

Endoxifen, But Not 4-hydroxytamoxifen, Degrades The Estrogen Receptor In Breast Cancer Cells: A Differential Mechanism Of Action Potentially Explaining CYP2D6 Effect

DISCLOSURES

- Dr. Hawse has no relevant financial relationships with commercial interests to disclose.
- Dr. Wu has no relevant financial relationships with commercial interests to disclose.
- Dr. Subramaniam has no relevant financial relationships with commercial interests to disclose.
- Dr. Goetz has no relevant financial relationships with commercial interests to disclose.
- Dr. Spelsberg has no relevant financial relationships with commercial interests to disclose.
- Dr. Ingle has no relevant financial relationships with commercial interests to disclose.

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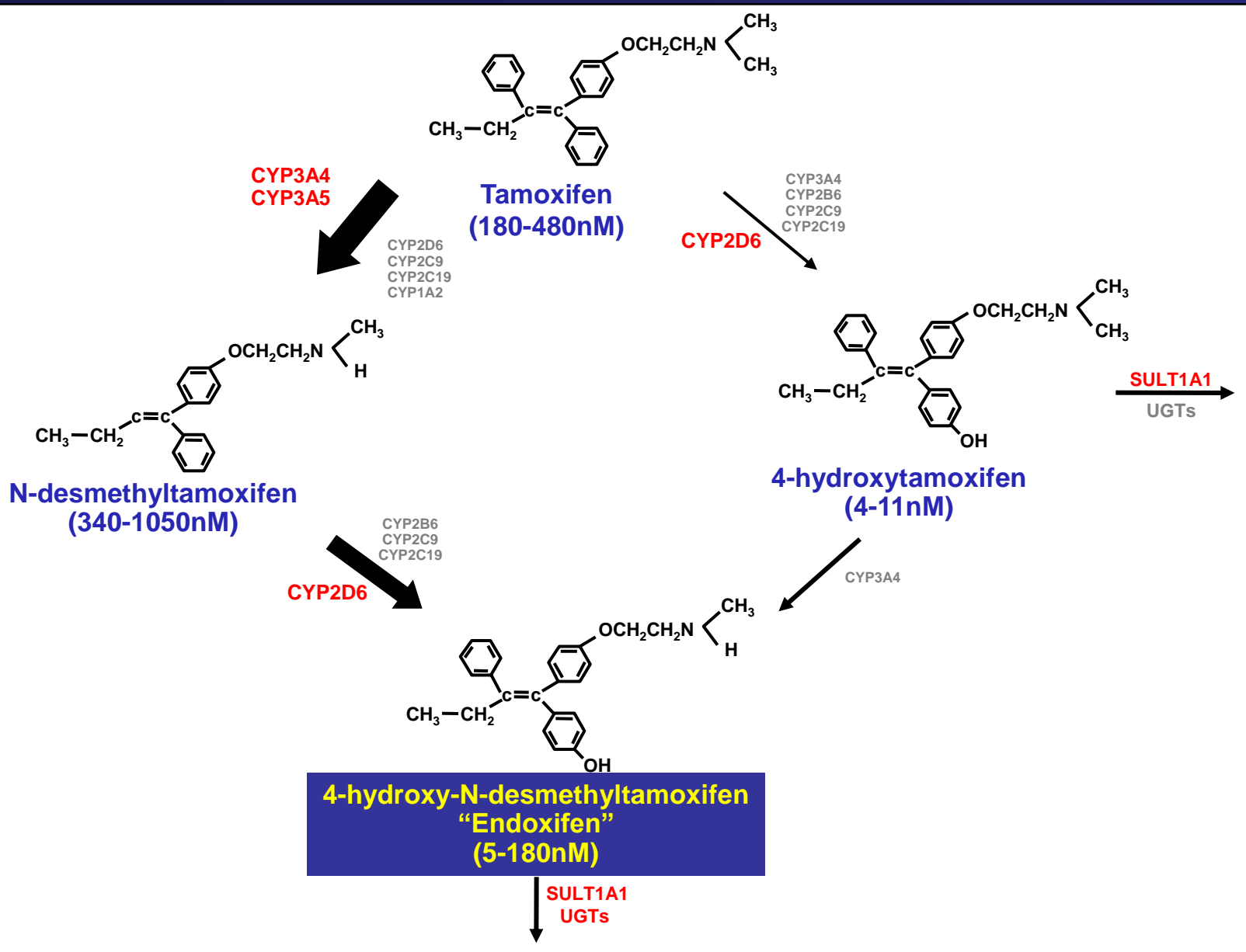
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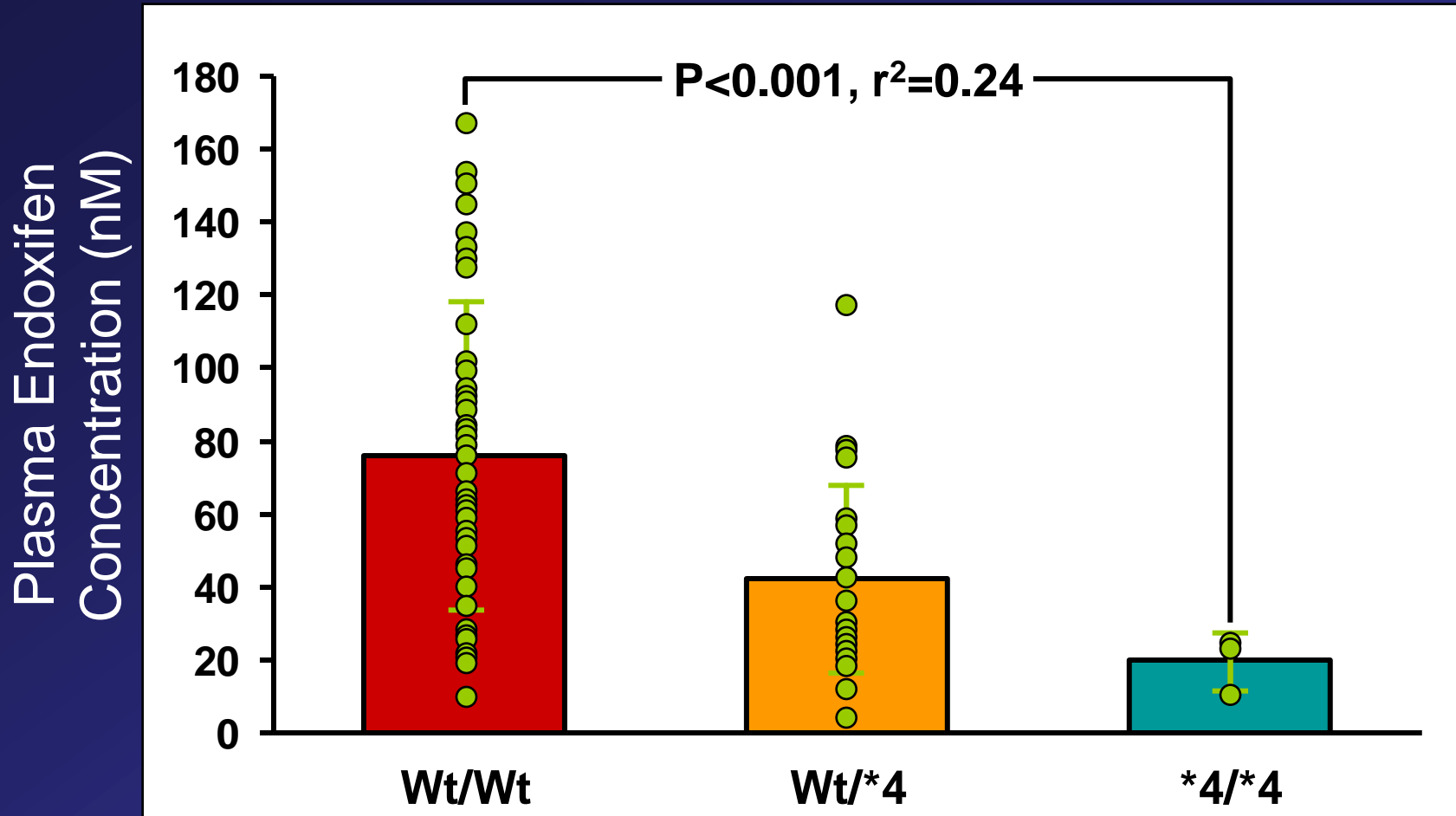
Tamoxifen and Breast Cancer

- Tamoxifen is approved for the prevention and treatment of pre- and post-menopausal ER positive cancer
- Tamoxifen is a pro-drug, and is converted to potent anti-estrogens: 4HT and endoxifen
- Tamoxifen and its metabolites are considered SERMs
- There are few data regarding the mechanism of action of endoxifen

Tamoxifen: Metabolic Pathway

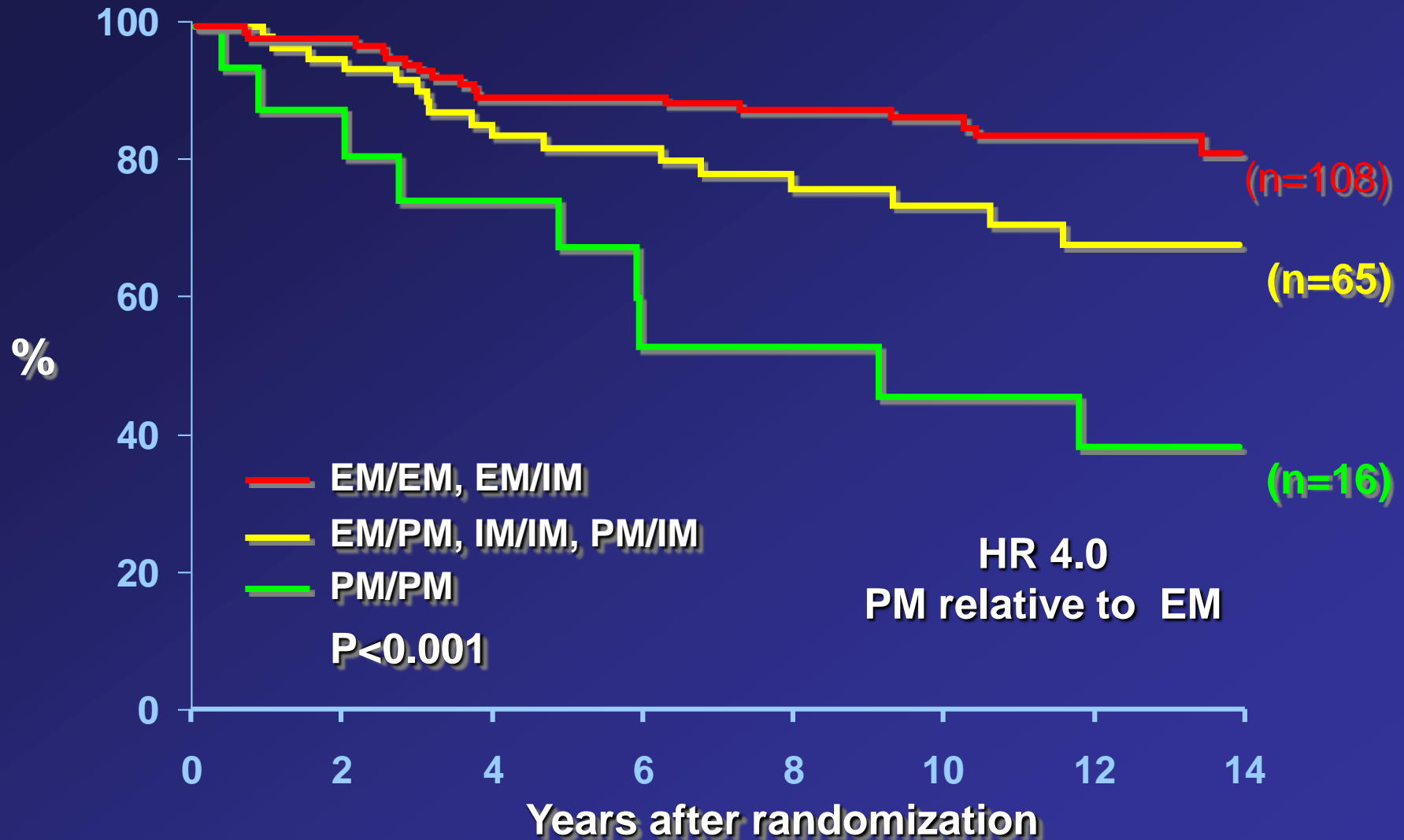


CYP2D6 Genotype and Endoxifen



CYP2D6*4 (most common genetic variant associated with the CYP2D6 poor metabolizer state)

Relapse-free time According to CYP2D6 Metabolizer Status



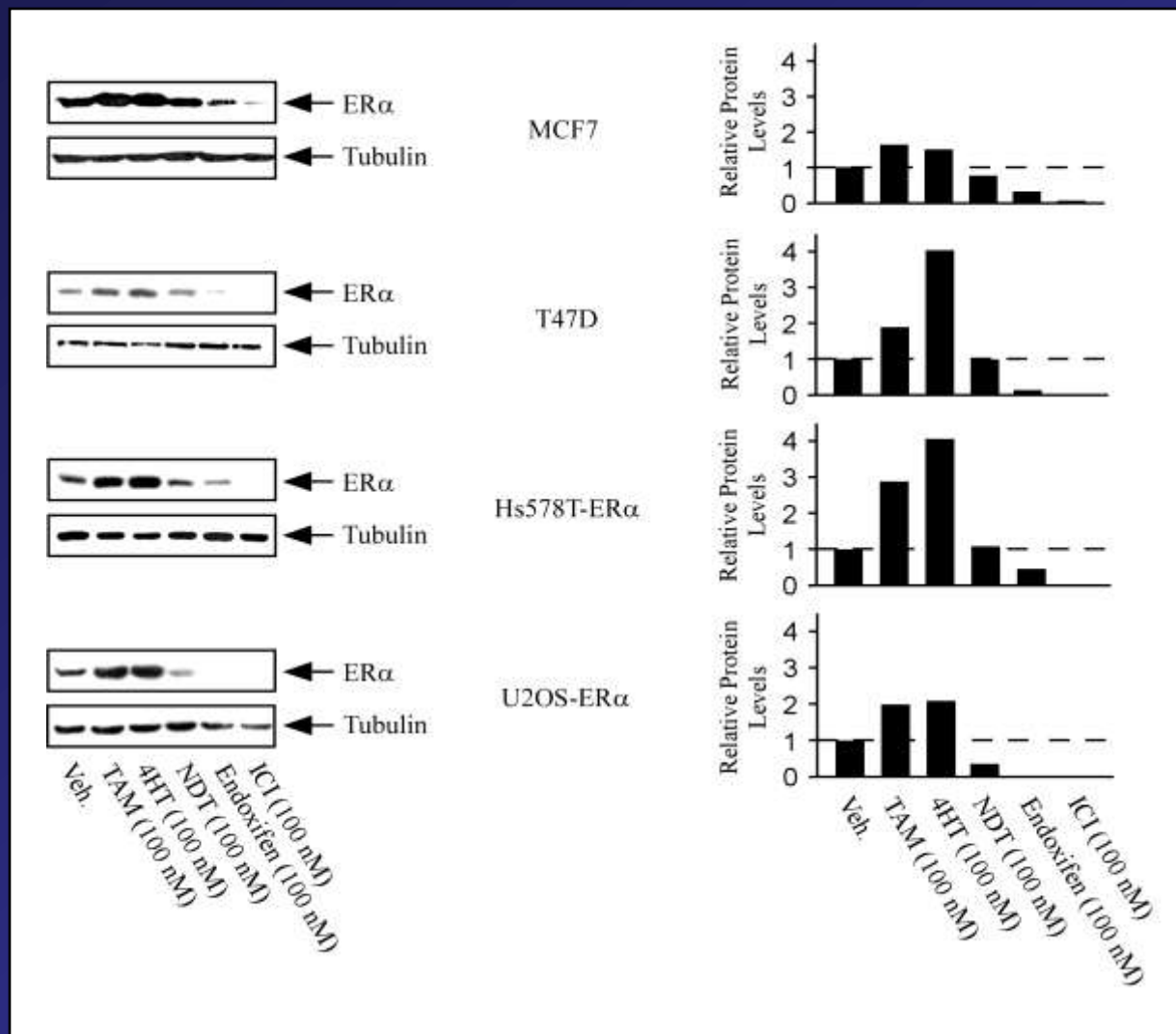
Questions Regarding Endoxifen

- Is there a potential mechanistic explanation for the clinical observations that suggest endoxifen levels relate to anti-breast cancer activity?
- Do variations in endoxifen concentration matter in the setting of clinically relevant concentrations of TAM, 4HT and NDT?
- Is the mechanism of action of endoxifen different from 4HT and other anti-estrogen drugs such as fulvestrant (ICI)?

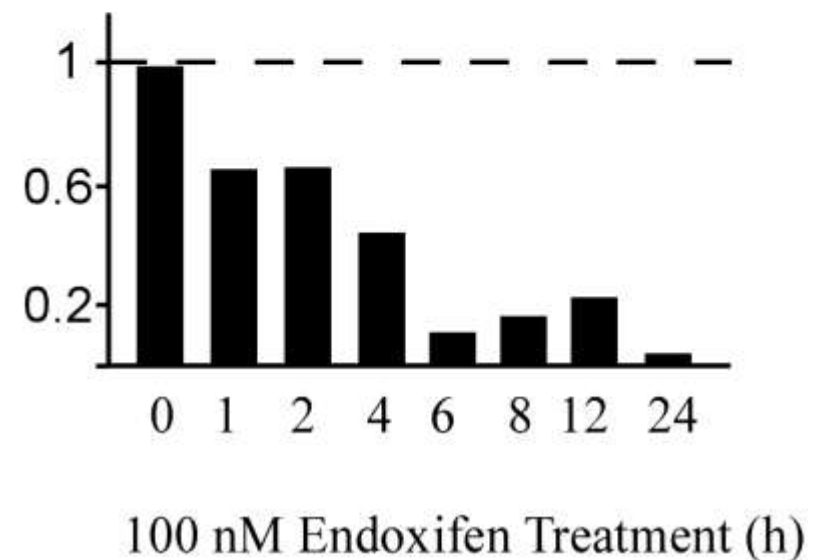
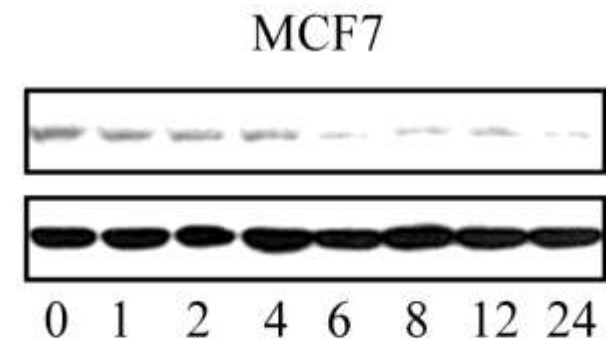
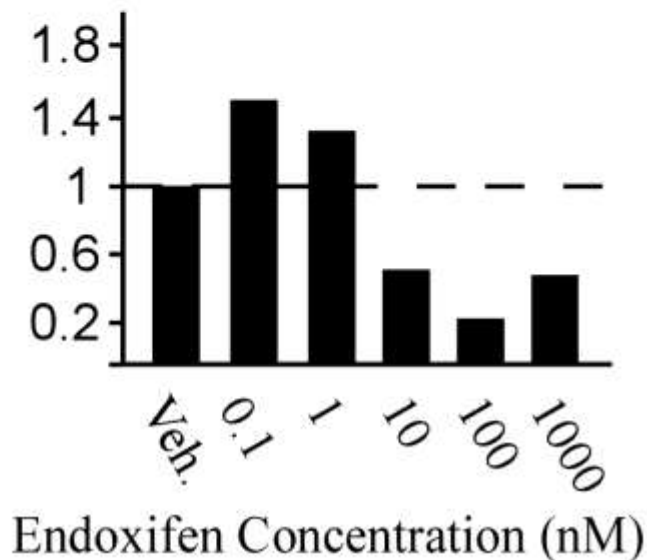
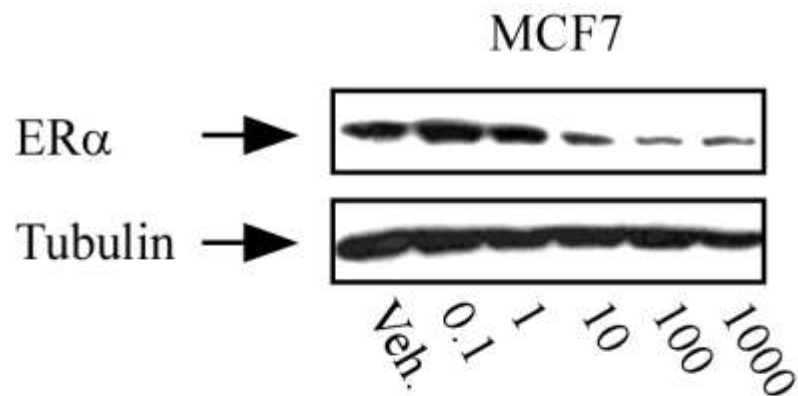
Methods

- Determine if endoxifen causes accumulation or degradation of the estrogen receptor by western blotting
- Analyze the ability of endoxifen to block estrogen receptor transcriptional activity using luciferase assays and RT-PCR
- Verify if endoxifen concentration is an important factor in the suppression of breast cancer cell proliferation using a cell viability assay
- Identify the gene expression profiles of breast cancer cells treated with endoxifen and compare these to 4HT and ICI using Illumina microarrays containing over 25,000 genes

Endoxifen Treatment Results in ER α Protein Degradation



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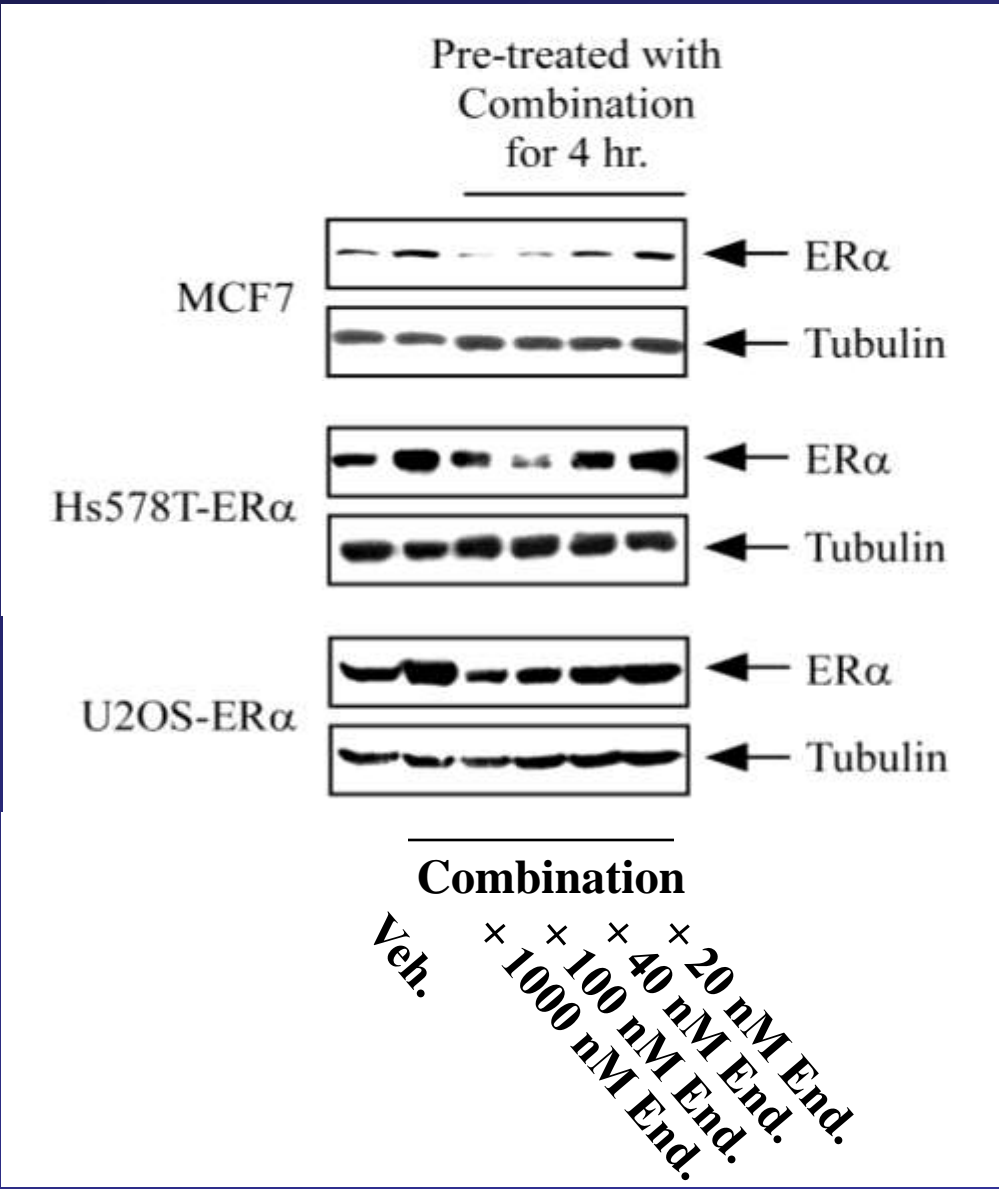
Tamoxifen Metabolite Concentrations in Patients

Metabolite Concentrations

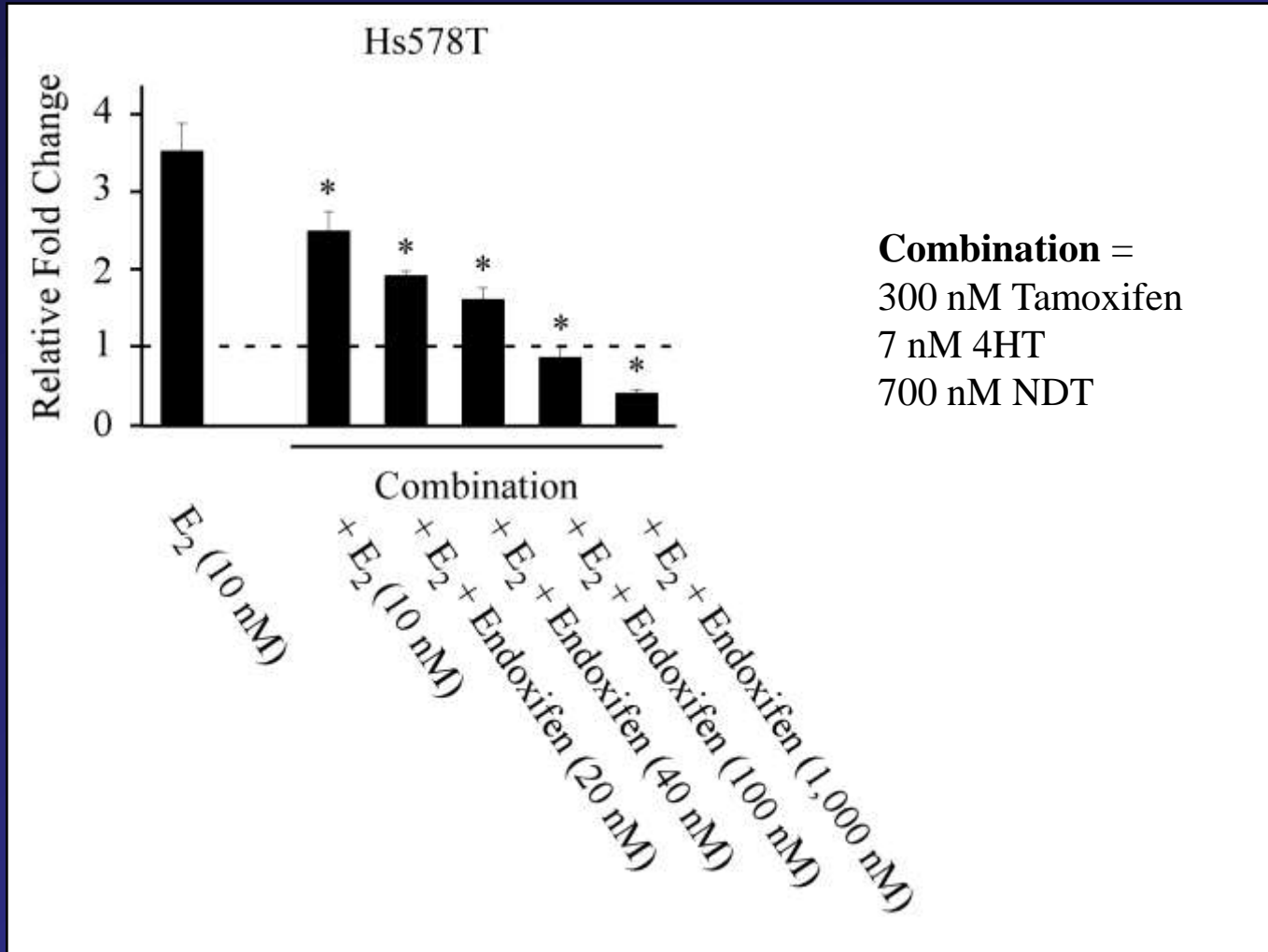
Metabolizer Status TAM NDT 4HT Endoxifen

Ultra-rapid	~ 300 nM	~ 700 nM	~ 7 nM	>90 nM
Extensive	~ 300 nM	~ 700 nM	~ 7 nM	~ 90 nM
Intermediate	~ 300 nM	~ 700 nM	~ 7 nM	~ 40-60 nM
Poor	~ 300 nM	~ 700 nM	~ 7 nM	< 30 nM

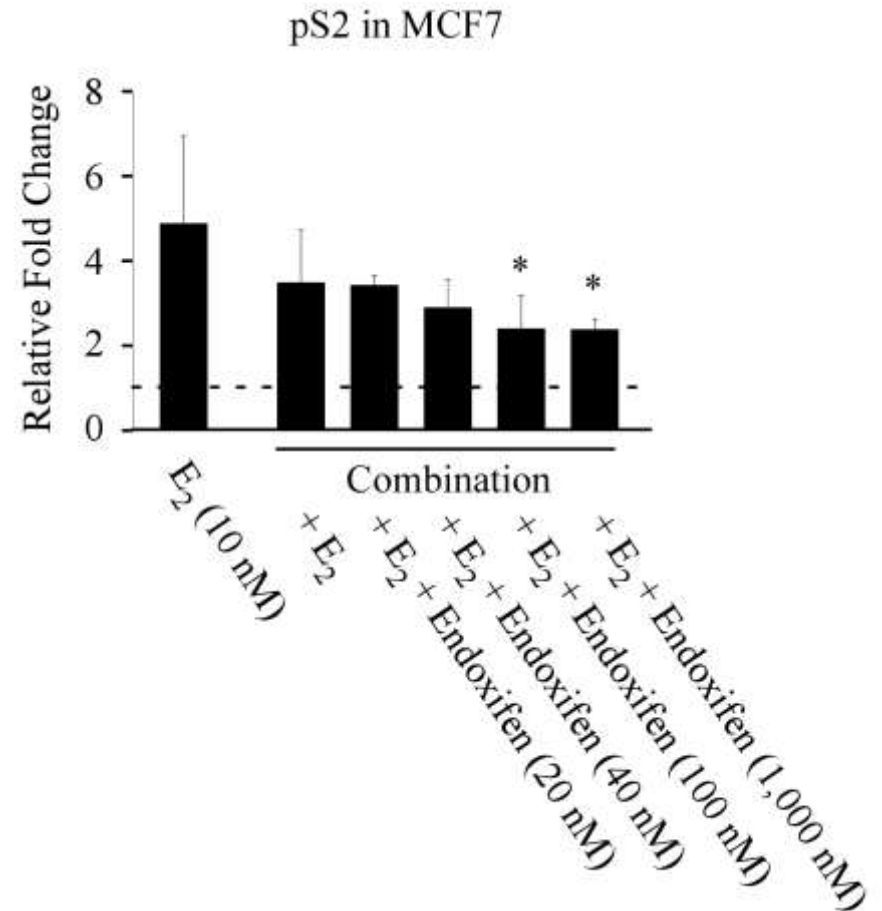
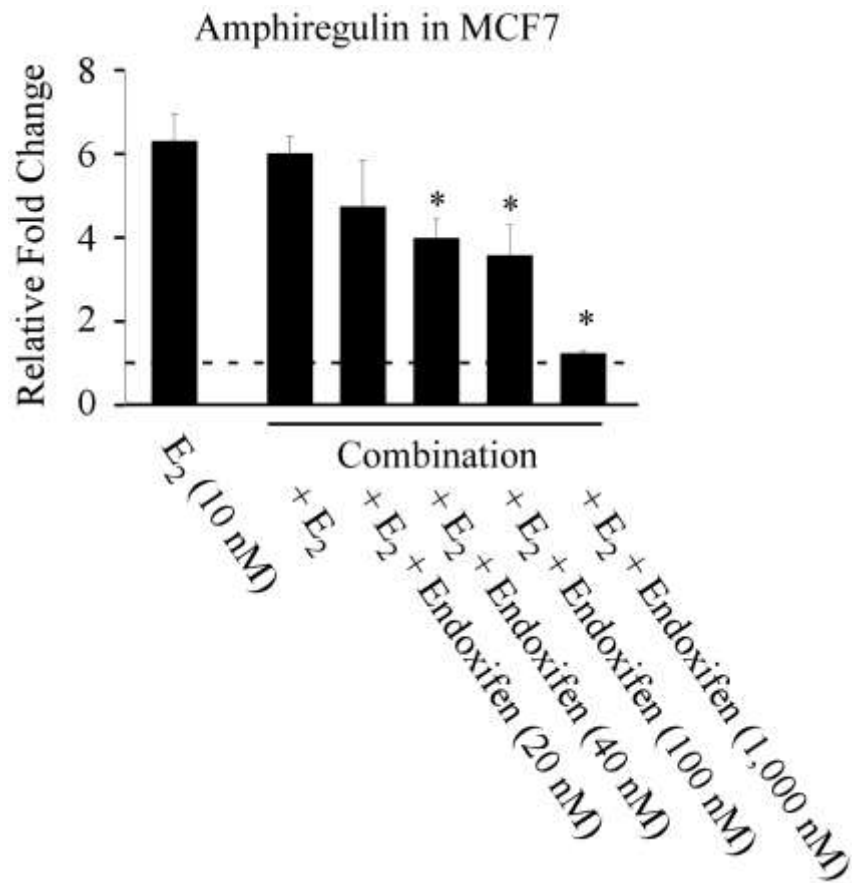
ER α Protein Levels are Reduced by Endoxifen Under Clinically Relevant Conditions



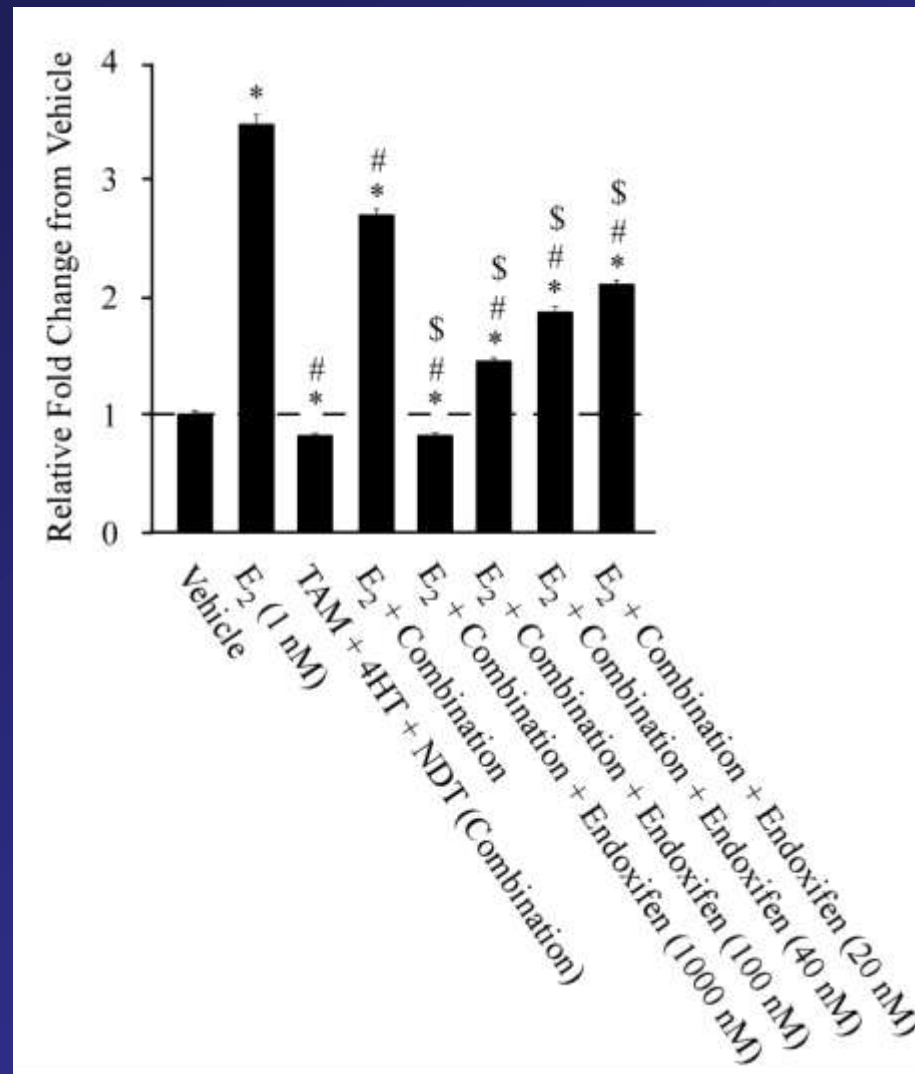
ER α Transcriptional Activity is Blocked by High Concentrations of Endoxifen Under Clinically Relevant Conditions



Endogenous Gene Expression is Blocked by High Concentrations of Endoxifen Under Clinically Relevant Conditions



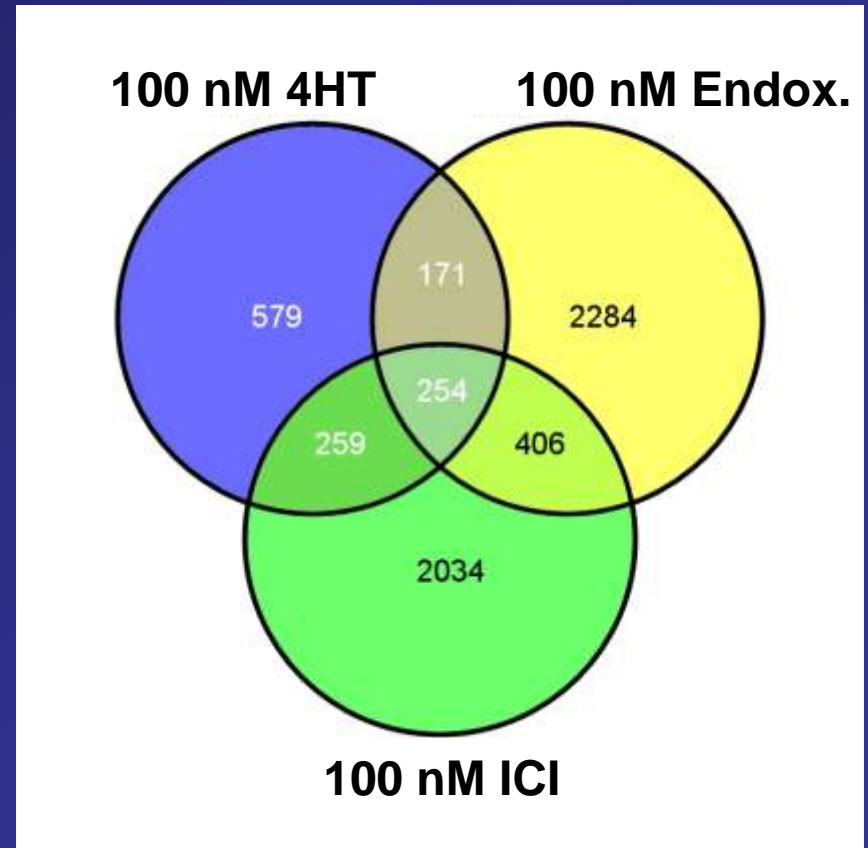
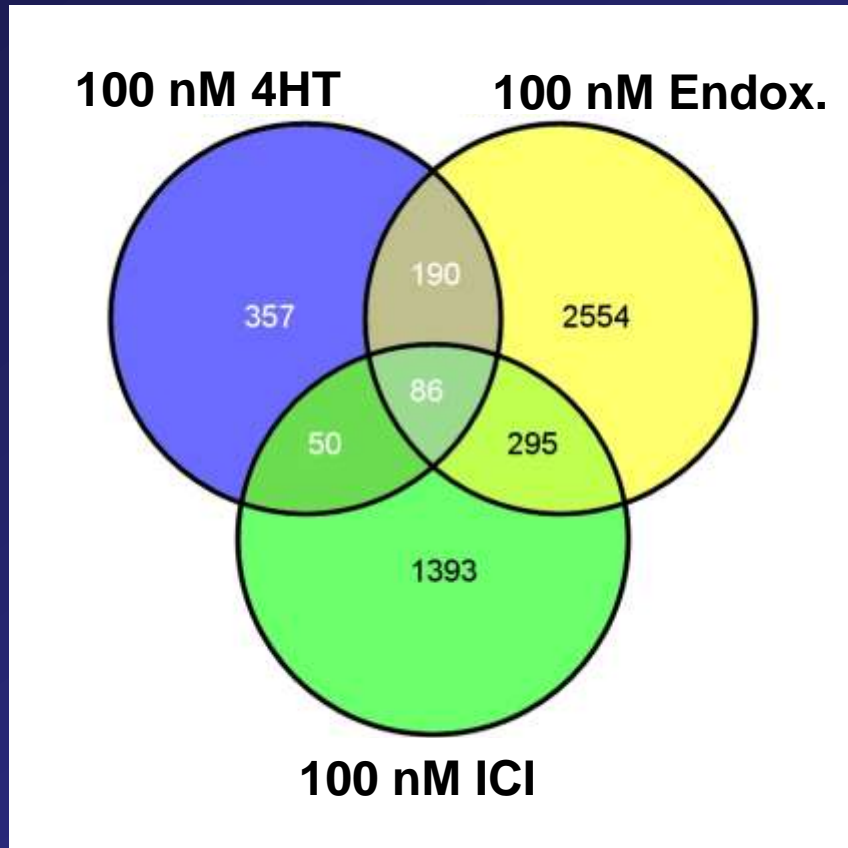
High Concentrations of Endoxifen Suppress Cell Proliferation Under Clinically Relevant Conditions



Venn Diagrams of MCF7 Cell Gene Expression Following Indicated Treatments

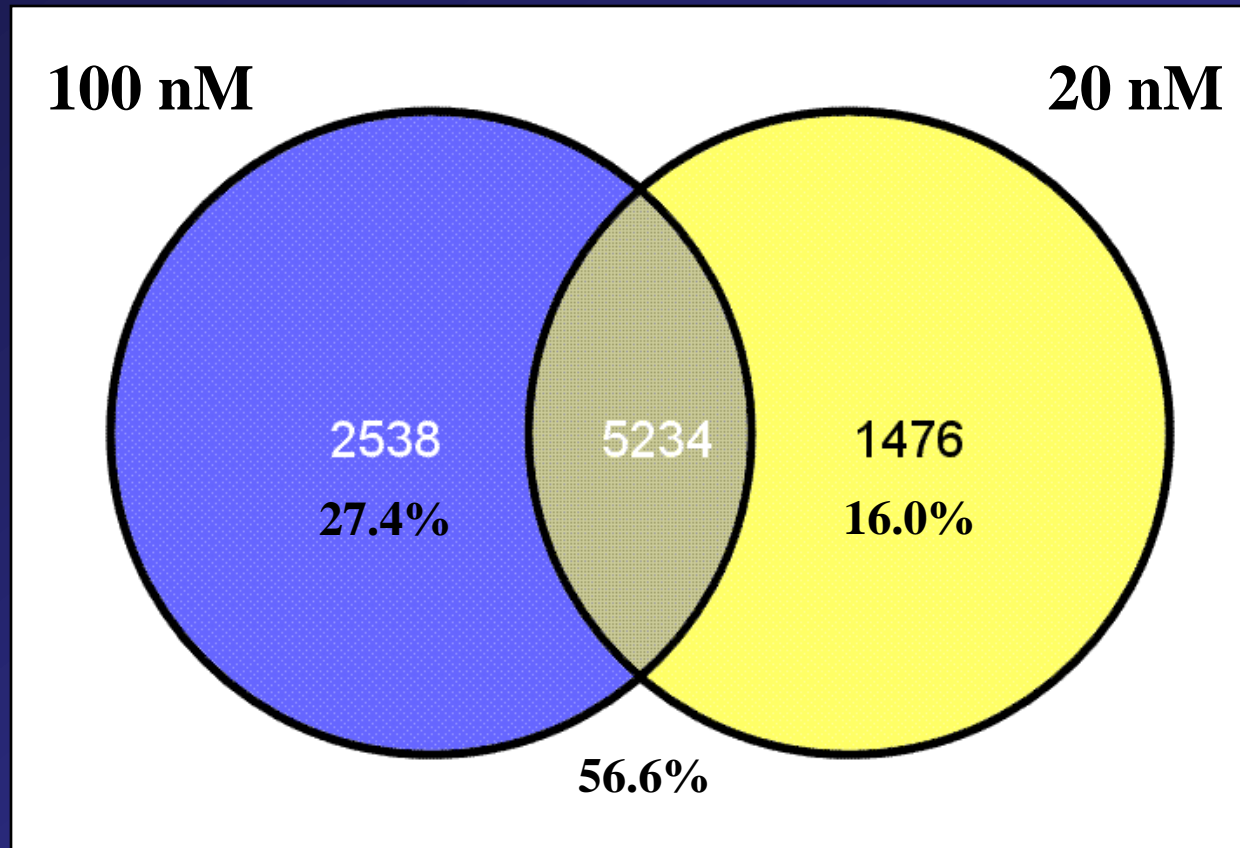
90 Minutes

24 Hours



Gene Expression Profiles of MCF7 Cells Under Conditions Mimicking an Extensive and Poor Metabolizer

Clinically relevant concentrations of estrogen, TAM, 4HT and NDT, plus indicated concentrations of endoxifen



10 nM Estrogen
300 nM Tamoxifen
7 nM 4HT
700 nM NDT

Conclusions

- In contrast to TAM and 4HT, endoxifen is a potent anti-estrogen that targets ER α for proteasomal degradation
- Endoxifen degrades ER α , blocks ER α transcriptional activity, and inhibits E2-induced breast cancer cell proliferation even at clinically relevant concentrations of TAM, NDT and 4HT
- Tamoxifen's optimal drug effect in breast cancer cells may be related to the ability of endoxifen to degrade rather than stabilize ER α
- Endoxifen regulates unique patterns of genes relative to 4HT and ICI

Conclusions (2)

- Concentrations of endoxifen mimicking high metabolizers (100nM) regulate markedly different patterns of genes compared to that of low metabolizers (20nM)
- Our data support the clinical findings regarding the importance of genetic and drug-induced variation that alters CYP2D6 enzyme activity
- These data support that endoxifen is the key metabolite responsible for the effectiveness of tamoxifen therapy

Acknowledgements

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