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Inhibiting androgen receptor-associated Src signaling by VAL201 inhibits prostate cancer metastasis in an orthotopic mouse model

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**Introduction**

VAL201 is a specific inhibitor of androgen receptor (AR) and estrogen receptor (ER) associated signaling that has promising effects to reduce prostate cancer growth and the compound is considered as a potential treatment for castration-resistant prostate cancer (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. The ER positive human prostate cancer cell line PC-3 is usually cited as AR negative, but there is evidence of low levels of AR expression as a form that has transcriptional activity that could associate with src (2).

**Aim of the Study**

We have studied the effects of VAL201 on PC-3 prostate cancer cell proliferation in vitro and growth and metastasis in vivo in an orthotopic xenograft model.

**Materials and Methods**

The proliferation effects were studied for 100 µM, 1 µM, 10 nM, 100 nM and 1 µM concentrations of VAL201 by measuring WST-1 values at days 3, 5, 7 and 9. Groups with 1 µM genistein (Centrall, El Lilly, Indianapolis, IN) as reference compound were included in the study. The xenograft study was performed with 8-week-old immunodeficient BALB/c nude mice (Harlan Laboratories, B.V., Horst, the Netherlands) that were allocated to 6 groups according to the body weight (with n=15/group), one group receiving vehicle and the others VAL201 at doses 0.04, 0.4, 4, 10 and 20 mg/kg. PC-3 cells (ATCC, Manassas, VA) in Matrigel (BD, Franklin Lakes, NJ) were inoculated orthogonally into the prostate. Subcutaneous dosing was started at day 1 and continued daily for 28 days. The mice were weighed twice a week. Orthotopic tumors were measured by caliper and the prostate and the lymph nodes were harvested at sacrifice. Metastases in lymph nodes were determined from H&E stained paraffin sections. Statistical analysis was performed using linear fixed effect model, one-way ANOVA or non-parametric Kruskal-Wallis test and Fischer’s exact test.

**Body weight**

**Tumor size and metastasis**

**Summary**

- VAL201 is a novel decapeptide representing the first example of a specific inhibitor of steroid-receptor-dependent signal transduction with the 0.4 mg/kg dose.
- VAL201 showed dose-dependent inhibition of PC-3 cell proliferation that was statistically significant with all doses above 100 µM.
- In the xenograft study VAL201 had no effect on body weight.
- Statistically significant effects on orthotopic tumor growth were not observed despite of a 35% decrease observed in tumor volumes with the 0.4 mg/kg dose.
- Most importantly, 0.04 and 0.4 mg/kg doses of VAL201 showed a significant 50% inhibition on the development of lymph node metastases.

**Conclusions**

VAL1 inhibited proliferation of PC-3 cells in vitro and development of lymph node metastases in a xenograft model, demonstrating its potential for inhibiting prostate cancer growth and metastasis without adverse effects associated with androgen deprivation.

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**References**


**FIGURE 1.** The structure of VAL201, the first example of a specific inhibitor of steroid-receptor-dependent signal transducing activity. VAL201 is a synthetic decapeptide made using standard solid phase techniques. The peptide corresponds to amino acids 377-385 (Ac-PPPYPHRK-NH2) of the human AR, and it was specifically selected as the smallest size that specifically and strongly inhibits the SHD domain mediated binding of Src to the AR.

**FIGURE 2. A:** Proliferation of PC-3 cells during a seven day treatment with either Gemcitabine (1µM or VAL201 (0.016-20µM). (A) Proportion of 4 days of treatment (ABS. 450nm, mean ± SEM). B: Proliferation after seven days of treatment (ABS. 450nm, mean ± SEM). Notation: " vs p<0.05, ** vs p<0.01, *** vs p<0.001.

**FIGURE 3.** A: Body weight during the study (g, mean ± SEM). Statistical differences were not observed. B: The proportion of animals with lymph node metastasis. The incidence of lymph node metastasis. There were no metastases in the groups VAL201: 0.04 mg/kg and VAL201: 0.4 mg/kg compared with vehicle group (VAL201: 0.04 mg/kg p=0.016 and VAL201: 0.4 mg/kg p=0.039). A trend of decreased metastases was observed also in group VAL201: 10 mg/kg (p=0.052). Notation: " vs p<0.05.

**FIGURE 4. A:** Tumor volume at sacrifice (mm³, mean ± SEM). Statistical differences were not observed (p<0.45). B: The proportion of animals with lymph node metastasis. The incidence of lymph node metastasis. There were no metastases in the groups VAL201: 0.04 mg/kg and VAL201: 0.4 mg/kg compared with vehicle group (VAL201: 0.04 mg/kg p=0.016 and VAL201: 0.4 mg/kg p=0.039). A trend of decreased metastases was observed also in group VAL201: 10 mg/kg (p=0.052). Notation: " vs p<0.05.