The Pharmacological Effects of Estrogen Receptor alpha Y537S and D538G Mutations

Steven J. Hartman[1], Tracy Kleinheinz[1], Jonathan White[2], Stephen Daly[2], Ria Goodwin[2], Wei Zhou[1], Jun Liang[1], Gina Wang[1], Lori Friedman[1], Martin O'Rourke[2], Ciara Metcalfe[1], Robert A. Blake[1]. (1 - Genentech, South San Francisco, CA 94080; 2- Charles River Laboratories).

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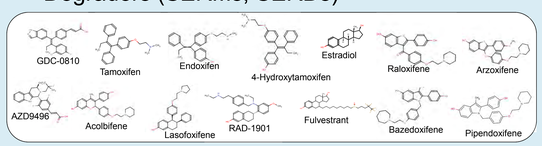
INTRODUCTION

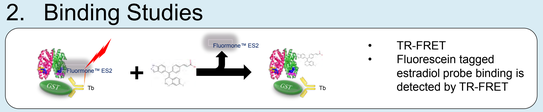
The frontline therapy for estrogen receptor alpha (ERα) positive Breast Cancer (ER$^+$BC) involves various forms of endocrine therapy, consisting of either Selective Estrogen Receptor Modulators (SERMs) or aromatase inhibitors. An emerging mechanism of ER$^+$BC resistance to endocrine therapy, and consequently disease relapse, has been associated with a set of "hotspot" mutations in and near to helix-12 of the ERα ligand binding domain. Selective Estrogen Receptor Degraders/Down-regulators (SERDs), such as GDC-0810 and AZD9496 [1,2], represent an important pharmacological strategy being applied to develop treatments for resistant ER$^+$BC. Here, we compare 2 of the most frequent ERα hotspot mutations (Y537S and D538G), with ERα wildtype (WT) and the ability of a set of SERM/SERDs and other ERα ligands to bind, antagonize, degrade/stabilize ERα and affect cell proliferation.

Brief methods section

1. Selective Estrogen Receptor Modulators and Degraders (SERMs, SERDs)

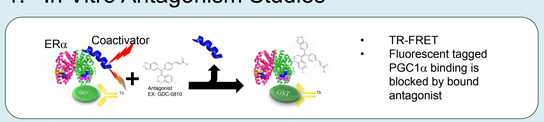


2. Binding Studies



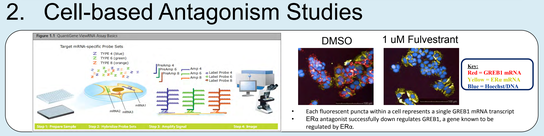
- TR-FRET
- Fluorescein tagged estradiol probe binding is detected by TR-FRET

1. In Vitro Antagonism Studies



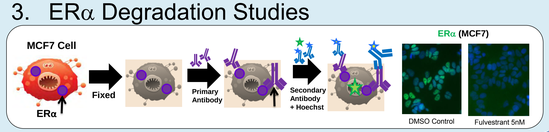
- TR-FRET
- Fluorescent tagged PGC1α binding is blocked by bound antagonist

2. Cell-based Antagonism Studies



- Each fluorescent puncta within a cell represents a single GREB1 mRNA transcript
- ERα antagonist successfully down regulates GREB1, a gene known to be regulated by ERα.

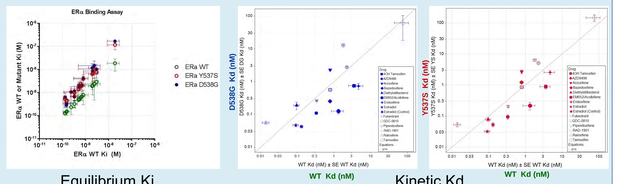
3. ERα Degradation Studies



RESULTS

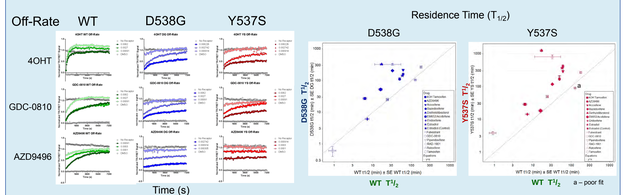
1. ERα Binding

Equilibrium and Kinetic Binding Studies



Equilibrium Ki | Kinetic Kd

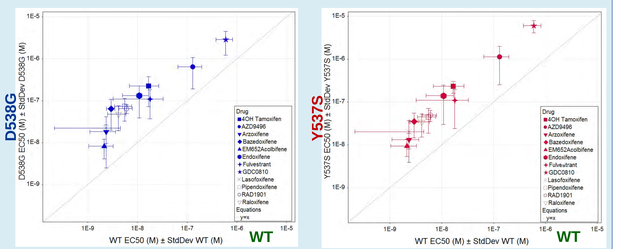
ERα Y537S and D538G Mutations Increase Drug Residence Time



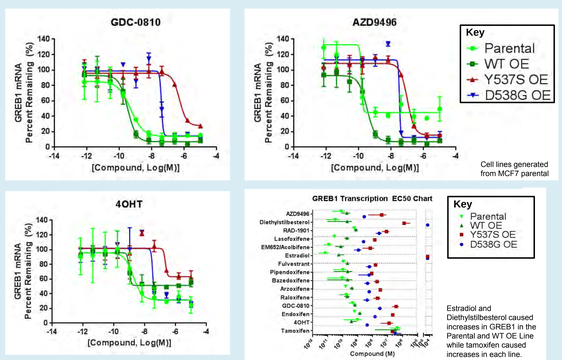
2.ERα Antagonism

a) *In Vitro* Analysis of Antagonism

ERα D538G and Y537S Mutants Require Higher Concentrations of SERM/Ds To Antagonize Co-Activator Peptide Binding. (Similar results have been reported for 4OHT and E2 [3]).



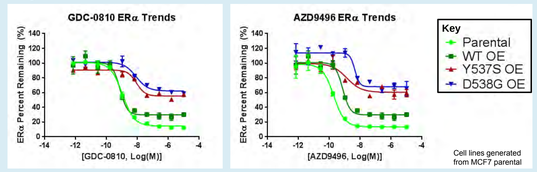
b) Cell-Based Analysis of Antagonism



- GDC-0810 and AZD9496 have similar antagonism profiles.
- In most cases SERM/D antagonism EC_{50}: WT < D538G < Y537S.

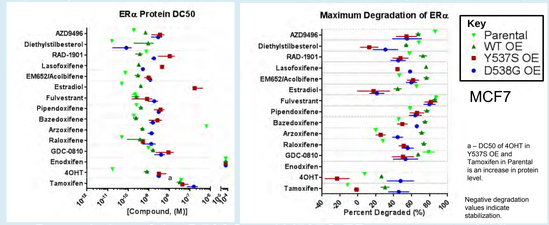
3. ERα Degradation

a) GDC-0810 and AZD9496



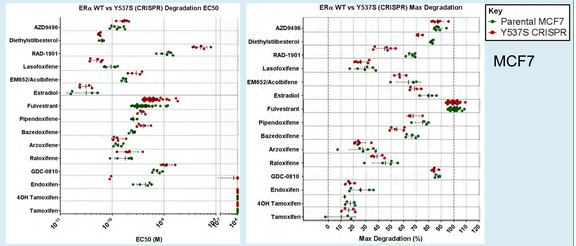
- Both GDC-0810 and AZD9496 are strong degraders of endogenous ERα WT and over-expressed ERα WT, but are weak degraders of the over-expressed Y537S and D538G ERα mutants.

b) Degradation of ERα mutants (Over-Expressed; OE)



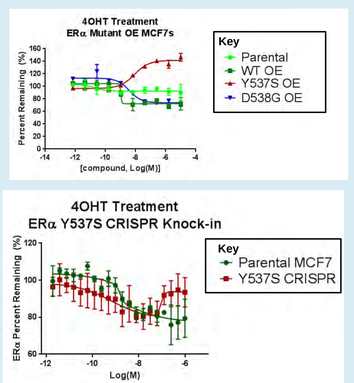
- Degradation of the D538G and Y537S ERα mutants typically requires higher drug concentrations than WT ERα.
- The shift in EC_{50} between WT and mutants degradation is typically less than the shift in antagonism EC_{50}.

c) Degradation of ERα Y537S (CRISPR Knock-In)



- The most significant relative shift in degradation EC_{50} between ERα WT and Y537S was seen for RAD-1901.

d) 4OHT Stabilization of ERα Y537S

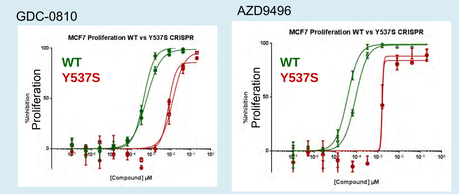


Section 3C:
- ERα Y537S represents 10-40% of total ERα in the Y537S knock-in cell line
- Degradation EC_{50} values are higher and maximum degradation is lower in the ERα Y537S MCF7 relative to MCF7 ERα WT

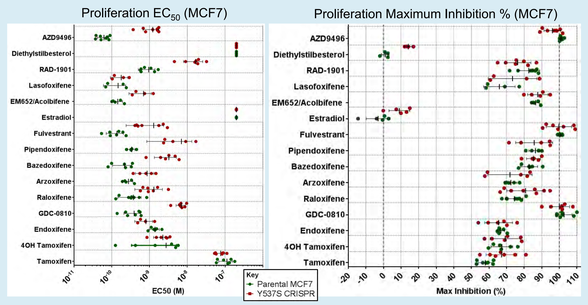
Section 3D:
- 4OHT stabilizes Y537S in the OE line, while weakly degrading the other forms of ERα.
- 4OHT has a biphasic effect on ERα levels in the Y537S CRISPR line, with an increase at higher concentrations. This is likely due to a mix of stabilization of Y537S and weak degradation of WT ERα.

4. *In Vitro* Proliferation Effects

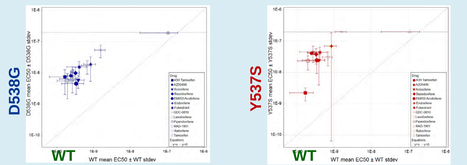
a) MCF7 ERα Y537S knock-in vs Wildtype MCF7



The proliferation EC_{50} values of GDC-0810 and AZD9496 are higher in MCF7 cells expressing ERα Y537S relative to ERα WT only.



b) *In Vitro* Proliferation Effects of ERα Y537S and D538G Mutations vs ERα Wildtype (Over-Expressed in MCF7 cells)



The majority of SERM/Ds required higher concentrations to inhibit proliferation in MCF7 cells over-expressing ERα Y537S (or CRISPR knock-in) and D538G relative to WT.

CONCLUSION:

- **Y537S and D538G mutations of ERα :**
  - **Increase the residence time of most SERM/Ds, relative to WT**
  - **Require higher concentrations to antagonize and degrade ERα**
  - **Require higher concentrations to inhibit ERα dependent proliferation**
- **4OHT stabilizes ERα Y537S through an unknown mechanism**
- **GDC-0810 and AZD9496 have similar *in vitro* profiles, achieve similar maximum degradation, antagonism and inhibition of proliferation.**

ACKNOWLEDGEMENTS

ERα kinetic binding studies: Art Wittwer, Jim Gierse (Confluence Discovery Technologies)

REFERENCES

1) Joseph, J.D. *et al.* The selective estrogen receptor downregulator GDC-0810 is efficacious in diverse models of ER+ breast cancer. *eLife* **5** (2016).
2) 2) De Savi, C. *et al.* Optimization of a novel binding motif to (E)-3-(3,5-difluoro-4-((1R,3R)-2-(2-fluoro-2-methylpropyl)-3-methyl-2,3,4,9-tetra hydro-1H-pyrido[3,4-b]indol-1-yl)phenyl)acrylic acid (AZD9496), a potent and orally bioavailable selective estrogen receptor downregulator and antagonist. *Journal of medicinal chemistry* (2015).
3) Fanning, S.W. *et al.* Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation. *eLife* **5** (2016).