Inhibiting androgen receptor-associated Src signaling with VAL201 inhibits breast cancer growth in an orthotopic xenograft model

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Inhibiting androgen receptor (AR) and estrogen receptor (ER) associated Src signaling has shown promising effects to reduce prostate cancer growth in cancer cell cultures and animal models (1). Inhibition of src by Val201 takes place after androgen binding, allowing inhibition of growth without blocking desirable receptor-dependent transcriptional activity, and thereby eliminating the majority of side effects associated with androgen deprivation therapies. Majority of breast cancers are ER positive, and the hormonal treatment includes depletion of estrogen by antiestrogens or aromatase inhibitors (2). However, up to 90% of ER positive and 20-50% of ER negative breast cancers are AR positive (2, 3), and recently some have been shown to be dependent on AR signaling (4). Furthermore, AI therapy can increase the androgen levels, as androgens are no longer converted to estrogens.

Aim of the Study

We have studied the effects of VAL201 on breast cancer growth in a xenograft model using estrogen-dependent, AR expressing MCF-7 breast cancer cells.

Materials and Methods

Five-week old athymic nude mice (Harlan Laboratories, S.R., Horst, the Netherlands) were allocated to groups according to body weight (n=5-15/group). Slow releasing pellets containing 0.72 mg of 17β-estradiol for 60-day release (Innovative Research of America, Sarasota, FL) were implanted one day before the cell inoculation. MCF-7 human mammary adenocarcinoma cells (ATCC, Manassas, VA) were cultured in standard cell culture conditions, until semi-confluency and inoculated into the inguinal mammary fat pad at day 0. Vehicle and VAL201 at doses 0.004, 0.04, and 4.0 mg/kg were administered daily for 28 days starting at day 1. Tumor growth was monitored by caliper measurements three times a week, and the tumors were weighed at sacrifice. Ki-67 immunohistochemical staining (H-9012-95, Novocastra Laboratories Ltd, Newcastle upon Tyne, UK) was performed on formalin fixed paraffin-embedded tumor sections of control and highest dose groups. The amount of positive cells was quantified from three microscopic fields in each sample with 200x magnification using thresholding techniques to calculate the proportion of positively stained cells. (Bioquant Imaging Software, Nashville, TN; Symbionics, CA). Statistical analysis was performed using linear fixed effect model, one-way ANOVA or non-parametric Friedman-Mann-Whitney test. After preliminary data checking, the 0.004 and 0.04 mg/kg groups were combined to a low-dose group and 0.4 and 4 mg/kg groups to a high-dose group.

VAL201 is a specific inhibitor of androgen receptor (AR) and estrogen receptor (ER) associated Src signaling that has shown promising effects to reduce prostate cancer growth in cancer cell cultures and animal models. Inhibition of src by VAL201 takes place after androgen binding, allowing inhibition of growth without blocking desirable receptor-dependent transcriptional activity, and thereby eliminating the majority of side effects associated with androgen deprivation therapies. Majority of breast cancers are ER positive, and the hormonal treatment includes depletion of estrogen by antiestrogens or aromatase inhibitors (2). However, up to 90% of ER positive and 20-50% of ER negative breast cancers are AR positive (2, 3), and recently some have been shown to be dependent on AR signaling (4). Furthermore, AI therapy can increase the androgen levels, as androgens are no longer converted to estrogens.

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Summary

VAL201 is a novel decapeptide representing the first example of a specific inhibitor of steroid-receptor-dependent signal transducing activity.

- Tumor volume growth curves demonstrated a dose-dependent inhibition of tumor growth by VAL201.
- VAL201 had a dose dependent effect on the tumor volume growth curves compared with vehicle group.
- Similar responses enabled combining the treatment groups to a low-dose group (including the groups receiving 0.004 and 0.04 mg/kg VAL201) and a high-dose group (including the groups receiving 0.4 and 4.0 mg/kg VAL201) to increase statistical power in further analyses.
- High dose of VAL201 increased body weight at sacrifice.
- The combined groups demonstrated that VAL201 decreased tumor volume and weight.

Conclusions

VAL201 showed inhibition of breast cancer growth in this orthotopic xenograft model, demonstrating its potential as a novel therapeutic agent for breast cancer. The growth inhibition was established in the presence of estrogen, underlining the importance of androgen receptor-associated src signaling even without estrogen depletion.

Acknowledgements

We thank Ms Johanna Rantanen and Ms Anniina Luostarinen for their skillful technical assistance.

References