

Identification of Oral Estrogen Receptor PROTAC Degraders for Breast Cancer

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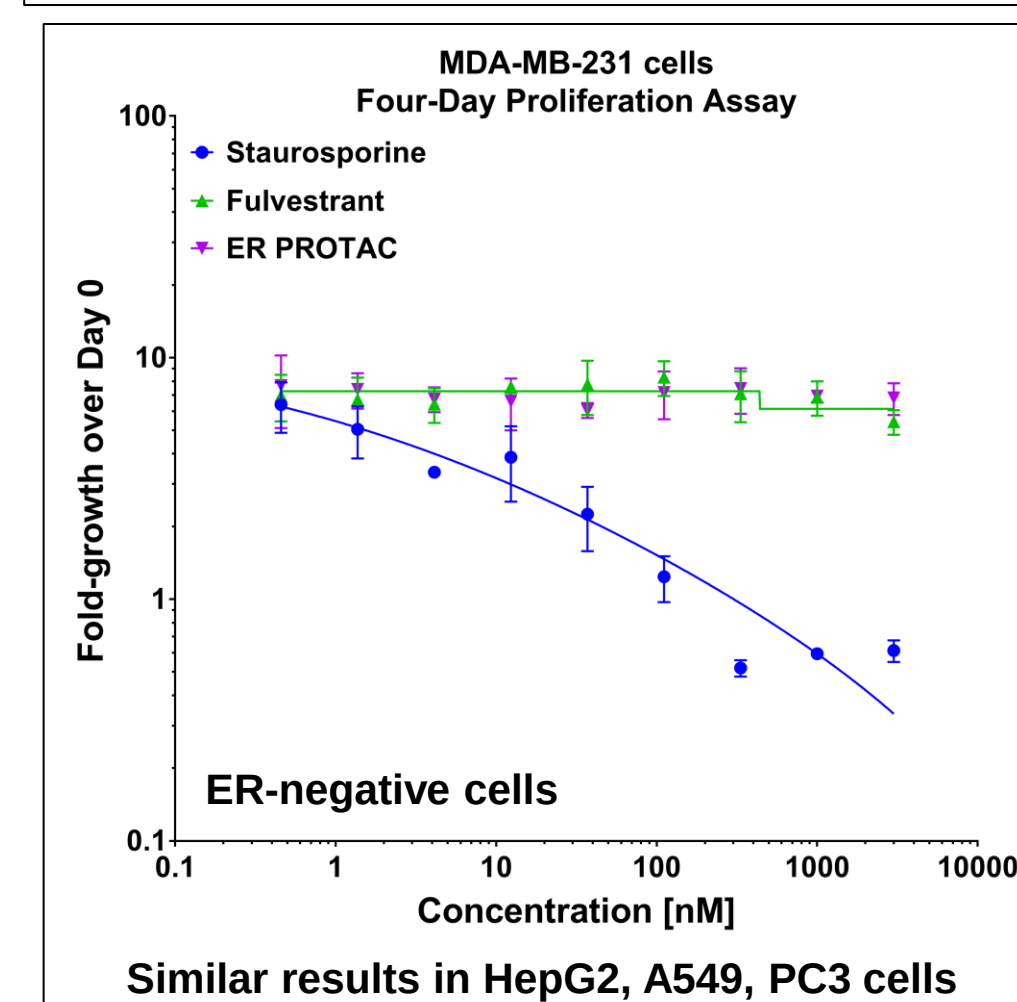
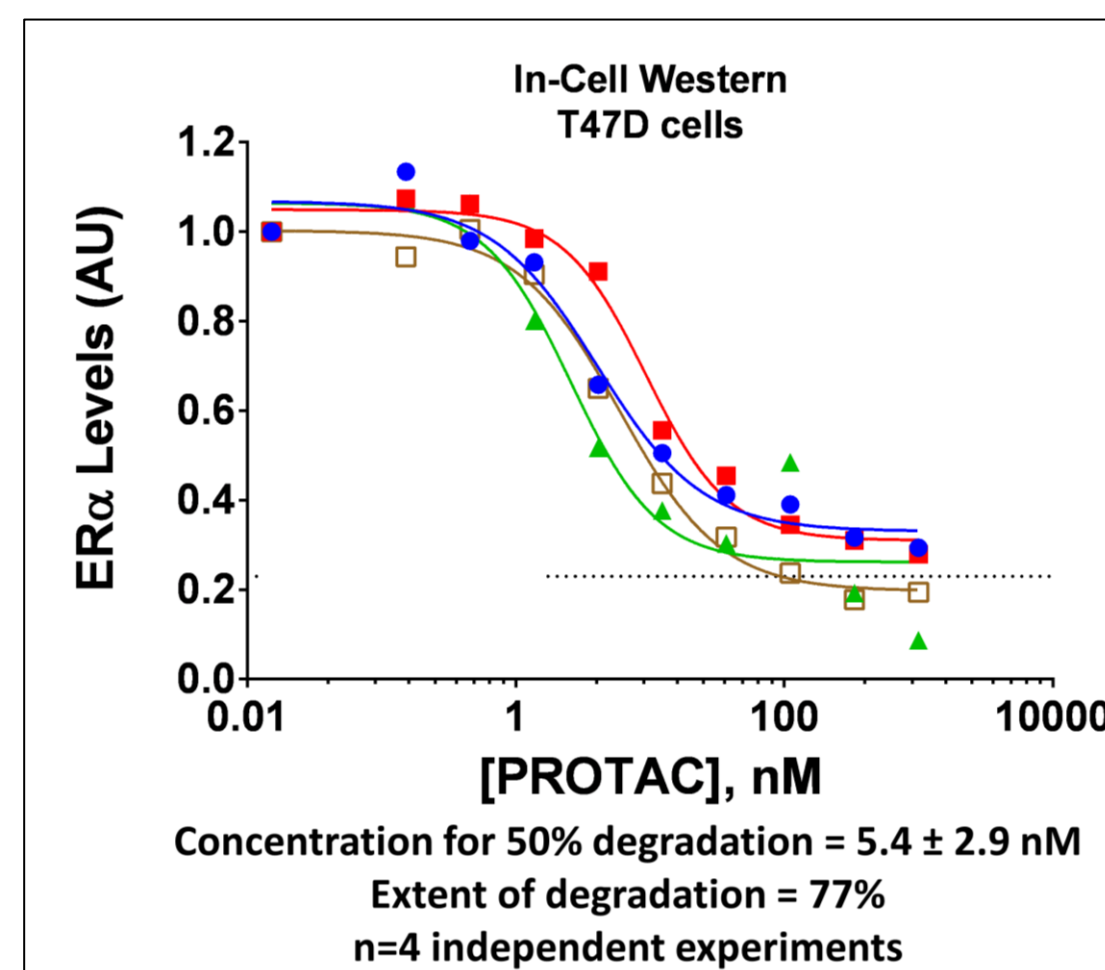
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Abstract

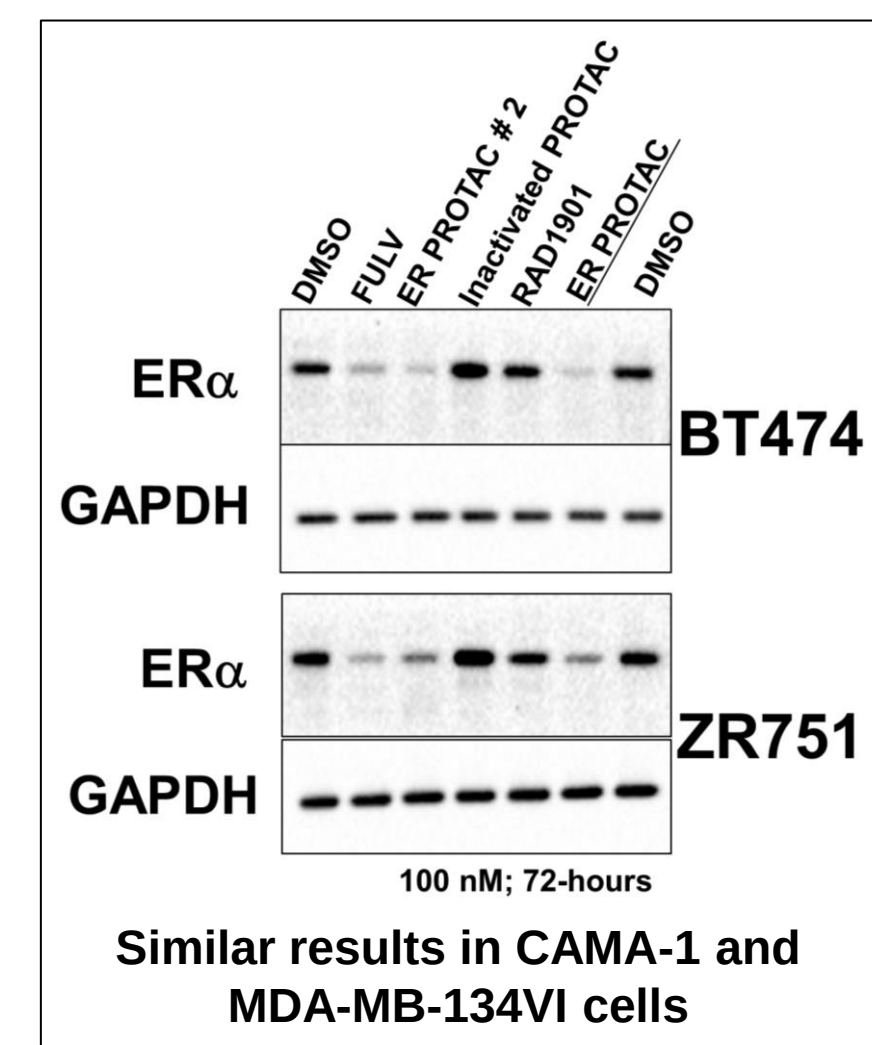
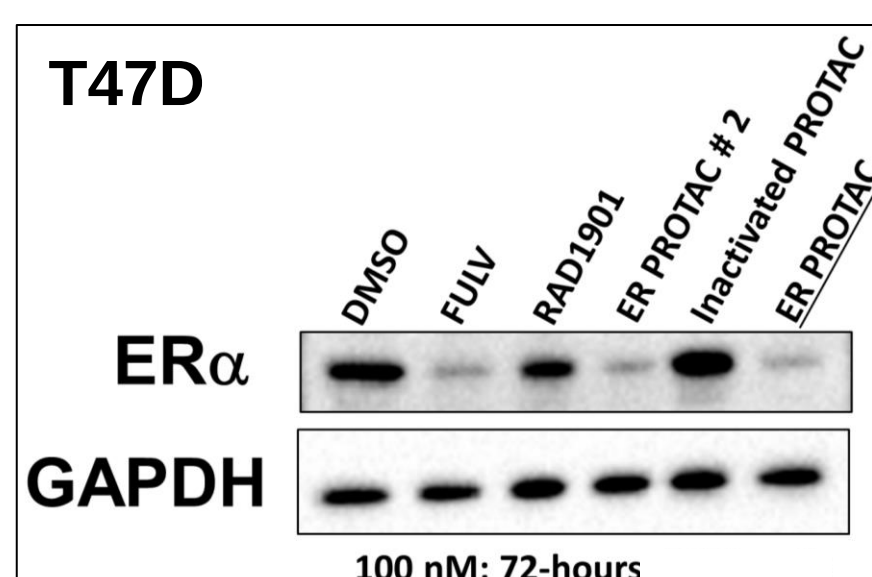
ER-positive breast cancers comprise approximately 70-80% of all newly diagnosed cases. Downregulation or degradation of ER is a treatment approach currently used in the clinic to target estrogen receptor signaling. Faslodex, the only clinically-approved ER-downregulator, is administered as a monthly 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.

In vitro characterization of ER α PROTAC



ER PROTACs degrade ER α across multiple ER+ cell lines



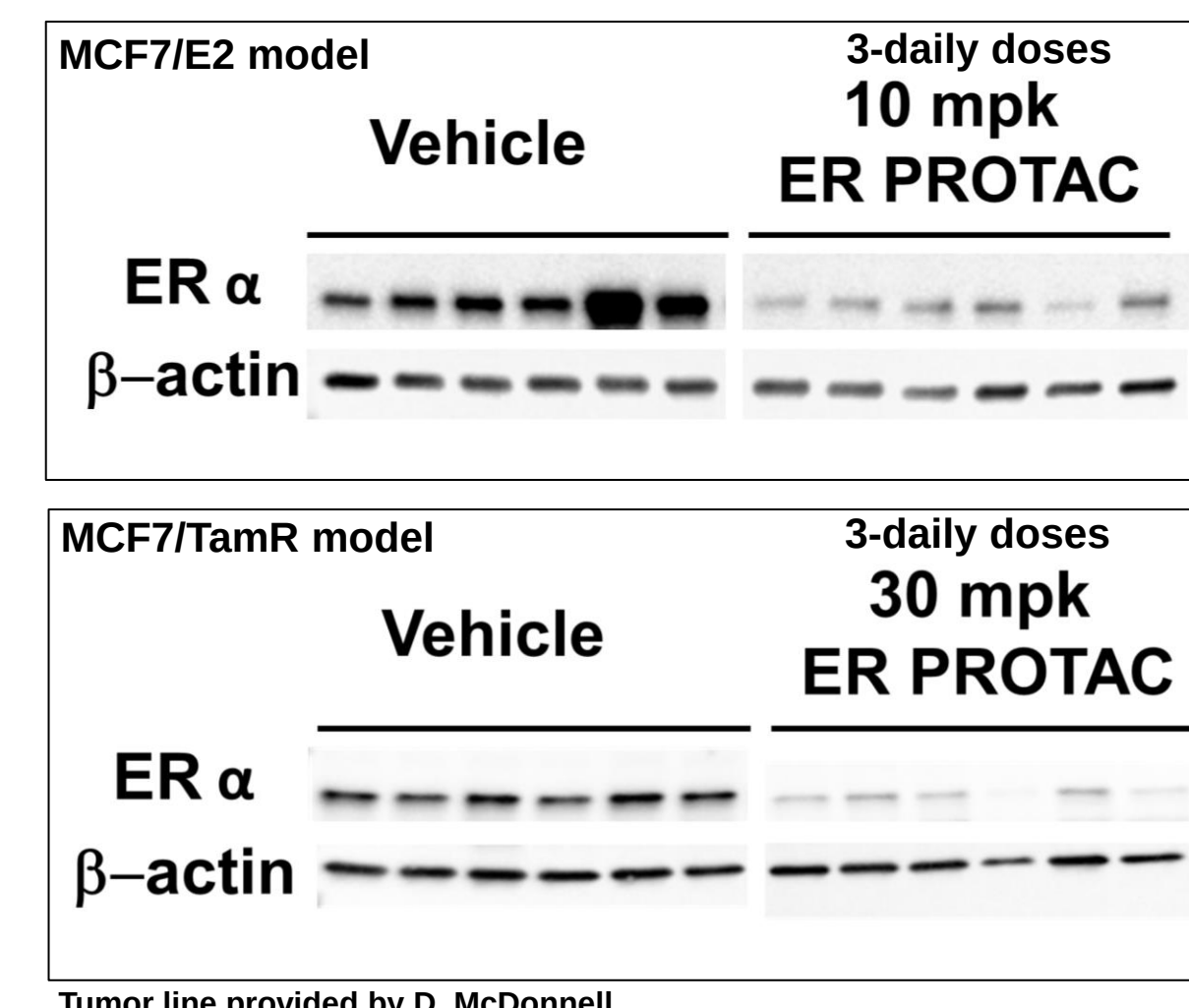
PK summary of ER α PROTAC

Species	% F	AUC/Dose (μ M $\cdot$ hr)/(mg/kg)
Mouse	42	0.8 $\pm$ 0.3
Dog	57	4.0 $\pm$ 1.3
Rat	26	0.6 $\pm$ 0.3

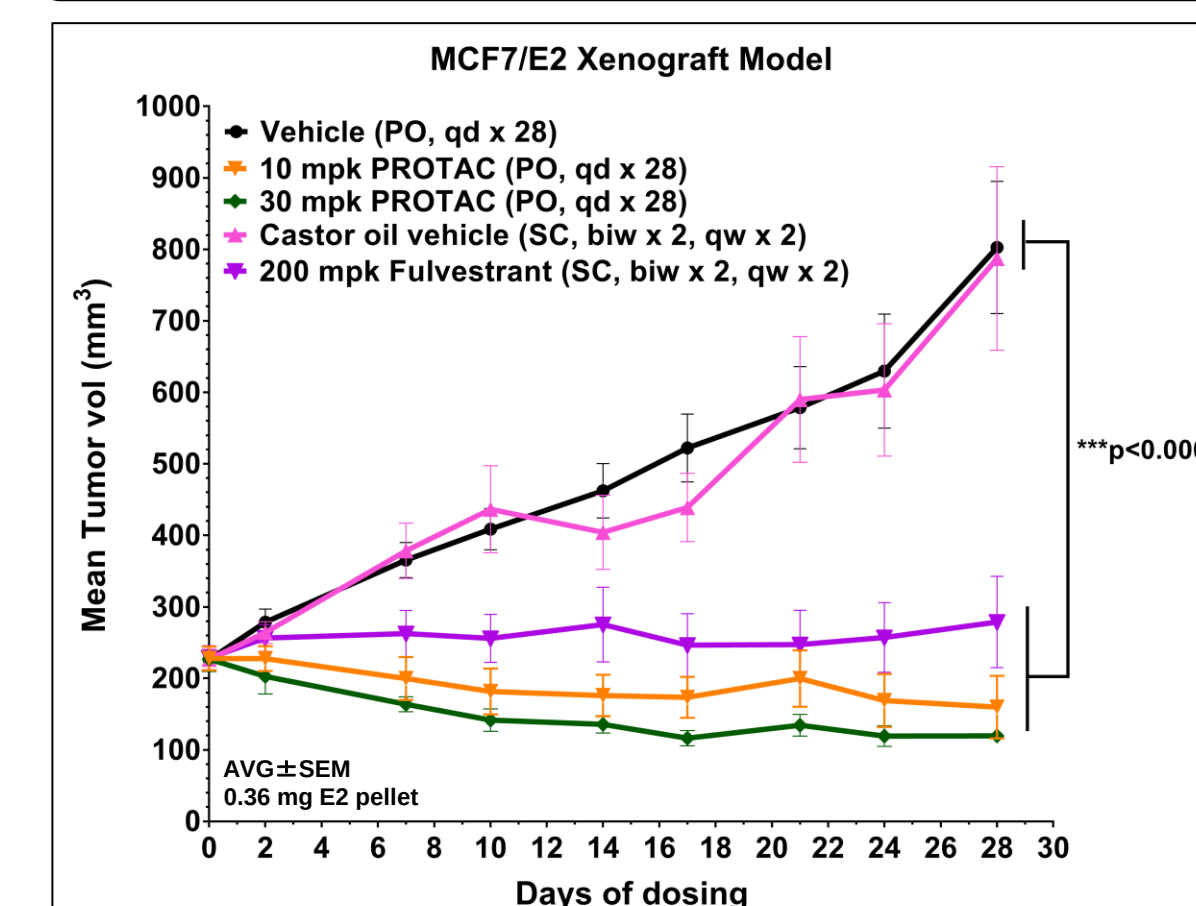
PO, 3 mpk

In vivo ER α degradation

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



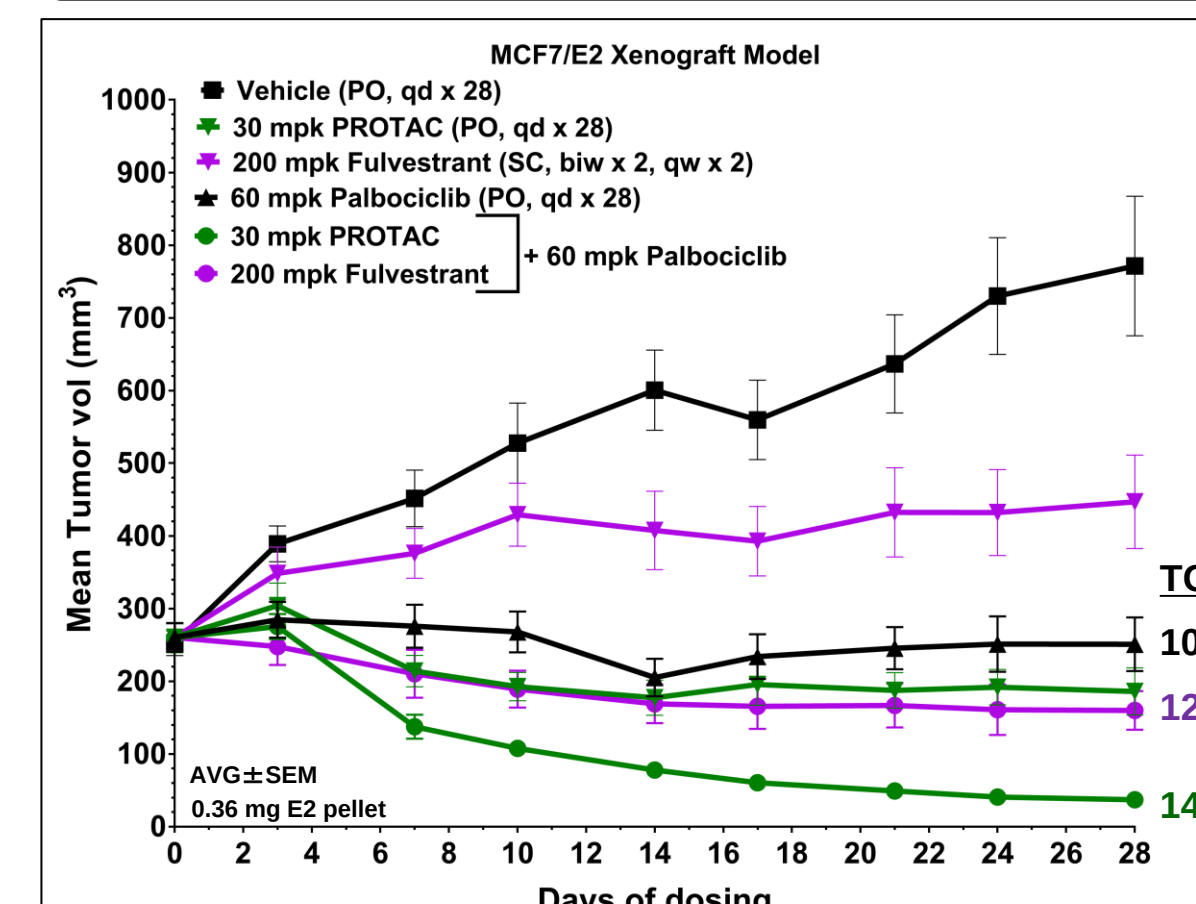
In vivo efficacy with ER α PROTAC



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

Arm	% TGI	% ER α decrease
10 mpk PROTAC	112	86
30 mpk PROTAC	120	82
200 mpk Fulvestrant	91	58

ER α PROTAC plus CDK4/6i

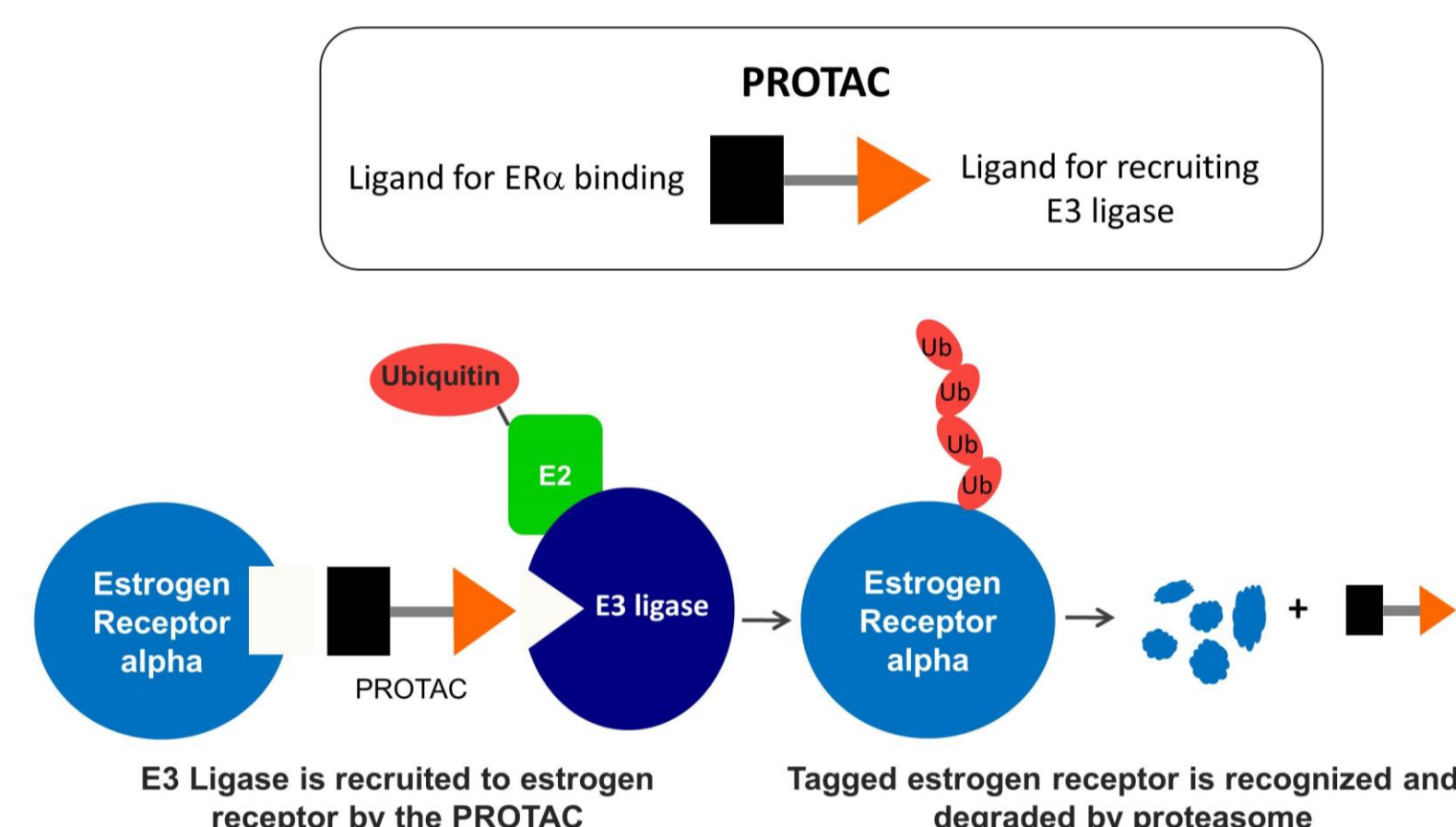


Combination of ER PROTAC and CDK4/6 inhibitor palbociclib provides superior tumor growth inhibition than fulvestrant plus palbociclib

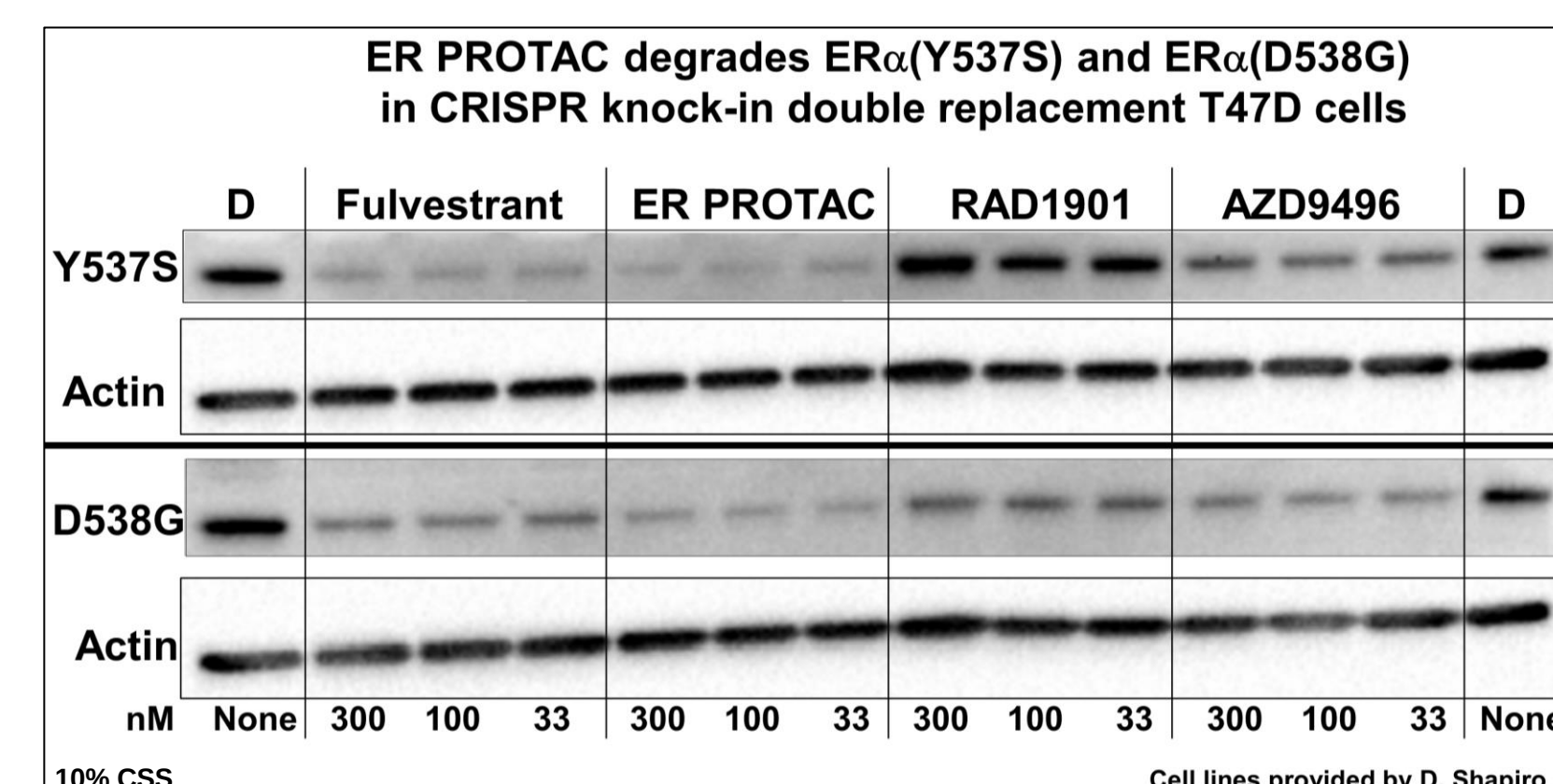
Comparison	Adj p-value
ER PROTAC/palbociclib vs palbociclib	<0.0001
ER PROTAC/palbociclib vs fulvestrant/palbociclib	0.0052
Fulvestrant/palbociclib vs palbociclib	0.0499

One-way ANOVA; Tukey's Multiple Comparisons Test

PROTAC: PROteolysis TArgeting Chimera



ER α PROTAC activity against ER α mutants



GI₅₀ (nM) values against T47D cell lines

Compound	Y537S	D538G
ER PROTAC	33 $\pm$ 11	8 $\pm$ 4
Fulvestrant	8 $\pm$ 4	4 $\pm$ 1
RAD1901	29 $\pm$ 8	13 $\pm$ 1
AZD9496	36 $\pm$ 1	7 $\pm$ 6

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

Summary

- Orally-bioavailable ER PROTACs demonstrate nanomolar ER α degradation potency and growth inhibition in a variety of wild-type ER α -expressing cell lines
- ER PROTACs degrade and inhibit growth of cells expressing clinically-relevant ER α variants, suggesting that ER PROTACs will be active in that resistance setting
- Oral administration of ER PROTAC provided more robust tumor growth inhibition and ER α 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