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MONOGRAPH

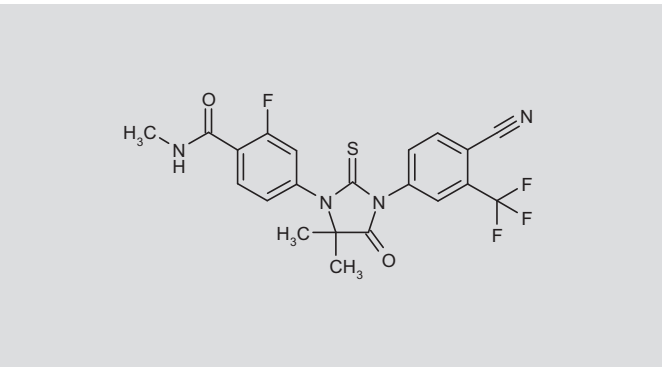
MDV-3100

Androgen Receptor Antagonist
Prostate Cancer Therapy

NC-54

4-[3-[4-Cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]-2-fluoro-*N*-methylbenzamide

InChI: 1S/C21H16F4N4O2S/c1-20(2)18(31)28(12-5-4-11(10-26)15(8-12)21(23,24)25)19(32)29(20)13-6-7-14(16(22)9-13)17(30)27-3/h4-9H,1-3H3,(H,27,30)



$C_{21}H_{16}F_4N_4O_2S$

Mol wt: 464.436

EN: 438422

SUMMARY

Prostate cancer is the most common cancer in men in the U.S. and the second most lethal. Nearly all prostate cancer deaths occur due to castration-resistant prostate cancer (CRPC) that has progressed despite androgen deprivation therapy (ADT). These therapies are designed to lower levels of androgens that can activate the androgen receptor (AR), the central protein in prostate cancer. We now know that androgens and androgen-dependent signaling pathways modulated by AR persist in CRPC cells despite ADT. However, until recently, effective agents to antagonize these persistent androgens and to disrupt AR signaling in CRPC cells were not available. MDV-3100 is a new antiandrogen that effectively inhibits androgen binding to and activation of AR in preclinical studies in CRPC cells. A phase I/II clinical trial with MDV-3100 in CRPC patients also showed encouraging results, which led to the initiation of two ongoing phase III clinical trials. In this review, we will focus on its preclinical development and summarize the clinical results to date.

L. Gao and J. Alumkal. Oregon Health & Science University – Medicine, Division of Hematology and Oncology, 3303 SW Bond Ave., CH14R, Portland, OR 97239, USA. E-mail: alumkalj@ohsu.edu.
*Synthesis prepared by R. Pandian, J. Bolòs, R. Castañer. Thomson Reuters, Provença 398, 08025 Barcelona, Spain.

SYNTHESIS*

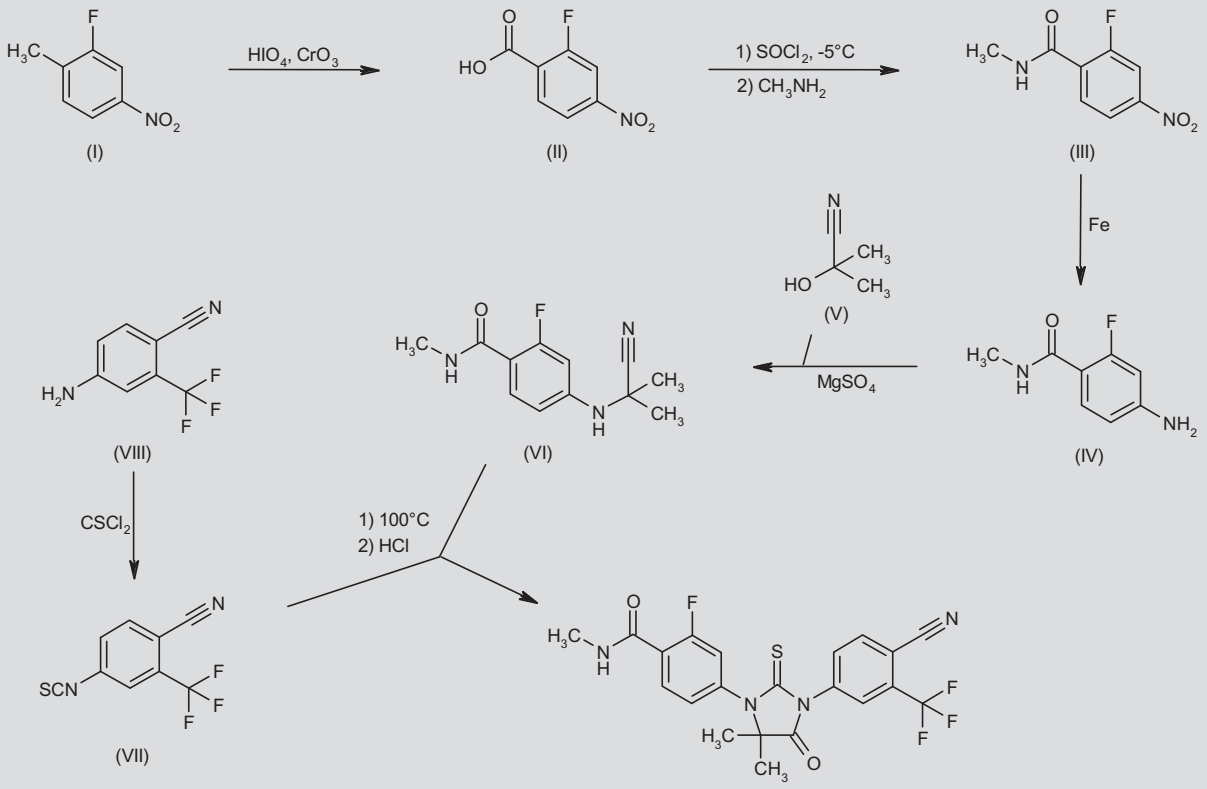
Oxidation of 2-fluoro-4-nitrotoluene (I) by means of periodic acid and CrO_3 affords 2-fluoro-4-nitrobenzoic acid (II), which by chlorination with $SOCl_2$ in DMF at −5 °C, followed by condensation with methylamine, yields 2-fluoro-*N*-methyl-4-nitrobenzamide (III). Reduction of the nitro group in compound (III) with Fe in refluxing AcOH/EtOAc gives amine (IV), which is then condensed with pure acetone cyanohydrin (V) in the presence of anhydrous $MgSO_4$ at 80 °C to provide 4-(1-cyano-1-methylethylamino)-2-fluoro-*N*-methylbenzamide (VI). Finally, compound (VI) is condensed with 4-isothiocyanato-2-(trifluoromethyl)benzonitrile (VII) (prepared by reaction of 4-amino-2-(trifluoromethyl)benzonitrile (VIII) with thiophosgene in H_2O) in DMF heated to 100 °C under microwave irradiation, and then cyclized by means of HCl in refluxing MeOH (1-3). Scheme 1.

BACKGROUND

Every year in the U.S., 218,000 men are diagnosed with prostate cancer and 32,000 men die from metastatic castration-resistant prostate cancer (CRPC) that has progressed despite androgen deprivation therapy (ADT), such as surgical castration or chemical castration with luteinizing hormone-releasing hormone (LHRH) agonists (4). These forms of ADT are designed to lower androgen production in the body and hence to prevent activation of the androgen receptor (AR), the expression of which is ubiquitous in prostate cancer. AR is a member of the nuclear steroid receptor family. Like other members of this family, AR is a ligand-dependent transcription factor that is activated by the binding of its ligand androgens (5). Among AR's androgen ligands are testosterone, which is produced primarily by the testes, and its more potent derivative 5α-dihydrotestosterone (DHT), which is converted from testosterone in the prostate (6).

Upon androgen binding, AR undergoes a conformational change, self-dimerizes and translocates into the nucleus, where it binds to consensus sequences in the genome that are called androgen-response elements (AREs) (6). AR also recruits transcriptional coactivators that facilitate gene transcription of AR target genes. These target genes include *KLK3*, which encodes for the prostatic-specific antigen (PSA) protein, the *TMPRSS2-ERG* oncogene, and many other genes that are essential for prostate cancer progression (6, 7). For that reason, AR is the primary therapeutic target in prostate cancer (8).

Scheme 1. Synthesis of MDV-3100



A widely used therapy in CRPC patients after progression despite initial ADT are antiandrogens, including bicalutamide. These drugs compete with residual intratumoral androgens for binding to AR. While tumor responses can be seen with bicalutamide or related antiandrogens, these responses are not long-lasting. Additionally, over time, bicalutamide can exhibit partial agonist properties that may promote prostate cancer growth (9, 10). These agonist effects of bicalutamide are most pronounced in prostate cancers with elevated AR protein levels, which commonly occurs in CRPC (10-12).

Multiple mechanisms may promote the evolution of castration-sensitive prostate cancer cells (CSPCs) to CRPC. While androgen-independent activation of AR has been described, most resistance mechanisms involve continued androgen dependence of AR in CRPC cells (9, 13, 14). In an earlier effort by Charles Sawyers' group to identify mechanisms that drive the evolution of CSPC cells to CRPC cells, they chronically grew AR-expressing CSPC cell lines in the absence of androgens. AR overexpression was seen in every single CRPC derivative cell line that emerged after chronic androgen deprivation. Moreover, elevated AR protein levels were essential and sufficient to render these cells resistant to bicalutamide. Overexpression of a mutant AR protein lacking a functional ligand (androgen)-binding domain (LBD) did not lead to bicalutamide resistance, which demonstrated the importance of this region, where both androgens and antiandrogens bind (10).

Other AR resistance mechanisms to ADT include: AR gain-of-function mutations, expression of ligand-independent AR transcript variants, and overexpression of AR coactivators (15-17). An example of the latter includes nuclear receptor coactivator 3 (steroid receptor coactivator protein 3, SRC-3), the overexpression of which in prostate cancer samples correlates with increased proliferation (18). SRC-3 is not only a coactivator for activated AR but also upregulates the insulin-like growth factor (IGF)/Akt pathway to promote cell proliferation and survival of prostate cancer cells despite androgen deprivation (19-21).

It is now clear that androgens and androgen-dependent AR target gene expression persist in CRPC patient samples (22, 23). Thus, AR remains a therapeutic target even after disease progression on ADT. Indeed, most of the efforts in prostate cancer therapeutics today are focused on developing better therapies to lower androgen production or to interfere with androgen binding to AR. Abiraterone acetate is a compound that has recently gained approval by the U.S. Food and Drug Administration (FDA). Abiraterone acetate reduces the production of androgens not only in the testes and the adrenal

glands, which are a sanctuary site of androgen production that are unaffected by chemical castration, but it may also reduce androgen production within prostate cells (24, 25). MDV-3100, on the other hand, is an antiandrogen and has shown promising results in both preclinical testing in CRPC cells and in phase I/II clinical trials in CRPC patients (26-28).

PRECLINICAL PHARMACOLOGY

As described earlier, androgens induce a conformational change in AR upon binding, which leads to a series of events: AR translocation into the nucleus, AR binding to androgen response elements (AREs), recruitment of transcription coactivators, and gene transcription of AR target genes (8). In an effort to identify better antiandrogens that more effectively compete for binding to AR, Tran et al. performed a screen of lead compounds. MDV-3100 was further pursued because of its potent antagonism of androgen binding and AR activation (26).

The primary model system used in subsequent experiments was the LNCaP/AR prostate cancer cell line that these authors engineered to overexpress AR at levels commonly seen in CRPC patients. MDV-3100 showed greater suppression of the growth of these cells than bicalutamide both in vitro and in vivo in xenograft studies (100% of LNCaP/AR tumors implanted in castrated male mice regressed when treated with MDV-3100 compared to 8% treated with bicalutamide). The authors determined that MDV-3100 has unique mechanisms of action that account for these effects, which are described below (Table I and Fig. 1) (26).

Affinity for AR

In order for androgens to promote prostate cancer growth, they must first bind to AR. In an in vitro competition assay, the affinity of MDV-3100 for AR was only two- to threefold less than DHT. Bicalutamide, on the other hand, had an affinity for AR that was five- to eightfold less than MDV-3100. This is important therapeutically because the greater potency of MDV-3100 may allow for lower doses than conventional antiandrogens like bicalutamide (26).

Nuclear localization

In order for AR to bind to DNA, it must first translocate to the nucleus. In LNCaP cells, bicalutamide reduced nuclear AR localization by 50% compared to the synthetic androgen R-1881. MDV-3100, on the other hand, reduced nuclear AR localization by 90% (26).

DNA binding

In order for AR to turn on the expression of its target genes, it must bind to its AREs. To examine the effect of MDV-3100 on DNA binding by AR, cells were transfected with an AR expression plasmid bearing a nuclear localization sequence and a synthetic plasmid with an ARE sequence upstream of the luciferase gene. The cells were treated with R-1881, bicalutamide or MDV-3100. In this system, luciferase reporter activity represents the ability of AR to bind to its ARE and drive transcription. Both R-1881 and bicalutamide (even at concentrations as low as 1 μM) activated the reporter. MDV-3100, on the other hand, reduced luciferase reporter activity, which indicates that MDV-3100 disrupted AR function (26).

Table I. *Comparison of the effects of the antiandrogens bicalutamide and MDV-3100 on disrupting androgen receptor (AR) function.*

	Bicalutamide	MDV-3100
Interferes with AR nuclear localization	+	++
Interferes with AR DNA binding in AR-overexpressing cells	No	Yes
Prevents AR coactivator binding	No	Yes
Pure antagonist in AR-overexpressing cells	No	Yes

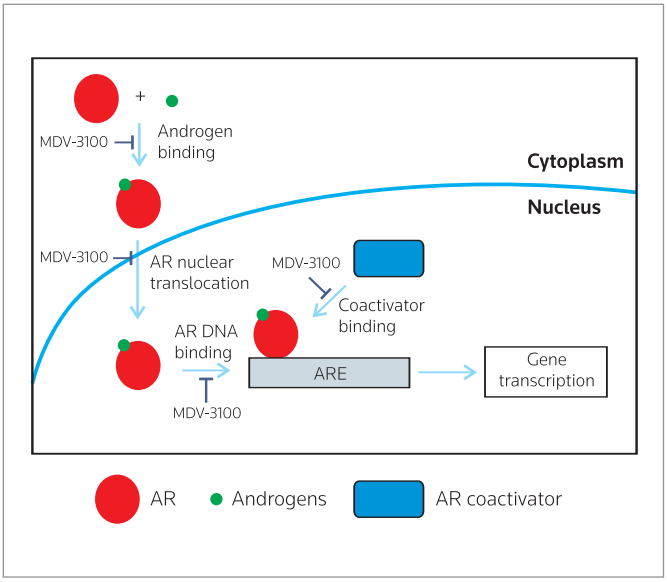


Figure 1. MDV-3100 disrupts androgen receptor (AR) signaling at multiple control points. MDV-3100 prevents androgen binding to AR, AR nuclear translocation, AR binding to DNA and AR binding to its coactivators. ARE, androgen response element.

Recruitment to enhancer elements

Rather than restricting their analysis of AR binding to a synthetic plasmid sequence, the authors also determined the effects of MDV-3100 treatment on AR localization to AREs in the genome by chromatin immunoprecipitation (ChIP) assays. In LNCaP/AR cells, bicalutamide promoted AR binding to its target genes' AREs, which resulted in transcriptional activation of these genes (10, 26). However, MDV-3100 prevented AR recruitment to AREs of its target genes (26).

Coactivator binding

In order to determine the effect on assembly of AR coactivators, the authors performed fluorescence resonance energy transfer (FRET) experiments. These experiments showed that bicalutamide, like DHT, promoted the interaction between AR and FxxLF motif-containing peptides, while MDV-3100 did not. These FxxLF motifs are present in many AR coactivators, and these results demonstrate that MDV-3100, unlike bicalutamide, disrupts the interaction between AR and its coactivators (26).

All of these properties of MDV-3100 demonstrate that it is a pure androgen antagonist, and unlike bicalutamide, MDV-3100 does not

have any agonist activity. Finally, a recent report demonstrated that AR transcript variants that lack a ligand-binding domain promote CRPC progression by enhancing the function of the full-length AR protein. However, MDV-3100 remains effective in suppressing AR function and the growth of CRPC cell lines expressing these constitutively active AR splice variants (27). This further demonstrates the ability of MDV-3100 to overcome another newly characterized and common form of castration resistance.

SAFETY

MDV-3100 was well tolerated in a phase I/II study. Fatigue was the most common grade 3 or 4 side effect, occurring in 11% of patients. Other common grade 3-4 side effects included: anemia (3%), arthralgia (2%), asthenia (2%) and seizures (2%). All of the three possible seizures occurred in patients receiving doses of 360 mg/day or higher. Two of these seizures were witnessed, and it is still unclear whether MDV-3100 was responsible, as these two patients had other risk factors for seizures, including the use of other medications known to lower the seizure threshold (28). However, given the seriousness of this adverse event, 240 mg was chosen as the safe and effective dose for subsequent phase III studies.

CLINICAL STUDIES

Based upon the wealth of encouraging preclinical data, MDV-3100 began clinical testing in patients with CRPC in 2007. The phase I/II study of MDV-3100 included patients with CRPC who had not received prior docetaxel chemotherapy (pre-chemotherapy group with or without overt metastases) or those with metastatic CRPC who had received prior docetaxel chemotherapy (post-chemotherapy group) (28). Dose cohorts included: 30, 60, 150, 240, 360, 480 and 600 mg once per day. A standard 3+3 design was initially employed. The maximum tolerated dose (MTD) was not reached in the first two dose cohorts, and all six of these patients experienced PSA declines. Therefore an additional 12 pre-chemotherapy and 12 post-chemotherapy patients were treated in subsequent dose cohorts ranging from 60 to 360 mg. Eventually, because a future phase III trial in the post-chemotherapy setting was planned, only post-chemotherapy patients were included in the highest-dose cohorts of 480 and 600 mg/day. Following oral administration, MDV-3100 was rapidly absorbed, and the half-life was 1 week (range: 3-13 days for individual patients) (28). While full pharmacokinetic (PK) data were not reported, the profile appeared to be linear across the tested dose range. Of note, the steady-state concentration of MDV-3100 in patients treated with the 150 mg/day dose –10 μg/mL– was similar to concentrations that were effective in CRPC xenograft models (26, 28).

The authors also determined the effect of MDV-3100 on androgen binding within tumor sites with 16β-[^{18}F]-fluoro-5α-dihydrotestosterone (FDHT) and 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG) PET-CT scans in 22 patients. All patients showed a reduction in FDHT uptake (range: 20-100%), which indicates androgen displacement, at doses ranging from 60 to 480 mg/day (28). These data suggest that MDV-3100 was capable of interfering with androgen action within prostate tumors in vivo, which recapitulates the preclinical findings (26, 28).

PSA is the protein product of the AR target gene *KLK3* that is secreted into the bloodstream. Declines in serum PSA levels either indicate attenuated AR signaling or, when the PSA declines are sustained, death of prostate cancer cells. Nearly all patients, regardless of prior chemotherapy exposure, experienced a decline in PSA levels while on study. At 12 weeks, the proportion of patients with > 50% declines in PSA levels was not statistically different between the two groups –62% of pre-chemotherapy patients and 51% of post-chemotherapy patients achieved this degree of PSA decline (28).

PSA response rates did not differ between those who had received three or more prior hormonal therapies versus those who had received two or fewer treatments. However, the rate of PSA decline (> 50% decrease from baseline) in patients who had received prior ketoconazole, an inhibitor of adrenal androgen synthesis, was lower than those who had not received prior ketoconazole (37%; 95% confidence interval [CI]: 25-50% vs. 71%, 60-81; $P = 0.0007$). Thus, subsequent phase III trials with MDV-3100 excluded patients with prior ketoconazole exposure. In the post-chemotherapy patients, rates of PSA decline were similar in those who had received one versus two prior chemotherapy regimens (28).

The time to PSA progression was also determined for all patients. In the pre-chemotherapy patients, the median time to PSA progression had not been reached at the time of publication but exceeded the 7.7-month time to PSA progression reported in the phase III study of docetaxel, which was approved by the FDA due to its demonstrated survival benefit (28, 29). For post-chemotherapy patients, the median time to PSA progression was 27 weeks (28). This contrasts with data from another study in a similar patient population with the drug mitoxantrone, another FDA-approved agent used in prostate cancer patients after progression on docetaxel. In that study, the median time to progression was only 2.2 months (30).

The median time to radiographic progression was not reached in pre-chemotherapy patients and was 29 weeks in patients who had received prior chemotherapy (28). Soft tissue partial responses (> 50% tumor shrinkage) were seen in 36% of pre-chemotherapy patients and 12% of post-chemotherapy patients. At 12 weeks, bone scans remained stable in the majority of pre-chemotherapy (63%) and post-chemotherapy patients (51%). FDG-PET scans were also performed in a subset of patients, and ≥ 25% reductions in FDG avidity were seen in 33% of pre-chemotherapy patients and 60% of post-chemotherapy patients. MDV-3100 also reduced circulating tumor cell counts, which are an emerging predictive marker (28, 31).

In 2009, the phase III AFFIRM study designed to evaluate MDV-3100 versus placebo in CRPC patients who had received prior docetaxel chemotherapy opened. The primary endpoint is overall survival. This trial recently closed to accrual, and results are awaited. The phase III PREVAIL study in asymptomatic patients with CRPC who have not received prior docetaxel chemotherapy was recently launched and is expected to complete accrual in late 2011.

CONCLUSIONS

Androgen deprivation therapy has been the principal treatment for metastatic prostate cancer for over 70 years since the Nobel prize-winning work of Charles Huggins (32). More recent work demon-

strates mechanisms of resistance to ADT, including persistent androgen activation of AR in CRPC cells (22, 23). Results thus far with MDV-3100 demonstrate that disruption of residual androgens and androgen-dependent AR signaling in CRPC is both rational and that it may provide clinical benefit to patients with CRPC. We await results of the two ongoing MDV-3100 phase III trials to determine if MDV-3100 improves survival for CRPC patients.

If a comparable survival benefit to abiraterone acetate is seen with MDV-3100 treatment and if MDV-3100 is well tolerated, it may become the standard first-line treatment after disease progression on docetaxel chemotherapy. This is due to the fact that treatment with MDV-3100, as opposed to abiraterone acetate, does not require concomitant administration of corticosteroids, the long-term use of which is associated with an increased risk of infections and osteoporosis. If MDV-3100 does prolong survival and receives approval by the U.S. FDA, further preclinical studies will be necessary to determine the mechanisms of resistance to MDV-3100, so that the next generation of antiandrogens may be developed.

SOURCES

Medivation, Inc. (US); licensed worldwide for codevelopment and co-commercialization to Astellas Pharma, Inc. (JP).

DISCLOSURES

Dr. Alumkal is a coinvestigator in the phase I/II MDV-3100 trial and both phase III MDV-3100 trials, but he receives no financial or research support from the manufacturer. Dr. Gao states no conflicts of interest.

REFERENCES

1. Sawyers, C.L., Jung, M.E., Chen, C.D., Ouk, S., Welsbie, D., Tran, C., Wongvipat, J., Yoo, D. (University of California, Oakland). *Diarylhydantoin compounds*. EP 1893196, JP 2008540523, US 2007004753, US 7709517, WO 2006124118.
2. Jung, M., Yoo, D., Sawyers, C.L., Tran, C. (University of California, Oakland). *Diarylthiohydantoin compounds*. EP 2013187, JP 2009531449, US 2007254933, US 2008139634, WO 2007127010.
3. Jung, M.E., Ouk, S., Yoo, D., Sawyers, C.L., Chen, C., Tran, C., Wongvipat, J. *Structure-activity relationship for thiohydantoin androgen receptor antagonists for castration-resistant prostate cancer (CRPC).* J Med Chem 2010 53(10): 2779-96.
4. Jemal, A., Siegel, R., Xu, J., Ward, E. *Cancer statistics,* 2010. CA Cancer J Clin 2010, 60(5): 277-300.
5. O'Malley, B.W., Kumar, R. *Nuclear receptor coregulators in cancer biology.* Cancer Res 2009, 69(21): 8217-22.
6. Dehm, S.M., Tindall, D.J. *Molecular regulation of androgen action in prostate cancer.* J Cell Biochem 2006, 99(2): 333-44.
7. Tomlins, S.A., Rhodes, D.R., Perner, S. et al. *Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer.* Science 2005, 310(5748): 644-8.
8. Taplin, M.E. *Drug insight: Role of the androgen receptor in the development and progression of prostate cancer.* Nat Clin Pract Oncol 2007, 4(4): 236-44.
9. Chen, Y., Sawyers, C.L., Scher, H.I. *Targeting the androgen receptor pathway in prostate cancer.* Curr Opin Pharmacol 2008, 8(4): 440-8.
10. Chen, C.D., Welsbie, D.S., Tran, C. et al. *Molecular determinants of resistance to antiandrogen therapy.* Nat Med 2004, 10(1): 33-9.
11. Culig, Z., Hoffmann, J., Erdel, M. et al. *Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system.* Br J Cancer 1999, 81(2): 242-51.
12. Taplin, M.E., Rajeshkumar, B., Halabi, S. et al. *Androgen receptor mutations in androgen-independent prostate cancer: Cancer and Leukemia Group B Study 9663.* J Clin Oncol 2003, 21(14): 2673-8.
13. Culig, Z., Bartsch, G. *Androgen axis in prostate cancer.* J Cell Biochem 2006, 99(2): 373-81.
14. Wang, Q., Li, W., Zhang, Y. et al. *Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer.* Cell 2009, 138(2): 245-56.
15. Scher, H.I., Sawyers, C.L. *Biology of progressive, castration-resistant prostate cancer: Directed therapies targeting the androgen-receptor signaling axis.* J Clin Oncol 2005, 23(32): 8253-61.
16. Hu, R., Dunn, T.A., Wei, S. et al. *Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer.* Cancer Res 2009, 69(1): 16-22.
17. Guo, Z., Yang, X., Sun, F. et al. *A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth.* Cancer Res, 2009, 69(6): 2305-13.
18. Zhou, H.J., Yan, J., Luo, W. et al. *SRC-3 is required for prostate cancer cell proliferation and survival.* Cancer Res 2005, 65(17): 7976-83.
19. Zhou, X.E., Suino-Powell, K.M., Li, J. et al. *Identification of SRC3/AIB1 as a preferred coactivator for hormone-activated androgen receptor.* J Biol Chem 2010, 285(12): 9161-71.
20. Yan, J., Yu, C.T., Ozen, M., Ittmann, M., Tsai, S.Y., Tsai, M.J. *Steroid receptor coactivator-3 and activator protein-1 coordinately regulate the transcription of components of the insulin-like growth factor/AKT signaling pathway.* Cancer Res 2006, 66(22): 11039-46.
21. Zou, J.X., Zhong, Z., Shi, X.B. et al. *ACTR/AIB1/SRC-3 and androgen receptor control prostate cancer cell proliferation and tumor growth through direct control of cell cycle genes.* Prostate 2006, 66(14): 1474-86.
22. Mohler, J.L. *Castration-recurrent prostate cancer is not androgen-independent.* Adv Exp Med Biol 2008, 617: 223-34.
23. Mostaghel, E.A., Page, S.T., Lin, D.W. et al. *Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: Therapeutic implications for castration-resistant prostate cancer.* Cancer Res 2007, 67(10): 5033-41.
24. Attard, G., Reid, A.H., A'Hern, R. 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(23): 3742-8.
25. Attard, G., Reid, A.H., Yap, T.A. et al. *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 2008, 26(28): 4563-71.
26. Tran, C., Ouk, S., Clegg, N.J. et al. *Development of a second-generation antiandrogen for treatment of advanced prostate cancer.* Science 2009, 324(5928): 787-90.
27. Watson, P.A., Chen, Y.F., Balbas, M.D. et al. *Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor.* Proc Natl Acad Sci U S A 2010, 107(39): 16759-65.
28. Scher, H.I., Beer, T.M., Higano, C.S. et al. *Antitumour activity of MDV3100 in castration-resistant prostate cancer: A phase 1-2 study.* Lancet 2010, 375(9724): 1437-46.

29. Tannock, I.F., de Wit, R., Berry, W.R. et al. *Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer.* N Engl J Med 2004, 351(15): 1502-12.

30. Rosenberg, J.E., Weinberg, V.K., Kelly, W.K. et al. *Activity of second-line chemotherapy in docetaxel-refractory hormone-refractory prostate cancer patients: Randomized phase 2 study of ixabepilone or mitoxantrone and prednisone.* Cancer 2007, 110(3): 556-63.

31. de Bono, J.S., Scher, H.I., Montgomery, R.B. et al. *Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer.* Clin Cancer Res 2008, 14(19): 6302-9.

32. Huggins, C., Hodges, C.V. *Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate.* Cancer Res 1941, 1: 293-7.