Original Paper

Urologia Internationalis

Urol Int 2016;97:112–117
DOI: 10.1159/000445251

Received: July 22, 2015
Accepted after revision: March 3, 2016
Published online: March 24, 2016

Cytochrome P450 17A1 Inhibitor Abiraterone Acetate Counteracts the Heat Shock Protein 27's Cell Survival Properties in Prostate Cancer Cells

Martin Weiss[a] Hannes Ahrend[a] Hannah Grossebrummel[a] Patrick Ziegler[c] Lars-Ove Brandenburg[d] Reinhard Walther[b] Uwe Zimmermann[a] Martin Burchardt[a] Matthias B. Stope[a]

[a]Department of Urology and [b]Department of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Greifswald, [c]Institute for Occupational and Social Medicine and [d]Department of Anatomy and Cell Biology, RWTH Aachen University, Aachen, Germany

Key Words
Prostate cancer · Abiraterone · Heat shock protein 27 · Cytoprotection · Chemotherapy

Abstract
Introduction: Inhibition of androgen synthesis by abiraterone acetate (AA) entails enhanced overall survival rates and clinical benefit for patients with locally advanced and metastasized prostate cancer (PC). The expression of heat shock protein 27 (HSP27) is generally associated with cytoprotection and was demonstrated to mediate chemoresistance under cytostatic therapy, for instance, docetaxel treatment. In this study, we investigated the impact of AA treatment on HSP27 expression and PC cell growth. ***Materials and Methods:*** HSP27 expression levels in docetaxel and AA-treated PC cell lines LNCaP and PC-3 were determined by SDS PAGE and Western blot analysis. Proliferation assays were performed using a CASY Cell Counter and Analyzer Model TT (Roche Applied Science). ***Results:*** Despite significantly increased HSP27 expression in PC cells incubated with docetaxel, Western blot analysis implicated a significant reduction of the cytoprotective HSP27 in AA-treated PC cells. Notably, HSP27 stably overexpressed in PC-3-HSP27 cells did not appear as an HSP27-mediated proliferation benefit in the presence of AA as shown in docetaxel incubation studies. ***Conclusion:*** In contrast to repeatedly demonstrated HSP27-driven chemoresistance related to chemotherapeutics, our results may constitute a broader molecular mode of action of AA chemotherapy. AA efficacy may exert an HSP27 suppressive role that goes beyond the primarily assumed inhibition of androgen biosynthesis.
© 2016 S. Karger AG, Basel

Introduction

Abiraterone acetate (AA) is a leading substance among the currently developed and promising antiandrogen drugs for the treatment of metastatic castration-resistant prostate cancer (mCRPC) [1–3]. mCRPC is yet one of the most diagnosed malignant diseases and remains the second-leading cause of tumour-associated deaths in male in the Western hemisphere [4]. Depending on local tumor progress and the presence of lymph node and bone metastasis, overall survival of mCRPC averaged 1.5 years

KARGER

E-Mail karger@karger.com
www.karger.com/uin

© 2016 S. Karger AG, Basel
0042–1138/16/0971–0112$39.50/0

Dr. Matthias B. Stope
Department of Urology
University Medicine Greifswald, Ferdinand-Sauerbruch-Strasse
DE–17475 Greifswald (Germany)
E-Mail matthias.stope@uni-greifswald.de

during the last 2 decades [5, 6]. Before 2010, docetaxel was the only available substance, which improved overall survival [7, 8]. This implicates the necessity of targeting additional molecular pathways in prostate cancer (PC). PC progression is usually accompanied with the loss of physiological paracrine androgen dependence, often related with a shift to autocrine supply with androgens, and an increase of further factors being important for tumor growth and survival [9, 10]. Moreover, mCRPC showed higher testosterone and dihydrotestosterone concentrations as well as a significantly induced cytochrome P450 17A1 (CYP17A1) expression within the tumour [1, 11, 12]. CYP17A1 is the key enzyme of gonadal and extragonadal androgen synthesis by conversion of progesterone and pregnenolone to 17-OH progesterone and 17-OH pregnenolone. Recently, AA-driven inhibition of CYP17A1 improved overall survival by the 4th month, and additionally increased the clinical outcome and quality of life of patients, illustrated by a fundamentally optimized suppression of serum androgen concentration [1, 13]. However, currently available therapy regimes, including AA, have not been fully satisfactory as mCRPC remains uncurable and still implies great disabilities for affected men. Among the existing challenges, overcoming drug resistance represents one of the most critical factors limiting the pharmacological benefit of androgen depletion and other androgen receptor (AR)-directed therapies. Recently discussed resistance mechanisms include up-regulation of CYP17A1 enzyme activity, AR alterations, such as mutations and AR splice variants, as well as arising AR-independent signalling pathways [14–16]. Besides, there is growing evidence, that the small heat shock protein 27 (HSP27) is associated with drug resistance, AR signaling, epithelial-to-mesenchymal transition and metastasis, with all representing pivotal anti-therapeutic mechanisms in mCRPC. Consequently, HSP27 itself was identified as a promising target in anticancer therapy [17, 18]. Interestingly, HSP27 has been demonstrated to considerably regulate AR expression levels, resulting in altered AR functionality [19–21]. It is reasonable to assume, that HSP27 could be involved in – so far largely unknown – mechanisms of treatment resistance during AA therapy. Previously, in vitro experiments in PC cells showed, that (1) stable overexpression of HSP27 revealed significantly enhanced resistance to docetaxel treatment and that (2) docetaxel incubation was followed by a significant induction of HSP27 protein expression [17]. Thus, HSP27-mediated cytoprotection may importantly impact therapy relapse and overall survival.

In this study, we examined a possible correlation between the expression of cytoprotective HSP27 and AA treatment in PC cells.

Materials and Methods

Cell Culture and Drug Treatment

Human epithelial PC cell lines LNCaP and PC-3 (Cell Lines Service, Eppelheim, Germany) were propagated in RPMI 1640 medium with 10% foetal calf serum and 100 units/ml penicillin/streptomycin (PAN Biotech) at 37°C and 5% CO_2 atmosphere. PC-3-HSP27 cells stably overexpressing HSP27 were selected with 400 μg/ml G418 (Carl Roth, Karlsruhe, Germany) as previously described [17]. LNCaP cells were treated with 10 nM docetaxel (Sigma-Aldrich, Munich, Germany) and 10 μM AA (Janssen-Cilag, Neuss, Germany). PC-3 cells were treated with 10 nM docetaxel and 30 μM AA, respectively. Both cell lines were incubated with dimethyl sulfoxide (DMSO, Sigma-Aldrich) as appropriate vehicle control. Drug treatment was generally proceeded to adherent cells seeded 24 h prior.

Proliferation Assay

Cellular proliferation was analysed in 24-well cell culture plates (1 ml/well) using a CASY Cell Counter and Analyzer Model TT (Roche Applied Science, Mannheim, Germany). Adherent cells were detached by 0.1% trypsin/0.04% ethylenediaminetetraacetic acid (EDTA) and resuspended in CASYton solution (Roche Applied Science). Measurement was performed using a capillary of 150 μm in diameter and cell line–specific gate settings to discriminate between living cells, dead cells and cellular debris. The number of living cells was determined in duplicates for each passage.

Western Blot Analysis

PC cells plated onto a 6-well cell culture plate containing 3 ml RPMI medium were incubated with AA and docetaxel for indicated time points. Adherent cells were detached by 0.1% trypsin/0.04% EDTA and lysed in RIPA buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 10 mM K_2HPO_4, 5 mM EDTA, 10% glycerol, 1% Triton X-100, 0.05% sodium dodecyl sulphate, 1 mM Na_3VO_4, 20 mM NaF, 0.1 mM phenylmethylsulfonyl fluoride, 20 mM 2-phosphoglycerate, and complete protease inhibitor cocktail from Roche Applied Science). Protein levels of all cell extracts were determined using Bradford solution (BioRad, München, Germany). Equal amounts of protein (10–100 μg) were separated by SDS-PAGE Mini-Protean system (BioRad) and transferred onto a protean nitrocellulose membrane (Whatman, Dassel, Germany) propagated with the Trans-Blot SD semi-dry transfer cell (BioRad). Membranes were blocked with 1 × Roti-Block (Carl Roth). Proteins of interest were detected by primary antibodies directed against HSP27 and glyceraldehyde 3-phophate dehydrogenase (GAPDH) and peroxidase-coupled secondary antibodies, all obtained from Cell Signaling Technology (Danvers, Mass., USA). Protein signals were visualized with the ChemiDoc system (BioRad) using SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific, Rockford, Ill., USA) and were quantified using Image Lab 3.0 (BioRad) software.

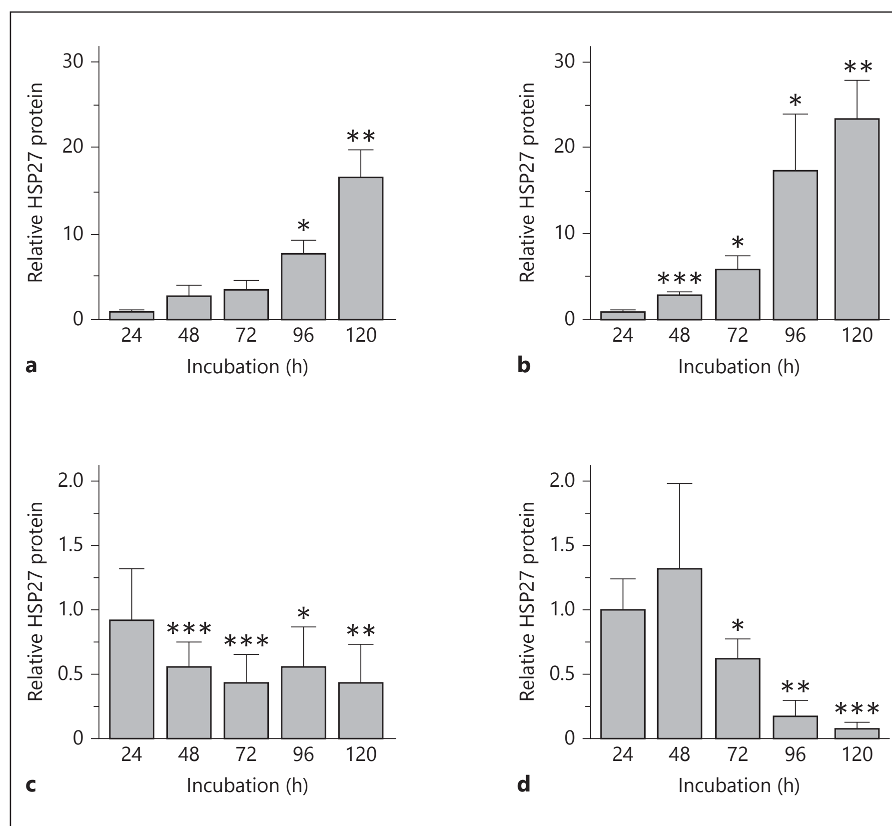

Fig. 1. AA suppresses pro-oncogenic HSP27 expression in LNCaP and PC-3 PC cells. Western blot analysis of HSP27 protein expression. LNCaP (**a**) and PC-3 (**b**) cells were incubated with 10 nM docetaxel for 120 h, LNCaP (**c**) and PC-3 (**d**) cells were incubated with 10 μM (PC-3) and 30 μM (LNCaP) AA for 120 h. All data were standardized to vehicle-treated control cells, GAPDH served as loading control. Docetaxel incubation in LNCaP and PC-3 was followed by a significant increase of HSP27 expression. Significantly decreased HSP27 levels were detected after AA incubation in both PC cell lines LNCaP and PC-3. Error bars indicate mean ± SD. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, as determined by Student's t test.

Statistics

Statistical analyses of at least 3 independent experiments were performed using the unpaired Student's t test. Results of * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ were considered significant. Data are given as mean ± SD.

Results

AA Treatment Diminished HSP27 Expression in PC-3 and LNCaP Cells

We have previously demonstrated that HSP27 expression in PC-3 cells significantly increased after docetaxel incubation [17]. In this context, we aimed at analysing the effects of AA treatment on HSP27 protein expression in human PC cell lines LNCaP and PC-3 compared to docetaxel incubation. LNCaP and PC-3 cells were incubated with 10 nm docetaxel or 10 μM (LNCaP) and 30 μM (PC-3) AA over a period of 120 h. HSP27 protein amounts were analysed by Western blot analysis and standardised to vehicle-treated controls. Incubation with docetaxel was followed by a significantly increased HSP27 protein expression in both cell lines LNCaP (fig. 1a; 24 h: 1; 48 h: 3.14, $p = 0.1108$; 72 h: 3.50, $p = 0.0717$; 96 h: 7.47, $p = 0.0187$; 120 h: 16.66, $p = 0.0063$) and PC-3 (fig. 1b; 24 h: 1; 48 h: 2.70, $p = 0.0008$; 72 h: 5.70, $p = 0.0213$; 96 h: 17.24, $p = 0.0358$; 120 h: 23.54, $p = 0.0015$) compared to vehicle-treated control cells. In contrast, however, AA treatment was associated with a significant reduction in total HSP27 levels in LNCaP (fig. 1c; 24 h: 0.91; 48 h: 0.59, $p < 0.0001$; 72 h: 0.47, $p = 0.0001$; 96 h: 0.62, $p = 0.0208$; 120 h: 046, $p = 0.0063$) and PC-3 cells (fig. 1d; 24 h: 1.01; 48 h: 1.33, $p = 0.4464$; 72 h: 0.62, $p = 0.0175$; 96 h: 0.28, $p = 0.0038$; 120 h: 0.11, $p = 0.0002$) compared to vehicle-treated controls.

AA Incubation Attenuated Cellular Proliferation of Human PC Cell Line PC-3 Independently from HSP27 Expression Levels

HSP27 was frequently characterized as a cytoprotective factor present in numerous molecular mechanisms causing chemoresistance. We previously demonstrated that overexpressed HSP27 lead to enhanced docetaxel resistance in PC-3 cells, which stably express HSP27 at high levels (PC-3-HSP27) [17]. To proof the impact of HSP27 on cell growth of AA-treated PC cells, we used PC-3-HSP27 cells compared to maternal low HSP27-level cell

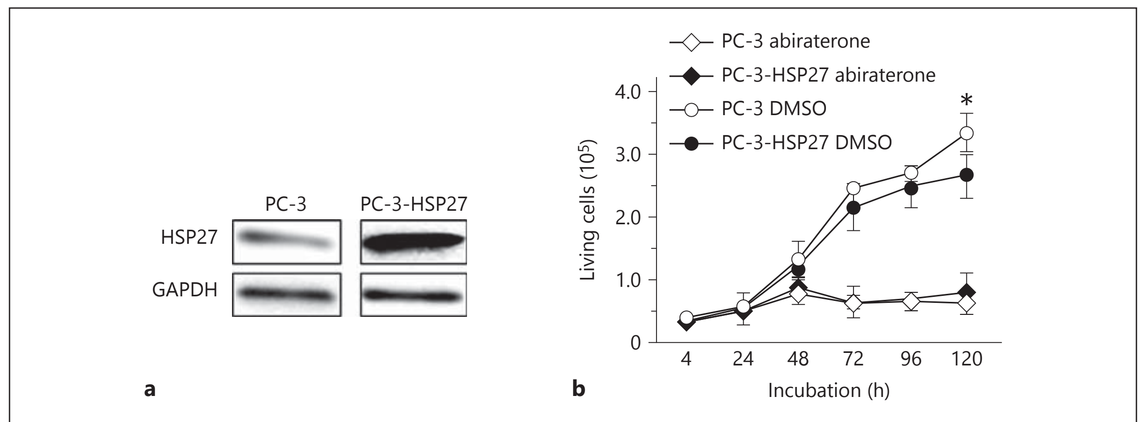

Fig. 2. Overexpression of HSP27 reveals no difference in cell growth during AA incubation. **a** Representative western blot of basal HSP27 expression in PC-3 and PC-3-HSP27 cells. GAPDH served as loading control. PC-3 cells showed a low basal HSP27 protein amount. PC-3-HSP27 cells exerted a stable HSP27 overexpression with high HSP27 protein levels. **b** Living cell number of 30 nM AA treated low-HSP27-level PC-3 cells (◇), PC-3-HSP27 cells stably overexpressing HSP27 (♦), as well as vehicle-treated PC-3 (○) and PC-3-HSP27 cells (●), respectively. Cells were analysed using a CASY Cell Counter and Analyzer Modell TT at indicated time points over a period of 120 h. HSP27 overexpression have not resulted in any statistically significant changes of cell growth during AA treatment. Results are expressed as the mean ± SD of cell count.

line PC-3 [19] (for a representative Western blot analysis see insert fig. 2a). Cellular proliferation was measured over a period of 120 h. PC-3 and PC-3-HSP27 cells were incubated with 30 μM AA and were compared to vehicle-treated controls. As demonstrated in figure 2b, vehicle-treated cells showed exponential cell growth, exhibiting no statistically significant differences between PC-3 and PC-3-HSP27 cells, except 120 h after the begin of vehicle incubation (fig. 2b; PC-3/vehicle: ○; PC-3-HSP27/vehicle; ●; 4 h: $p = 0.9105$; 24 h: $p = 0.9166$; 48 h: $p = 0.5547$; 72 h: $p = 0.1695$; 96 h: $p = 0.3305$; 120 h: $p = 0.0208$). AA incubation was followed by obviously diminished cell numbers of low HSP27-level PC-3 cells and high HSP27-level PC-3-HSP27 cells. There were no statistically significant differences between cell numbers of both cell lines (fig. 2b; PC-3/AA: ○; PC-3-HSP27/AA; ●; 4 h: $p = 0.9534$; 24 h: $p = 0.7919$; 48 h: $p = 0.7021$; 72 h: $p = 0.9511$; 96 h: $p = 0.8502$; 120 h: $p = 0.3146$).

Discussion

The introduction of AA as a first-line drug was celebrated as outstanding progress for anticancer therapy of locally advanced PC and mCRPC, however, many questions about the underlying molecular mechanisms of overcoming drug resistance still remain unsolved. Recently, we identified AA as an inhibitor of proliferative and pro-oncogenic pathways in AR-negative and therefore androgen-insensitive PC cells [22]. Thus, apart from blockade of androgen biosynthesis, AA may control further AR-independent mechanisms involved in cytostasis and chemoresistance control. The cytoprotective factor HSP27 has repeatedly been proven being a substantial factor in many pro-oncogenic pathways in numerous tumor entities including PC. Recently, its imposing cytoprotective properties were demonstrated in a cell culture model system conceived to investigate the molecular mechanisms of first-line cytostatic therapy of mCRPC with docetaxel [17]. HSP27 overexpression thereby was followed by a significant decrease of docetaxel-sensitivity in PC-3 cells. Notably, accompanied with the increase of viability and proliferation of PC-3 cells, a docetaxel-dependent induction of HSP27 was detected. In this study, we could verify the docetaxel-dependent HSP27 overexpression in PC-3 cells and could further demonstrate similar results for docetaxel treated LNCaP cells (fig. 1a, b). In line with this, a number of recent studies showed further oncogenic pathways, which are stimulated by AR-inhibition therapies. These oncogenic proteins are the most suppressed by high AR concentrations in the pre-therapeutical setting. Thus, drug resistance is probably enforced by oncogenic factors, such as the enhancer of zeste homolog 2, signal transducer and activator of transcription 3 and hepatocyte growth factor receptor (c-Met) pathway [23–25]. From this, it is crucial to analyse a potential AA-specific modulation of HSP27 during anticancer therapy. Notably, in this study a significant HSP27 suppression was detectable in PC cells in the presence of AA (fig. 1c, d),

which may explain why AA demonstrates high efficacy even after docetaxel therapy failure.

Our examination contains further experimental approaches in which (1) 3 days of AA incubation were followed by three days of docetaxel incubation, (2) 3 days of docetaxel incubation were followed by 3 days of AA incubation, and (3) AA incubation was combined with docetaxel incubation (data not shown). The aim of this setup was to provide experimental evidence for our hypothesis of AA counteracting docetaxel-mediated HSP27 induction in an in vitro cell culture model. However, neither combination nor sequential regimen of AA and docetaxel has demonstrated an antithetic regulation of HSP27 expression. Primarily due to enhanced cytotoxic effects of both drugs as well as to the short-termed incubation, the established in vitro model failed to appear suitable for this type of studies. Additionally, the significance of sequential incubation experiments is restricted to intracellular drug accumulation and activity even after change-over of incubation conditions.

Currently, there are no other studies reporting about a downregulation of striking pro-oncogenic factors like HSP27 in the course of AA treatment. This argues strongly in favour for a so far unknown reverse interconnection between AA treatment and HSP27 expression. Moreover, we could demonstrate that HSP27 stably overexpressed in PC-3 cells has no significant impact on PC cell growth during AA incubation (fig. 2b). This is notable as it implies that AA treatment is independent from HSP27-driven cytoprotection. Moreover, HSP27 suppression by AA could be of great interest, as HSP27 was already shown to be a critical cytoprotective factor counter-acting various anti-proliferative cancer therapies [26]. Consequently, a specific HSP27 inhibitor, Apatorsen (OGX-427, OncoGenex Pharmaceuticals, Bothell, Wash., USA), has been developed to address the problem of HSP27-driven survival mechanisms in PC therapy. This compound is currently part of phase 2 clinical trials in combination with prednisone (pre-chemo) and in combination with AA (pre- and post-chemo) [27–29]. AA's HSP27 inhibitory activity may enhance the anti-androgen activity of AA itself as well as the cytostatic efficacy of other anticancer drugs. Moreover, AA generally attenuates HSP27-driven cell survival, which may represent a promising approach for the prevention of chemoresistance and unspecific toxicity as well as for enhanced therapy duration and drug efficacy in anticancer therapy.

Conclusion

This study indicated that antiandrogen therapy with AA is independent from HSP27-mediated cytoprotection. Despite HSP27 is a prevalent cell-survival factor and mediator of chemoresistance, HSP27 overexpression had no consequence for proliferation of PC cells in the presence of AA. This could be of great importance for future mCRPC treatment strategies, for example, this could be important in the estimation of future sequential therapy approaches integrating new drugs. Besides the anti-androgen efficacy by CYP17A1 inhibition, AA appears to exhibit a concomitant accessory anti-HSP27 activity, which may facilitate AA's outstanding anticancer efficacy in PC therapy.

Disclosure Statement

By way of disclosure of conflict of interest, the compound AA was provided by the Janssen-Cilag GmbH, Neuss, Germany.

Acknowledgements

The authors thank Anne Brandenburg and Katja Wittig for their excellent technical assistance.

References

1 de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OB Jr, Saad F, Staffurth JN, Mainwaring P, Harland S, Flaig TW, Hutson TE, Cheng T, Patterson H, Hainsworth JD, Ryan CJ, Sternberg CN, Ellard SL, Fléchon A, Saleh M, Scholz M, Efstathiou E, Zivi A, Bianchini D, Loriot Y, Chieffo N, Kheoh T, Haqq CM, Scher HI; COU-AA-301 Investigators: Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.

2 Kaku T, Hitaka T, Ojida A, Matsunaga N, Adachi M, Tanaka T, Hara T, Yamaoka M, Kusaka M, Okuda T, Asahi S, Furuya S, Tasaka A: Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer. Bioorg Med Chem 2011;19:6383–6399.

3 Vasaitis T, Belosay A, Schayowitz A, Khandelwal A, Chopra P, Gediya LK, Guo Z, Fang HB, Njar VC, Brodie AM: 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 2008;7:2348–2357.

4 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ: Cancer statistics, 2008. CA Cancer J Clin 2008;58:71–96.

5 Wu JN, Fish KM, Evans CP, Devere White RW, Dall'Era MA: No improvement noted in overall or cause-specific survival for men presenting with metastatic prostate cancer over a 20-year period. Cancer 2014;120:818–823.

6 Froehner M, Hölscher T, Hakenberg OW, Wirth MP: Treatment of bone metastases in urologic malignancies. Urol Int 2014;93:249–256.
7 Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D, Crawford ED: Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 2004;351:1513–1520.
8 Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Théodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA; TAX 327 Investigators: Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004;351:1502–1512.
9 Logothetis CJ, Gallick GE, Maity SN, Kim J, Aparicio A, Efstathiou E, Lin SH: Molecular classification of prostate cancer progression: foundation for marker-driven treatment of prostate cancer. Cancer Discov 2013;3:849–861.
10 Turo R, Tan K, Thygesen H, Sundaram SK, Chahal R, Prescott S, Cross WR: Diethylstilboestrol (1 mg) in the management of castration-resistant prostate cancer. Urol Int 2015; 94:307–312.
11 Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD, Nelson PS: Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447–4454.
12 Chang KH, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ, Sharifi N: Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. Proc Natl Acad Sci U S A 2011;108: 13728–13733.
13 Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, Carles J, Mulders PF, Basch E, Small EJ, Saad F, Schrijvers D, Van Poppel H, Mukherjee SD, Suttmann H, Gerritsen WR, Flaig TW, George DJ, Yu EY, Efstathiou E, Pantuck A, Winquist E, Higano CS, Taplin ME, Park Y, Kheoh T, Griffin T, Scher HI, Rathkopf DE; COU-AA-302 Investigators: Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138–148.
14 Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, Nelson PS, Montgomery RB: Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011;17:5913–5925.
15 Joseph JD, Lu N, Qian J, Sensintaffar J, Shao G, Brigham D, Moon M, Maneval EC, Chen I, Darimont B, Hager JH: A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. Cancer Discov 2013;3:1020–1029.
16 Gao L, Schwartzman J, Gibbs A, Lisac R, Kleinschmidt R, Wilmot B, Bottomly D, Coleman I, Nelson P, McWeeney S, Alumkal J: Androgen receptor promotes ligand-independent prostate cancer progression through c-Myc upregulation. PLoS One 2013; 8:e63563.
17 Stope MB, Weiss M, Preuss M, Streitbörger A, Ritter CA, Zimmermann U, Walther R, Burchardt M: Immediate and transient phosphorylation of the heat shock protein 27 initiates chemoresistance in prostate cancer cells. Oncol Rep 2014;32:2380–2386.
18 Ischia J, Saad F, Gleave M: The promise of heat shock protein inhibitors in the treatment of castration resistant prostate cancer. Curr Opin Urol 2013;23:194–200.
19 Stope MB, Schubert T, Staar D, Rönnau C, Streitbörger A, Kroeger N, Kubisch C, Zimmermann U, Walther R, Burchardt M: Effect of the heat shock protein HSP27 on androgen receptor expression and function in prostate cancer cells. World J Urol 2012;30:327–331.
20 Stope MB, Stender C, Schubert T, Peters S, Weiss M, Ziegler P, Zimmermann U, Walther R, Burchardt M: Heat-shock protein HSPB1 attenuates microRNA miR-1 expression thereby restoring oncogenic pathways in prostate cancer cells. Anticancer Res 2014;34:3475–3480.
21 Weiss M, Burchardt M, Stope M: Master of puppets in prostate cancer: heat shock protein 27 is pulling androgen receptor's strings. Can Cell Microenviron 2014;1:e287.
22 Grossebrummel H, Peter T, Mandelkow R, Weiss M, Muzzio D, Zimmermann U, Walther R, Jensen F, Knabbe C, Zygmunt M, Burchardt M, Stope MB: Cytochrome P450 17A1 inhibitor abiraterone attenuates cellular growth of prostate cancer cells independently from androgen receptor signaling by modulation of oncogenic and apoptotic pathways. Int J Oncol 2016;48:793–800.
23 Bohrer LR, Chen S, Hallstrom TC, Huang H: Androgens suppress EZH2 expression via retinoblastoma (RB) and p130-dependent pathways: a potential mechanism of androgen-refractory progression of prostate cancer. Endocrinology 2010;151:5136–5145.
24 Schroeder A, Herrmann A, Cherryholmes G, Kowolik C, Buettner R, Pal S, Yu H, Müller-Newen G, Jove R: Loss of androgen receptor expression promotes a stem-like cell phenotype in prostate cancer through STAT3 signaling. Cancer Res 2014;74:1227–1237.
25 Verras M, Lee J, Xue H, Li TH, Wang Y, Sun Z: The androgen receptor negatively regulates the expression of c-Met: implications for a novel mechanism of prostate cancer progression. Cancer Res 2007;67:967–975.
26 Kostenko S, Moens U: Heat shock protein 27 phosphorylation: kinases, phosphatases, functions and pathology. Cell Mol Life Sci 2009;66:3289–3307.
27 Ischia J, Saad F, Gleave M: The promise of heat shock protein inhibitors in the treatment of castration resistant prostate cancer. Curr Opin Urol 2013;23:194–200.
28 A randomized, double-blind phase 2 study comparing gemcitabine and cisplatin in combination with OGX-427 or placebo in patients with advanced transitional cell carcinoma. http://clinicaltrials.gov/show/NCT01454089.
29 A randomized phase II study of OGX-427 (a second-generation antisense oligonucleotide to heat shock protein-27) in patients with castration resistant prostate cancer who have not previously received chemotherapy for metastatic disease. http://clinicaltrials.gov/ct2/show/NCT01120470?term=NCT01120470&rank=1.