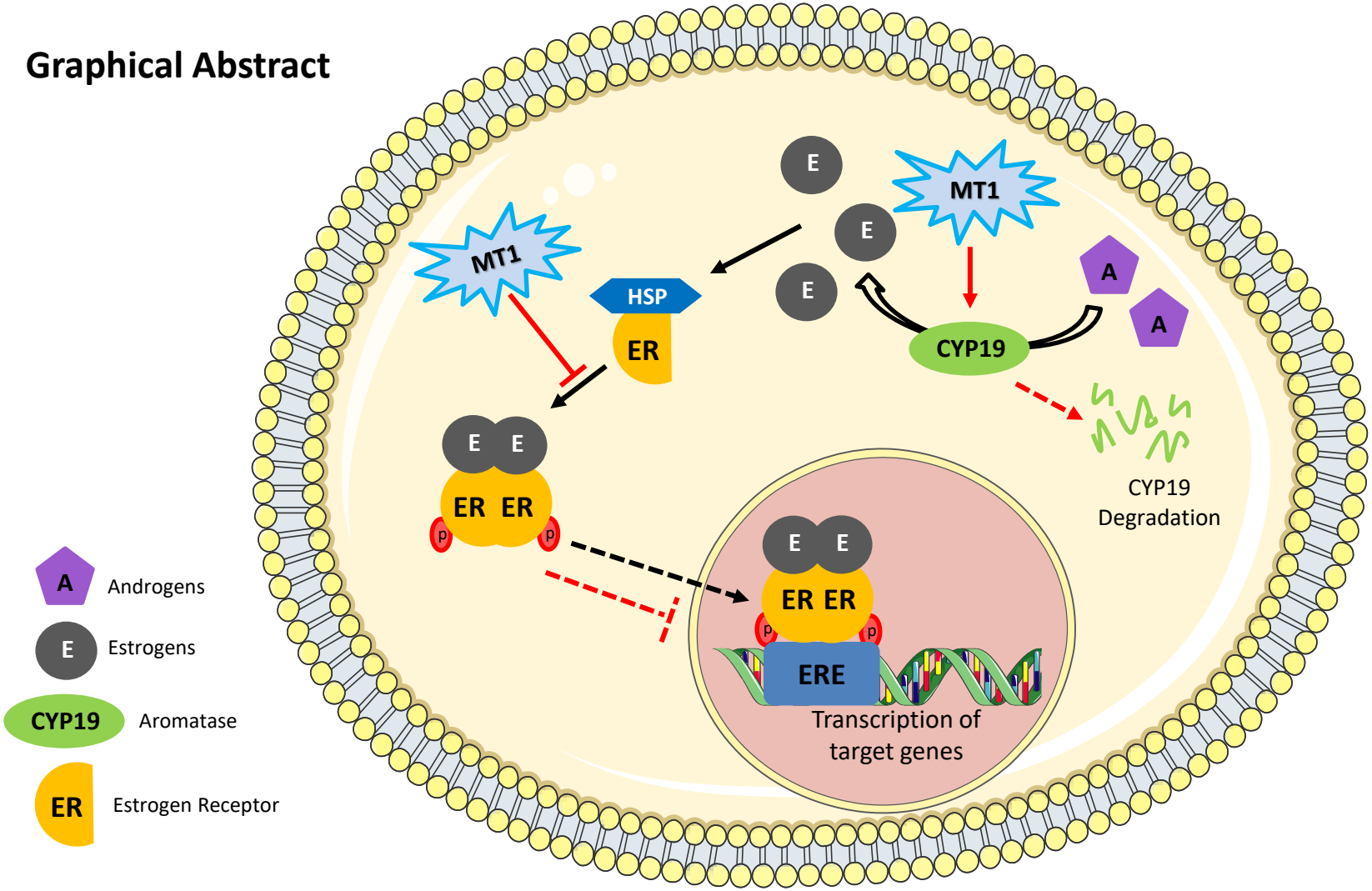
# A novel approach for ER+ breast cancer treatment: A new compound that modulates aromatase and ER

## Graphical Abstract



**Abstract**

Estrogen receptor-positive ($ER^+$) breast cancer is the most common subtype of breast cancer worldwide. Estrogens, after being synthetized by aromatase, bind to ERα promoting breast cancer proliferation. Besides the success of the already approved therapies, they induce several side effects, reason why it is crucial to discover novel therapeutic approaches. Considering this, our goal is to discover multi-target compounds able to simultaneously inhibit aromatase and modulate ERα. For that, the known aromatase inhibitors (AIs) and ERα antagonists were collected and chemical descriptors were constructed and organized in clusters. After that, the selected compounds were analyzed by molecular docking. Anti-aromatase activity was evaluated in human placental microsomes. Aromatase and ERα expression was assessed by Western-Blot in $ER^+$ an aromatase-overexpressing breast cancer cell line (MCF-7aro).

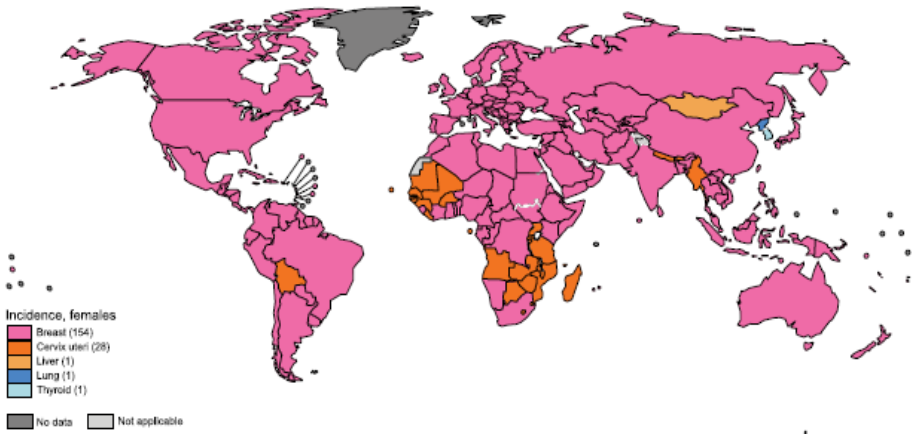
One compound (MT1) was selected to be studied in microsomes and in MCF-7aro cells. This compound was not able to inhibit aromatase in microsomes, but curiously, MT1 decreased aromatase protein levels in MCF-7aro cells. Furthermore, MT1 impaired ERα activation, acting as an ERα antagonist. This represents a great advantage for breast cancer treatment, since aromatase and ERα are key targets in this type of cancer.

**Keywords:** $ER^+$ Breast Cancer, Aromatase, ERα, Multi-target

# Introduction

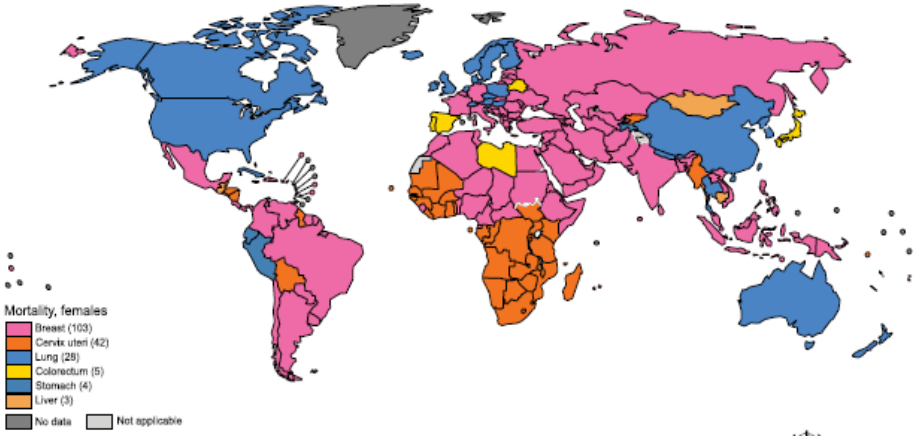
**Breast cancer**
2018

## Incidence




**New cases/2018:** 2 088 849

## Mortality

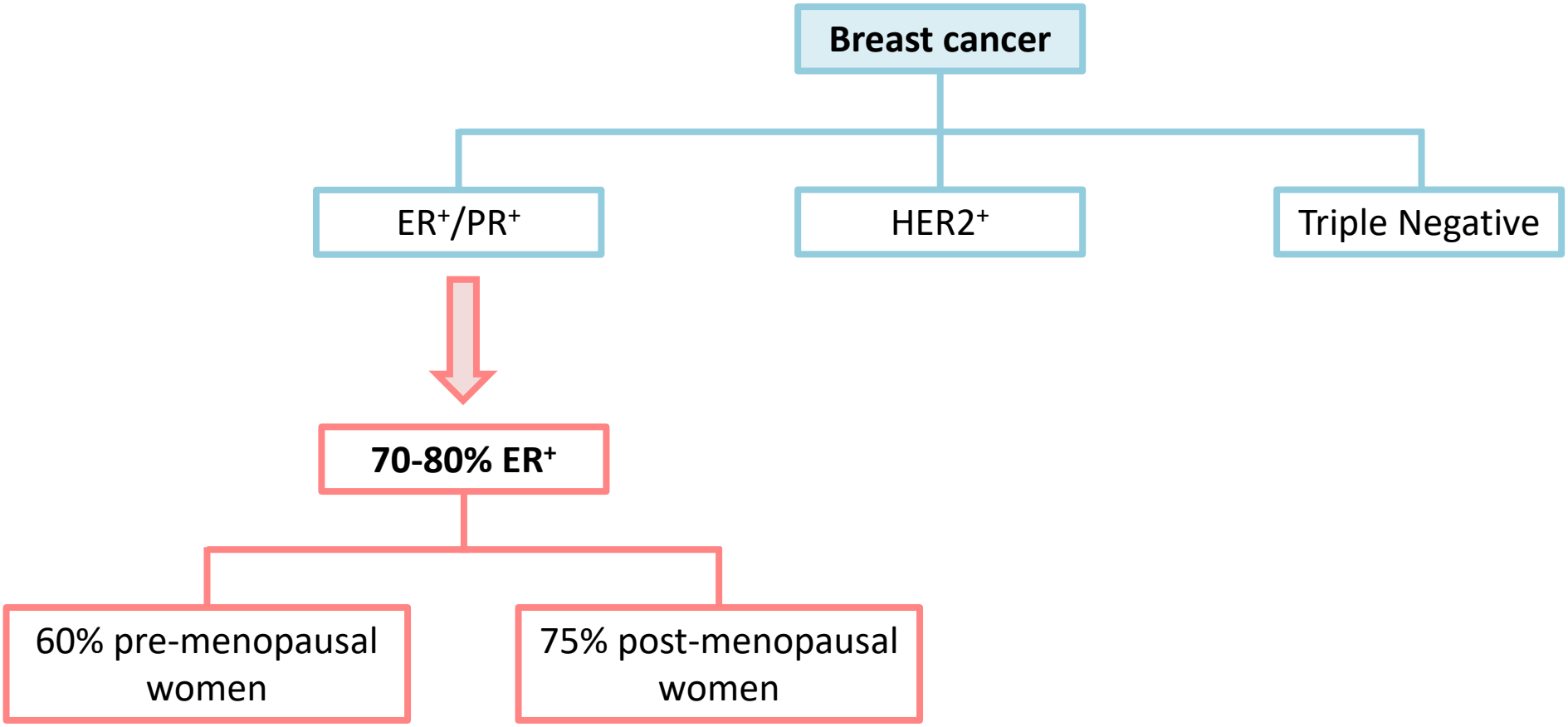



**Deaths/2018:** 626 679

Bray F. *et al* (2018) CA CANCER J CLIN, 68:394–424
The Global Cancer Observatory, March 2019

# Introduction

## Breast cancer

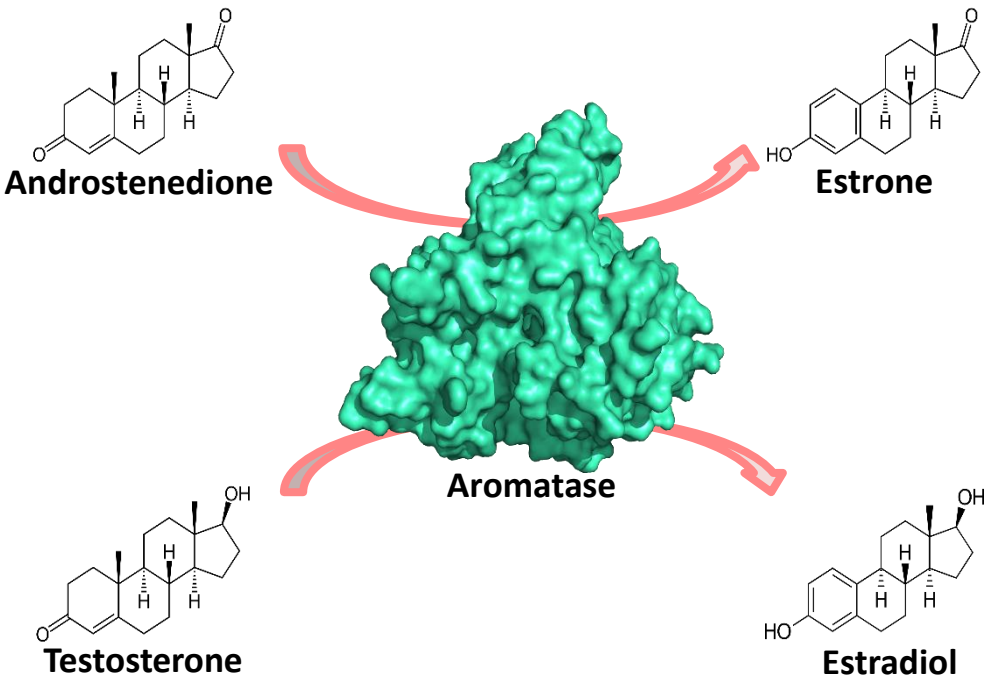



Amaral C et al. (2017) J Steroid Biochem Mol Biol;171:218-28

# Introduction

## Aromatase (CYP19)

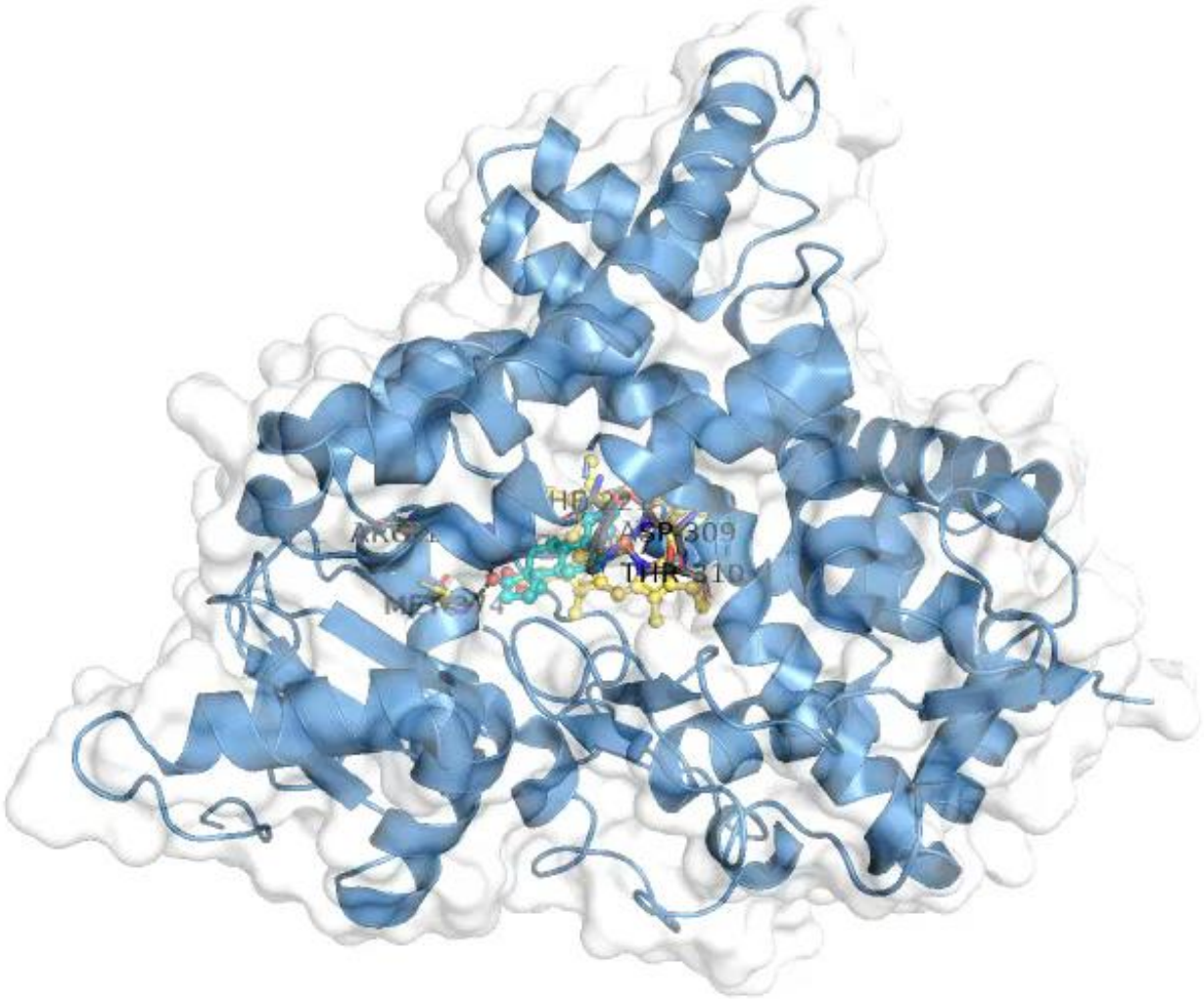
- Belongs to the cytochrome P450 family
- Product of the *CYP19A1* gene on chromosome 15
- Highly expressed in the ovaries of pre-menopausal women and in adipose cells of post-menopausal women
- In $ER^+$ breast cancer patients is overexpressed
- Responsible for the conversion of androgens into estrogens




Augusto TV et al. (2018) Endocr Relat Cancer;25(5):R283-R301

# Introduction

## Aromatase



# Introduction

## Estrogen Receptor (ER)

**ERs**

**ERα**

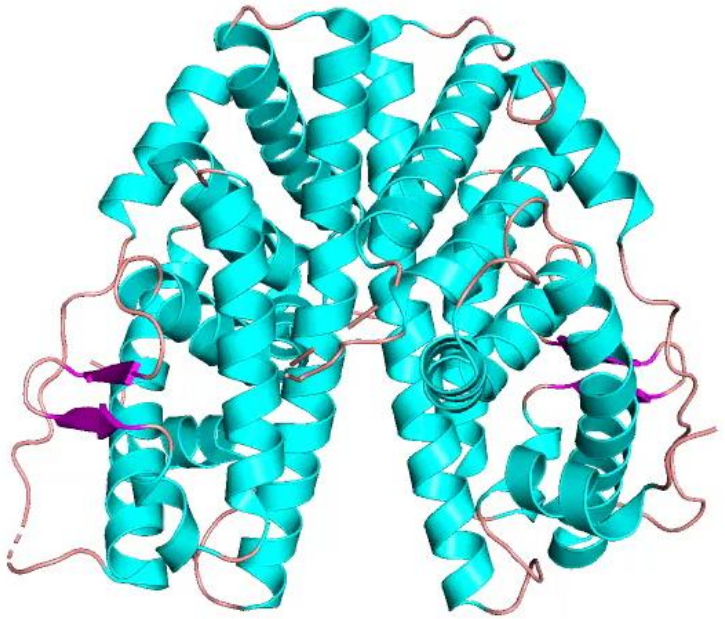
Growth and survival of breast epithelial cells, through cell proliferation and inhibition of apoptosis

**Tumor development**

**ERβ**

Anti-proliferative and pro-apoptotic properties

**Tumor suppression**

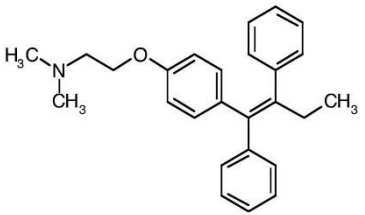


Augusto TV et al. (2018) Endocr Relat Cancer;25(5):R283-R301

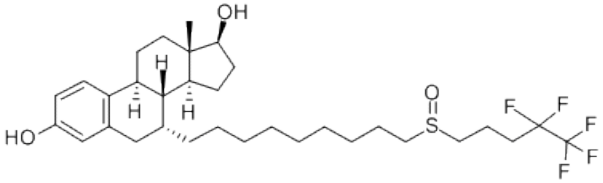
# Introduction

## Endocrine Therapy

**ER Modulators**




Tamoxifen




Fulvestrant

Pre-menopausal women

Pre- and post-menopausal women

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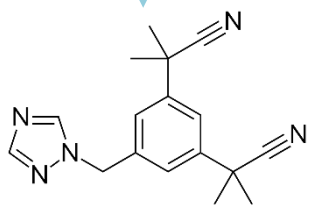
# Introduction

## Endocrine Therapy

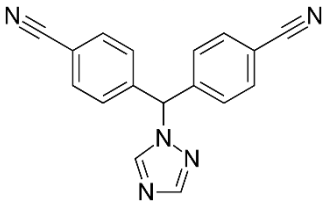
**Aromatase Inhibitors (AIs)**

**Non-Steroidal**

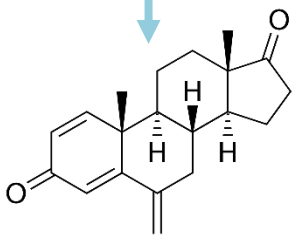
**Steroidal**



Anastrozole



Letrozole



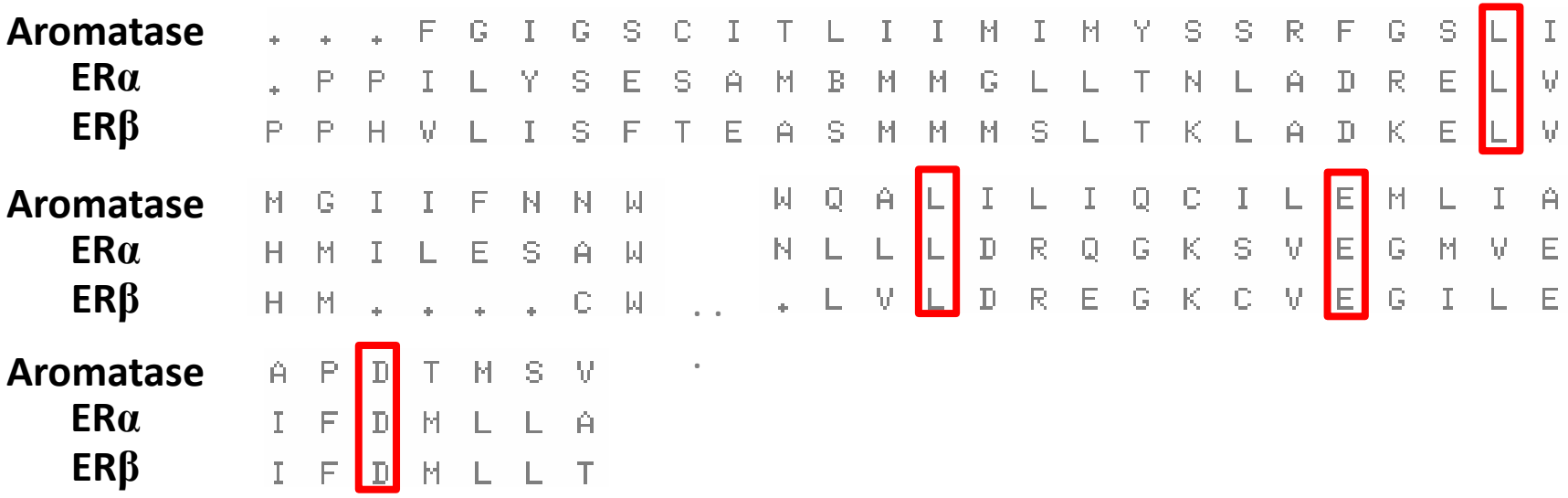
Exemestane

Post-menopausal women and pre-menopausal women after ovaries ablation

Augusto TV et al. (2018) Endocr Relat Cancer;25(5):R283-R301

**And if we find a compound able to inhibit aromatase and simultaneously modulate ERs activity?**

## Sequence Alignment




**Conservation of important residues**

# Are the ligands of these targets similar?

# Aim

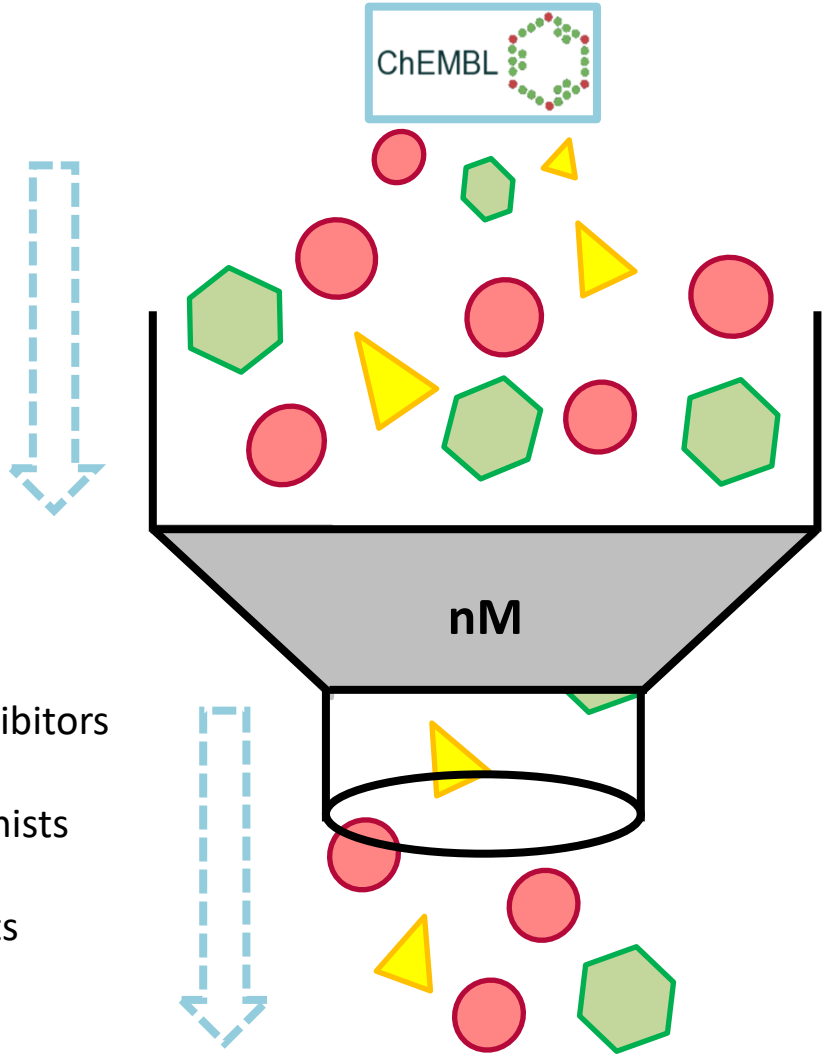
Understand the specificities of each set of compounds that interact with each one of the three targets

Find the common features

**Discover a multi-target compound**

# Results and discussion

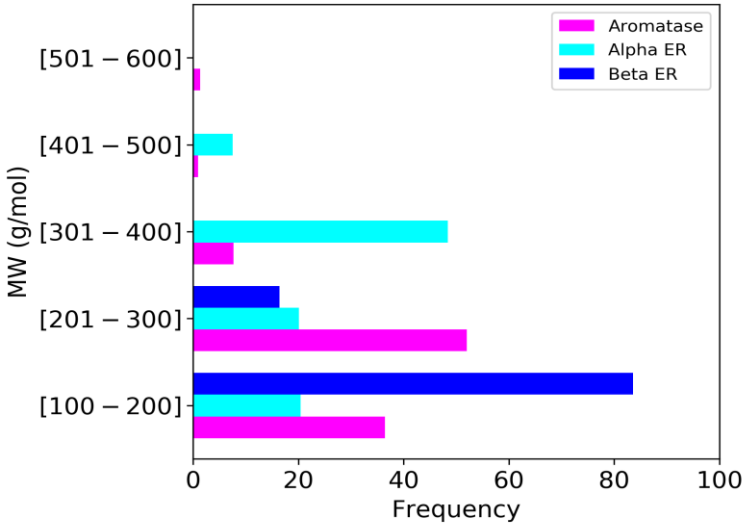
**2619** aromatase inhibitors
+
**3701** ERα antagonists
+
**665** ERβ agonists
=
**6985**

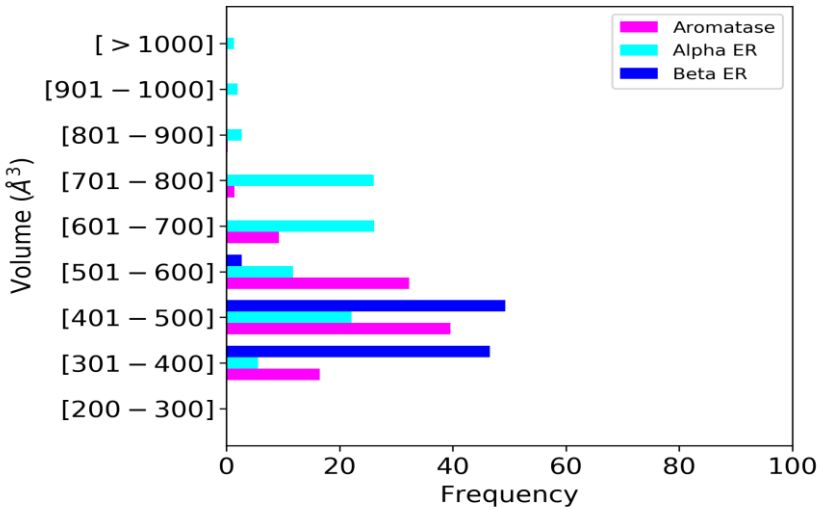



**1210** aromatase inhibitors
+
**1557** ERα antagonists
+
**73** ERβ agonists
=
**2840**

# Results and discussion

## Chemical Descriptors Evaluation – 1D Descriptors



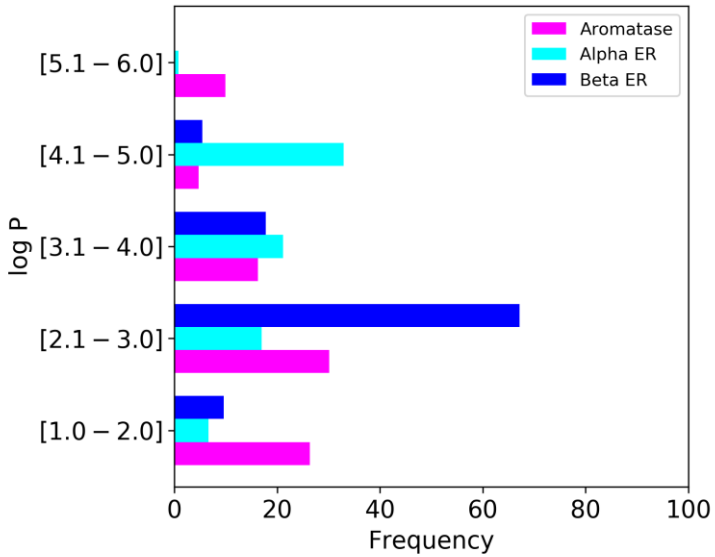



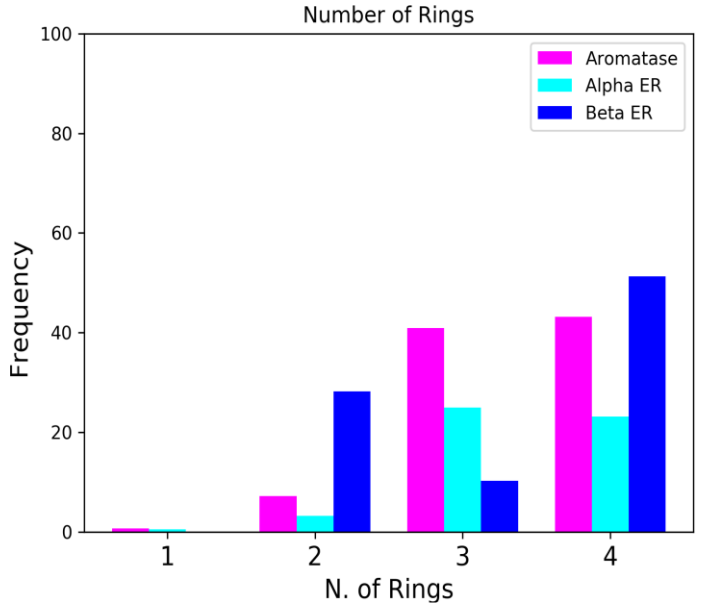

- The majority of the AIs has a molecular weight between 100 g/mol and 300 g/mol
- ERα antagonists have higher molecular weights

- AIs have volumes mainly between 301 Å$^3$ and 600 Å$^3$
- ERα antagonists have higher molecular volumes

# Results and discussion

## Chemical Descriptors Evaluation – 1D Descriptors



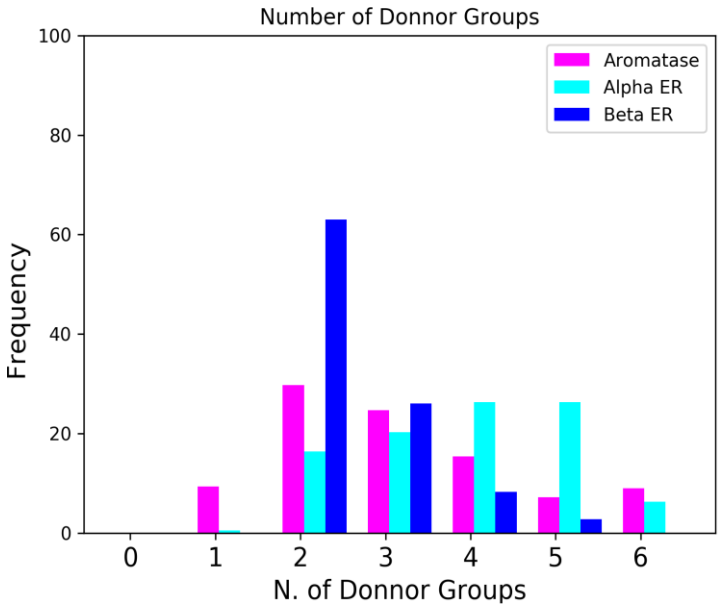



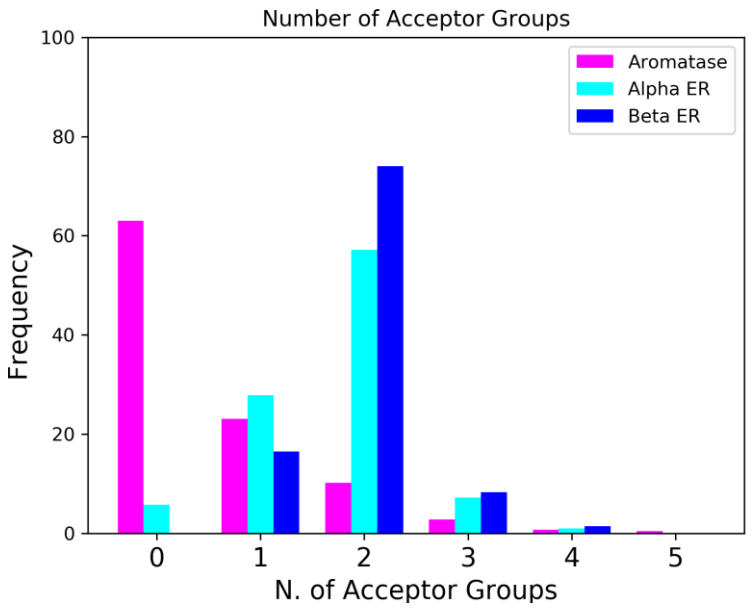

- The majority of AIs have log P values between 1.0 and 3.0
- ERα antagonists presente log P values between 2.1 and 5.0

- The majority of the compounds have 3 or 4 rings

# Results and discussion

## Chemical Descriptors Evaluation – 1D Descriptors




- The majority of the compounds have between 2 and 5 donor groups

- **More than 60% of AIs do not have any acceptor group**
- **ERα antagonists have tipically 2 acceptor groups**

# Results and discussion

## Extended-Connectivity Fingerprints – 2D Descriptors


***Source:*** *ChemAxon*

- Constructed with ChemAxon software
- Represent molecular structures by means of circular atom neighborhoods
- ECFPs are circular topological fingerprints designed for molecular characterization, similarity searching and structure-activity models
- Applied in VS studies

# Results and discussion

**2840 Compounds**

↓

Clusters

↓

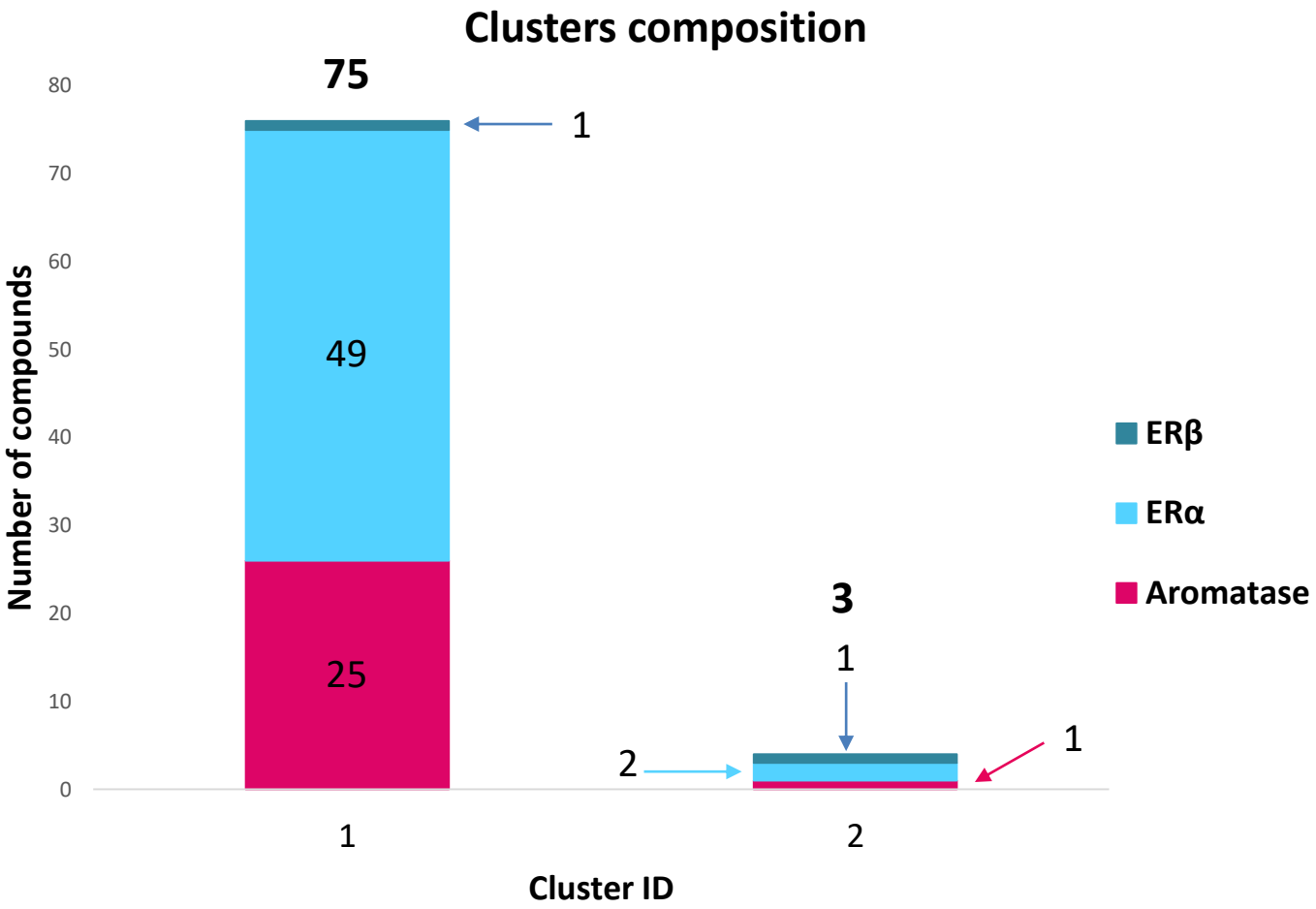
Similarity between 0.6 and 0.9

↓

175 clusters

↓

**2 clusters** containing compounds of the three targets



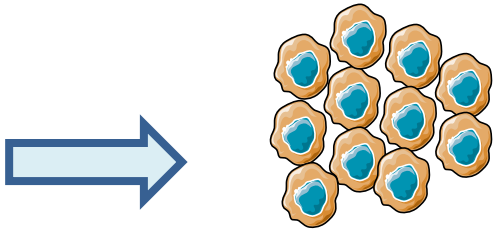
# Results and discussion

- The selected compound was designated as MT1

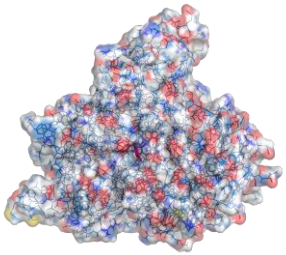
**Aromatase inhibition**

Human placental microsomes

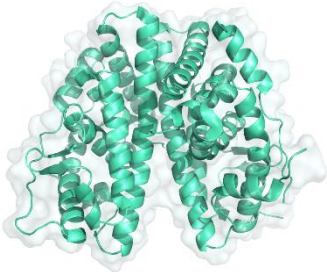
Radiometric assay



MCF-7aro cells



Aromatase



ERα

Western-Blot

qPCR

# Results and discussion

## Anti-aromatase activity of MT1

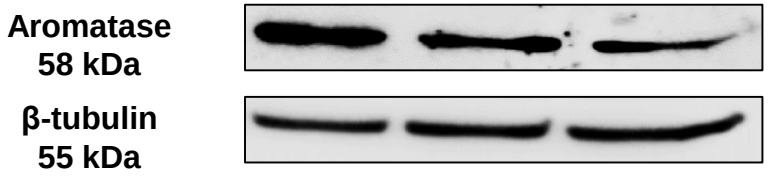
| Compound | Anti-aromatase activity (%) |
|---|---|
| **MT1** (2 µM) | -2.81 ± 3.05 |
| **Exe** (1 µM) | 97.86 ± 0.52 |
| **Ana** (1 µM) | 99.12 ± 0.02 |
| **Let** (1 µM) | 99.69 ± 0.06 |

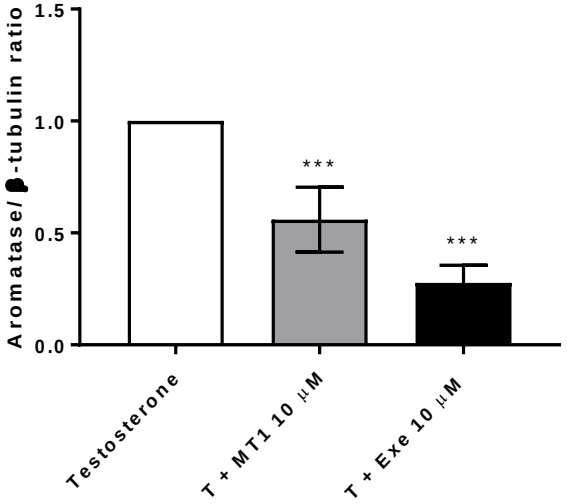
**MT1 is not able to inhibit aromatase**

# Results and discussion

## MT1 effects on aromatase expression levels in MCF-7aro cells

**Western-Blot of aromatase**



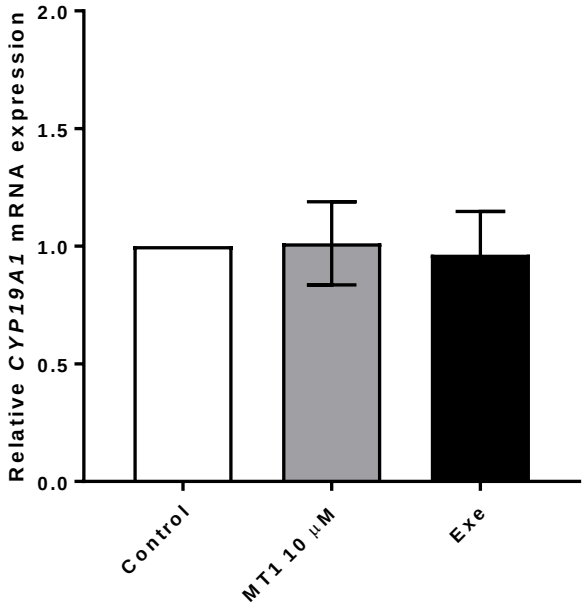

**MT1 induced a decrease of 44% on aromatase expression levels**

**Are the effects induced by MT1 on aromatase expression levels a result of a decreased *CYP19A1* gene expression or a consequence of aromatase degradation?**

# Results and discussion

## MT1 effects on *CYP19A1* transcription levels in MCF-7aro cells

**PCR analysis**



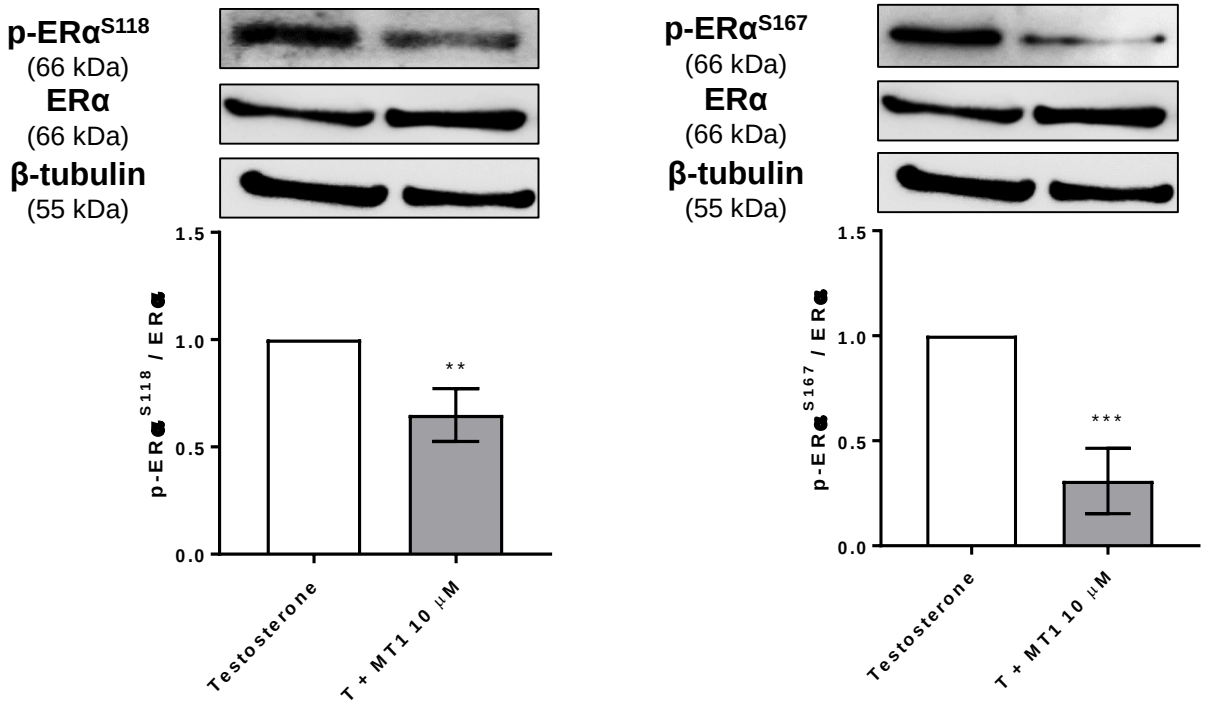

**Housekeeping gene:** *α-tubulin*

**MT1 did not induce any change in *CYP19A1* transcript levels**

# Results and discussion

## MT1 effects on ERα activation in MCF-7aro cells

**Western-Blot of ERα phosphorylation at Ser118 and Ser167**




**MT1 may act as an ERα antagonist**

# Conclusions

❖ Molecular Descriptors analysis:

✓ Aromatase inhibitors and ERα antagonists have similar values of molecular weight, volume, rings and donor groups.

✓ The main difference among all the compounds is the number of acceptor groups

❖ The compound selected, MT1:

✓ Did not inhibit aromatase but induces aromatase degradation

✓ Impairs ERα activation, acting as an ERα antagonist

**MT1 is able to modulate two key targets of $ER^+$ breast cancer, which represents a great advantage in this type of cancer**

## Hit to lead transformation

Alteration of the substituents

**Strong compound**

**Weak compound**

# Acknowledgments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Pedro A. Fernandes | Maria J. Ramos | Ana Oliveira

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SFRH/BPD/98304/2013 attributed to Cristina Amaral

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