# AVA-291 (d3-Testosterone) vs Testosterone in MCF-7 ER+ Cells: Lack of Aromatization Leads to a Highly Differentiated Cellular Proliferation Profile

Abstract # 2297 / 19

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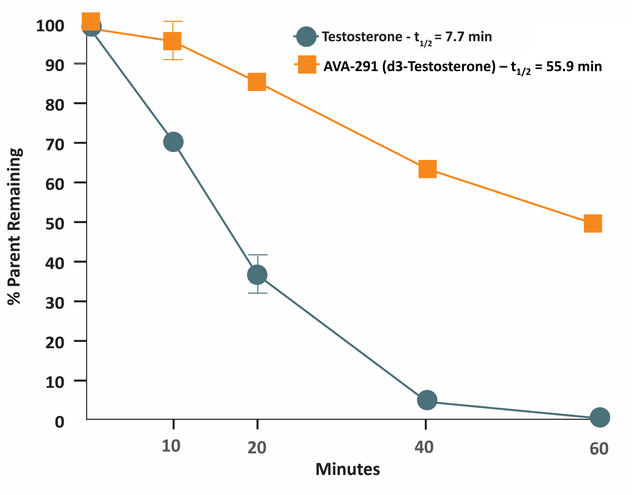
## BACKGROUND

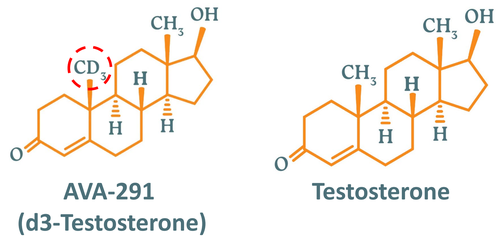
- Aromatization of androgens to estrogens within breast tumors plays a key role in the proliferation of estrogen receptor positive ($ER^+$)breast cancer cells
- Testosterone (T) has been shown to promote breast cancer cell proliferation *in vitro* which can be ameliorated by an aromatase inhibitor[1]
- Clinically, testosterone has been associated with a breast cancer signal in postmenopausal women[2]
- Increasing use of T therapy in women raises concerns regarding potential risk for breast cancer risk, the most prevalent cancer in women
- T formulations have not been approved for use in women in the US due to safety concerns[3]
- AVA-291 (d3-Testosterone [d3-T]) is a structurally identical, deuterium-substituted isotopologue of T designed to resist aromatization to estradiol (E2)
- AVA-291 has a similar metabolic profile and androgen receptor potency as T but resists aromatization

## AROMATIZATION PROPERTIES

- AVA-291 is highly resistant to aromatization in the presence of recombinant human aromatase
  - AVA-291 demonstrated substantially longer stability compared with T in the presence of pure, concentrated aromatase (worst-case considering human aromatase concentrations *in vivo*)

**Figure 1.** ***In Vitro*** **Metabolism of AVA-291 vs Testosterone in Presence of Pure Recombinant Aromatase (CYP19)**




AVA-291 (d3-Testosterone)

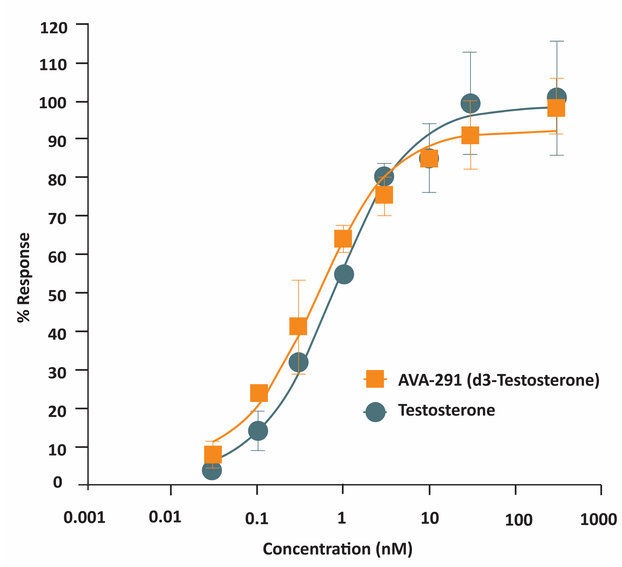



Testosterone

## ANDROGEN RECEPTOR TRANSLOCATION

- d3-T and T are equipotent and equi-efficacious (similar maximum effect) in inducing androgen receptor translocation

**Figure 2.** **Comparison of Androgen Receptor Translocation AVA-291 vs Testosterone**



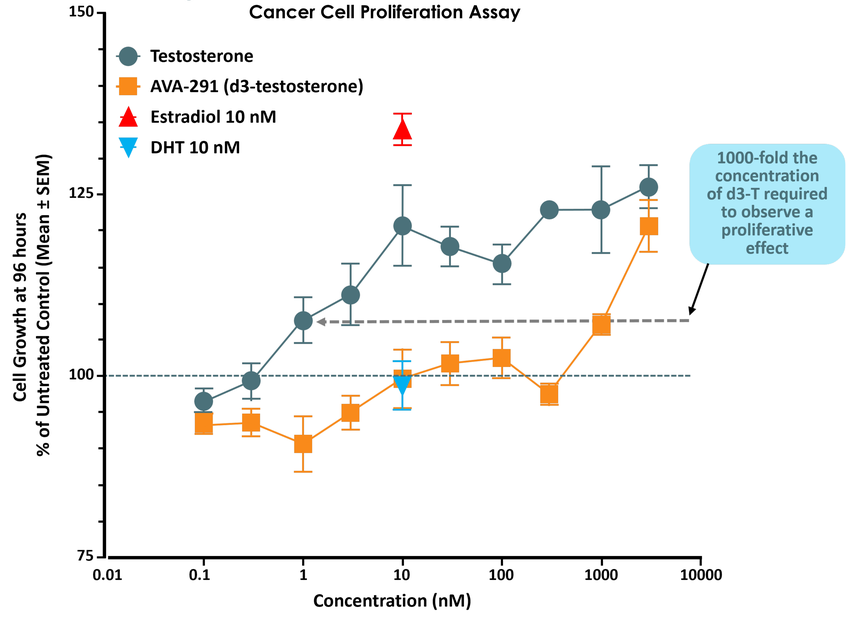

## OBJECTIVE

- **To compare the proliferative effects of AVA-291 and testosterone in MCF-7, an $ER^+$ breast cancer cell line**

## RESULTS

- As expected, T had a proliferative effect on MCF-7 $ER^+$ breast cancer cells starting at 1 nM
- AVA-291 did not induce proliferation of MCF-7 cells until the concentration was increased 1000-fold to 1 µM
- E2 and DHT (positive and negative controls) behaved as expected

**Figure 3.** **AVA-291 vs Testosterone in an MCF-7 $ER^+$ Breast Cancer Cell Proliferation Assay**




- MCF-7 cells were seeded at 4000 cells/well in RPMI-1640 (no phenol red throughout) + 10% fetal bovine serum into 96-well plates to attach overnight at 37°C
- On Day 0, media was removed, cells were washed with media only and replaced with RPMI-1640 + 5% heat inactivated newborn calf serum containing T or AVA-291
  - AVA-291 and T dosing ranged from 0.1 nM to 3 uM
  - E2 and dihydrotestosterone (DHT) included as positive and negative controls, respectively, at 10 nM
  - Cell growth was measured on Day 0 and Day 6 while cells were in the exponential growth phase

## CONCLUSIONS

- **AVA-291 (d3-T), a structurally identical isotopologue of T, is resistant to aromatization to estradiol**
- **In $ER^+$ MCF-7 breast cancer cells, AVA-291 requires a 1000-fold higher concentration than T to induce cellular proliferation**
- **These findings support aromatization as a key driver of testosterone-mediated proliferation in ER+ breast cancer**
- **AVA-291 (d3-T) demonstrates a distinct, highly differentiated cellular proliferation profile compared with T**
- **AVA-291 (d3-T) may be a useful alternative to T in clinical situations where aromatization of T limits its therapeutic potential such as hormone replacement therapy in menopausal women**

[1]Sonne-Hansen K, et al. *J. Steroid Biochem. Mol. Biol.* 2005;93:25-34 [2]Davis S, et al., *N Engl J Med* 2008;359:2005-2017 [3]Shames D et al. *Int. J. Impot. Res.* 2007;19:30-36.