**ODM-201 – New generation androgen receptor inhibitor targeting resistance mechanisms to androgen signalling-directed prostate cancer therapies**

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**Abstract # 674**

**Background**

Growth of prostate cancer is dependent on stimulation of the androgen receptor (AR). Remarkably, also castration-resistant state of prostate cancer (CRPC) is still dependent on functional AR, and several molecular mechanisms have been proposed to explain the addiction on a functional AR. Known causes of CRPC include gene amplification and overexpression of AR mRNA and protein, point mutations of AR, resulting in promiscuous ligand usage, and increased expression of steroidogenic enzymes, leading to increased intratumoral androgen levels.

AR mutations have also been found in approximately 10–30% of the CRPCs treated with androgen-depleted media for 24 h and luciferase activity was measured.

Testosterone analyses: Serum testosterone levels were measured from intact nude male mice with VCaP orthotopic tumors after 3 week treatment of test compounds using RIA (radioimmunoassay) method.

AR nuclear translocation: AR overexpressing HEK293 cells were treated with 1 µM test compounds together with 0.3 nM testosterone in steroid depleted medium. AR subcellular localisation was studied by immunolabeling of AR with polyclonal AR ab (N-20) (Santa Cruz). DNA was depleted medium. AR subcellular localisation was studied by

**Materials and Methods**

**AR binding affinity:** Binding affinity to wild type AR was determined in cyclospeptic titrations obtained from ventral prostates of castrated rats using a competition binding assay.

**Antagonism of ODM-201 to wt AR:** Functional activity and potency to wtAR were determined in HEK293 cells stably transfected with full-length hAR and androgen-responsive luciferase reporter gene constructs. The cells were treated with test compound and 0.45 nM testosterone in steroid depleted media for 24 h and luciferase activity was measured.

**Transactivation assays with mutant ARs:** Inhibition of mutant ARs (AR(T877A), AR(W741L) and AR(F876L)) by test compounds was studied using RIA (radioimmune assay) method.

**VCaP proliferation assay:** Androgen-sensitive VCaP prostate cancer cells containing endogenous AR gene amplification were treated with sub-maximal concentration (0.1 %) of mibolerone in steroid depleted growth medium. Cell growth was measured using WST-1 Cell Proliferation Assay (Roche) according to manufacturer’s instructions.

**Castration resistant VCaP xenograft:** Tumors were established by subcutaneous injection of VCaP cells into male nude mice. After initial tumor growth, when the average tumor volume reached ~20 mm³, mice were castrated. Oral treatment (ODM-201 50 mg/kg bid, or enzalutamide 20 mg/kg qd for 37 days) was initiated upon tumor regrowth. In case of bicalutamide, oral dose was 20 mg/kg for 43 days. Mean tumor volumes were calculated for each treatment group.

**Testosterone analyses:** Serum testosterone levels were measured from intact nude male mice with VCaP orthotopic tumors after 3 week treatment of test compounds using RIA (radioimmunoassay) method.

**Brain/plasma ratios:** ODM-201 and ORM-1531 concentrations were studied in mouse plasma and brain homogenates after 7-day oral dosing of ODM-201 25-100 mg/kg bid. Enzalutamide concentrations were studied after 7 day oral dosing of 20 mg/kg and ARN-509 concentrations after single oral dose of 10 mg/kg. AUC values for plasma and brain were determined and brain/plasma ratio was calculated.

**Results**

**1. Superior potency of ODM-201 and its active metabolite**

Comparison of AR binding affinity, antagonism and anti-tumor activity to other antiandrogens

**2. Overcoming prostate cancer resistance by suppressing mutant androgen receptors by ODM-201**

a) Altered ligand specificity of AR mutations identified in PCs

**3. Inhibition of AR nuclear translocation by ODM-201**

**4. Low or negligible seizure risk with ODM-201**

**Conclusions**

**ODM-201, a new generation AR inhibitor, overcomes prostate cancer resistance via multiple mechanisms.**

- Superior affinity and activity to wild-type and overexpressed AR
- Excellent antagonistic and antitumor activity both in vitro and in vivo models of CRPC
- Suppression of T877A, W741L and F876L mutant ARs
- Inhibition of androgen-mediated nuclear translocation of AR in AR overexpressing cells
- No brain entry and testosterone elevation in preclinical models

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