Asian Journal of Urology (2019) 6, 50–56

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ajur

Review

Potential impact of combined inhibition of 3α-oxidoreductases and 5α-reductases on prostate cancer

Michael V. Fiandalo [a], Daniel T. Gewirth [b], James L. Mohler [a,*]

[a] Department of Urology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA
[b] Hauptman-Woodward Medical Research Institute, Buffalo, NY, USA

Received 2 June 2018; received in revised form 2 July 2018; accepted 2 August 2018
Available online 26 September 2018

KEYWORDS
Androstanediol;
Dihydrotestosterone;
Dutasteride;
3α-oxidoreductases;
Androgen deprivation therapy;
Abiraterone

Abstract Prostate cancer (PCa) growth and progression rely on the interaction between the androgen receptor (AR) and the testicular ligands, testosterone and dihydrotestosterone (DHT). Almost all men with advanced PCa receive androgen deprivation therapy (ADT). ADT lowers circulating testosterone levels, which impairs AR activation and leads to PCa regression. However, ADT is palliative and PCa recurs as castration-recurrent/resistant PCa (CRPC). One mechanism for PCa recurrence relies on intratumoral synthesis of DHT, which can be synthesized using the frontdoor or primary or secondary backdoor pathway. Androgen metabolism inhibitors, such as those targeting 5α-reductase, aldo-keto-reductase family member 3 (AKR1C3), or cytochrome P450 17A1 (CYP17A1) have either failed or produced only modest clinical outcomes. The goal of this review is to describe the therapeutic potential of combined inhibition of 5α-reductase and 3α-oxidoreductase enzymes that facilitate the terminal steps of the frontdoor and primary and secondary backdoor pathways for DHT synthesis. Inhibition of the terminal steps of the androgen metabolism pathways may be a way to overcome the shortcomings of existing androgen metabolism inhibitors and thereby delay PCa recurrence during ADT or enhance the response of CRPC to androgen axis manipulation.

* Corresponding author.
E-mail address: James.mohler@roswellpark.org (J.L. Mohler).
Peer review under responsibility of Second Military Medical University.

https://doi.org/10.1016/j.ajur.2018.09.002

1. Prostate cancer and androgen deprivation therapy

Prostate cancer (PCa) growth and recurrence relies on the interaction between the androgen receptor (AR) and testicular ligands, either testosterone (T) or dihydrotestosterone (DHT). Men with advanced PCa receive androgen deprivation therapy (ADT). The goal of ADT is to lower levels of circulating T and impair AR activation in order to induce PCa regression. However, ADT is palliative and PCa recurs as lethal castration-recurrent/resistant PCa (CRPC) [1—5]. Several mechanisms contribute to PCa persistence during ADT and the transition to CRPC, such as AR mutations, AR hypersensitivity, natural or iatrogenic-induced expression of AR splice variants [6], and intratumoral synthesis of T or DHT [7—9]. Intratumoral T or DHT synthesis produces tissue T levels similar to androgen-stimulated PCa and benign prostate, and tissue DHT levels, although reduced, remain sufficient to activate AR [7,8,10].

PCa cells have available up to three androgen metabolism pathways to produce DHT [8,11—16]. The frontdoor pathway uses the two adrenal androgens, dehydroepiandrosterone (DHEA) and androstenedione (ASD), to synthesize T. DHEA and ASD are synthesized in the adrenal gland using a combination of cytochrome P450 17A1 (CYP17A1) and HSD3B2 enzymes. The terminal step of the frontdoor pathway uses 5α-reductases (SRD5A1, 2, 3) to 5α-reduce T to DHT. The terminal steps of the primary and secondary backdoor pathways do not use T to make DHT. Instead, the primary and secondary backdoor pathways use a combination of SRD5A, CYP17A1 and 3α-oxidoreductase enzymes to produce 5α-androstane-3α,17β-diol (androstanediol) or 5α-androstane-3,17-dione (androstanedione; 5α-dione). The terminal step of the primary backdoor pathway uses 3α-oxidoreductases to oxidize androstanediol to DHT. The secondary backdoor pathway uses 3α-oxidoreductases to reduce 5α-dione to DHT (Fig. 1). The secondary backdoor pathway, named for its discovery after the primary backdoor pathway, is synonymous with the Sharifi "alternative" [11] and Penning "alternate" [13] backdoor pathways, and Corcoran "5α-dione" pathway [17].

The goal of this review is to describe the shortcomings of available androgen metabolism inhibitors and to discuss the therapeutic potential of combined inhibition of the 5α-reductases and 3α-oxidoreductases that catalyze the terminal steps of the frontdoor and primary and secondary backdoor pathways for DHT synthesis.

2. CYP17A1 activity, inhibitors and mechanisms of PCa resistance

The concept of inhibition of adrenal androgen production began with Huggins and Scott in 1945 [18], who reported that adrenalectomy depleted testicular and adrenal androgens. However, adrenalectomy also produced adrenal insufficiency, which was lethal to patients without glucocorticoid replacement therapy [19,20]. Since 1945, several adrenal androgen biosynthesis inhibitors were identified, such as aminoglutethimide [21,22], which impaired 11β-hydroxylase, and ketoconazole [23—25], which targeted 21- and/or 11β-hydroxylase, 17α-hydroxylase and 17, 20-lyase activity, to deplete circulating adrenal steroid levels, such as DHEA-sulfate [24]. However, aminoglutethimide and ketoconazole were not ideal inhibitors for a number of reasons including drug toxicity [26,27].

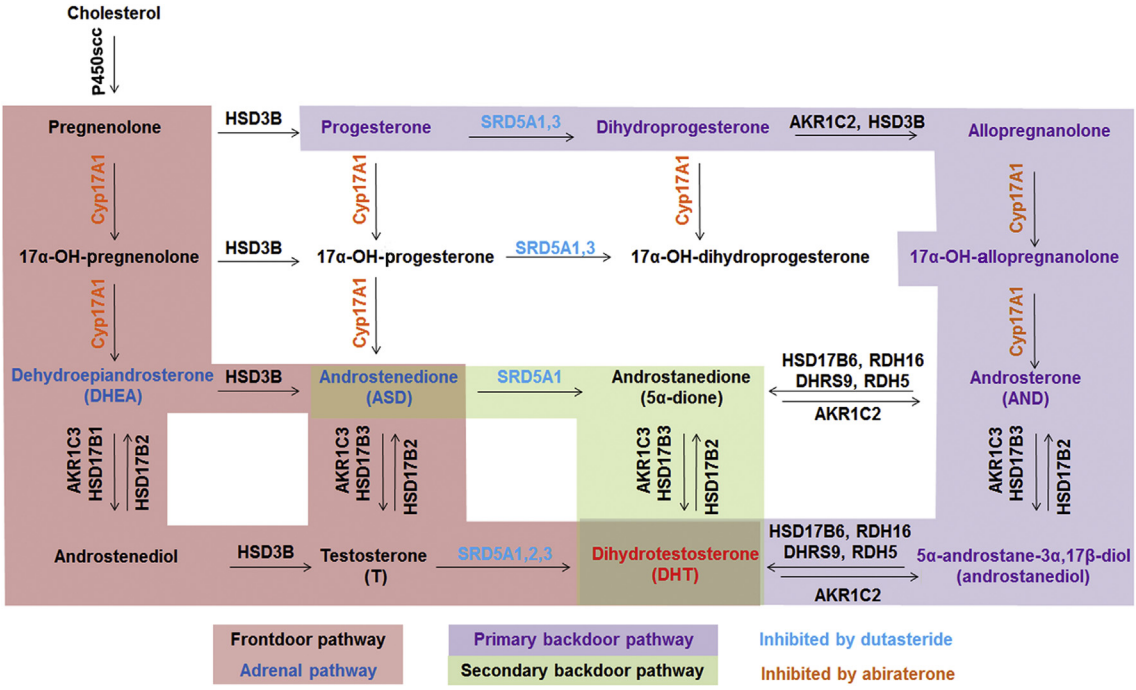

Figure 1 Pathways to DHT synthesis (modified from Ref. [62]). 5α-reductase (SRD5A) 1, 2 or 3 (frontdoor pathway; pink) metabolize testosterone to dihydrotestosterone (DHT). 3α-oxidoreductase (primary backdoor pathway; purple) or aldo-keto reductase (secondary backdoor pathway; green) enzymes convert androstanediol or androstanedione, respectively to DHT.

The CYP17A1 inhibitor, abiraterone, impairs both the 17α-hydroxylase and 17,20-lyase activities of the enzyme, thus blocking adrenal androgen synthesis [28,29]. Abiraterone depletes PCa or CRPC tissue androgen levels and extends patient survival approximately 4 months when used for CRPC, which garnered Food and Drug Administration (FDA) approval [30—33]. However, inhibition of CYP17A1 by abiraterone can lead to glucocorticoid insufficiency and mineralocorticoid excess. Hypertension, hypokalemia and peripheral edema occur with sufficient frequency that abiraterone is co-administered with prednisone [34—36].

The earlier clinical failure of abiraterone (and other CYP17A1 inhibitors) has been attributed to several mechanisms that involve AR splice variants [37], circulating DHEA-SO_4 that remains in spite of abiraterone treatment [38], increased expression of CYP17A1 [39,40], or other primary [37,41] and/or secondary backdoor pathway [39] androgen metabolism enzymes. CYP17A1 inhibition induces progesterone accumulation, which competes with abiraterone for CYP17A1 [42]. CYP17A1 inhibition may result in up-regulation of CYP11A1 and the aldo-keto reductase family 1 member C3 (AKR1C3); both enzymes are capable of increasing intra tumoral *de novo* androgen synthesis by activating different androgen metabolism pathways [42]. Chang et al. [43] reported HSD3B1 mutations promote DHT synthesis despite therapeutic intervention. The concept of interrupting intratumoral androgen metabolism has merit, but CYP17A1 inhibition occurs too early in the DHT synthesis pathways, which enables PCa cells to alter the pathways used to produce DHT.

Therefore, inhibition of the terminal steps of the primary and secondary backdoor pathways using a combination of SRD5A and 3α-oxidoreductase enzyme inhibitors may improve PCa response. Inhibition of the steps immediately proximal to DHT synthesis will render pathway switching ineffective, which may lower DHT levels better than any androgen metabolism inhibitor used alone.

3. 5α-reductase inhibition alone is insufficient to deplete intratumoral DHT levels

The 5α-reductases (types I—III) encoded by SRD5A1, 2 or 3 play an essential role in metabolism of progestagens, glucocorticoids and androgens. These enzymes irreversibly reduce the double bond at C4 and C5 in Ring A (Fig. 2; purple) [44—46] of substrates such as progesterone, cortisolor T, to produce dihydroprogesterone, dihydrocortisolor DHT, respectively [46]. The type II reductase encoded by SRD5A2 is predominant in benign hyperplastic prostate and is the target of finasteride. Dutasteride inhibits type I and type II 5α-reductase, and may also inhibit type III as well [47]. PCa may depend more on SRD5A1 and SRD5A3 [48]. Finasteride treatment enhanced the extent of response to ADT when initiated simultaneously with ADT [49—53], which suggests 5α-reductase inhibition may be useful when used earlier for advanced PCa. However, finasteride and dutasteride both have proven ineffective in CRPC because of variable patient response, accumulation of T that enables AR activation, and/or insufficient depletion of intratumoral DHT levels due to active SRD5A3 or primary or secondary backdoor pathway metabolism [54—57].

4. 3α-oxidoreductases inhibition lowers intratumoral DHT levels *in vitro*

Redox enzymes, such as hydroxysteroid dehydrogenases (HSD), aldo-keto reductases or retinol dehydrogenases (RDH) oxidize or reduce steroid substrates [8,9,58]. These enzymes are responsible for steroid metabolism, which

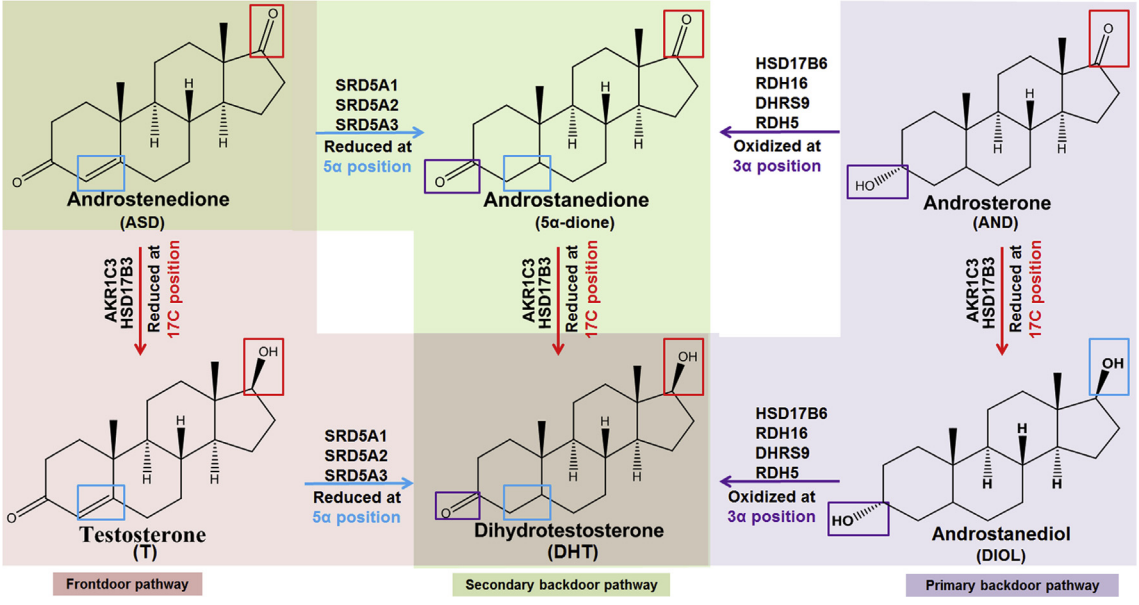

Figure 2 5α-reductase (SRD5A) (blue) and 3α-oxidoreductase (oxidation [purple]; reduction [red]) target sites on steroid rings. SRD5A activity (blue) occurs at the 5α position of Ring A, 3α-oxidoreductase oxidation sites (purple) occur at the 3α position of Ring A and aldo-keto reduction (red) occurs at the 17β position of Ring D.

includes glucocorticoid, mineralocorticoid and androgen synthesis or degradation [59—61].

The terminal step in the primary backdoor pathway of androgen metabolism uses one of four 3α-oxidoreductases (HSD17B6; RDH5; RDH16; and dehydrogenase/reductase family member 9 [DHRS9]) to convert androstanediol to DHT [8,62]. The 3α-oxidoreductases have conserved catalytic amino acids (Fig. 3) and co-factor binding sites [63—66], and carry out similar reactions, despite having different K_m values [62,67]. Preclinical studies using castration-recurrent CWR-R1 human xenografts demonstrated that androstanediol is converted to DHT [68]. The secondary backdoor pathway requires a combination of SRD5A, to 5α-reduce ASD to 5α-dione, the four 3α-oxidoreductases, AKR1C3 or HSD17B3, to finally reduce 5α-dione to DHT [11—13,17].

A pre-clinical study using the AKR1C3 inhibitor, indomethacin, demonstrated a proof of principle that AKR1C3 inhibition overcame PCa resistance to abiraterone and enzalutamide. The study provided evidence for the necessity for identification and development of AKR1C3 inhibitors [13,69]. A Phase 1/2 clinical trial that tested the efficacy of an AKR1C3 inhibitor, ASP9521, ended without evidence of clinical response. The authors suggested that insufficient PCa cell expression of AKR1C3 may have caused therapeutic failure [70].

No 3α-oxidoreductase inhibitors are used in the clinic to inhibit the four enzymes that metabolize androstanediol to DHT or 5α-dione to DHT. Recently, our group showed that combined inhibition of the four 3α-oxidoreductases and SRD5A1 or SRD5A2 lowered DHT levels better than inhibition of either enzyme group alone using *in vitro* PCa cell line models [62].

One challenge with using 3α-oxidoreductase inhibition therapy is enzyme redundancy. Dutasteride treatment and expression of catalytically inactive 3α-oxidoreductases impaired PCa cell line production of DHT, but did not reduce DHT levels completely in all experimental conditions [62]. Expression data showed that clinical PCa specimens and PCa cell lines express at least one or more 3α-oxidoreductase enzymes [62]. Inhibition of one 3α-oxidoreductase enzyme may impair DHT production, but remain insufficient to deplete DHT levels and inactivate AR. An ideal inhibitor would target all four 3α-oxidoreductases. However, the enzymes have low protein sequence homology. This implies that there are variations in the precise substrate recognition regions of the enzymes despite their sharing 100% identity among their catalytic residues [62,63].

Finally, the role of post-translational modification and activity regulation in 3α-oxidoreductase remains unclear in clinical prostate specimens. These regulators of 3α-

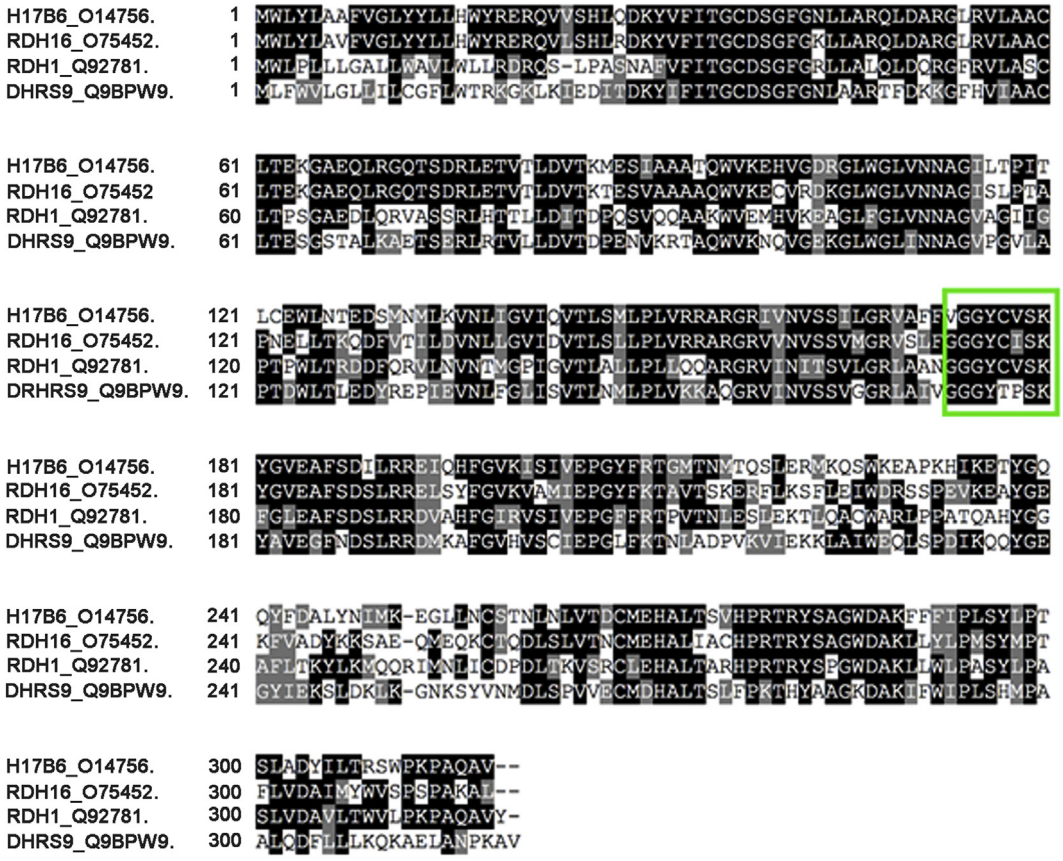

Figure 3 Sequence alignment of the four 3α-oxidoreductases showed that their catalytic amino acid residues are conserved (green box). COBALT protein sequence alignment shows the conserved catalytic amino acid residues among the four 3α-oxidoreductases.

oxidoreductase activity may provide an alternative target for catalytic inhibition.

5. Conclusion

The inhibition of androgen metabolism enzymes to improve PCa response to ADT or impair or prolong PCa transition to CRPC has merit. Despite success with CYP17A1 inhibition (an upstream androgen metabolism enzyme), PCa recurs or CRPC persists during treatment and remains lethal [37,39—41]. Inhibition of SRD5A to block the terminal step of the frontdoor pathway (conversion of T to DHT) has proven ineffective against CRPC [55—57]. AKR1C3 inhibition also has been unsuccessful [70]. Inhibition of the enzymes that catalyze terminal stepsin the primary backdoor androgen metabolism (conversion androstanediol to DHT) or the secondary backdoor androgen metabolism pathways (conversion androstanedione to DHT) has not been tested clinically because of a lack of candidate inhibitors.

Once inhibitors of the four 3α-oxidoreductases are developed, the lead candidate can be tested alone or combined with ADT, CYP17A1 and SRD5A1 inhibitors (Fig. 4) in order to impair upstream and downstream androgen metabolism and thereby minimize the ability of PCa cells to adapt their androgen metabolism pathways during ADT. This therapy is similar to the proposed androgen annihilation therapy [71], however, this new "complete" androgen annihilation includes 3α-oxidoreductase inhibition to impair both terminal steps of the primary and secondary backdoor androgen metabolism pathways. 3α-oxidoreductase inhibition may need to be combined with anti-androgens or AR splice variant therapeutics if 3α-oxidoreductase inhibition alone reveals AR is stimulated despite inhibition of the backdoor pathway or if AR-splice variants develop. Complete androgen annihilation should improve ADT and delay or prevent death from CRPC.

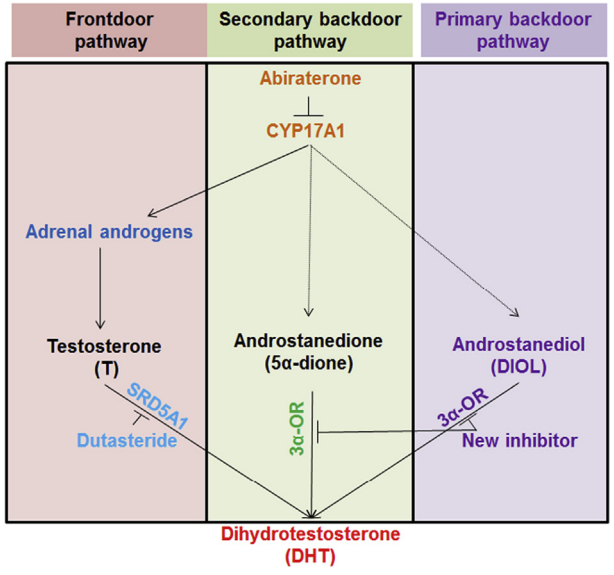

Figure 4 Model of a coordinated attack on enzymes that drive dihydrotestosterone (DHT) production that should lower DHT levels better than an upstream attack of any specific enzyme. Androgen metabolism inhibitors against CYP17A1, 3α-oxidoreductase, aldo-keto reductase and SRD5A used simultaneously should lower DHT levels better than individual enzyme inhibition alone. CYP17A1, cytochrome P450 17A; SRD5A, 5-reductases; 3α-OR, 3α-oxidoreductase.

Author contributions

Study design: James L. Mohler and Michael V. Fiandalo.
Data acquisition: Michael V. Fiandalo.
Data analysis: James L. Mohler and Michael V. Fiandalo.
Drafting of manuscript: James L. Mohler, Michael V. Fiandalo and Daniel T. Gewirth.
Figure development: All figures were developed by Drs. Mohler, Fiandalo and Gewirth.
Critical revision of the *manuscript*: James L. Mohler, Michael V. Fiandalo and Daniel T. Gewirth.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

We thank N. Que for a careful reading of the manuscript. This study was supported by the DoD Prostate Cancer Research Program Award (No. W81XWH-16-1-0635) and the National Cancer Institute (NO. P01CA77739 and NO. R21CA205108) to James L. Mohler; Post-doctoral Training Award (No. W81XWH-15-1-0409) to Michael V. Fiandalo; DoD Synergistic Idea Development Award (No. W81XWH-14-1-0520) to Dan T. Gewirth, and NCI Cancer Center Support Grant to Roswell Park Comprehensive Cancer Center for the Bioanalytics, Metabolomics and Pharmacokinetics, Pathology Network, and Genomics Shared Resources (No. P30CA016056).

References

[1] Ryan CJ, Shah S, Efstathiou E, Smith MR, Taplin ME, Bubley GJ, et al. Phase II study of abiraterone acetate in chemotherapy-naive metastatic castration-resistant prostate cancer displaying bone flare discordant with serologic response. Clin Cancer Res 2011;17:4854—61.
[2] Ryan CJ, Smith MR, Fong L, Rosenberg JE, Kantoff P, Raynaud F, et al. Phase I clinical trial of the CYP17 inhibitor abiraterone acetate demonstrating clinical activity in patients with castration-resistant prostate cancer who received prior ketoconazole therapy. J Clin Oncol 2010;28:1481—8.
[3] Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. Lancet 2010;375:1437—46.
[4] Scher HI, Fizazi K, Saad F, Taplin ME, Steinburg C, Miller K, et al. Effect of MDV3100, an androgen receptor signaling inhibitor (ARSI), on overall survivial in patients with prostate cancer postdocetaxel: results from the phase III AFFIRM study. J Clin Oncol 2012;30(5_suppl). https://doi.org/10.1200/jco.2012.30.5_suppl.lba1.
[5] Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in

patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 2006;24:3089—94.
[6] Fiandalo MV, Wu W, Mohler JL. The role of intracrine androgen metabolism, androgen receptor and apoptosis in the survival and recurrence of prostate cancer during androgen deprivation therapy. Curr Drug Targets 2013;14:420—40.
[7] Mohler JL, Gregory CW, Ford 3rd OH, Kim D, Weaver CM, Petrusz P, et al. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004;10:440—8.
[8] Mohler JL, Titus MA, Bai S, Kennerley BJ, Lih FB, Tomer KB, et al. Activation of the androgen receptor by intratumoral bioconversion of androstanediol to dihydrotestosterone in prostate cancer. Cancer Res 2011;71:1486—96.
[9] Auchus RJ. The backdoor pathway to dihydrotestosterone. Trends Endocrinol Metab 2004;15:432—8.
[10] Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. Clin Cancer Res 2005;11:4653—7.
[11] Chang KH, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ, et al. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. Proc Natl Acad Sci USA 2011;108:13728—33.
[12] Chang KH, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. Cell 2013;154:1074—84.
[13] Penning T. Androgen biosynthesis in castration resistant prostate cancer. Endocr Relat Cancer 2014;21:T67—78.
[14] Chang KH, Sharifi N. Prostate cancer—from steroid transformations to clinical translation. Nat Rev Urol 2012;9:721—4.
[15] Attard G, Parker C, Eeles RA, Schroder F, Tomlins SA, Tannock I, et al. Prostate cancer. Lancet 2016;387:70—82.
[16] Mostaghel EA, Plymate SR, Montgomery B. Molecular pathways: targeting resistance in the androgen receptor for therapeutic benefit. Clin Cancer Res 2014;20:791—8.
[17] Fankhauser M, Tan Y, Macintyre G, Haviv I, Hong MK, Nguyen A, et al. Canonical androstenedione reduction is the predominant source of signaling androgens in hormone-refractory prostate cancer. Clin Cancer Res 2014;20:5547—57.
[18] Huggins C, Scott WW. Bilateral adrenalectomy in prostatic cancer: clinical features and urinary excretion of 17-ketosteroids and estrogen. Ann Surg 1945;122:1031—41.
[19] Thorne GW, Forsham PH, Frawley TF, Hill Jr SR, Roche M, Staehelin D, et al. The clinical usefulness of ACTH and cortisone. N Engl J Med 1950;242:824—34.
[20] Huggins C, Bergenstal DM. Surgery of the adrenals. J Am Med Assoc 1951;147:101—6.
[21] Havlin KA, Trump DL. Aminoglutethimide: theoretical considerations and clinical results in advanced prostate cancer. Cancer Treat Res 1988;39:83—96.
[22] Dexter RN, Fishman LM, Ney RL, Liddle GW. Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. J Clin Endocrinol Metab 1967;27:473—80.
[23] Trump DL, Havlin KH, Messing EM, Cummings KB, Lange PH, Jordan VC. High-dose ketoconazole in advanced hormone-refractory prostate cancer: endocrinologic and clinical effects. J Clin Oncol 1989;7:1093—8.
[24] De Coster R, Caers I, Coene MC, Amery W, Beerens D, Haelterman C. Effects of high dose ketoconazole therapy on the main plasma testicular and adrenal steroids in previously untreated prostatic cancer patients. Clin Endocrinol (Oxf) 1986;24:657—64.
[25] Trachtenberg J, Zadra J. Steroid synthesis inhibition by ketoconazole: sites of action. Clin Invest Med 1988;11:1—5.
[26] Ahmann FR, Crawford ED, Kreis W, Levasseur Y. Adrenal steroid levels in castrated men with prostatic carcinoma treated with aminoglutethimide plus hydrocortisone. Cancer Res 1987;47:4736—9.
[27] Keizman D, Huang P, Carducci MA, Eisenberger MA. Contemporary experience with ketoconazole in patients with metastatic castration-resistant prostate cancer: clinical factors associated with PSA response and disease progression. Prostate 2012;72:461—7.
[28] Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkerd E, et al. Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol 2009;27:3742—8.
[29] Jarman M, Barrie SE, Llera JM. The 16,17-double bond is needed for irreversible inhibition of human cytochrome p45017alpha by abiraterone (17-(3-pyridyl)androsta-5, 16-dien-3beta-ol) and related steroidal inhibitors. J Med Chem 1998;41:5375—81.
[30] de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995—2005.
[31] Scher HI, Heller G, Molina A, Kheoh T, Attard G, Moreira J, et al. Evaluation of circulating tumor cell (CTC) enumeration as an efficacy response biomarker of overall survivial (OS) in metastatic castration-resistant prostate cancer (mCRPC); Planned final analysis (FA) of Cou-AA-301, a randomized double-blind, placebo-controlled phase III study of abiraterone acetate (AA) plus low-dose prednisone (P) post docetaxel. J Clin Oncol 2011;29(18_suppl). https://doi.org/10.1200/jco.2011.29.18_suppl.lba4517.
[32] Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138—48.
[33] Reid AH, Attard G, Danila DC, Oommen NB, Olmos D, Fong PC, et al. Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. J Clin Oncol 2010;28:1489—95.
[34] Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017;377:352—60.
[35] Cavo A, Rubagotti A, Zanardi E, Fabbroni C, Zinoli L, Di Meglio A, et al. Abiraterone acetate and prednisone in the pre- and post-docetaxel setting for metastatic castration-resistant prostate cancer: a mono-institutional experience focused on cardiovascular events and their impact on clinical outcomes. Ther Adv Med Oncol 2018;10. https://doi.org/10.1177/1758834017745819.
[36] Attard G, Reid AH, Auchus RJ, Hughes BA, Cassidy AM, Thompson E, et al. Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. J Clin Endocrinol Metab 2012;97:507—6.
[37] Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. 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—25.
[38] Tamae D, Mostaghel E, Montgomery B, Nelson PS, Balk SP, Kantoff PW, et al. The DHEA-sulfate depot following P450c17 inhibition supports the case for AKR1C3 inhibition in high risk localized and advanced castration resistant prostate cancer. Chem Biol Interact 2015;234:332—8.
[39] Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, et al. Intratumoral *de novo* steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011;71:6503—13.
[40] Bremmer F, Jarry H, Strauss A, Behnes CL, Trojan L, Thelen P. Increased expression of CYP17A1 indicates an effective targeting of the androgen receptor axis in castration resistant prostate cancer (CRPC). Springerplus 2014;3:574.

[41] Silverman RB. The potential use of mechanism-based enzyme inactivators in medicine. J Enzyme Inhib 1988;2:73—90.
[42] Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, et al. Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011;71:6503—13.
[43] Chang KH, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. Cell 2013;154:1074—84.
[44] Nixon M, Andrew R, Chapman KE. It takes two to tango: dimerisation of glucocorticoid receptor and its anti-inflammatory functions. Steroids 2013;78:59—68.
[45] Roy AB. The steroid 5 alpha-reductase activity of rat liver and prostate. Biochimie 1971;53:1031—40.
[46] Azzouni F, Godoy A, Li Y, Mohler J. The 5alpha-reductase isozyme family: a review of basic biology and their role in human diseases. Adv Urol 2012;2012:530121.
[47] Titus MA, Li Y, Kozyreva OG, Maher V, Godoy A, Smith GJ, et al. 5alpha-reductase type 3 enzyme in benign and malignant prostate. Prostate 2014;74:235—49.
[48] Godoy A, Kawinski E, Li Y, Oka D, Alexiev B, Azzouni F, et al. 5alpha-reductase type 3 expression in human benign and malignant tissues: a comparative analysis during prostate cancer progression. Prostate 2011;71:1033—46.
[49] Fleshner NE, Trachtenberg J. Combination finasteride and flutamide in advanced carcinoma of the prostate: effective therapy with minimal side effects. J Urol 1995;154:1642—5. discussion 1645—6.
[50] Leibowitz RL, Tucker SJ. Treatment of localized prostate cancer with intermittent triple androgen blockade: preliminary results in 110 consecutive patients. Oncologist 2001;6:177—82.
[51] Dutkiewicz SA. Comparison of maximal and more maximal intermittent androgen blockade during 5-year treatment of advanced prostate cancer T3NxMx-1. Int Urol Nephrol 2012;44:487—92.
[52] Tay MH, Kaufman DS, Regan MM, Leibowitz SB, George DJ, Febbo PG, et al. Finasteride and bicalutamide as primary hormonal therapy in patients with advanced adenocarcinoma of the prostate. Ann Oncol 2004;15:974—8.
[53] Ornstein DK, Rao GS, Johnson B, Charlton ET, Andriole GL. Combined finasteride and flutamide therapy in men with advanced prostate cancer. Urology 1996;48:901—5.
[54] Shah S, Trump D, Sartor O, Tan W, Wilding G, Mohler J. Phase II study of dutasteride for recurrent prostate cancer during androgen deprivation therapy. J Urol 2009;181:621—6.
[55] Wurzel R, Ray P, Major-Walker K, Shannon J, Rittmaster R. The effect of dutasteride on intraprostatic dihydrotestosterone concentrations in men with benign prostatic hyperplasia. Prostate Cancer Prostatic Dis 2007;10:149—54.
[56] Yamana K, Labrie F, Luu-The V. Human type 3 5alpha-reductase is expressed in peripheral tissues at higher levels than types 1 and 2 and its activity is potently inhibited by finasteride and dutasteride. Horm Mol Biol Clin Invest 2010;2:293—9.
[57] Shah SK, Trump DL, Sartor O, Tan W, Wilding GE, Mohler JL. Phase II study of Dutasteride for recurrent prostate cancer during androgen deprivation therapy. J Urol 2009;181:621—6.
[58] Day JM, Tutill HJ, Purohit A, Reed MJ. Design and validation of specific inhibitors of 17beta-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. Endocr Relat Cancer 2008;15:665—92.
[59] Auchus RJ, Yu MK, Nguyen S, Mundle SD. Use of prednisone with abiraterone acetate in metastatic castration-resistant prostate cancer. Oncologist 2014;19:1231—40.
[60] Li J, Alyamani M, Zhang A, Chang KH, Berk M, Li Z, et al. Aberrant corticosteroid metabolism in tumor cells enables GR takeover in enzalutamide resistant prostate cancer. Elife 2017;6. https://doi.org/10.7554/eLife.20183.
[61] Snaterse G, Visser JA, Arlt W, Hofland J. Circulating steroid hormone variations throughout different stages of prostate cancer. Endocr Relat Cancer 2017;24:R403—20.
[62] Fiandalo MV, Stocking JJ, Pop EA, Wilton JH, Mantione KM, Li Y, et al. Inhibition of dihydrotestosterone synthesis in prostate cancer by combined frontdoor and backdoor pathway blockade. Oncotarget 2017;9:11227—42.
[63] Tanaka N. SDR: structure, mechanism of action, and substrate recognition. Curr Org Chem 2001;5. 89—11.
[64] Biswas MG, Russell DW. Expression cloning and characterization of oxidative 17beta- and 3alpha-hydroxysteroid dehydrogenases from rat and human prostate. J Biol Chem 1997;272:15959—66.
[65] Kavanagh KL, Jornvall H, Persson B, Oppermann U. Medium- and short-chain dehydrogenase/reductase gene and protein families: the SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. Cell Mol Life Sci 2008;65:3895—906.
[66] Persson B, Kallberg Y, Bray JE, Bruford E, Dellaporta SL, Favia AD, et al. The SDR (short-chain dehydrogenase/reductase and related enzymes) nomenclature initiative. Chem Biol Interact 2009;178:94—8.
[67] Fiandalo MV, Wilton J, Mohler JL. Roles for the backdoor pathway of androgen metabolism in prostate cancer response to castration and drug treatment. Int J Biol Sci 2014;10:596—601.
[68] Mohler JL, Titus MA, Wilson EM. Potential prostate cancer drug target: bioactivation of androstanediol by conversion to dihydrotestosterone. Clin Cancer Res 2011;17:5844—9.
[69] Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, et al. Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. Cancer Res 2015;75:1413—22.
[70] Loriot Y, Fizazi K, Jones RJ, Van den Brande J, Molife RL, Omlin A, et al. Safety, tolerability and anti-tumour activity of the androgen biosynthesis inhibitor ASP9521 in patients with metastatic castration-resistant prostate cancer: multi-centre phase I/II study. Invest New Drugs 2014;32:995—1004.
[71] Mohler JL. Concept and viability of androgen annihilation for advanced prostate cancer. Cancer 2014;120:2628—37.