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# Identification of Oral Estrogen Receptor PROTAC Degraders for Breast Cancer

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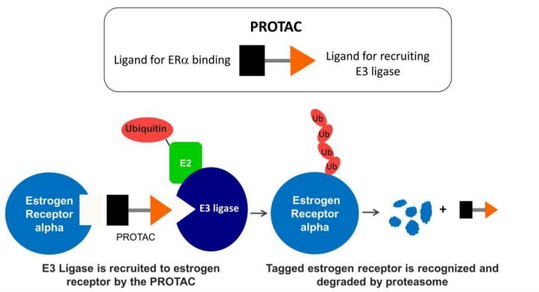
ARVINAS

## Abstract

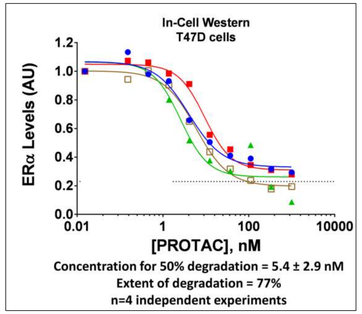
ER-positive breast cancers comprise approximately 70-80% of all newly diagnosed cases. Downregulation or degradation of ER is a treatment approach currently used in the clinic to target estrogen receptor signaling. Faslodex, the only clinically-approved ER-downregulator, is administered as a monthly intramuscular injection with limiting pharmaceutical properties. Reasoning that an orally-available estrogen receptor degrader would be beneficial to patients, we have leveraged our experience in targeted protein degradation to generate and characterize novel proteolysis targeting chimeras (PROTACs) against estrogen receptor alpha. PROTACs are heterobifunctional molecules that facilitate the formation of a ternary complex comprised of the PROTAC, a pathogenic target protein of interest and an E3 ligase, which catalyzes the ubiquitylation and subsequent degradation of the target protein via the proteasome.

To identify novel ER degraders (ER PROTACs), we have used several in vitro assays to characterize the extent of target engagement and receptor degradation. Potent ER PROTACs with excellent oral exposure in multiple pre-clinical species were further evaluated in a breast cancer xenograft model. Orally-administered ER PROTACs achieved >80% degradation of estrogen receptor alpha and demonstrated single agent tumor growth inhibition in this disease model. Further, ER PROTAC combination with a CDK4/6 inhibitor resulted in robust anti-tumor activity.

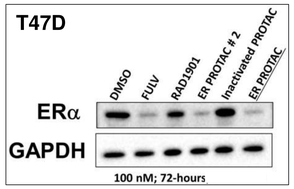
PROTAC: PROteolysis TArgeting Chimera

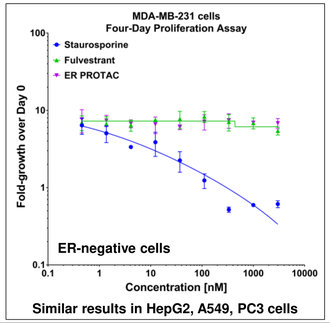



## In vitro characterization of ERα PROTAC

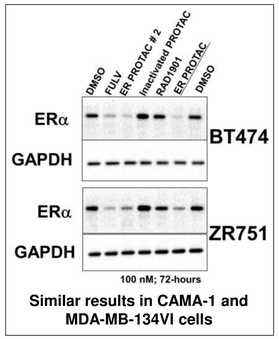



ER PROTACs degrade ERα across multiple ER+ cell lines




Similar results in HepG2, A549, PC3 cells




Similar results in CAMA-1 and MDA-MB-134VI cells

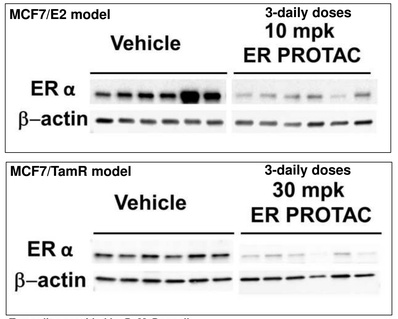
## PK summary of ERα PROTAC

| Species | % F | AUC/Dose (μM•hr)/(mg/kg) |
|---|---|---|
| Mouse | 42 | 0.8 ± 0.3 |
| Dog | 57 | 4.0 ± 1.3 |
| Rat | 26 | 0.6 ± 0.3 |

PO, 3 mpk

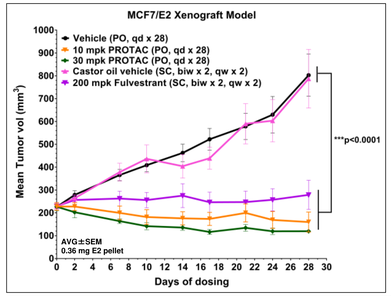
## In vivo ERα degradation

ER PROTAC demonstrates significant ERα degradation in MCF7/E2 and MCF7/TamR xenograft models




Tumor line provided by D. McDonnell

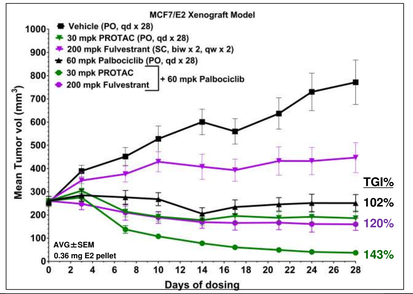
## In vivo efficacy with ERα PROTAC




ER PROTAC demonstrates robust tumor growth inhibition and better ERα degradation than fulvestrant in MCF7/E2 model

| Arm | % TGI | % ERα decrease |
|---|---|---|
| 10 mpk PROTAC | 112 | 86 |
| 30 mpk PROTAC | 120 | 82 |
| 200 mpk Fulvestrant | 91 | 58 |

## ERα PROTAC plus CDK4/6i

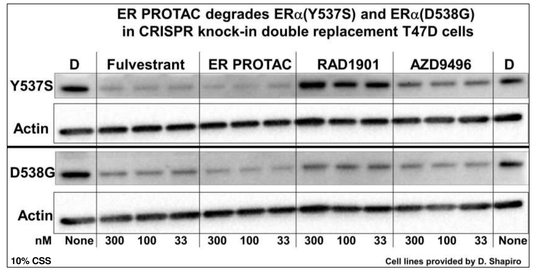



Combination of ER PROTAC and CDK4/6 inhibitor palbociclib provides superior tumor growth inhibition than fulvestrant plus palbociclib

| Comparison | Adj p-value |
|---|---|
| ER PROTAC/palbociclib vs palbociclib | <0.0001 |
| ER PROTAC/palbociclib vs fulvestrant/palbociclib | 0.0052 |
| Fulvestrant/palbociclib vs palbociclib | 0.0499 |

One-way ANOVA; Tukey's Multiple Comparisons Test

## ERα PROTAC activity against ERα mutants




$GI_{50}$ (nM) values against T47D cell lines

| Compound | Y537S | D538G |
|---|---|---|
| ER PROTAC | 33 ± 11 | 8 ± 4 |
| Fulvestrant | 8 ± 4 | 4 ± 1 |
| RAD1901 | 29 ± 8 | 13 ± 1 |
| AZD9496 | 36 ± 1 | 7 ± 6 |

n =2 independent biological experiments; 10-point dose-response curves; three replicates per data point

## Summary

- Orally-bioavailable ER PROTACs demonstrate nanomolar ERα degradation potency and growth inhibition in a variety of wild-type ERα-expressing cell lines
- ER PROTACs degrade and inhibit growth of cells expressing clinically-relevant ERα variants, suggesting that ER PROTACs will be active in that resistance setting
- Oral administration of ER PROTAC provided more robust tumor growth inhibition and ERα degradation compared to fulvestrant in an orthotopic MCF7/E2 xenograft model
- Combination of ER PROTAC and CDK4/6 inhibitor demonstrated superior tumor growth inhibition when compared to fulvestrant and CDK4/6 inhibitor combination
- Data supports the clinical development of ER PROTACs for advanced breast cancer