

## PULSATILE ESTROGEN MODULATES ER $\alpha$ ACTIVITY, LIMITING BREAST TUMOR GROWTH WHILE ALLEVIATING MENOPAUSAL SYMPTOMS

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**Background:** Menopause hormone treatment (MHT) is the most effective therapy to alleviate menopause-related symptoms. However, its use remains controversial because it is associated with several side effects, including an increased risk of breast cancer. Various estrogens can be used in the composition of MHTs. However, it remains questionable whether their pharmacokinetic profile could differently impact their efficacy and breast cancer risk.

**Methods:** We compared three natural estrogens (E2, E3, and E4) administered to produce two distinct pharmacokinetic profiles—pulsed or continuous—and assessed their effects in vitro and in vivo across breast cancer, mammary gland, bone, brain, interscapular brown adipose tissue (iBAT), and endometrium. We validated our findings using patient-derived xenografts (PDXs) from ER<sup>+</sup> breast cancer and healthy endometrium. The modulation of ER $\alpha$  transcriptional activity was assessed by RNA-seq and Chip-qPCR, confirmed at the protein level, and the dissociation kinetics of ER $\alpha$ –ligand complexes were quantified.

**Results:** Our data clearly demonstrate that pulsed E3 and E4 treatments, which dissociate faster from ER $\alpha$  than E2, induce a transient activation of ER $\alpha$  signaling and transcriptional activity leading to reduced mammary gland development and breast cancer neutrality, while maintaining thermoregulation and biological responses in bone, brain, and uterus.

**Conclusions:** In the context of MHT, our results emphasize that adapting the nature of the estrogen and its route of administration modulates ER $\alpha$  signaling and could be key to achieving a good balance between effectively relieving menopausal symptoms and minimizing excessive estrogenic action on the mammary gland and breast cancer.