Lasofoxifene decreases breast cancer lung and liver metastasis in a mammary intraductal (MIND) xenograft model of mutant ERα+ breast cancer

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Introduction

The standard of care for early postmenopausal ERα+ breast cancer patients is adjuvant endocrine therapy, typically an aromatase inhibitor or tamoxifen, and endocrine therapy with or without a CDK 4/6 inhibitor in the metastatic setting. However, a number of these patients are not sensitive to endocrine therapy or experience breast cancer recurrence 10 to 15 years after endocrine treatment, and all eventually progress in the metastatic setting. Metastasis of ER+ therapy-resistant breast cancer correlates with the acquisition of ESR1 activating mutations in up to 40% or more of affected patients. The two most common ERα mutations are Y537S and D538G, both of which confer ERα constitutive activity. Lasofoxifene is a SERM originally developed to treat vulvovaginal atrophy and osteoporosis. Studies have shown that lasofoxifene reduces vertebral and non vertebral fractures in postmenopausol women with osteoporosis. Lasofoxifene also showed a 79% reduction in total breast cancer incidence and an 83% reduction in ERα+ breast cancer. In this study, we analyzed the efficacy of lasofoxifene in the treatment of MCF7 tumor explant models that were engineered with CRISPR/Cas9 to express Y537S or D538G ERα. To better mimic the natural micro-environment of infiltrating ductal breast cancer, we used the mammary intraductal mouse model (MIND), in which tumor cells are introduced into the ducts via the nipple. With this model we are able to show that lasofoxifene is effective at inhibiting metastasis development in breast cancer.

Experimental design

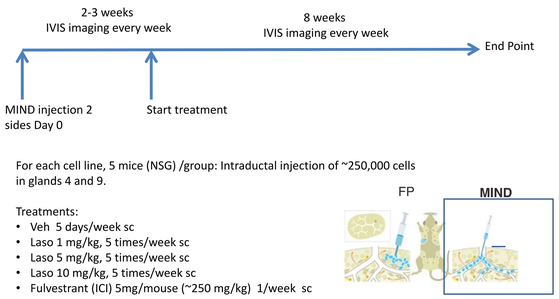


Fig 1: MCF7 ERα-WT, MCF7 ERα Y537S, and MCF7 ERα D538G cells were labeled with Luciferase-GFP plasmid via Lentiviral infection. Animal treatments were started 2-3 weeks after cell injection. Mice were imaged weekly until sacrifice at day 70.

Results

Lasofoxifene effectively inhibited tumor growth of MCF7 WT cells as well as MCF-7 Y537S and D538G ER mutants.

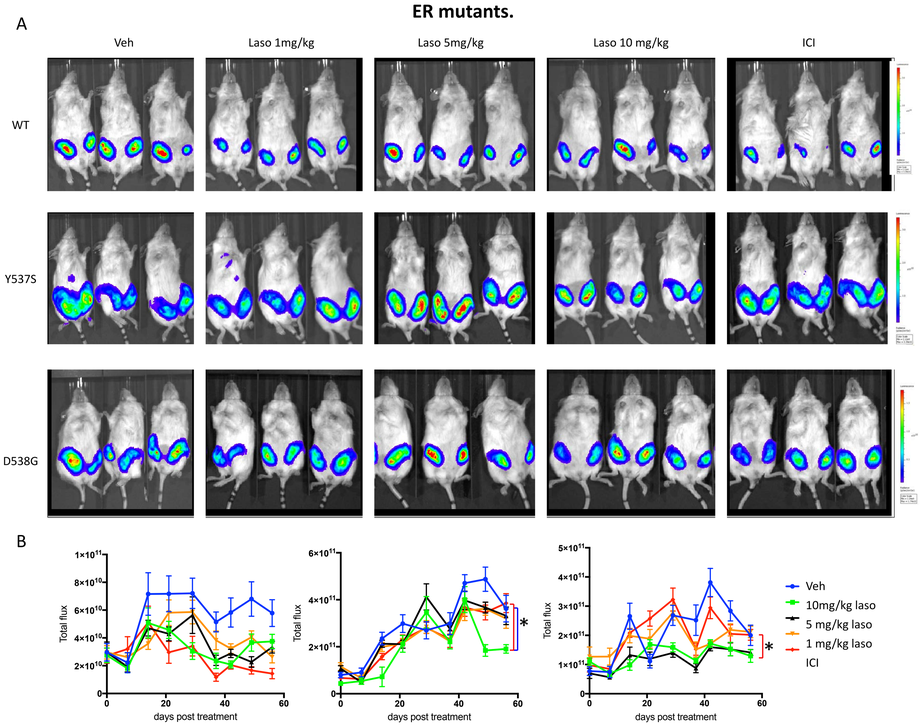


Fig 2: A. IVIS images at day 56 of 3 representative mice for each treatment. **B** Quantification of the total photon flux measured over time on a Xenogen IVIS 200 imager, demonstrating that lasofoxifene is as effective as fulvestrant (ICI) at inhibiting tumor growth for the MCF7 WT, Y537S and D538G mutant cells. P values are calculated with a student t-test. * p<0.05, n=8-10

Tumor weights at sacrifice were significantly lower for MCF-7 Y537S and D538G ER mutants

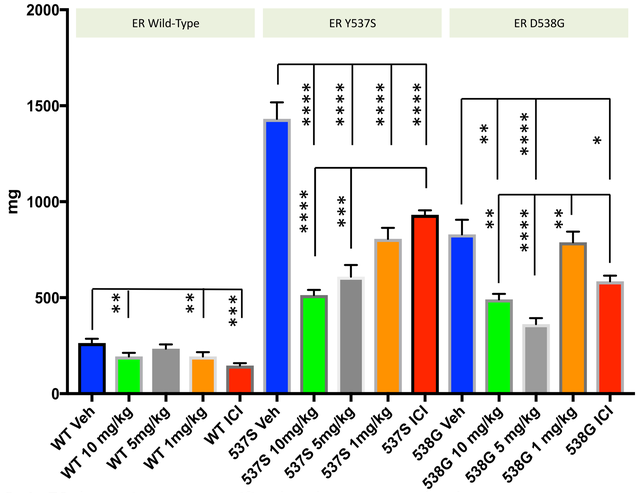


Fig 3: At 70 days mice were sacrificed and mammary gland tumors were removed and weighed. Tumor weights were significantly lower for 5 and 10 mg/kg lasofoxifene-treated MCF7 Y537S and D538G mutants compared to vehicle treatment. For the Y537S mutant, tumor weights for mice treated with 5 and 10 mg/kg lasofoxifene were also significantly lower compared to ICI treatment. P values are calculated with a student t-test. * p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001

Lasofoxifene at 5 and 10 mg/kg is effective at inhibiting lung metastasis of WT, Y537S and D538G MCF-7 tumors compared to fulvestrant (ICI)

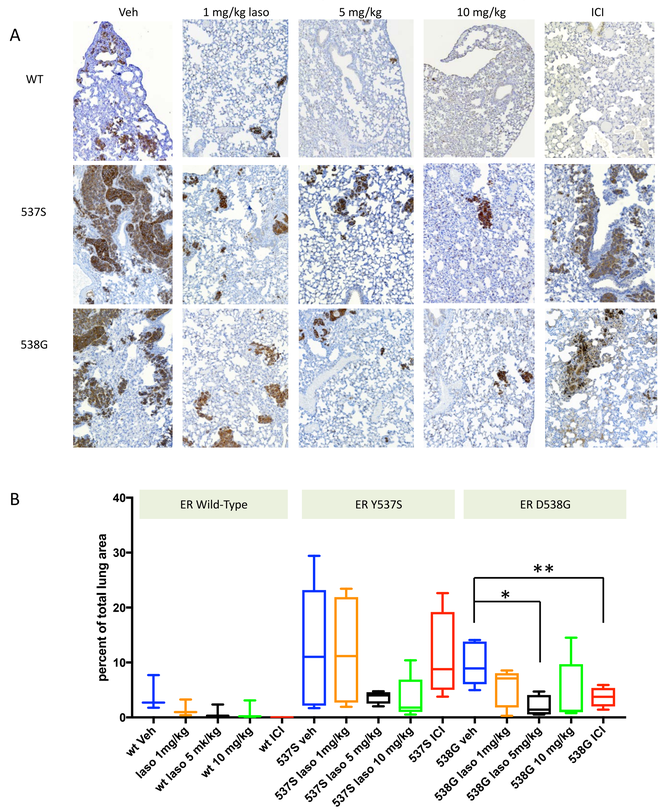


Fig 4: **A** IHC staining of lungs with human cytokeratin AE1/AE3. **B** Box plot quantification of IHC staining, showing the area of stained cells normalized to the total lung area. n= 4-5. P values are calculated with a student t-test. * p<0.05, **p<0.005, n=4-5

Lasofoxifene at 5 and 10 mg/kg was significantly more effective at inhibiting lung metastasis of Y537S and D538G MCF-7 tumors compared to fulvestrant (ICI).

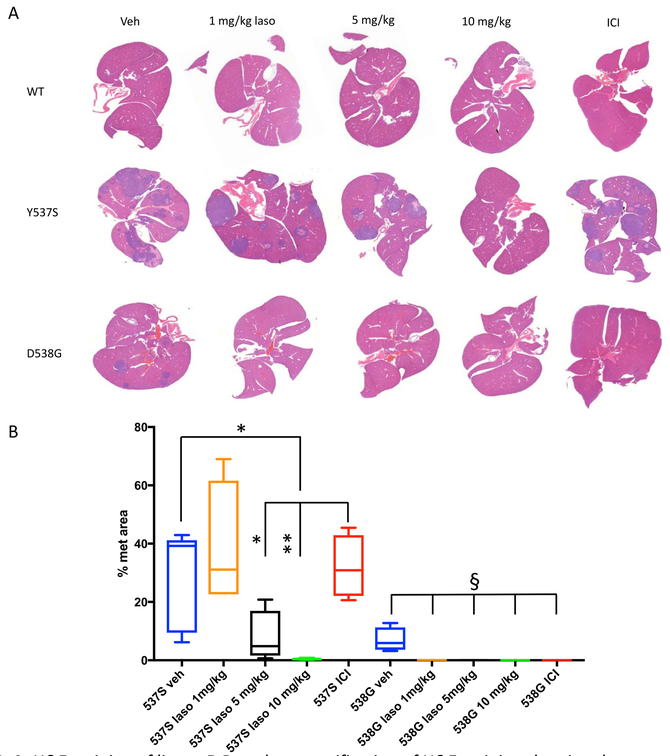
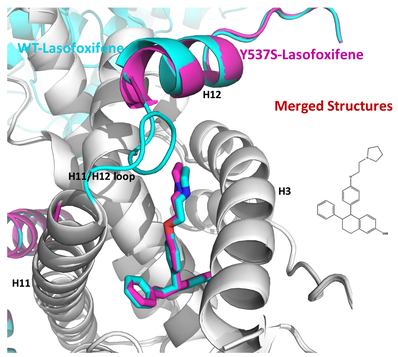


Fig 5: **A**. H&E staining of livers. **B** Box plot quantification of H&E staining showing the area of tumor cells normalized to the total liver area. For 537S p values are calculated with a student t-test. * p<0.05, **p<0.005. For 538G p values (§) p=0.05 were calculated with a fisher exact test, n= 3-5



Merged structures for WT ER LBD (**blue**) and Y537S ER LBD (**magenta**) bound to lasofoxifene. Note the absence of a visible loop between H11 and H12 for for **Y537S ER LBD**. It also appears that H11 is shorter for Y537S than for WT ER LBD. Note that the side chain of lasofoxifene is shifted toward where the loop should be.

Fig 6 Lasofoxifene distorts antagonist structure of Y537S ER LBD compared to WT ER LBD.

CONCLUSIONS

- Lasofoxifene at 5 and 10 mg/kg is significantly effective at reducing tumor growth for the MCF7 Y537S and D538G tumors.
- Lasofoxifene is effective at inhibiting the metastasis of both MCF7 Y537S and D538G mutants to the lungs and liver.
- Lasofoxifene is more effective than fulvestrant at inhibiting metastasis for both MCF7 Y537S and D538G mutants, whereas fulvestrant only inhibited metastasis of the MCF7 D538G mutant to both organs.
- Lasofoxifene may have the potential to be utilized as a treatment for metastatic breast cancers, including those that express constitutively active ERα mutations, such as D538G and Y537S.