Review

Discovery and development of ODM-204: A Novel nonsteroidal compound for the treatment of castration-resistant prostate cancer by blocking the androgen receptor and inhibiting CYP17A1

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A B S T R A C T

We report the discovery of a novel nonsteroidal dual-action compound, ODM-204, that holds promise for treating patients with castration-resistant prostate cancer (CRPC), an advanced form of prostate cancer characterised by high androgen receptor (AR) expression and persistent activation of the AR signaling axis by residual tissue androgens. For ODM-204, has a dual mechanism of action. The compound is anticipated to efficiently dampen androgenic stimuli in the body by inhibiting CYP17A1, the prerequisite enzyme for the formation of dihydrotestosterone (DHT) and testosterone (T), and by blocking AR with high affinity and specificity. In our study, ODM-204 inhibited the proliferation of androgen-dependent VCaP and LNCaP cells in vitro and reduced significantly tumour growth in a murine VCaP xenograft model in vivo. Intriguingly, after a single oral dose of 10–30 mg/kg, ODM-204 dose-dependently inhibited adrenal and testicular steroid production in sexually mature male cynomolgus monkeys. Similar results were obtained in human chorionic gonadotropin-treated male rats. In rats, leuprolide acetate-mediated (LHRH agonist) suppression of the circulating testosterone levels and decrease in weights of androgen-sensitive organs was significantly and dose-dependently potentiated by the co-administration of ODM-204. ODM-204 was well tolerated in both rodents and primates. Based on our data, ODM-204 could provide an effective therapeutic option for men with CRPC.

1. Introduction

Prostate cancer (PCa) is the most common cancer and among the three leading causes of cancer deaths in men in the United States [1] and in Europe [2]. Even if the 5-year survival rate of patients with localized prostate cancer is high, the prognosis for those patients (10–20%) who develop castration-resistant prostate cancer (CRPC) within that 5-year follow-up period is poor [3]. The androgen receptor (AR) signaling pathway plays a major role in PCa, and androgen deprivation therapy (ADT) by surgical or chemical castration is the standard treatment for patients with advanced PCa. Most of the patients initially respond to androgen deprivation therapy, but eventually their cancer progresses and develops into a more aggressive form of the disease. In recent years several clinical trials have shown that the castration-resistant growth is driven, to a large extent, by continued AR signaling, and CRPC remains sensitive to androgens because of

sensitization and amplification of AR [4–9]. Thus, hormonal manipulation with AR blockers or steroid biosynthesis inhibitors reducing the activity of AR-mediated pathway [10], remains the core of CRPC treatment.

Although castration leads to very low levels of circulating testosterone, androgens are still present in the prostate cancer tissues at clinically relevant concentrations, sufficient to activate AR and promote tumour growth [11]. These androgens are most likely synthesized by adrenal glands [12] regulated by the hypothalamic-pituitary-adrenal axis and/or via intratumoral de novo steroidogenesis. Synthesis of testosterone and dihydrotestosterone (DHT), the primary androgens, requires a cascade of oxidative enzymes, one of the key enzymes being CYP17A1, catalyzing two essential steps in the steroidogenic pathway, namely 17-alpha-hydroxylation and 17,20-lyation in the endoplasmic reticulum.

CYP17A1 inhibitors have been designed for the treatment of

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prostate cancer with an androgen-dependent disease. So far, the only CYP17A1-inhibitor approved for clinical treatment of men with CRPC is the small molecule inhibitor Zytiga® (Abiraterone), which has been shown to exhibit a significant overall survival benefit of 4.6 months and objective tumour responses in up to 60% of patients in a post-chemotherapy phase III study [13]. Currently, only one drug candidate inhibiting CYP17A1 is in clinical trials, namely VT-464 (Viamet Pharmaceuticals). VT-464 is shown to be a selective C17,20-lyase inhibitor [14], and it is currently studied in a phase I/II trial in patients with CRPC (EudraCT number: 2011-004103-20). Recently, one phase III study with CYP17A1 inhibitor galeterone, ARMOR3-SV (galeterone compared against enzalutamide) with AR-V7-positive patients was discontinued after a data monitoring committee determined that the trial was unlikely to meet its endpoint. Galeterone claimed to be a multi-targeted steroidal agent and demonstrated to be of being a potent selective CYP17A1 inhibitor and a potent AR antagonist [15] in nonclinical studies. However, the mechanisms of the anticancer effects of galeterone are not completely understood [16].

In addition, a second-generation AR antagonist, Xtandi® (enzalutamide), [17] has been approved for the treatment of metastatic CRPC. In a phase III trial, enzalutamide significantly increased overall survival by 4.8 months [18], decreased the risk of radiographic progression and death and delayed the initiation of chemotherapy in male patients with metastatic PCa [19]. Also, several other AR antagonists are under development, apalutamide (ARN-509) and darolutamide (ODM-201) being the most advanced ones with on-going phase III trials [20,21].

ODM-204, the compound described in the present study, is a novel nonsteroidal compound designed to inhibit the CYP17A1 enzyme and to block AR mediated signaling at the receptor level, specifically developed for the treatment of CRPC. Effects on the growth of prostate cancer cell lines (VCaP and LNCaP) and on steroidogenesis in testicular microsomes and in an adrenocortical tumour cells (H295R) were used as in vitro models. In vivo, the effects of ODM-204 on AR dependent signaling and androgen biosynthesis were studied in primates and rodents, and its anti-tumour properties were assessed in a VCaP xenograft mouse model. On the basis of this preclinical data phase I/II clinical (DUALIDES) was initiated with metastatic CRPC (Fig. 1).

2. Materials and methods

2.1. Chemicals

ODM-204, abiraterone and galeterone were synthesised by Orion Pharma. Several of antiandrogen series were evaluated using structural models of androgen receptor and CYP17A1, to find whether a typical cytochrome heme binding moiety, like imidazole or pyridine derivatives, could be incorporated into their structures without losing the antiandrogen activity. For five of these compound series a number of such imidazole and pyridine derivatives were synthesized, and the series to which ODM-204 belongs was chosen for further optimization because it exhibited the most balanced inhibition of both targets. Synthesis is provided in detail as a supplemental material (Supplement 1). Stock solutions of testosterone (Fluka), DHEA and A-dione (Sigma-Aldrich) for mass spectrometry (MS) standards were prepared in 100% methanol. For in vitro and in vivo functional assays testosterone was purchased from Fluka, mibolerone and ^{3}H-mibolerone from Perkin Elmer Life Sciences Inc., DHT from Sigma, and luteinizing hormone

releasing hormone (LHRH) agonist leuprolide acetate from Takeda Pharmaceutical Company Ltd.

2.2. Cell lines

LNCaP cells were purchased from the European Collection of Cell Cultures (ECACC) in 2003. VCaP cells were obtained in 2006 and both DU-145 and H295R cells in 2008, all from the American Type Cell Culture (ATCC). VCaP cells were (last) tested and authenticated by short-tandem repeat analysis in January 2014. AR-HEK293 cells used in transactivation studies were created by stably transfecting HEK293 cells with an expression vector encoding full-length AR (pSG5-hAR) and an androgen-responsive reporter gene construct (pcDNA3.1/GRE2-TK-Luc). AR nuclear translocation was monitored in a cell clone of AR-HEK293 having over 5-fold overexpression of AR (HS-HEK293, data not shown).

VCaP, AR-HEK293, HS-HEK293, DU-145, and H1581 cells were cultured as described previously [22]. H295R cells were cultured in a mixture (1:1) of DMEM and F12 Nutrient mixture supplemented with 2.5% Nu-Serum, 100 UI/ml penicillin, 100 μg/ml streptomycin, 2 mM GlutaMAX, and certain additives (insulin, transferrin, selenium, BSA and linoleic acid) in the form of ITS + Premix (1%, BD Biosciences). LNCaP cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 UI/ml penicillin, 100 μg/ml streptomycin, HEPES (10 mM) and sodium pyruvate (1 mM) and 2 mM of GlutaMAX. Corresponding phenol red-free media supplemented with steroid-depleted FBS were used in AR activity assays. If not otherwise stated, cell culture reagents were purchased from Gibco. Cells were grown in a humidified incubator with 5% CO_2 at 37 °C.

2.3. Inhibition of CYP17A1 in vitro

Formation of 17α-hydroxyprogesterone from progesterone was tested using human, monkey, and rat testicular microsomes. Human testicular tissue samples were collected at the Turku University Hospital (Finland) from five male patients with testicular tumours or prostate cancer (Clinical study protocol 3102001). Monkey testicular tissue was collected from four sexually mature male monkeys (Mauritius) at Covance Inc. (Munster, Germany) and rat testicular tissue from four sexually mature rats (Bkl:WISTAR and HsdHan:Wistar rats) at Orion Pharma (Turku, Finland). Tissue samples were snap-frozen in liquid nitrogen and stored at –70 °C. Further preparing the microsomes testicular tissues of human and monkey were homogenized 1:3 in ice-cold 0.1 M phosphate buffer (pH 7.4, containing 5 mM $MgCl_2$) in individual tubes with homogenizing probes (Omni International) and rat testicular tissue in cold phosphate buffer (1:4) using Potter-S homogenizer (Sartorius Stedim Biotech).

Microsomal fractions were isolated by differential ultra-centrifugation at + 4 °C. Microsomal pellets were re-suspended in homogenization buffer and protein concentrations measured with a protein assay kit (human and monkey samples with protein assay dye reagent concentrate, Bio-Rad, and rat samples with Pierce micro BCA protein assay kit, (Thermo Scientific).

For CYP17A1 inhibition studies, microsomal pools were prepared for each species by combining equal amounts of protein from each individual sample. Progesterone (0.5 μM for human and monkey, 1.5 μM for rat, final incubation concentrations), 0.1 mM phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, and microsomes (0.05 mg/ml of final
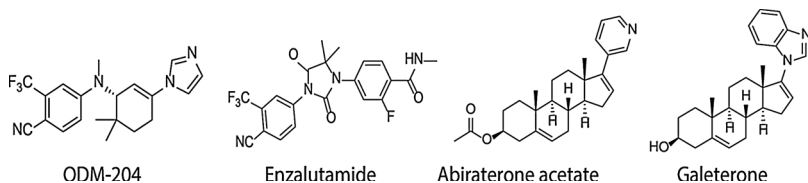

Fig. 1. "Molecular structures of ORM-204 and reference compounds: enzalutamide, abiraterone (Abi) and galeterone (Gal)".

ODM-204   Enzalutamide   Abiraterone acetate   Galeterone

incubation) were preincubated (4 min) in the absence and presence of the study compounds (ODM-204, abiraterone, and galeterone) at six concentrations. Enzymatic reactions were started by adding NADPH (Sigma Aldrich) and terminated after 15 min by adding ice-cold acetonitrile. The samples were analysed using liquid chromatography-mass spectrometry (LC/MS-MS) to determine the level of 17α-hydroxyprogesterone.

Conversion of 17α-[21-^{3}H]hydroxypregnenolone (American Radiolabelled Chemicals) into dehydroepiandrosterone (DHEA) and ^{3}H-labeled acetic acid was studied with the human adrenal cortex cell line H295R, which has been shown to express all the key steroidogenic enzymes [23]. Cells were seeded on poly-D-lysine 96-well plates (Corning) and incubated overnight at + 37 °C before starting the assay. Different concentrations of ODM-204, abiraterone, and galeterone were added on cells in four replicates. Immediately thereafter, ^{3}H-labeled 17-α-hydroxypregnenolone was added to each well resulting in a final concentration of 3 nM and cells were incubated overnight at + 37 °C, 5% CO_2.

To determine the inhibitory effect of the test compounds, we measured the amount of ^{3}H-labeled acetic acid [24], in the supernatant samples. This was accomplished by extracting all steroids from the reaction mixture with dextran-coated charcoal suspension [24], adding two volumes of scintillation liquid (OptiPhase Supermix, PerkinElmer) to each sample, and determining radioactivity with a microplate counter (1450 MicroBeta Trilux, Liquid Scintillation & Luminescence Counter, Wallac) the next day.

2.4. AR antagonism

AR antagonism of ODM-204 was studied with previously described assays [22]. Briefly, Ki values were determined by letting the test compounds compete against a radio ligand, [^{3}H]mibolerone, in cytosolic lysates from ventral prostates of castrated rats. AR-HEK293 cells, expressing full-length AR (pSG5-hAR) and an androgen-responsive reporter gene construct (pcDNA3.1/GRE2-TK-Luc) were used in transactivation studies and AR-dependent luciferase activity was measured with a Centro LB 960 microplate luminometer (Berthold Technologies) using a luciferase assay system (Promega Corporation) according to the manufacturer's instructions.

Finally AR nuclear translocation was tested in a cell clone of AR-HEK293 having over 5-fold overexpression of AR. Cells were treated with ODM-204 or enzalutamide together with testosterone for 4 h. Fixed cells were incubated with polyclonal AR antibody conjugated with Alexa Fluor® 488 (Santa Cruz, sc-816, dilution 1:75) and DNA was labeled with DAPI (Sigma, 1 μg/mL). The images were analysed with a NucTrans V3 assay algorithm (Thermo).

2.5. VCaP and LNCaP proliferation assays

Androgen-responsive VCaP (AR overexpression) and LNCaP (AR mutation T878A) PCa cells were treated with an previously optimized concentration of mibolerone (0.06 nM for VCaP and 0.3 nM for LNCaP) and increasing concentrations of ODM-204 in steroid-free medium. After 4-day incubation with the compounds, cell viability was measured using a WST-1 assay (Roche), according to the manufacturer's instructions. To rule out other than AR- mediated toxicity, AR-negative prostate cancer cells (DU-145) and lung cancer cells (H1581) were treated with an increasing concentration of ODM-204, and cell viability was measured as described above (Supplement 2).

2.6. Inhibition of testosterone, DHEA, and A-dione production in H295R cells and measuring the steroids by LS-MS/MS

Metabolism of the main adrenal androgenic hormones (A-dione, DHEA, and testosterone) was studied in detail using a human adrenal cortex cell line H295R. Cells were seeded (60,000 cells/well) on poly-D-

lysine 96-well plates (Corning) and incubated overnight at + 37 °C, 5% CO2 before starting the assay. Different concentrations of ODM-204, abiraterone, and galeterone were added on cells in duplicates. Media were collected and concentrations of testosterone, DHEA, and A-dione were determined by an ultra-performance liquid chromatography and tandem mass spectrometer.

The method was performed in terms of specificity, sensitivity, calibration range, precision, and accuracy. The calibration range of the method was 0.05–100 ng/ml for testosterone and A-dione, and 1–500 ng/ml for DHEA. The sample preparation was carried out by using liquid-liquid extraction. An aliquot of acidified medium was extracted using ethyl acetate:hexane (20:80, v:v). The analytes were isolated with gradient elution in an Acquity UPLC® BEH C18, 100 × 2.1 (1.7 μm) column followed by an electrospray ionisation and detected using the selected reaction monitoring by UPLC-MS/MS. For quantification of testosterone, DHEA, and A-dione, an internal standardization based on peak area ratios was used.

The quantitative analyses of ODM-204 in monkey plasma, the plasma samples were prepared by liquid-liquid extraction and analysed with a triple quadrupole mass spectrometer (Waters Acquity UPLC-TQD). The analyte was detected using selected reaction monitoring and quantified by external standardisation. The calibration range of the method was 0.5–3000 ng/ml and the lower limit of quantification (LLOQ) was 0.5 ng/ml.

All in vivo studies were conducted in accordance with EU legislation (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Official Journal of the European Union L276, 33–79 (2010 Sep 2022) (2010)), and approved by the local Institutional Animal Care and Use Committees (Finnish and German) Animal Experiment Board.

2.7. Inhibition of CYP17A1 in rats in vivo

The effect of a single oral dose of ODM-204 (3, 10, 30, or 100 mg/kg) on the testosterone production was studied in intact male rats (8 weeks of age, 234–297 g HsdRCCHanWist, Harlan, The Netherlands: n = 5/group). Animals were housed in IVC-cages, with aspen chips as a housing material, maximum 3 animals/cage, a temperature and humidity-controlled room (22 ± 2 °C) with a 12 h:12 h light/dark cycle. Water and standard rodent diet available ad libitum. For comparison, reference compounds abiraterone and galeterone were also studied in the assay with the dose of 30 mg/kg. Test compounds were orally administered, followed one hour later by intramuscular (i.m.) injection of the hCG (Chorulon, 100 U/kg). Two hours after the hCG dosing, blood samples were taken from tail vein, and serum was separated by centrifugation. Serum testosterone concentrations were determined by ELISA kit (Demeditec Diagnostics, Germany) according to the manufacturer's instructions.

Repeated dosing: Sprague Dawley male rats (6 weeks of age, 200–241 g in the beginning of the study); Harlan, The Netherlands: n = 9/group. Animals, were housed in IVC-cages, with aspen chips as a housing material, maximum 3 animals/cage, a temperature and humidity-controlled room (22 ± 2 °C) with a 12 h:12 h light/dark cycle. Water and standard rodent diet were available ad libitum. Rats were treated with a single dose of the LHRH agonist leuprolide acetate (Enanton®, 0.525 mg/animal), and 7 days later an oral administration with ODM-204 at doses (10, 30, or 50 mg/kg/day) was initiated and continued for 14 days. After the treatment period serum testosterone levels and weights of androgen-sensitive tissues were measured. A group of rats was also castrated, under isoflurane inhalation anaesthesia, carprofen were used for postoperative pain relief, to compare the effects of surgical castration and chemical castration induced by the LHRH agonist. The effects of treatments on the weights of prostate (ventral and dorsolateral), seminal vesicles, testis, adrenal gland and liver were determined. In addition, the effects of treatments on pituitary-gonadal axis and steroid synthesis pathways were evaluated by
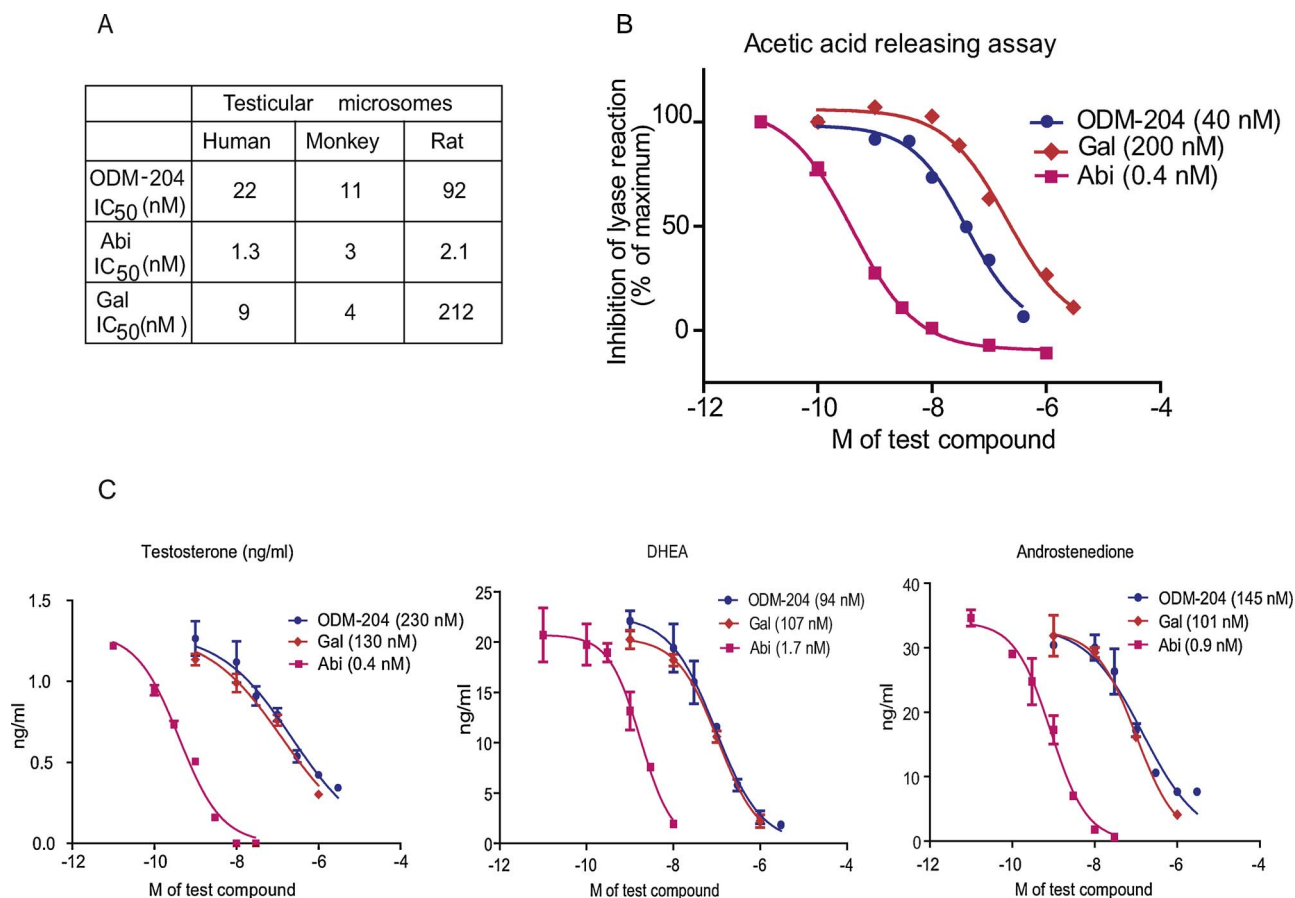
A

	Testicular microsomes		
	Human	Monkey	Rat
ODM-204 IC_{50} (nM)	22	11	92
Abi IC_{50} (nM)	1.3	3	2.1
Gal IC_{50} (nM)	9	4	212

Fig. 2. "ODM-204, abiraterone (Abi) and galeterone (Gal) inhibit CYP17A1 in low nanomolar level, and reduce testosterone, DHEA and androstenedione in vitro".
A) Inhibition of 17-alpha-hydroxylase in human monkey and rat testicular microsomes. B) Inhibition of 17,20-lyase reaction in NCl-H295R cells, C) Inhibition of testosterone, DHEA and androstenedione production the CYP17A1-inhibitors ODM-204, abiraterone (Abi) and galeterone (Gal).

analysing testosterone, LH, cortisol, estradiol, progesterone, and ACTH concentrations in serum.

Testosterone radioimmunoassay and LH immunofluorometric assay were performed using previously described methods [25,26]. ACTH was measured as a part of the MAP rat pituitary magnetic bead panels (Milliplex) and steroid /thyroid hormone magnetic bead panel (Milliplex), were used for cortisol, estradiol, and progesterone.

2.8. VCaP xenografts

In intact or CRPC VCaP models Hsd:Athymic Nude-Foxn1nu male mice (7 weeks of age, Harlan, Netherlands, n = 10–12/group) (Animals were housed in IVC-cages, with aspen chips as a housing material, maximum 3 animals/cage, a temperature and humidity-controlled room (22 ± 2 °C) with a 12 h:12 h light/dark cycle. Water and standard rodent diet available ad libitum), were subcutaneously injected with 2 million VCaP cells in 150 µl of RPMI-1640 medium and Matrigel (BD) (1:2). Tumour growth was monitored twice weekly by calliper measurements. The volume of the tumours was calculated according to the formula W^2 x L/2 (mm^3), where W is the shorter and L the longer diameter of the tumour. Oral treatments with ODM-204 50 mg/kg/day in (0.5% w/v methyl cellulose suspension) or abiraterone acetate 33.7 mg/kg/day in (5% Dimethylamine(DMA) :50% Polyethyleneglycol 300 : 10% Tween80: 35% glucosteril) solution were started, when the average tumour volume reached ~150 mm^3, and continued for 21 days.

In CRPC VCaP model animals were castrated under isoflurane anaesthesia, when the average tumor volume reached 200 mm^3. Oral treatments with ODM-204 and equivalent human dose of abiraterone

acetate, both with physiological dose of the glucocorticoid supplement prednisone (2 mg/kg/day p.o.) or vehicle, were started when the tumors were re-growing (~2 weeks after castration) and continued 24–32 days.

2.9. ODM-204 pharmacokinetic and pharmacodynamics effects of ODM-204 in primates

Sixteen purpose-bred cynomolgus monkeys (Macaca fascicularis) of Mauritian origin were included in the study. The pre-dose body weight of the animals was 7–10.4 kg and the age range 5–9 years. Maturity of males was proven by the presence of sperm in the ejaculate. Animals were housed in a temperature-controlled room (22 ± 2 °C) with a 12 h:12 h light/dark cycle.

Animals were divided into four groups and blood samples for ODM-204 analytics were collected 1, 2, 5, 8, 10, 12 and 24 h after single once or twice daily drug administration (vehicle, 10 mg/kg q.d. or b.i.d. 8 h apart and 30 mg/kg in 0.5% w/v methyl cellulose suspension). The samples were collected from the femoral vein and sentrifuged to obtain plasma that was kept frozen at –20 °C ± 4 °C prior to analysis. Pharmacodynamic effects of ODM-204 were studied by measuring the following hormones with immunoassays: testosterone (Beckmann Coulter 335 B), DHEA (DSL 8900 Beckmann Coulter 130819) cortisol (Beckmann Coulter 130 722) from individual plasma samples taken at 5 and 10 h time points after first dose of ODM-204; progesterone (Beckmann Coulter TPG1 1329) and luteinizing hormone (Siemens TK TT5 1810) from pooled plasma samples (5 and 10 h time points) after the first dose of ODM-204.
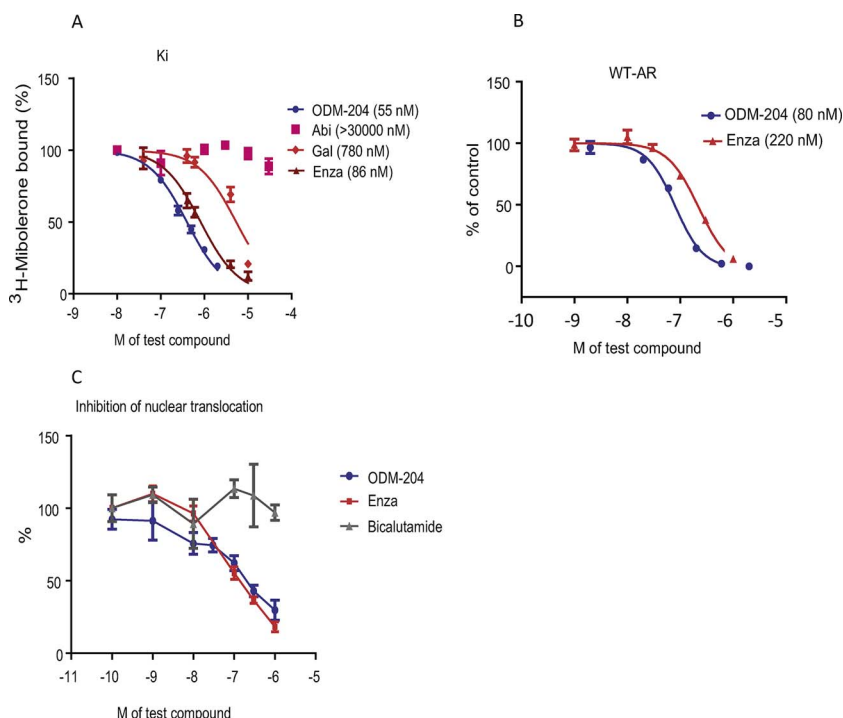
A

B

C

Fig. 3. "ODM-204 binds to AR, shows AR antagonism and inhibits nuclear translocation ≥ than enzalutamide (Enza). Abiraterone (Abi) does not bind to AR".
A) Competitive binding affinities of ODM-204, abiraterone (Abi), galeterone (Gal) and enzalutamide (Enza) to wtAR. All data points are means of quadruplicates ± SEM. B) Antagonism of ODM-204 or enzalutamide (Enza) to wtAR. All data points are means of triplicates ± SEM. C) AR nuclear localization of ODM-204, enzalutamide or bicalutamide as percentage of the control. All data points are means of triplicates ± EM.

2.10. Statistics

For in vitro data (if not otherwise stated) were analyzed with GraphPad Prism 7 (software version 7.03) to obtain Ki and IC50 values. In vivo murine data were analysed with SPSS 19.0 Statistical Package (SPSS Inc., Chigaco, IL, USA). Data were reported as the mean ± SEM for each group. In rat and mouse efficacy studies one-way-analysis of variance (ANOVA) was used to evaluate statistically significant differences ($p < 0.05$) between treatment groups. If significant differences were found, Dunnett's post hoc t-test was carried out. In monkey PK/PD study plasma hormone results were expressed as percentages of the mean pre-treatment values (mean of values 72, 48, 24 h before dosing), because it is well known, that plasma hormone levels variate a lot among individuals, and in addition circadian rhythm and possible stress mediated variation may cause effects.

3. Results

3.1. ODM-204 inhibits CYP17A1 in vitro

The inhibitory effect of ODM-204 on the CYP17A1-mediated hydroxylation required for the formation of 17α-hydroxyprogesterone from progesterone was analyzed using testicular microsomes. The IC_{50} values for ODM-204 were 22, 11, and 92 nM for human, monkey, and rat testicular microsomes, respectively. The corresponding IC_{50} values for human monkey and rat for abiraterone were 1.3, 3, and 2.1 nM and for galeterone, 9, 4, and 212 nM, respectively (Fig. 2a).

ODM-204 was shown to inhibit the 17,20-lyase catalysed conversion of 17α-hydroxypregnenolone to dehydroepiandrosterone and acetic acid, which was measured by acetic acid release assay (AARA) in the H295R human adrenocortical carcinoma cell line. The IC_{50} for ODM-204 was 40 nM, whereas for abiraterone and galeterone, the IC_{50} values were 0.4 and 200 nM, respectively (Fig. 2b). Furthermore ODM-204 inhibited the production of DHEA, A-dione, and testosterone with IC_{50} values of 94, 145, and 230 nM, respectively in cultured H295R cells. Under identical conditions the IC_{50} values for abiraterone, were 1.7, 0.9, and 0.4 nM, and for galeterone, 107, 101, and 130 nM, respectively (Fig. 2c).

3.2. ODM-204 is a potent androgen receptor antagonist

In a competitive AR binding assay, the inhibition constant (Ki) value for ODM-204 was 55 nM, whereas the corresponding Ki value for galeterone was 780 nM. Abiraterone was found to show only 13% inhibition at a 30 μM concentration, and therefore, no Ki value could be determined. For enzalutamide, the Ki value was 86 nM (Fig. 3A). The most potent AR binding compounds, ODM-204 and enzalutamide, were further tested with transactivation assays in AR-HEK293 cells stably expressing full-length human AR and an androgen-responsive luciferase reporter gene construct. ODM-204 was a potent and full antagonist for human AR with an IC_{50} value of 80 nM. In this assay, enzalutamide had an IC50 value of 220 nM, suggesting that ODM-204 has a higher AR antagonist potency than enzalutamide (Fig. 3B). Immunocytochemical labelling with an anti-AR antibody in AR overexpressing HS-HEK293 cells was used to investigate the effect of ODM-204 on the subcellular localization of AR. In this model, in the absence of the AR-ligand immunofluorescence is predominantly cytoplasmic and treatment of cells either with testosterone or bicalutamide induces nuclear translocation of AR. On the contrary, both ODM-204 and enzalutamide were equipotent in inhibiting the testosterone-induced nuclear translocation of AR (Fig. 3C).

3.3. ODM-204 significantly inhibits steroid production in intact rats

To study the potency of ODM-204 in vivo, we studied serum testosterone concentrations after oral administration of ODM-204 at doses 3, 10, 30, and 100 mg/kg in intact hCG induced male rats (n = 5). The study showed that serum testosterone levels were significantly (***p < 0.001) decreased by all doses of ODM-204, as with the reference compounds, abiraterone and galeterone by the studied dose of 30 mg/kg (Fig. 4). Furthermore, both surgical and chemical castration (LHRH agonist) significantly decreased the weights of androgen-sensitive organs (seminal vesicles and ventral and dorsolateral prostates) in rats. ODM-204 was observed to potentiate the inhibitory effects of an LHRH agonist on androgen-sensitive parameters by further decreasing serum testosterone levels and reducing the weight of androgen-sensitive organs in male rats (Fig. 4). The treatments had no effect on the weights
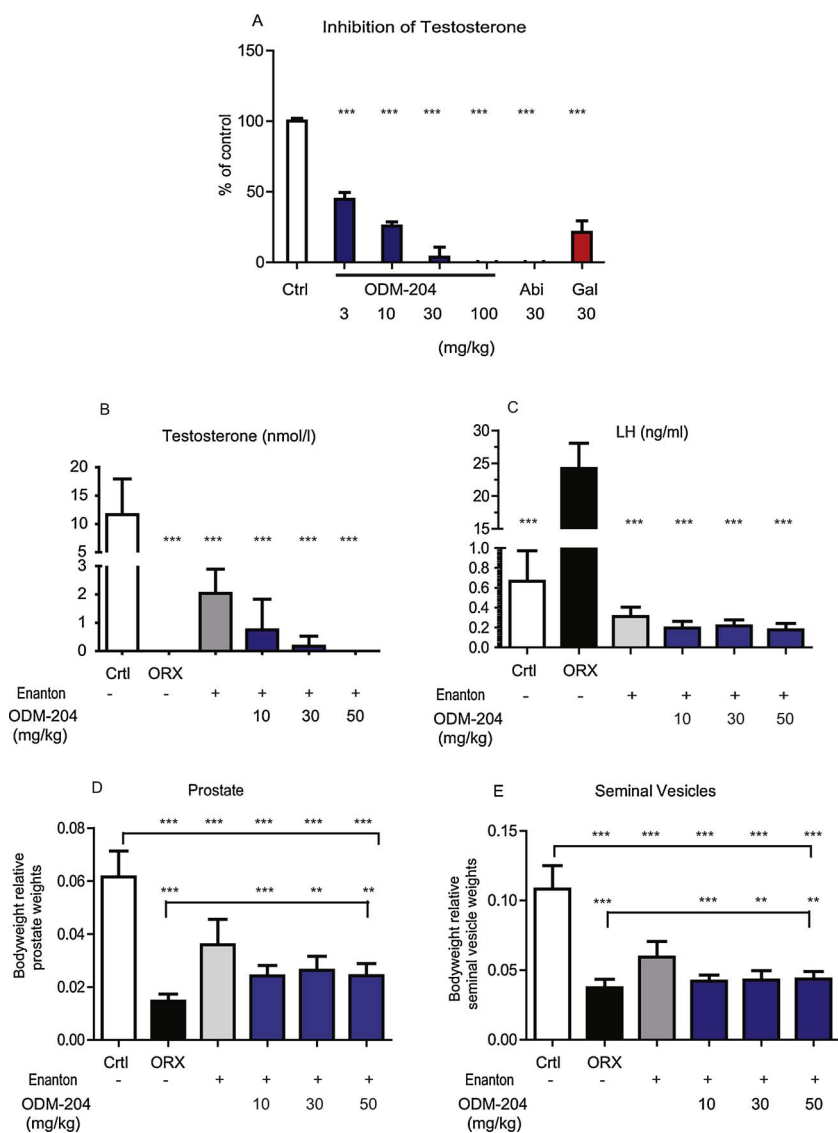
Fig. 4. "ODM-204 dose dependently inhibits testosterone production in vivo. Combination of ODM-204 with Enanton® potentiates the antagonistic activity in intact rats".

A) The effect of ODM-204 (3, 10, 30 or 100 mg/kg), abiraterone (Abi) (30 mg/kg) and galeterone (Gal) (30 mg/kg), on testosterone-synthesis pathway in hCG-injected male rats. Mean + SD, n = 5. Statistically significant differences were observed between the vehicle control and the treatment groups by Dunnett´s test. In intact male rat model ODM-204 suppresses the testosterone (B) and the LH (C) production and suppresses the weight of prostate (D) and seminal vesicles (E). Mean + SEM, n = 9. ** p < 0.01, *** p < 0.001 versus intact or ORX control by Dunnett's test.

of adrenal gland or liver, nor did they affect the amount of cortisol or estradiol in serum or adrenocorticotropic hormone (ACTH) in plasma (data not shown).

3.4. ODM-204 inhibits prostate cancer cell proliferation in vitro and tumour growth in a VCaP xenograft model

The anti-proliferative effect of ODM-204 was studied in vitro using in two prostate cancer cell lines VCaP and LNCaP (Fig. 5A and B). ODM-204 suppressed androgen-induced proliferation of LNCaP cells more efficiently than enzalutamide, abiraterone or galeterone (IC$_{50}$ values 200 nM, 880 nM, 1300 nM and 2600 nM, respectively). Proliferation of VCaP cells was inhibited by ODM-204 and enzalutamide with equal potency (260 nM and 315 nM), while galeterone and abiraterone were less efficient (2000 and 9000 nM, respectively). To confirm that the anti-proliferative effect was antiandrogen-mediated, ODM-204 was shown not to have an effect on the viability of AR-negative DU-145 prostate cancer and H1581 lung cancer cell lines (Supplement 2).

To elucidate the efficacy of ODM-204 in vivo, male nude mice (n = 10–12/group) with subcutaneously injected VCaP cells were treated orally with ODM-204 (50 mg/kg) or abiraterone acetate (33.7 mg/kg) once daily for 21 or 24 days. The doses were selected based on our pharmacokinetic studies in intact male mice (data not shown), and the treatment was started when mean tumor volume

reached 130 mm^3. In this study ODM-204 significantly reduced the intact VCaP tumor growth as compared with the vehicle-treated mice (p < 0.001) (Fig. 5C and D). In similar setting, abiraterone acetate failed to show efficacy in the model. There were no signs of treatment-related toxicity, and the body weights of the mice did not differ during the ODM-204 treatment from the vehicle treated control animals (Supplement 3).

ODM-204 (60 mg/kg/day) and abiraterone acetate (33.7 mg/kg/day), both with a combination of the physiological dose of glucocorticoid supplement prednisone (2 mg/kg/day p.o.), were also further studied in the CRPC VCaP model in orchidectomised animals. The dosing was started when tumors started to re-growth after the orchidectomy. ODM-204 significantly (p < 0.05) inhibited VCaP CRPC tumor growth, whereas abiraterone acetate did not show any efficacy in the model (Fig. 5E and F).

3.5. ODM-204 decreases T and DHEA levels and shows favourable pharmacokinetic properties in sexually mature male monkeys

In order to better predict the potential efficacy of ODM-204 in human patients with CRPC, its pharmacokinetic properties were studied in primates. The data indicated that ODM-204 was well tolerated in sexually mature male cynomolgus monkeys after oral doses of 10–30 mg/kg. Exposures were dose-dependent and showed good drug
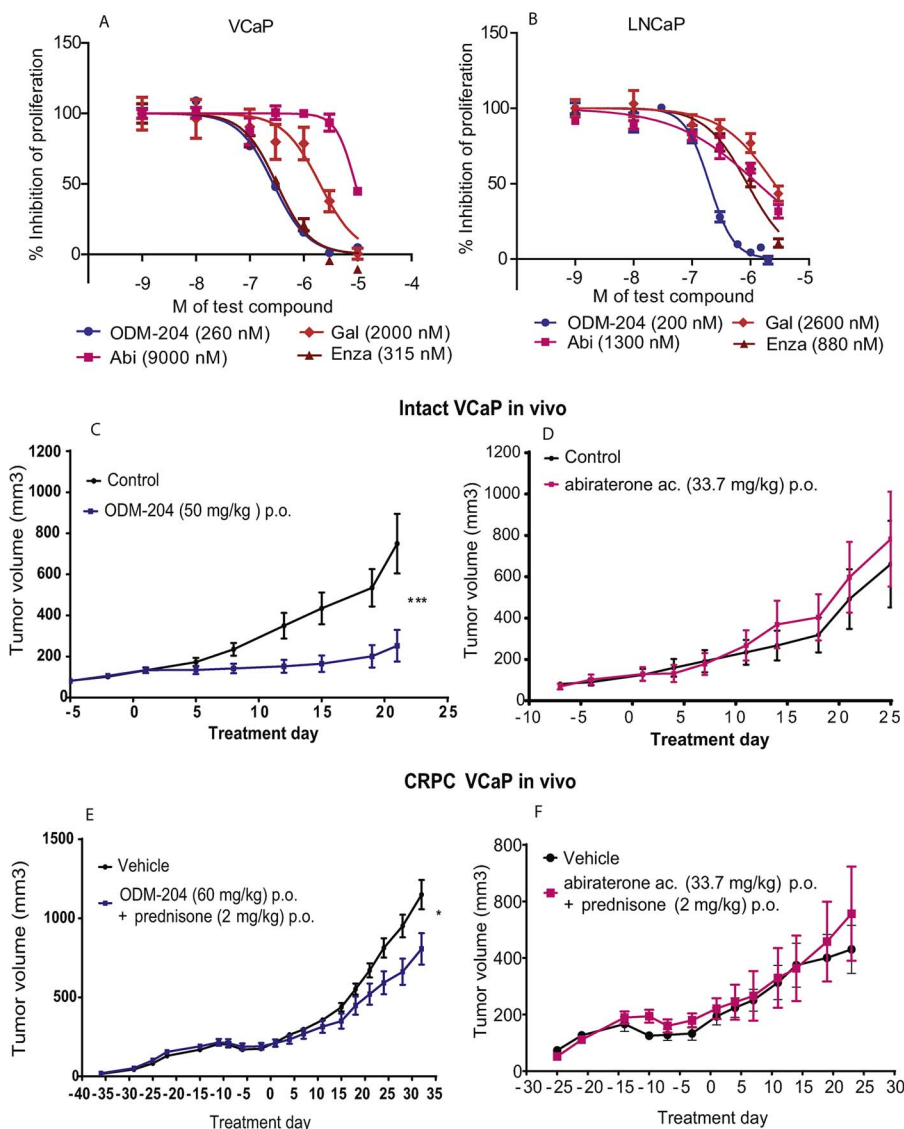
Fig. 5. "ODM-204 shows dose-dependent inhibition of proliferation in vitro, and significantly inhibits tumor growth in intact and CRPC VCaP models".

Inhibition of proliferation with ODM-204, abiraterone (Abi), galeterone (Gal) and enzalutamide (Enza) in VCaP (A) and LNCaP (B) prostate cancer cells. Representative graph of the inhibitory effects of test compounds on the viability of cells is shown. All data points are means of quadruplicates $\pm$ EM. IC_{50} values are presented in parentheses. Mean tumor volumes (mm^3, $\pm$ EM) in intact nude mice with subcutaneous VCaP tumors after the oral treatment with (C) ODM-204 (50 mg/kg/day) or (D) abiraterone acetate (33.7 mg/kg/day). ***$p < 0.001$ vs. intact. Mean tumor volumes (mm^3, $\pm$ EM) in castrated male nude mice with subcutaneous VCaP tumors after the oral treatment with (E) ODM-204 (60 mg/kg/day) or (F) abiraterone acetate (33.7 mg/kg/day) both with combination of physiological dose of Prednisone (2 mg/kg/day). *$p < 0.05$ vs vehicle.

absorption and similar metabolism at all used dose levels (Fig. 6F). Testosterone levels were markedly reduced (Fig. 6A) already after a single oral dose of ODM-204, irrespective of the dose group. DHEA levels decreased (Fig. 6B) immediately with the doses 10 mg/kg twice daily and 30 mg/kg/day and remained lower than the control throughout the study. Also the levels of cortisol decreased (Fig. 6C), however reduction was more distinct at later 10 h time point. As expected, the concentration of both LH and progesterone increased (Fig. 6D and E) dose-dependently as a function of time. While the inter-animal variation was fairly large as far as LH was concerned, the variation was in line with testosterone, and the effect on progesterone was unambiguous.

4. Discussion and conclusion

Castration-resistant prostate cancer (CRPC) is characterized by high androgen receptor (AR) expression and persistent activation of AR signaling axis by residual tissue androgens, which are mainly produced by adrenals via de novo synthesis from cholesterol [27]. On the other hand, overexpression of AR [28] and the increase in progesterone, a weak AR agonist [29], have been reported as one potential resistance mechanism to abiraterone therapy in metastatic CRPC [30]. Thereby, the dual inhibition of AR and CYP17A1 has been suggested to provide a more effective therapy than inhibition with either therapy alone [31].

Because of this, several phase III/IV clinical combination trials have been initiated with abiraterone and enzalutamide: ALLIANCE A031201 (abiraterone + enzalutamide in 1st line treatment of mCRPC, NCT 01949337), STAMPEDE arm J (abiraterone + enzalutamide in mCRPC, NCT00268476) [32], and PLATO (abiraterone + enzalutamide after progression on enzalutamide, NCT01995513), the last one of which was, however, recently reported to be negative for its primary endpoint, progression-free survival [33]

ODM-204, according to our preclinical models, exhibits AR antagonism at least on the similar level as enzalutamide [17]. In line with this, ODM-204 dose-dependently inhibits proliferation of two clinically relevant AR-dependent prostate cancer cell lines: 1) VCaP cells, which have an endogenous AR gene amplification leading to AR protein overexpression [34] typical for CRPC in vitro and in vivo, and 2) LNCaP cells, which express the T878A mutated androgen receptor [35,36]. Additionally, dose dependent inhibition of steroid biosynthesis both in rat and human testicular microsomes and in cellular assays indicate that the efficiency of ODM-204 to inhibit CYP17A1 is similar to what has been shown for galeterone. Whereas abiraterone had significantly better CYP17A1 inhibitory IC_{50}-values in vitro, we could not, however, demonstrate in vivo activity in VCaP prostate cancer models. This may be due to the finding that the preclinical VCaP model, and especially in the CRPC stage, high AR-levels in the tumors are observed [37]. This, combined with known species differences in the adrenal androgen
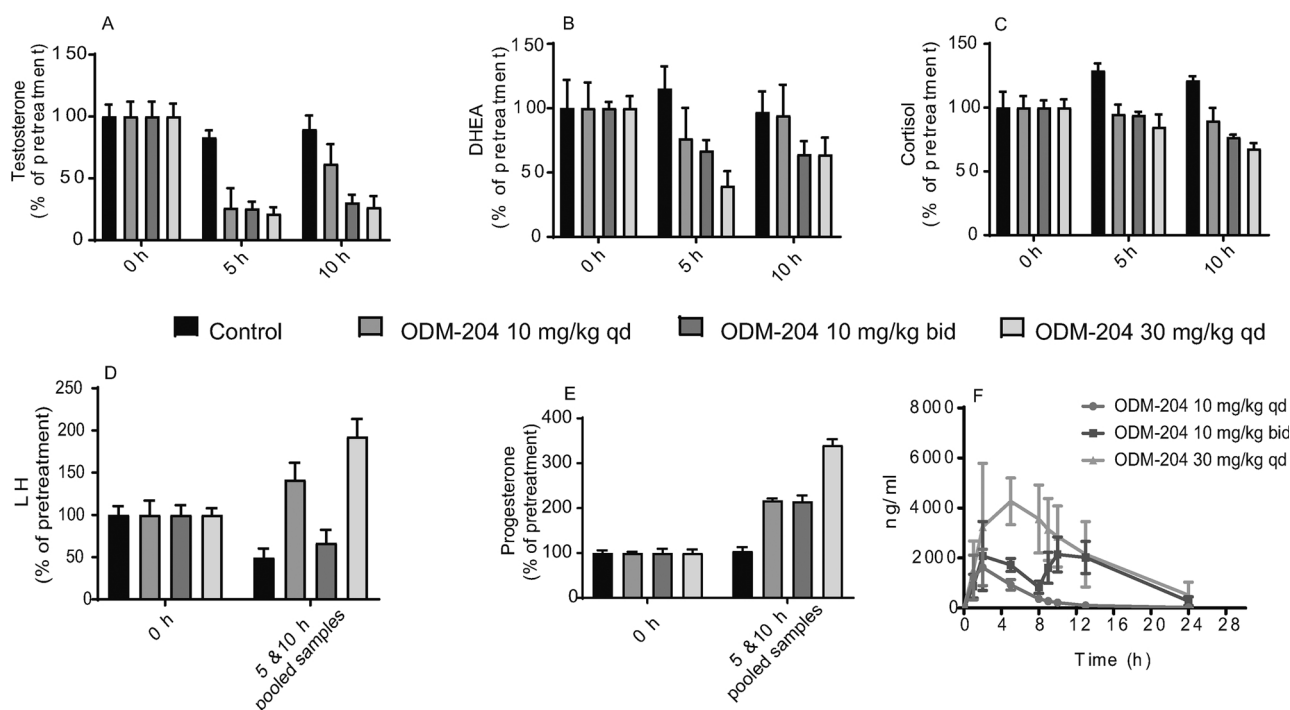
Fig. 6. "ODM-204 dose-dependently decreases testosterone, DHEA and cortisol, while increases in LH and progesterone plasma levels were detected in male monkeys".
Single (10 or 30 mg/kg) or repeated (10 mg/kg/bid) doses of ODM-204 in male mature monkeys decrease serum testosterone (A), DHEA (B), and cortisol (C) levels. Each value represents mean + SEM (n = 4) and is expressed as percentages of the mean pretreatment values. The levels of LH (D) and progesterone (E) increased ODM-204 exposure dependently (F). Each value represents mean + SD (n = 4).

synthesis [38], might make VCaP model better suited for studing antiandrogenic compounds. In rat, ODM-204 further potentiated the effect of leuprolide acetate by dose-dependently suppressing testosterone production and decreasing the weight of androgen-sensitive organs.

In primates, the endocrine regulation and secretion of adrenal steroids are very similar to that in humans [39], and, unlike other species, monkeys share very high homology with the human CYP17A1 enzyme [40,41]. In male cynomolgus monkeys, ODM-204 showed favourable oral bioavailability and PK properties already after single oral doses of 10–30 mg/kg. In line with in vitro data, ODM-204 induced dose-dependent inhibition of both principal androgens T and DHEA, secreted from testicles and adrenals already after a single oral dose. In the following toxicological studies, ODM-204 was shown to be well tolerated (data not shown).

In conclusion, ODM-204 is a potent dual inhibitor of CYP17A1 and AR. It has activity similar to that of galeterone towards CYP17A1, and it also blocks AR action to the same degree as enzalutamide. The compound was shown to efficiently inhibit steroid biosynthesis in rodents and primates, and it presented promising antitumor activity in animal models of human prostate cancer. Further, it is possible that single agent medication, like ODM-204 having dual inhibitory properties, may provide benefit in CRPC patients, due to the lack of drug interaction potential unlike with treatment combinations [42] and/or the pharmacokinetic exposure benefits. Taken together, ODM-204 has therapeutic potential to be an effective treatment of CRPC.

Conflict of interest

All authors are employed by Orion Pharma, Orion Corporation, Finland.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jsbmb.2018.02.004.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, CA Cancer J. Clin. 65 (2015) 5–29.
[2] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer 49 (2013) 1374–1403.
[3] M. Kirby, C. Hirst, E.D. Crawford, Characterising the castration-resistant prostate cancer population: a systematic review, Int. J. Clin. Pract. 65 (2011) 1180–1192.
[4] M.J. Linja, K.J. Savinainen, O.R. Saramaki, T.L. Tammela, R.L. Vessella, T. Visakorpi, Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer, Cancer Res. 61 (2001) 3550–3555.
[5] H.I. Scher, C.L. Sawyers, Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis, J. Clin. Oncol. 23 (2005) 8253–8261.
[6] L. Bubendorf, J. Kononen, P. Koivisto, P. Schraml, H. Moch, T.C. Gasser, N. Willi, M.J. Mihatsch, G. Sauter, O.P. Kallioniemi, Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays, Cancer Res. 59 (1999) 803–806.
[7] T. Visakorpi, E. Hyytinen, P. Koivisto, M. Tanner, R. Keinanen, C. Palmberg, A. Palotie, T. Tammela, J. Isola, O.P. Kallioniemi, In vivo amplification of the androgen receptor gene and progression of human prostate cancer, Nat. Genet. 9 (1995) 401–406.
[8] K.K. Waltering, A. Urbanucci, T. Visakorpi, Androgen receptor (AR) aberrations in castration-resistant prostate cancer, Mol. Cell. Endocrinol. 360 (2012) 38–43.
[9] C.S. Grasso, Y.M. Wu, D.R. Robinson, X. Cao, S.M. Dhanasekaran, A.P. Khan, M.J. Quist, X. Jing, R.J. Lonigro, J.C. Brenner, I.A. Asangani, B. Ateeq, S.Y. Chun, J. Siddiqui, L. Sam, M. Anstett, R. Mehra, J.R. Prensner, N. Palanisamy, G.A. Ryslik, F. Vandin, B.J. Raphael, L.P. Kunju, D.R. Rhodes, K.J. Pienta, A.M. Chinnaiyan, S.A. Tomlins, The mutational landscape of lethal castration-resistant prostate cancer, Nature 487 (2012) 239–243.

[10] J.S. de Bono, C.J. Logothetis, A. Molina, K. Fizazi, S. North, L. Chu, K.N. Chi, R.J. Jones, O.B. Goodman Jr, F. Saad, J.N. Staffurth, P. Mainwaring, S. Harland, T.W. Flaig, T.E. Hutson, T. Cheng, H. Patterson, J.D. Hainsworth, C.J. Ryan, C.N. Sternberg, S.L. Ellard, A. Flechon, M. Saleh, M. Scholz, E. Efstathiou, A. Zivi, D. Bianchini, Y. Loriot, N. Chieffo, T. Kheoh, C.M. Haqq, H.I. Scher, Abiraterone and increased survival in metastatic prostate cancer, N. Engl. J. Med. 364 (2011) 1995–2005.

[11] J.L. Mohler, K.E. Gaston, D.T. Moore, M.J. Schell, B.L. Cohen, C. Weaver, P. Petrusz, Racial differences in prostate androgen levels in men with clinically localized prostate cancer, J. Urol. 171 (2004) 2277–2280.

[12] J. Liu, J. Geller, J. Albert, M. Kirshner, Acute effects of testicular and adrenal cortical blockade on protein synthesis and dihydrotestosterone content of human prostate tissue, J. Clin. Endocrinol. Metab. 61 (1985) 129–133.

[13] E.A. Mostaghel, Abiraterone in the treatment of metastatic castration-resistant prostate cancer, Cancer Manage. Res. 6 (2014) 39–51.

[14] A.B. Alex, S.K. Pal, N. Agarwal, CYP17 inhibitors in prostate cancer: latest evidence and clinical potential, Ther. Adv. Med. Oncol. 8 (2016) 267–275.

[15] T. Vasaitis, A. Belosay, A. Schayowitz, A. Khandelwal, P. Chopra, L.K. Gediya, Z. Guo, H.B. Fang, V.C. Njar, A.M. Brodie, Androgen receptor inactivation contributes to antitumor efficacy of 17{alpha}-hydroxylase/17,20-lyase inhibitor 3beta-hydroxy-17-(1H-benzimidazole-1-yl)androsta-5,16-diene in prostate cancer, Mol. Cancer Ther. 7 (2008) 2348–2357.

[16] V.C. Njar, A.M. Brodie, Discovery and development of galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer, J. Med. Chem. 58 (2015) 2077–2087.

[17] C. Tran, S. Ouk, N.J. Clegg, Y. Chen, P.A. Watson, V. Arora, J. Wongvipat, P.M. Smith-Jones, D. Yoo, A. Kwon, T. Wasielewska, D. Welsbie, C.D. Chen, C.S. Higano, T.M. Beer, D.T. Hung, H.I. Scher, M.E. Jung, C.L. Sawyers, Development of a second-generation antiandrogen for treatment of advanced prostate cancer, Science 324 (2009) 787–790.

[18] C.N. Sternberg, J.S. de Bono, K.N. Chi, K. Fizazi, P. Mulders, L. Cerbone, M. Hirmand, D. Forer, H.I. Scher, Improved outcomes in elderly patients with metastatic castration-resistant prostate cancer treated with the androgen receptor inhibitor enzalutamide: results from the phase III AFFIRM trial, Ann. Oncol. 25 (2014) 429–434.

[19] T.M. Beer, A.J. Armstrong, D.E. Rathkopf, Y. Loriot, C.N. Sternberg, C.S. Higano, P. Iversen, S. Bhattacharya, J. Carles, S. Chowdhury, I.D. Davis, J.S. de Bono, C.P. Evans, K. Fizazi, A.M. Joshua, C.S. Kim, G. Kimura, P. Mainwaring, H. Mansbach, K. Miller, S.B. Noonberg, F. Perabo, D. Phung, F. Saad, H.I. Scher, M.E. Taplin, P.M. Venner, B. Tombal, Enzalutamide in metastatic prostate cancer before chemotherapy, N. Engl. J. Med. 371 (2014) 424–433.

[20] D. Rathkopf, Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer, J. Clin. Oncol. 31 (October (28)) (2013) 6.

[21] K. Fizazi, C. Massard, P. Bono, R. Jones, V. Kataja, N. James, J.A. Garcia, A. Protheroe, T.L. Tammela, T. Elliott, L. Mattila, J. Aspegren, A. Vuorela, P. Langmuir, M. Mustonen, Activity and safety of ODM-201 in patients with progressive metastatic castration-resistant prostate cancer (ARADES): an open-label phase 1 dose-escalation and randomised phase 2 dose expansion trial, Lancet Oncol. 15 (2014) 975–985.

[22] A.M. Moilanen, R. Riikonen, R. Oksala, L. Ravanti, E. Aho, G. Wohlfahrt, P.S. Nykanen, O.P. Tormakangas, J.J. Palvimo, P.J. Kallio, Discovery of ODM-201, a new-generation androgen receptor inhibitor targeting resistance mechanisms to androgen signaling-directed prostate cancer therapies, Sci. Rep. 5 (2015) 12007.

[23] V.J. Cobb, B.C. Williams, J.I. Mason, S.W. Walker, Forskolin treatment directs steroid production towards the androgen pathway in the NCI-H295R adrenocortical tumour cell line, Endocr. Res. 22 (1996) 545–550.

[24] V. Isomaa, A.E. Pajunen, C.W. Bardin, O.A. Janne, Nuclear androgen receptors in the mouse kidney: validation of a new assay, Endocrinology 111 (1982) 833–843.

[25] I. Huhtaniemi, H. Nikula, S. Rannikko, Treatment of prostatic cancer with a gonadotropin-releasing hormone agonist analog: acute and long term effects on endocrine functions of testis tissue, J. Clin. Endocrinol. Metab. 61 (1985) 698–704.

[26] A.M. Haavisto, K. Pettersson, M. Bergendahl, A. Perheentupa, J.F. Roser,

[27] I. Huhtaniemi, A supersensitive immunofluorometric assay for rat luteinizing hormone, Endocrinology 132 (1993) 1687–1691.

[27] R.B. Montgomery, E.A. Mostaghel, R. Vessella, D.L. Hess, T.F. Kalhorn, C.S. Higano, L.D. True, P.S. Nelson, Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth, Cancer Res. 68 (2008) 4447–4454.

[28] C.D. Chen, D.S. Welsbie, C. Tran, S.H. Baek, R. Chen, R. Vessella, M.G. Rosenfeld, C.L. Sawyers, Molecular determinants of resistance to antiandrogen therapy, Nat. Med. 10 (2004) 33–39.

[29] N. Sharifi, R.J. Auchus, Androstenedione is the preferred androgen source in hormone refractory prostate cancer–letter, Clin. Cancer Res. 20 (2014) 4971.

[30] G. Attard, A.H. Reid, T.A. Yap, F. Raynaud, M. Dowsett, S. Settatree, M. Barrett, C. Parker, V. Martins, E. Folkerd, J. Clark, C.S. Cooper, S.B. Kaye, D. Dearnaley, G. Lee, J.S. de Bono, Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven, J. Clin. Oncol. 26 (2008) 4563–4571.

[31] E.A. Mostaghel, B.T. Marck, S.R. Plymate, R.L. Vessella, S. Balk, A.M. Matsumoto, P.S. Nelson, R.B. Montgomery, Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants, Clin. Cancer Res. 17 (2011) 5913–5925.

[32] G. Attard, M.R. Sydes, M.D. Mason, N.W. Clarke, D. Aebersold, J.S. de Bono, D.P. Dearnaley, C.C. Parker, A.W. Ritchie, J.M. Russell, G. Thalmann, E. Cassoly, R. Millman, D. Matheson, F. Schiavone, M.R. Spears, M.K. Parmar, N.D. James, Combining enzalutamide with abiraterone, prednisone, and androgen deprivation therapy in the STAMPEDE trial, Eur. Urol. 66 (2014) 799–802.

[33] Y. Yamada, N. Matsubara, K.I. Tabata, T. Satoh, N. Kamiya, H. Suzuki, T. Kawahara, H. Uemura, A. Yano, S. Kawakami, Abiraterone acetate after progression with enzalutamide in chemotherapy-naive patients with metastatic castration-resistant prostate cancer: a multi-center retrospective analysis, BMC Res. Notes 9 (2016) 471.

[34] S. Korenchuk, J.E. Lehr, M.C.L. Y.G. Lee, S. Whitney, R. Vessella, D.L. Lin, K.J. Pienta, VCaP, a cell-based model system of human prostate cancer, In Vivo 15 (2001) 163–168.

[35] J. Veldscholte, C.A. Berrevoets, A.O. Brinkmann, J.A. Grootegoed, E. Mulder, Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation, Biochemistry 31 (1992) 2393–2399.

[36] J. Veldscholte, C. Ris-Stalpers, G.G. Kuiper, G. Jenster, C. Berrevoets, E. Claassen, H.C. van Rooij, J. Trapman, A.O. Brinkmann, E. Mulder, A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens, Biochem. Biophys. Res. Commun. 173 (1990) 534–540.

[37] M. Knuuttila, A. Mehmood, R. Huhtaniemi, E. Yatkin, M.R. Hakkinen, R. Oksala, T.D. Laajala, H. Ryberg, D.J. Handelsman, T. Aittokallio, S. Auriola, C. Ohlsson, A. Laiho, L.L. Elo, P. Sipila, S.I. Makela, M. Poutanen, Antiandrogens reduce intratumoral androgen concentrations and induce androgen receptor expression in castration-resistant prostate cancer Xenografts, Am. J. Pathol. (2017).

[38] W.M. van Weerden, H.G. Bierings, G.J. van Steenbrugge, F.H. de Jong, F.H. Schroder, Adrenal glands of mouse and rat do not synthesize androgens, Life Sci. 50 (1992) 857–861.

[39] D.H. Abbott, I.M. Bird, Nonhuman primates as models for human adrenal androgen production: function and dysfunction, Rev. Endocr. Metab. Disord. 10 (2009) 33–42.

[40] Y. Uno, S. Hosaka, H. Yamazaki, Identification and analysis of CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 in cynomolgus macaques, J. Vet. Med. Sci. 76 (2014) 1647–1650.

[41] A.C. Swart, N.W. Kolar, N. Lombard, J.I. Mason, P. Swart, Baboon cytochrome P450 17alpha-hydroxylase/17,20-lyase (CYP17), Eur J. Biochem. 269 (2002) 5608–5616.

[42] M. Del Re, S. Fogli, L. Derosa, F. Massari, P. De Souza, S. Crucitta, S. Bracarda, D. Santini, R. Danesi, The role of drug-drug interactions in prostate cancer treatment: focus on abiraterone acetate/prednisone and enzalutamide, Cancer Treat. Rev. 55 (2017) 71–82.