Preclinical characterization of imlunestrant, an oral brain-penetrant selective estrogen receptor degrader with activity in a brain metastasis (BM) model

Matthew VandeKopple[1], Cecilia Mur[2], Weihua Shen[1], Carlos Marugan[2], Andrew Capen[1], Lysiane Huber[1], Mark Castanares[3], David Garcia-Tapia[3], Brian Mattioni[3], Jolie Bastian[3], Jason Manro[3], Nicholas Pulliam[1], Michele Dowless[1], Maria Jesus Ortiz Ruiz[2], Maria Jose Lallena[2], Alfonso De Dios[3], Xueqian Gong[1]

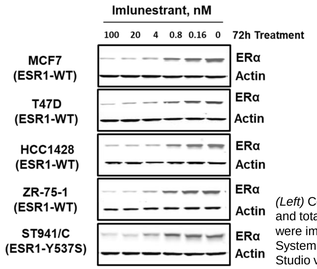
[1]Loxo@Lilly, Stamford, CT, USA. [2]Eli Lilly and Company, Alcobendas, Spain. [3]Eli Lilly and Company, Indianapolis, USA.

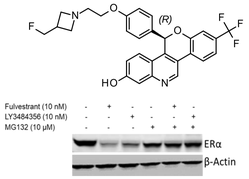
FPN# 41P

BACKGROUND

- Nearly 70% of newly diagnosed breast cancers (BC) are estrogen receptor (ERα) positive, for which endocrine therapy is the basis of treatment. (1)
- Until recently, the selective estrogen receptor degrader fulvestrant was the only approved SERD for ER+, HER2- BC, but usage is limited by suboptimal systemic pharmacology and poor drug solubility. (2)
- A predominant mechanism of endocrine therapy resistance is acquired mutation of ERα (*ESR1*), resulting in constitutive, ligand-independent activation of ERα (3).
- Up to 10% of patients with advanced ER+ BC are at risk of developing brain metastases (BM) (4), and targeted therapeutic options are limited.
- Here we describe the preclinical activity of imlunestrant, a next generation orally available SERD, with potent activity against both WT and mutant ERα, and importantly, brain penetrance and efficacy.

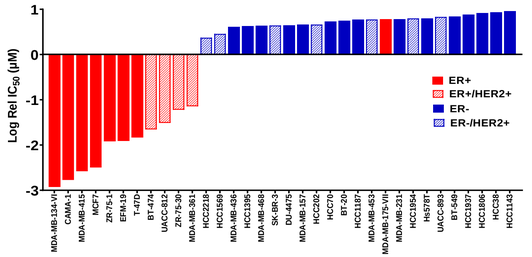
Figure 1. Imlunestrant displays potent degradation of ERα in both WT and mutant *ESR1* cell lines





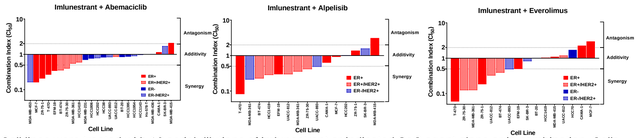
(Left) Cells were treated for 72 hours with imlunestrant *(top right)* and total cell lysate subjected to Western blot for ERα. Blots were imaged using LI-COR Odyssey Classic Infrared Imaging System. Images were processed and analyzed using Image Studio version 3.1. *(Bottom right)* Cells were treated with imlunestrant or fulvestrant with or without MG132, followed by Western blot analysis.

Figure 2. ER+ breast cancer cell lines are sensitive to imlunestrant

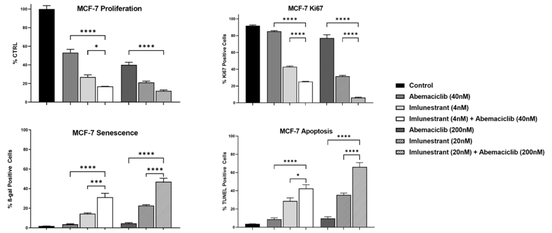


A panel of ER+ and ER- breast cancer cell lines were treated for two doubling times with 10 serial dilutions of imlunestrant. Cells were fixed, then stained with propidium iodide (PI). Plates were scanned with an *Acumen eX3* instrument and cell number was calculated with an Acumen algorithm. IC_{50} values were determined by curve fitting the cell number data to a four-parameter logistic using GENE DATA™.

Figure 3. Imlunestrant alone and in combination with SoC agents inhibits proliferation in breast cancer cell lines *in vitro*

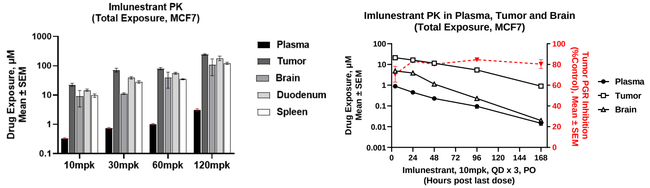


Cell lines were treated with 10 serial dilutions of imlunestrant or indicated SoC agent alone or in combination. Cells were then fixed, stained with PI, and scanned with an *Acumen eX3* instrument. IC_{50} values from the single agent and combination treatment were used to calculate the combination index at 50% inhibition (CI_{50}).

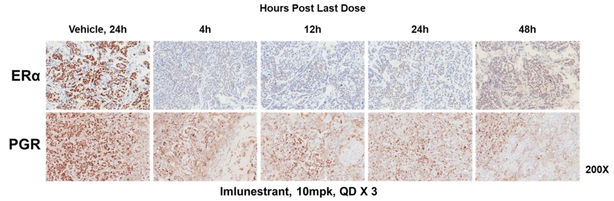


MCF7 cells were treated with imlunestrant, abemaciclib, or combination for 8 days. Cells were fixed, stained with primary antibody (KI67 or β-Gal), then a fluorescent secondary antibody and PI. TUNEL reagent was added to wells without antibody. Following wash, plates were read with an *Acumen eX3* instrument. Proliferation data was based on cell counts and % positive data based on control population.

Figure 4. Imlunestrant treatment demonstrates dose dependent exposure, prolonged tumor and brain exposure, and sustained PGR and ERα inhibition

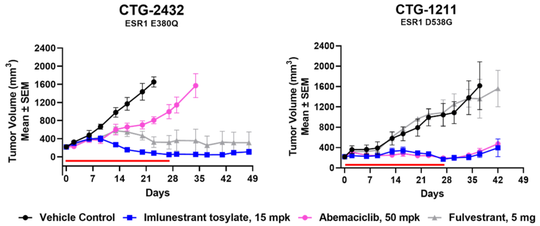


Mice bearing MCF7 xenografts were treated with imlunestrant for 3 days. Animals were sacrificed at the indicated time points, and organs and plasma collected for exposure analysis. *(Left)* 24 hrs post last dose, imlunestrant exposure demonstrated a dose dependent increase. *(Right)* RNA was prepared from half of each tumor to measure PGR inhibition by real-time qPCR analyses. GAPDH was used as the housekeeping gene to normalize results and PGR expression was calculated relative to the DMSO control for each sample.



Tumors were collected, fixed with 10% neutral buffered formalin, and embedded in paraffin blocks for ERα and PGR immunohistochemical analysis. Blocks were then transferred to ARUP laboratories for processing, staining and analysis.

Figure 5. Imlunestrant demonstrates single agent activity and response durability in *ESR1* mutant PDX models



Mice were treated with vehicle, imlunestrant tosylate (15 mpk PO), abemaciclib (50 mpk PO), or fulvestrant (5 mpk) for 28 days. Imlunestrant and abemaciclib were dosed orally once per day, fulvestrant was dosed by subcutaneous injection once weekly (n=5 mice/group). Red line on graph represents treatment period.

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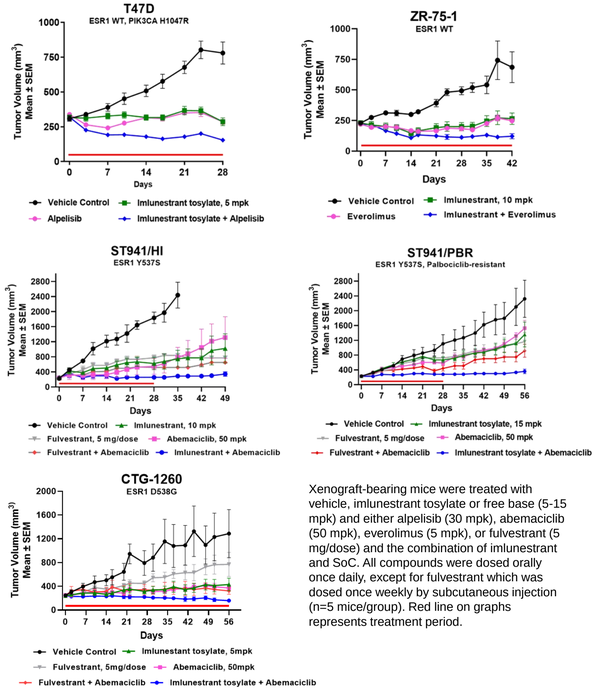
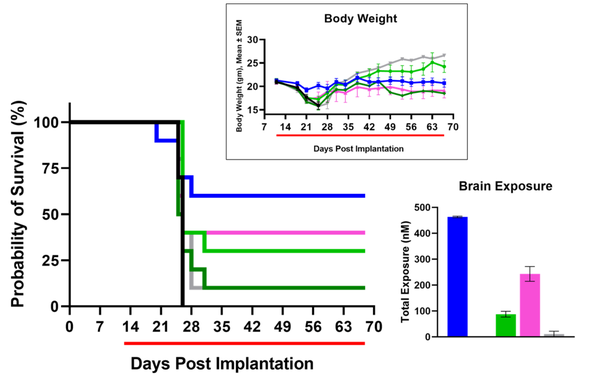


Xenograft-bearing mice were treated with vehicle, imlunestrant tosylate or free base (5-15 mpk) and either alpelisib (30 mpk), abemaciclib (50 mpk), everolimus (5 mpk), or fulvestrant (5 mg/dose) and the combination of imlunestrant and SoC. All compounds were dosed orally once daily, except for fulvestrant which was dosed once weekly by subcutaneous injection (n=5 mice/group). Red line on graphs represents treatment period.

Figure 7. Imlunestrant treatment increases survival probability in an ER+ brain orthotopic model



MCF-7 cells stably expressing luciferase (MCF7-luc) were implanted orthotopically into the brains of female NOD SCID mice supplemented with estrogen pellets. All compounds were dosed orally once daily, except for fulvestrant which was dosed once weekly by subcutaneous injection (n=10 mice/group). Red line on graphs represents treatment period. For exposure analysis, non-tumor bearing mice were dosed orally once daily for 7 days, except for fulvestrant, which was dosed once by SC injection. Brains from all treatment groups were harvested on day 8.

CONCLUSIONS

- Imlunestrant is a potent degrader of ERα, inhibits cell growth in both *ESR1* WT and mutant ER+ BC cell lines, and suppresses ER-mediated pathways
- Imlunestrant combined with BC SoC agents demonstrates additive or synergistic combination activity in multiple cell lines
- PK/PD analysis of imlunestrant *in vivo* shows dose dependent exposure in multiple tissues, persistent exposure over time, sustained *PGR* gene expression inhibition, and reduction of ERα and PGR by immunohistochemistry
- Imlunestrant is efficacious in *ESR1* WT and mutant CDX and PDX models and exhibits enhanced efficacy in both WT and mutant PDX models when combined with SoC agents
- Imlunestrant shows sustained exposure in the brain, demonstrating its ability to effectively cross the blood-brain barrier. In an ER+ brain orthotopic mouse model, imlunestrant treatment prolonged overall survival compared to control, fulvestrant and alternative SERD therapies

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1. Howlader N, *et al. J Natl Cancer Inst* 2014;**106**(5)
2. van Kruchten M, *et al. Cancer Discov* 2015;**5**(1):72-81
3. Jeselsohn R, *et al. Clin Cancer Res* 2014;**20**(7):1757-67
4. Darlix A, *et al. Br J. Cancer* 2019;121 991-1000

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Background

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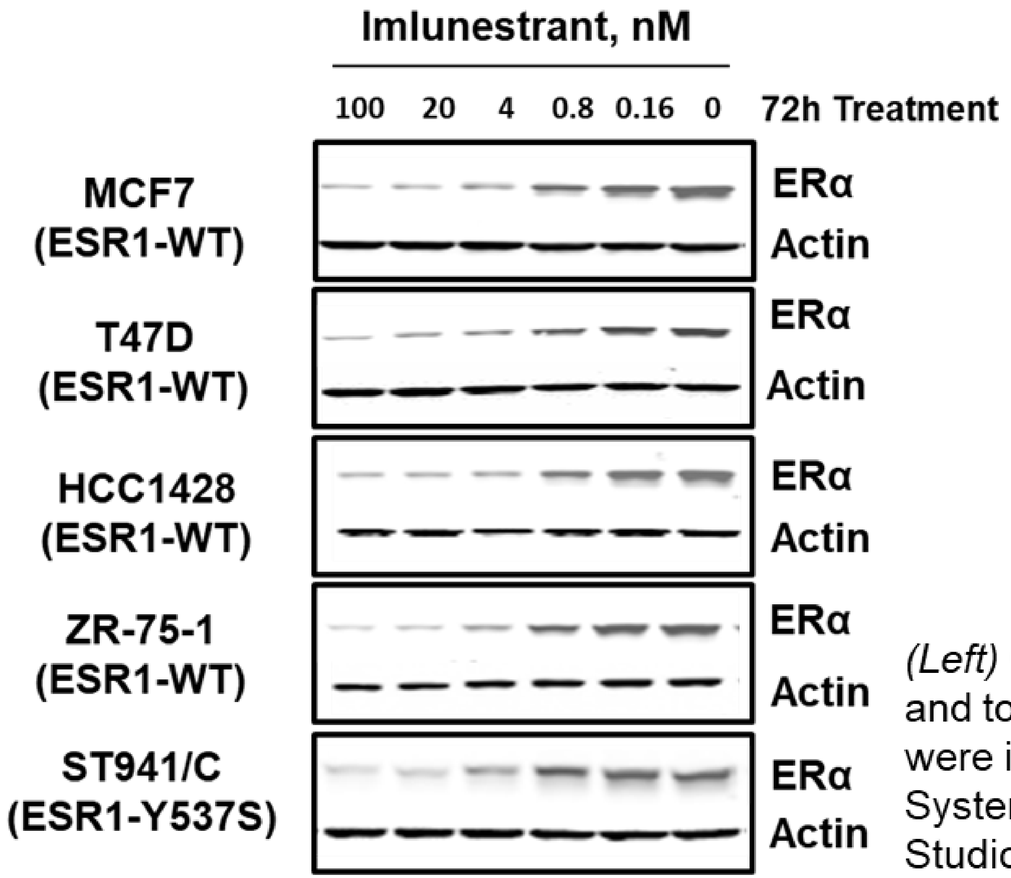

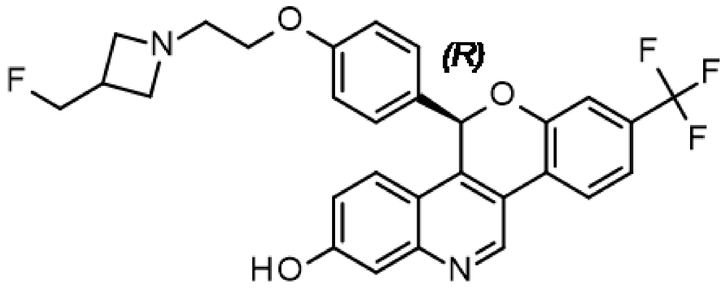

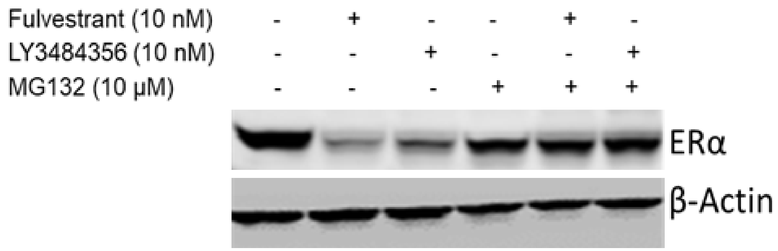

(Left) Cells were treated for 72 hours with imlunestrant *(top right)* and total cell lysate subjected to Western blot for ERα. Blots were imaged using LI-COR Odyssey Classic Infrared Imaging System. Images were processed and analyzed using Image Studio version 3.1. *(Bottom right)* Cells were treated with imlunestrant or fulvestrant with or without MG132, followed by Western blot analysis.

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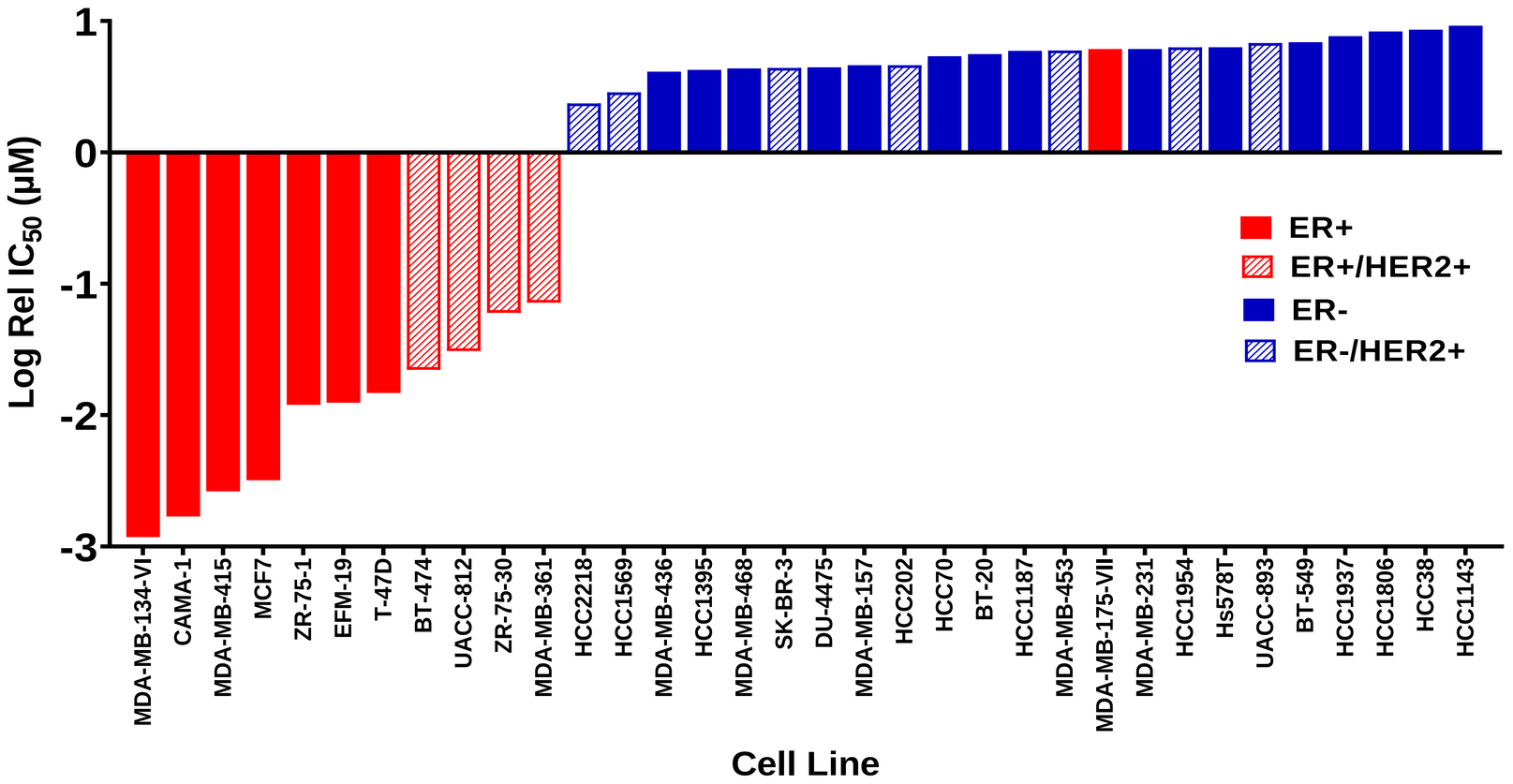

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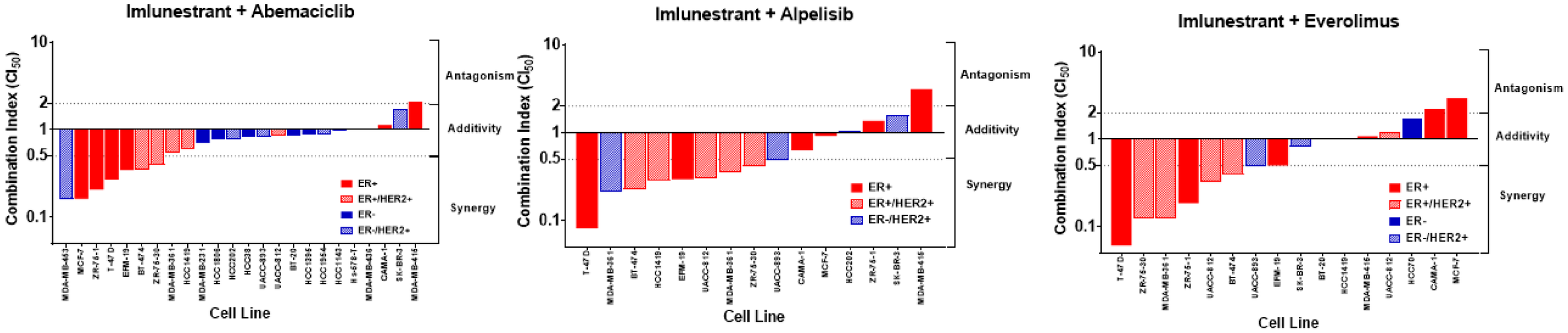
Figure 3. Imlunestrant alone and in combination with SoC agents inhibits proliferation in breast cancer cell lines *in vitro*



Cell lines were treated with 10 serial dilutions of imlunestrant or indicated SoC agent alone or in combination. Cells were then fixed, stained with PI, and scanned with an *Acumen eX3* instrument. IC_{50} values from the single agent and combination treatment were used to calculate the combination index at 50% inhibition (CI_{50}).

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Figure 3. Imlunestrant alone and in combination with SoC agents inhibits proliferation in breast cancer cell lines *in vitro* (continued)

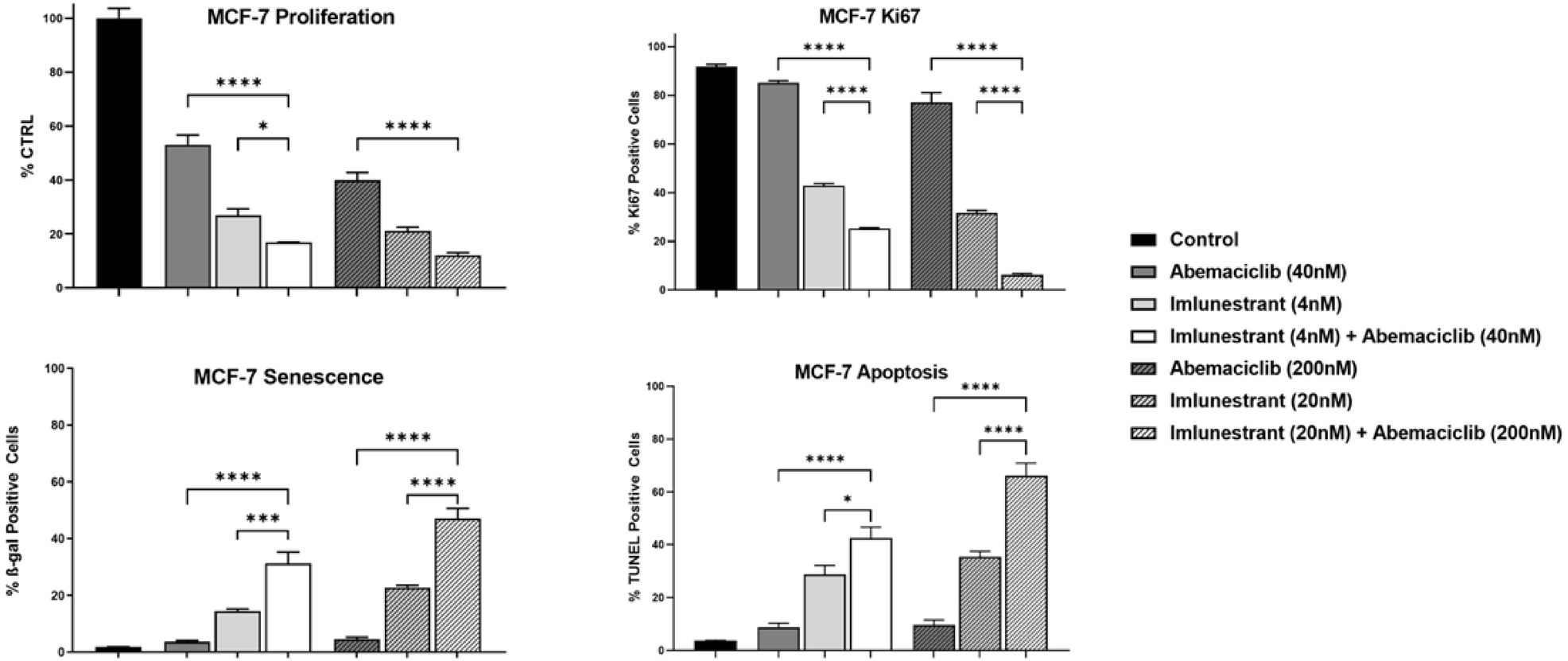

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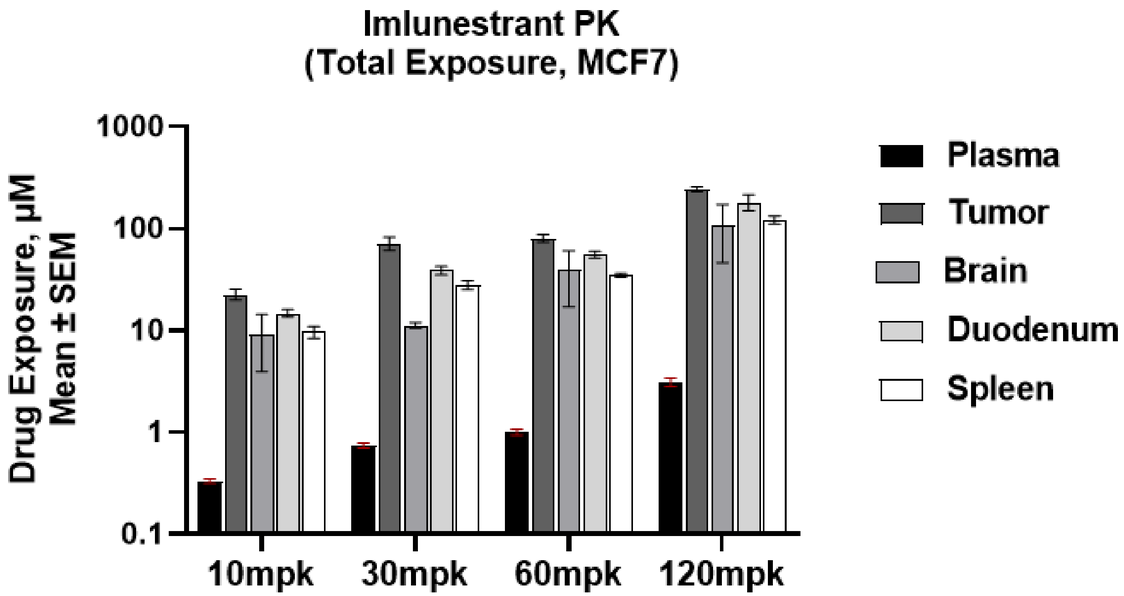
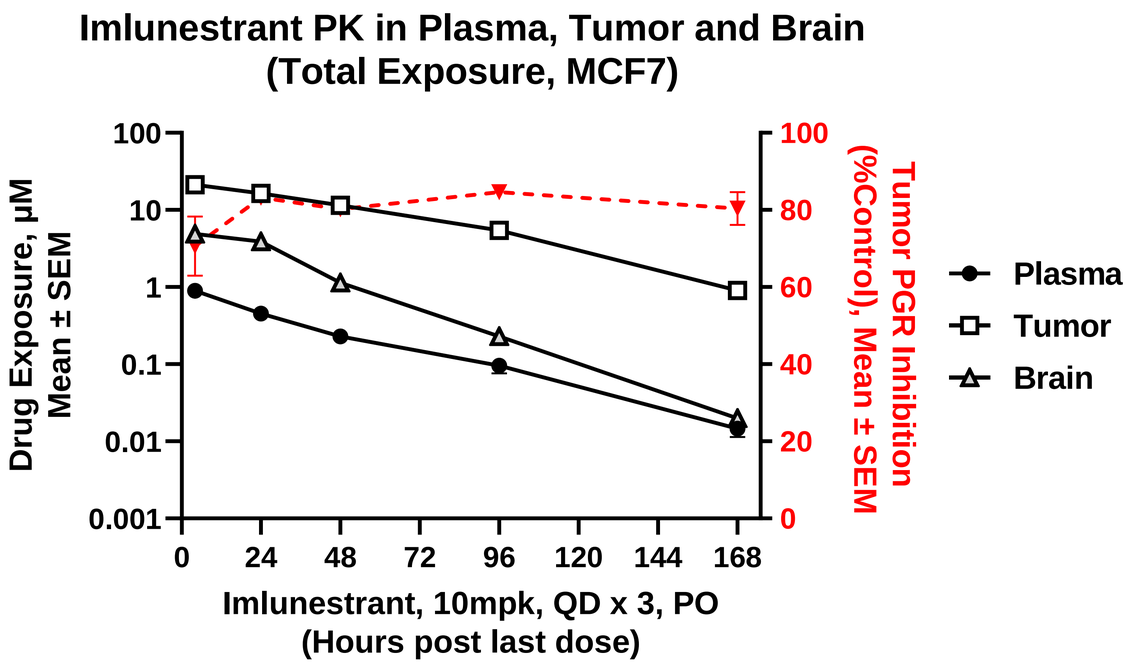
Figure 4. Imlunestrant treatment demonstrates dose dependent exposure, prolonged tumor and brain exposure, and sustained PGR and ERα inhibition





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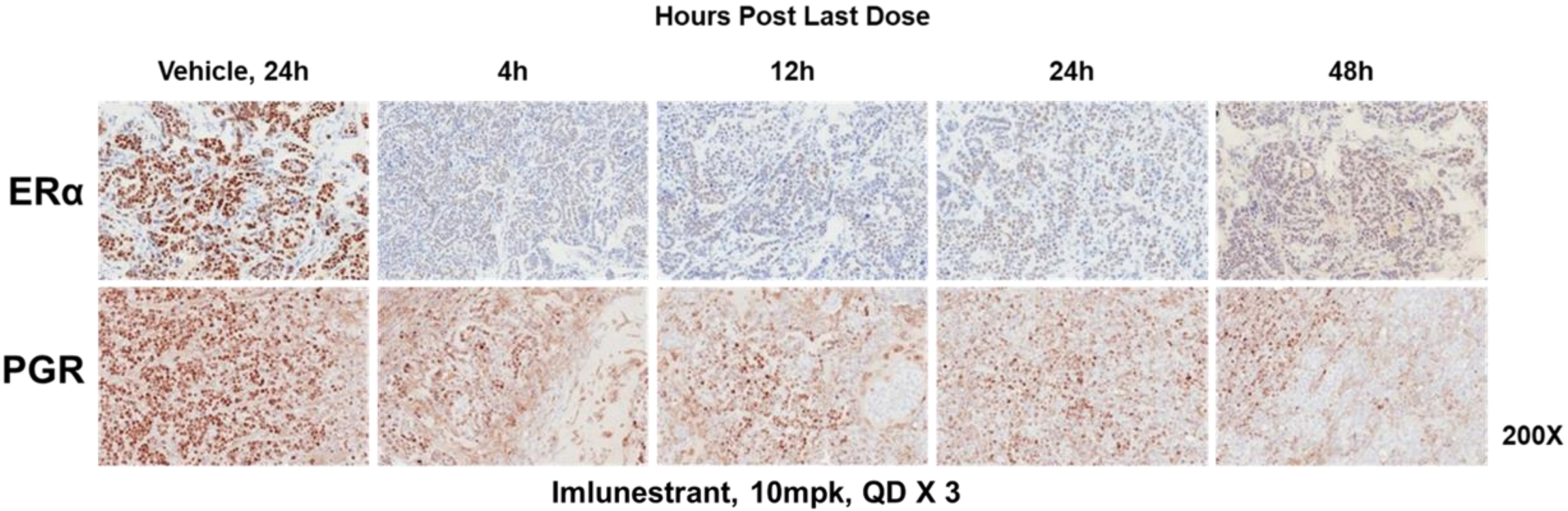

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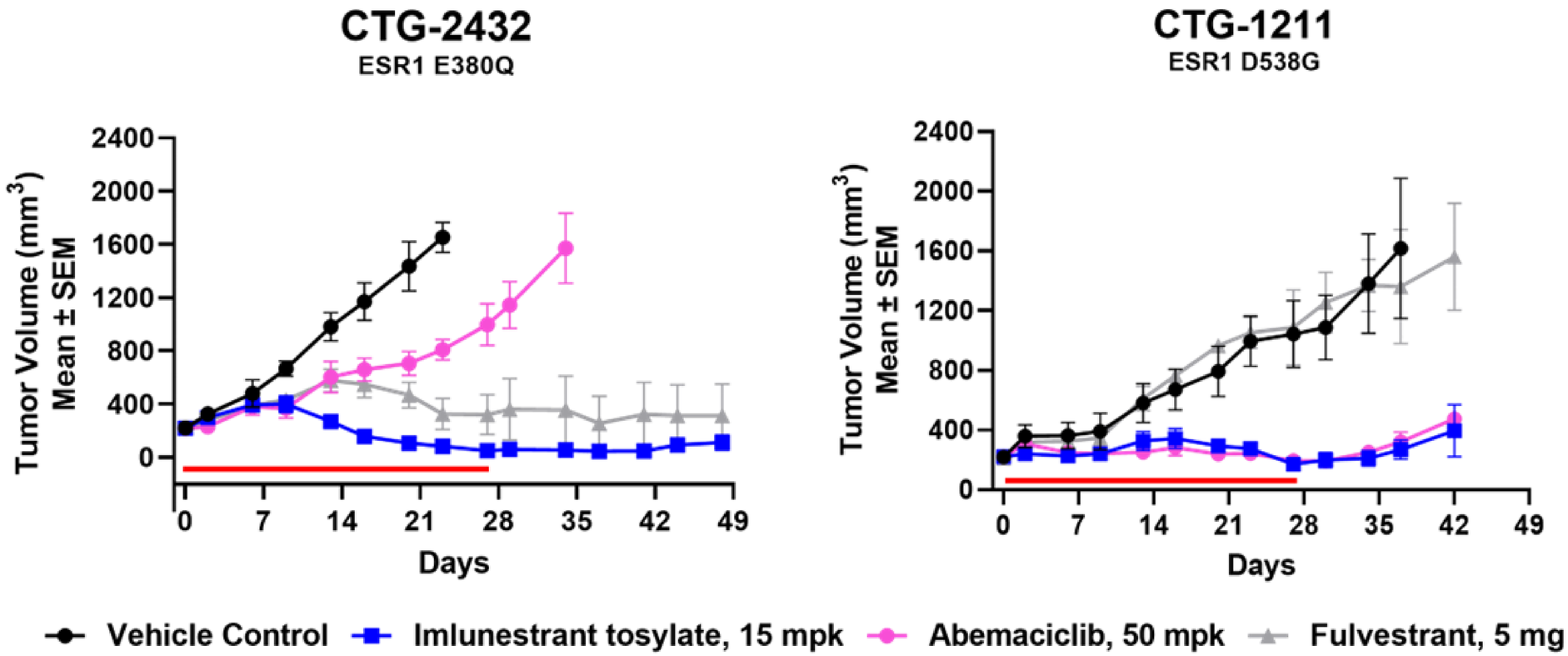

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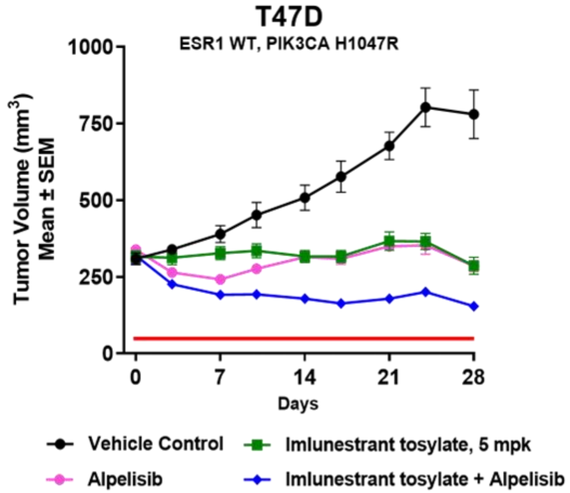

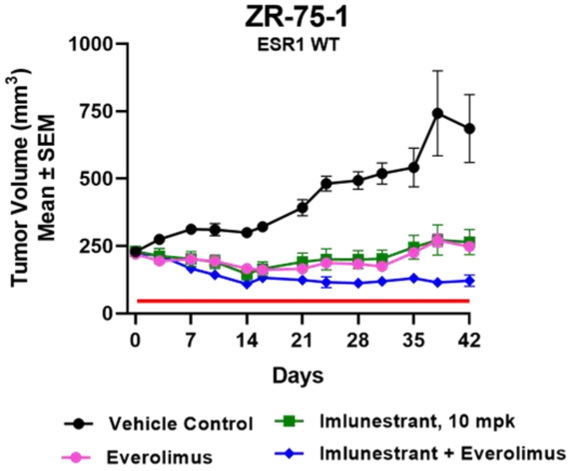

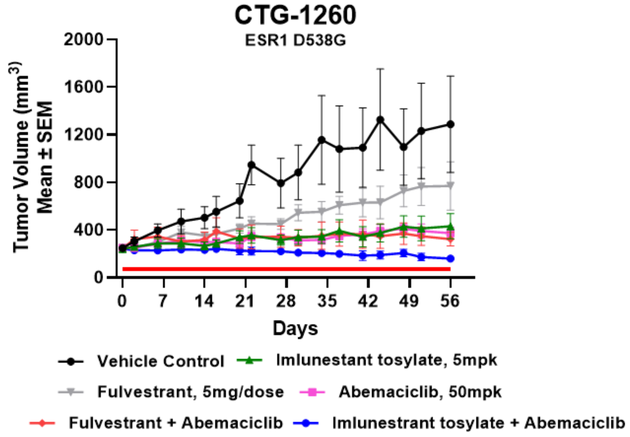

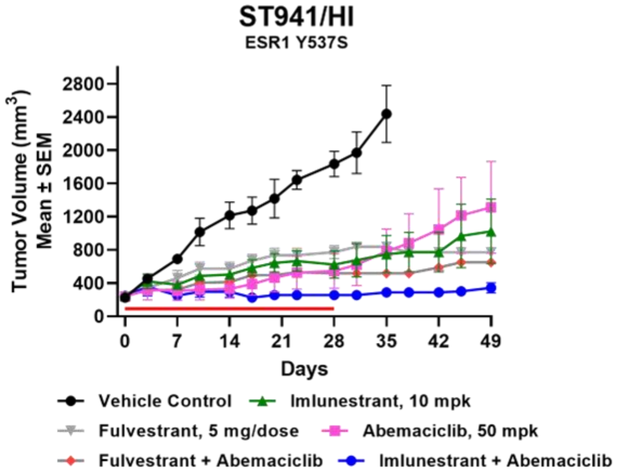

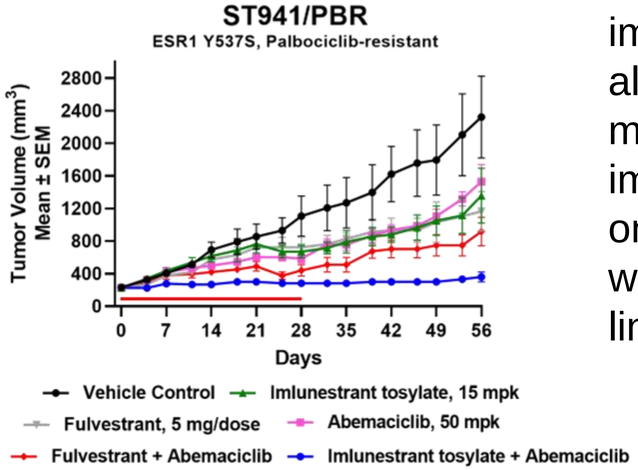

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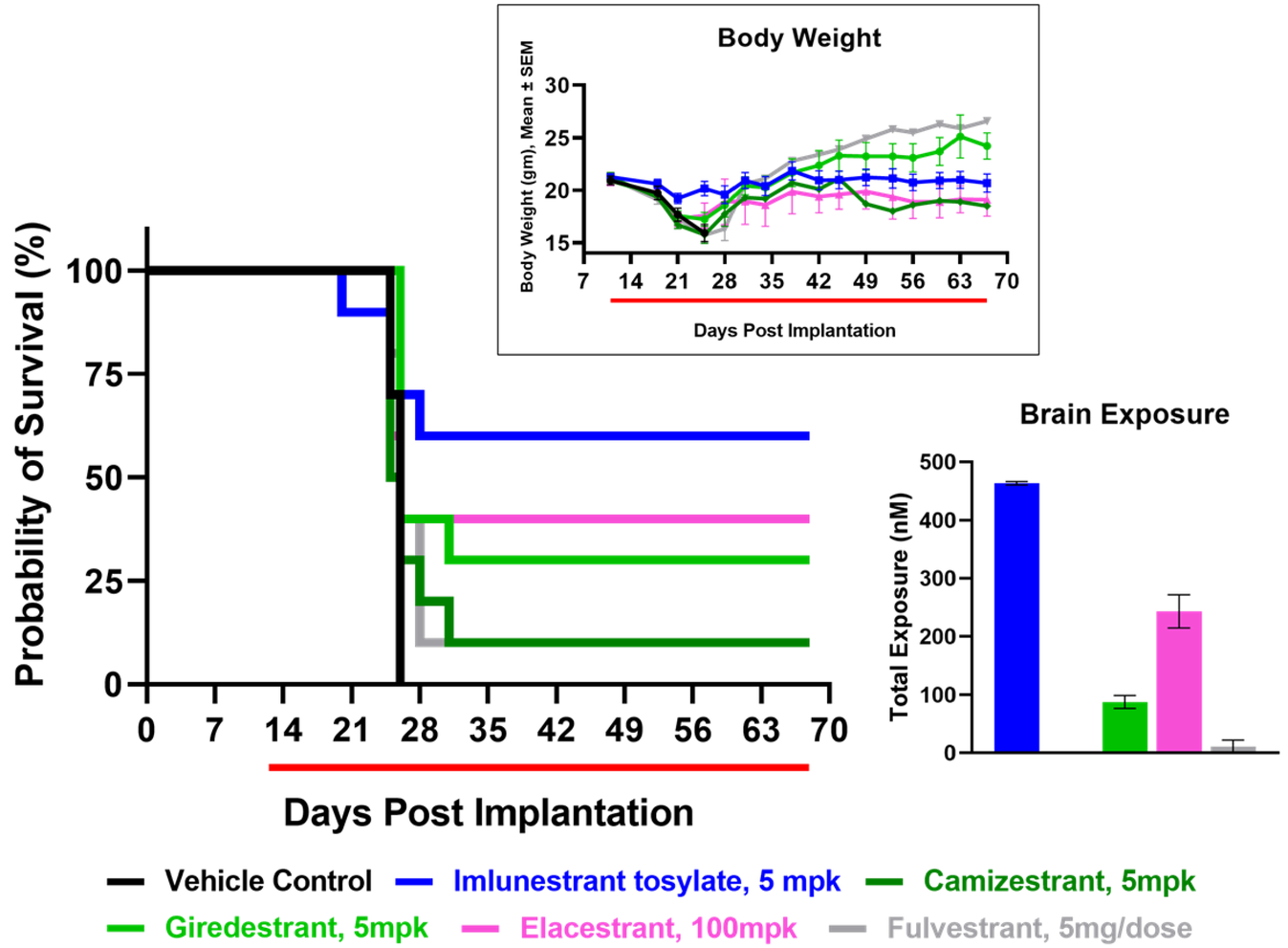
Figure 7. Imlunestrant treatment increases survival probability in an ER+ brain orthotopic model



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References, Acknowledgements, and Disclosure

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1. Howlader N, *et al. J Natl Cancer Inst* 2014;**106**(5)
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DISCLOSURES

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