Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Endocrine therapy for prostate cancer: review of the latest clinical evidence

Clin. Invest. (2011) 1(4), 533–541

Che-Kai Tsao[1], Sonia Seng[1], William K Oh[1] & Matthew D Galsky[†1]

[1]Mount Sinai School of Medicine, The Tisch Cancer Institute, 1 Gustave L Levy Place, NY 10029, USA

[†]Author for correspondence:
Tel.: +1 212 659 5412
Fax: +1 212 659 5599
E-mail: matthew.galsky@mssm.edu

Prostate cancer, the most common nonskin cancer in men, is estimated to cause 27,000 deaths annually in the USA. With the advent of prostate-specific antigen screening, most are diagnosed with local disease, but approximately one-third of patients ultimately develop recurrence despite definitive local therapy. Despite the high initial response rate to androgen deprivation, castration resistance eventually develops. In this article, we examine mechanisms of androgen resistance and further discuss current progress in the development of novel endocrine therapy in the treatment of castration-resistant prostate cancer. We also explore the rationale of complete androgen blockade, with current ongoing investigation of combination hormonal therapy in this context.

Keywords: androgen deprivation • androgen receptor • castration resistance • hormone refractory • post-translational modification • prostate cancer

Prostate cancer, the most common nonskin cancer in men, is estimated to cause 27,000 deaths annually in the USA [1]. While the introduction of widespread prostate-specific antigen (PSA) screening has significantly increased cancer detection, approximately 2% of men have metastatic disease at the time of initial presentation [2]. In addition, approximately one-third of men treated with definitive local therapy will experience recurrent disease [3]. Despite often being considered an 'indolent' disease, prostate cancer remains the second leading cause of cancer death in men in the USA, causing significant morbidity.

Since Huggins *et al.* reported the link between androgens and prostate cancer, maneuvers to deplete testosterone have been the standard first-line therapy for patients with metastatic disease [4]. The vast majority of patients achieve a response to androgen deprivation therapy, as measured by various parameters including declines in serum PSA, improvement in pain and regression of measurable disease [5,6]. However, androgen deprivation therapy in the setting of metastatic prostate cancer is not curative, and the vast majority of patients ultimately develop disease progression, despite castrate levels of serum testosterone.

While prostate cancer was once labeled 'hormone refractory' at the time of progression on androgen deprivation therapy, this term is now considered a misnomer, as many hormone refractory cancers are actually 'hormone ultrasensitive'. Over the past decade, advancement in the understanding of androgen receptor signaling axis have led to insights into the multiple mechanisms of resistance to androgen deprivation therapy. These insights have had direct clinical relevance, with the rapid development of new therapeutics, which specifically target mechanisms of resistance to androgen deprivation. Here, we describe the laboratory and clinical findings that are redefining the role of androgen receptor (AR) signaling in castration-resistant prostate cancer (CRPC).

FUTURE SCIENCE part of fsg

Redefining hormone refractory prostate cancer: clinical & laboratory evidence

The majority of androgens are produced in the testis, while the adrenal glands produce approximately 10% of physiologic serum androgen, particularly in the form of androstenediol and dehydroepiandrosterone. Testosterone, and its reduced metabolite dihydrotestosterone (DHT), are the principal stimulants of prostate cell growth. Testosterone is converted to DHT, via 5α-reductase, which binds to the AR. The DHT-bound AR then acts in the nucleus, where it functions as a DNA-binding transcription factor, regulating expression of genes that promote prostate cell growth and proliferation [7].

The majority of metastatic prostate cancers are initially dependent on testosterone synthesized by the testicles for growth and survival. However, as evidenced by clinical responses to chemical or surgical castration, small clones of resistant cells are likely present at baseline. During treatment with androgen deprivation therapy, selective pressure allows these clones to proliferate, while other clones with molecular changes will promote resistance to emerge.

Multiple mechanisms of resistance to androgen deprivation have recently been identified (Box 1). Importantly, several of these mechanisms may be involved in any given patient, and may play a differing role at various times during the natural history of the disease (Figure 1).

■ Persistent intratumoral androgens despite castration

Mounting evidence suggests that despite chemical or surgical castration, and resultant lowering of the serum testosterone, intratumoral androgen levels may not be sufficiently suppressed to inhibit prostate cancer cell growth. For example, following androgen deprivation, DHT levels in prostate tissue remained at approximately 25% of the amount measured before androgen deprivation therapy [8]. Furthermore, prostate cancer cells may contribute to the synthesis of androgens themselves, as evidenced by increased intraprostatic expression of multiple enzymes involved in steroid synthesis in the setting of castration [9–11].

Box 1. Mechanisms of castration resistance.

- Persistent systematic (e.g., adrenal) and intratumoral androgen levels
- AR gene amplification
- AR mutation
- Increased expression of AR coactivators
- AR splice variants
- Epigenetic AR modification

AR: Androgen receptor.

The contribution of adrenal androgens to the pathogenesis of CRPC has been well recognized clinically for decades. Ketoconazole, an inhibitor of cytochrome p17 (CYP17) and consequently adrenal androgens, has antitumor activity in approximately 30% of patients in this clinical state [12]. In addition, Ryan and colleagues demonstrated that higher baseline levels of serum adrenal androgens, including DHEA and androstenedione, were predictive of response to ketoconazole, and that these levels declined after treatment with ketoconazole and rose again at the time of treatment resistance [13]. This latter observation suggests a mechanism of resistance to ketoconazole that may be exploited with newer generation cytochrome p17 inhibitors.

■ AR gene amplification

Approximately 30% of CRPCs harbor amplifications of the *AR* gene. These tumors may be more sensitive resulting in enhanced tumor proliferation despite low levels of androgens [14,15]. Other mechanisms of 'adaptation' to low levels of androgens have also been described, including increased AR sensitivity to DHT [16], and increasing DHT levels via upregulation of 5-α reductase activity [17].

■ AR mutations

Mutated AR has been speculated to be activated by progesterone, estrogens, adrenal androgens and metabolic by-products of DHT [18–20]. In addition, it may also bind AR antagonists [21,22], as well as various corticosteroids [23]. For example, the clinical phenomenon of the antiandrogen withdrawal syndrome, a situation in which patients achieve a clinical response simply by discontinuing treatment with an antiandrogen, is hypothesized to occur due to mutations in the AR, causing antiandrogens to function as receptor agonists rather than antagonists [24].

■ Increased expression of AR coactivators/ decreased expression of AR corepressors

Co-activator and -repressor proteins can regulate ligand–AR binding and subsequent transcriptional activity, respectively. This has been demonstrated in several prostate cancer cell lines. One example of this phenomenon was reported by Rocchi *et al.*, reporting that androgen withdrawal in prostate cancer cell lines resulted in a significant increased hsp27 expression [25,26]. Furthermore, increasing expression of Her2/Neu tyrosine kinase [27], bcl-2 [28] and IGF-binding protein 5 [29] have all been associated with the development and growth of androgen-independent prostate cancer. Likewise, numerous corepressors have been described and hypothesized to contribute to AR regulation [30]. AR repression can be achieved with direct sequestration by DAX-1 [31], interruption of AR

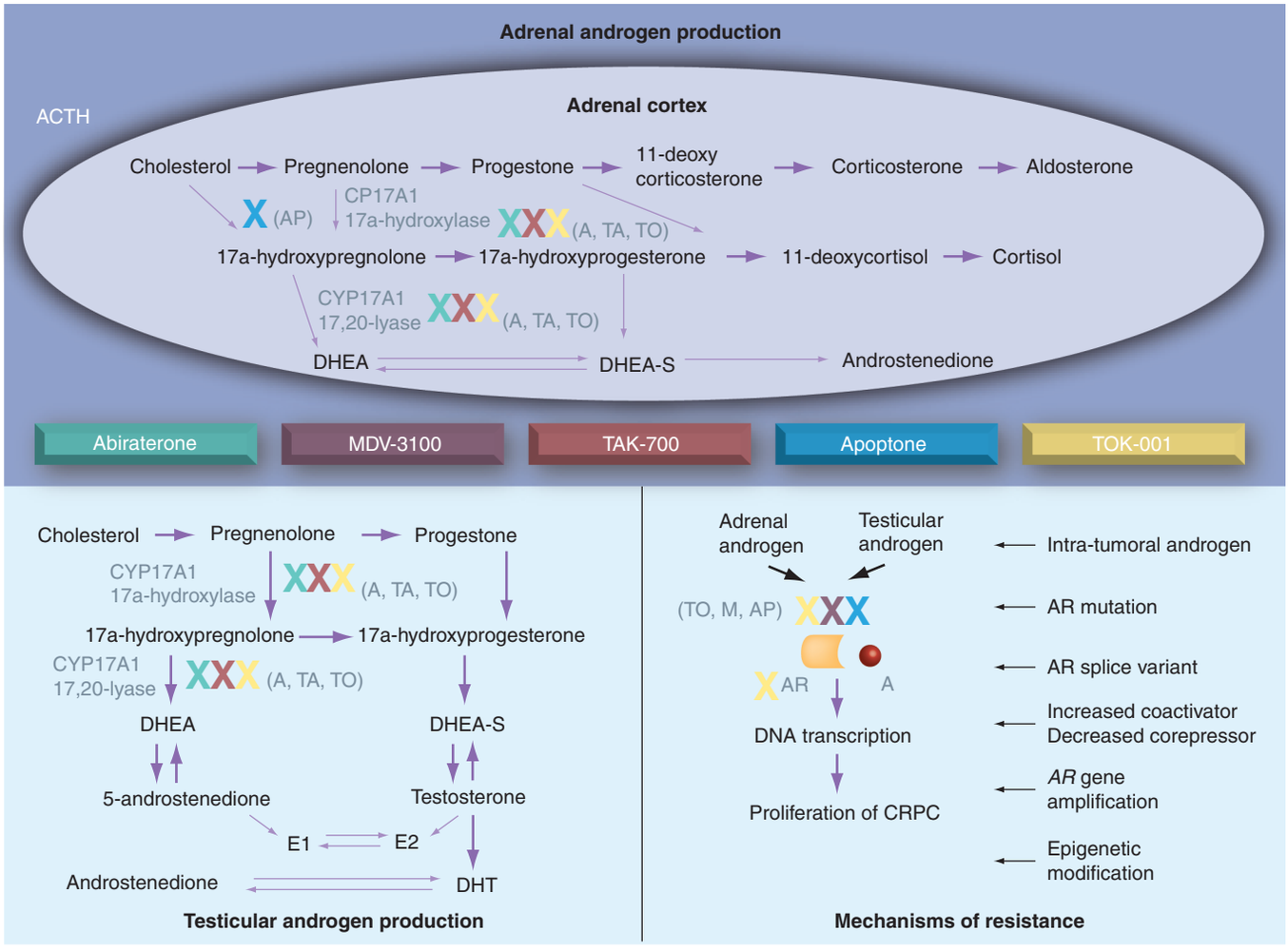

Figure 1. Androgen genesis and mechanisms of resistance in the evolution of castration-resistant prostate cancer. Represented are targets of novel endocrine therapeutic agents in the treatment of CRPC.
A: Abiraterone; AR: Androgen receptor; CRPC: Castration-resistant prostate cancer; DHT: Dihydrotestosterone; M: MDV-3100; TA: TAK-700; AP: Apoptone; TO: TOK-001.

C- and N-terminal interaction by filamin-A [32], manipulating another co-activator by PATZ [33], and well as other mechanisms involving corepressors.

■ AR splice variants

AR splice variants (ARv) have recently been described and have been hypothesized to contribute to the development of CRPC [34]. While under normal conditions, ligand binding is necessary to cause AR translocation into the nucleus and regulate expression of androgen-responsive genes, ARv isoforms are constitutively active, promoting tumor cell growth in a ligand-independent manner [35]. This mechanism of castration resistance is problematic, as most currently existing hormonal agents, and those in development, would not be expected to effectively block this ligand-independent signaling. However, recent preclinical work has demonstrated that the novel antiandrogen MDV3100 inhibits the growth of ARv prostate cancer *in vitro* [36].

■ Epigenetic AR modification

Ongoing work suggests that post-translational modifications may modulate AR function and stability. Among the most well-characterized post-translational modifications in prostate cancer are DNA methylation and histone modifications, both of which have been demonstrated in preclinical models to play an important role in progression to castration resistance [37]. In CRPC cells, the AR suppressor binding complex is downregulated secondary to hypermethylation, leading to high AR expression [38]. Conversely, DNA hypomethylation increases melanoma antigen gene protein-A11 expression during androgen deprivation, leading to increased AR signaling in CRPC [39].

The histone octamer has flexible N-terminal tails extending from the nucleosome, providing an amino acid-rich site for reversible post-translational modification. Mechanisms include acetylation, methylation, ubiquitinylation, phosphorylation and sumoylation, resulting in changes in chromatin structure and transcriptional activity [40]. Histone acetyl transferases and histone deacetylases, via regulation of chromatin–DNA interaction and acetylation of nonhistone proteins, exert control over AR transcription [41,42]. These enzymes have been implicated in the progression of CRPC, and several novel therapeutic strategies targeting these post-translational modifications are now being explored in clinical trials [37,43].

Clinical results with novel endocrine therapies

Several of the mechanisms of resistance to castration described previously have been readily 'actionable', resulting in the development of novel therapeutics. These agents targeting the androgen receptor signaling axis have rapidly entered the clinic, and moved forward to definitive randomized trials (Figure 1, Table 1).

■ Abiraterone

Abiraterone acetate is an irreversible oral inhibitor of the steroidal enzyme CYP17, a cytochrome p450 complex that is involved in adrenal steroid synthesis. In contrast to ketoconazole, abiraterone is much more potent and selective in its inhibition of CYP17. In a Phase I study, Attard *et al.* reported that abiraterone could be safely administered in patients with metastatic CRPC at escalating doses between 250 and 2000 mg in three patient cohorts, with peak therapeutic effect at 1000 mg [44]. The Phase I study provided proof of concept, demonstrating that serum testosterone and adrenal androgen levels quickly plummeted after initiation of treatment, while mineralocorticoids increased (the latter which could be offset by the addition of epleronone). Adverse events in this trial were mostly attributed to mineralocorticoid excess, as coadministration of corticosteroids was not mandated per protocol; these included hypertension, hypokalemia and lower limb edema. Furthermore, early evidence of clinical activity was observed, including declines in PSA, reduction in analgesic use, improvement in symptoms and regression of measurable disease. A second Phase I study demonstrated that responses were achieved even in patients previously receiving ketoconazole [45].

These encouraging results led two Phase II trials with abiraterone. In the study reported by Attard *et al.*, 47 patients with CRPC received daily doses of abiraterone 1000 mg and dexamethasone 0.5 mg [46]. In chemotherapy-naive patients, >50% declines in PSA were achieved in 67% of the patients; 37.5% of patients with measurable disease by Response Evaluation Criteria In Solid Tumors (RECIST) had partial response, and at 6 months follow-up, 66% of the patients had no radiographic progression. Furthermore, pretreatment levels of DHEA, DHEA-S, androstenedione and estradiol were associated with increased probability of a >50% PSA decline and increased time to PSA progression. As expected, secondary mineralocorticoid excess resulted in hypokalemia (88%), hypertension (40%) and fluid overload (31%), requiring eplerenone treatment.

Table 1. Ongoing and recently completed randomized Phase III trials of 'novel' hormonal therapies in prostate cancer.

Trial	Agent	Clinical state	Sample size (n)	Primary outcome	Secondary outcome	Status	Ref.
COU-AA-301	Abiraterone	Castration-resistant, postchemotherapy	1158	OS	PSAD	Final data collection for primary outcome 6/2011	[103]
COU-AA-302	Abiraterone	Castration-resistant, prechemotherapy	1000	OS	TBD	Final data collection for primary outcome 4/2011	[104]
AFFIRM	MDV3100	Castration-resistant, postchemotherapy	NA	OS	TBD	Ongoing, not recruiting	[105]
PREVAIL	MDV3100	Castration-resistant, prechemotherapy	1680	OS PFS	TTSE TTICT	Final data collection for primary outcome 9/2014	[106]
NCT01193244	TAK-700	Castration-resistant, chemo-naive	1454	rPFS OS	cCTC TTPP PSA50	Final data collection for primary outcome 1/2013	[107]
NCT01193257	TAK-700	Castration-resistant, postchemotherapy	1083	OS	PSA50 rPFS PR	Final data collection for primary outcome 9/2013	[108]

cCTC: Changes in circulating tumor cells; NA: Not available; OS: Overall survival; PFS: Progression-free survival; PR: Pain response; PSA50: 50% PSA response; PSAD: PSA decline >50%; rPFS: Radiographic progression-free survival; TBD: To be determined; TTICT: Time to cytotoxic therapy; TTPP: Time to pain progression; TTSE: Time to skeletal event.

Another multicenter Phase II trial by Danila *et al.* reported positive results with using abiraterone and prednisone in docetaxel-treated CRPC [47]. Given daily doses of abiraterone 1000 mg and prednisone 10 mg, >50% PSA declines were achieved in 36% of the patients, including 26% of the ketoconazole-treated patients. Additional measures of antitumor activity in this postchemotherapy-treated population included 18% partial radiographic response rate, 28% of patients demonstrating improved Eastern Cooperative Oncology Group (ECOG) functional status, and a median time to PSA progression of 169 days. Importantly, with the coadministration of prednisone, no clinical evidence of significant mineralocorticoid excess was observed. Based on these promising Phase I and II results, two large randomized trials of abiraterone were launched: COU-AA-301 for patients with progressive disease despite prior chemotherapy and COU-AA-302 for patients with chemotherapy-naive disease.

While COU-AA-302 is currently ongoing, intriguing results from COU-AA-301 were recently reported. COU-AA-301 was a 1180-patient, multicenter, double-blind, randomized Phase III study comparing abiraterone acetate plus prednisone versus prednisone plus placebo in CRPC patients who had previously received docetaxel. The primary end point of this trial was overall survival. The preliminary results of the COU-AA-301 trial were reported at the European Society for Medical Oncology (ESMO) 35th congress [48], demonstrating that treatment with abiraterone results in a statistically significant improvement in overall survival compared with the prednisone group (14.8 vs 10.9 months; HR: 0.65; $p < 0.0001$). Other secondary end points, including time to PSA progression (10.2 vs 6.6 months; HR: 0.58; $p < 0.0001$), radiographic progression-free survival (5.6 vs 3.6 months; $p < 0.0001$), and PSA response (38 vs 10%; $p < 0.0001$), showing statistically significant differences favoring the combination treatment. The combination treatment group experienced more adverse events: fluid retention (30.5 vs 22.3%), hypokalemia (17.1 vs 8.4%), grade 3/4 hypertension (1.3 vs 0.3%), transaminitis (10.4 vs 8.1%), and cardiac dysfunction (12.5 vs 9.4%). However, these were generally low-grade and manageable. This marks the first hormonal treatment that has shown a statistically significant improvement in survival in patients with metastatic CRPC following treatment with docetaxel.

MDV3100

Of the progressive changes in AR signaling in CRPC, the most frequent is AR amplification or overexpression [49]. In preclinical models, AR overexpression is sufficient to shorten tumor latency in castrate mice and confer resistance to bicalutamide [50]. MDV3100 is a potent AR antagonist optimized from a screen for novel antiandrogens that retain activity in the setting of increased androgen-receptor expression [51]. Compared with bicalutamide, MDV3100 has greater affinity for AR, has no detectable agonist effects and inhibits nuclear translocation of AR. In a Phase I–II study, escalating doses (30–600 mg/day) of MDV3100 were given to patients with progressive CRPC [52]. The most common grade 3 and 4 adverse event was fatigue (11%), with increased occurrence at daily dosage at or above 240 mg. In addition, three seizures (2%), occurred at doses higher than 360 mg daily or above. Consequently, a dose of 240 mg/day was selected for further investigation.

Given early evidence of antitumor activity, cohorts receiving between 60 and 480 mg daily expanded to enroll 20–30 patients each. Post-treatment PSA declines of >50% were achieved in 56% of patients, objective responses in measurable disease occurred in 22% of patients, and stabilization of bone metastases occurred in 56% of patients. In addition, PET imaging of 22 patients with the novel tracer, ^{18}F-fluoro-5α-dihydrotestosterone, revealed decreased binding, confirming the hypothesized mechanism of action of androgen receptor blockade with this agent. Based on these intriguing results, two randomized, placebo-controlled trials of MDV3100 have been initiated, in patients with chemotherapy-naive and chemotherapy-resistant disease.

TAK-700

TAK-700 is a selective 17,20-lyase inhibitor that substantially reduces adrenal androgen levels *in vivo* in preclinical models. Preliminary results of a Phase I/II, open-label, dose-escalation study were presented at the 2010 American Society of Clinical Oncology: Genitourinary (ASCO GU) symposium [53]. At the time of the initial data analysis, a total of 26 patients had received TAK-700 at five dose levels: 100, 200, 300, 400 or 600 mg twice daily. An additional cohort of patients received TAK-700 400 mg twice daily plus prednisone 5 mg twice daily. No dose-limiting toxicities were reported. Of the 14 patients who had received TAK-700 ≥300 mg for ≥3 cycles, 11 had PSA reductions ≥50% and four had PSA reductions ≥90%. Consequently, the Phase II portion proceeded with a dose of 400 mg twice daily. Based on observed antitumor activity, a Phase III trial is currently being planned.

Apoptone

Apoptone, also known as 17α-ethynyl-5α-androstane-3α, 17β-diol or HE3235, is a novel synthetic analogue of 3β-androstanediol that is active in rodent models against prostate and breast cancer [54]. HE3235 decreased AR expression in an androgen-sensitive human prostate adenocarcinoma cell line, and suppressed growth and

tumoral androgen synthesis in a CRPC xenograft. HE3235 appears to bind to AR, downregulate Bcl-2, and increase the expression of caspases. In addition, HE3235 inhibits conversion of D-cholesterol to D-pregnenolone independent of CYP17 inhibition. A Phase I/II study is now ongoing evaluating its safety and activity in patients with both chemo-naive and chemotherapy-treated metastatic CRPC [55–57].

■ TOK-001

TOK-001 is a multifunctional inhibitor of the AR signaling axis. In preclinical studies, TOK-001 demonstrated three distinct mechanisms: inhibition of CYP17, antagonism of AR and decreasing AR level in prostate cancer cells. A Phase I/II study is ongoing, evaluating the safety and activity of escalating doses of TOK-001. This trial is ongoing, with anticipated completion by July 2011 [101].

Future perspective: combination therapies

Controversy still remains over whether castration-resistant clones exist even prior to hormonal treatment. If not, achieving complete androgen blockade theoretically could provide durable control for castration-naive disease. When added to medical or surgical castration, antiandrogenic agents have not provided a definitive survival benefit in advanced prostate cancer [58], although when excluding trials with cyproterone acetate, the improvement in 5-year survival for combined androgen blockade was statistically superior to castration alone in a systematic meta-analysis (HR: 0.92, 2.9% improvement in overall survival). Akaza and colleagues reported a Phase III, multicenter, double-blind, controlled trial comparing combined androgen blockade using newer antiandrogenic agent bicalutamide versus castration alone in the treatment of metastatic prostate cancer, which demonstrated an improvement of overall survival at a median follow-up of 5.2 years (HR: 0.78; 95% CI: 0.60–60.99; p = 0.0498) [59]. Furthermore, recent efforts to block androgen production and binding at multiple levels, simultaneously, have shown promising results in early trials [60]. With more potent and selective means of blocking androgen production and binding, regimens combining the new generation of hormonal therapies in prostate cancer are of significant interest. For example, abiraterone is currently being evaluated in combination with leuprolide acetate in the neoadjuvant treatment of high-risk prostate cancer prior to radical prostatectomy (NCT00924469) [102]. This trial, with post-treatment tissue available for correlative analysis, will be valuable in not only determining the activity of this combination in castration-naive disease, but may shed light on mechanisms of synergy and resistance.

Another exciting approach involves combining endocrine therapy with cytotoxic chemotherapy. This strategy is based on the premise that androgen-deprivation therapy will exert selective pressure, resulting in proliferation of resistant clones and subsequently castration resistance. Addition of a cytoxic agent aimed at controlling the growth of these castration-resistant clones may lead to improved clinical outcome. This approach has shown promising early results in pilot studies using docetaxel plus conventional hormonal therapies [61,62]. Trials combining docetaxel plus TAK-700 (NCT01084655) are ongoing, while trials combining docetaxel with other novel hormonal therapies are planned.

Conclusion

With recent breakthroughs in the treatment of metastatic prostate cancer, therapeutic options beyond docetaxel for castration-resistant disease are rapidly expanding. In the past year, cabazitaxel, sipuleucel-T and abiraterone have all been reported in randomized Phase III trials to be effective in improving overall survival in this devastating disease. Based on improved understanding of the mechanisms of resistance to androgen deprivation, additional agents including MDV-3100, TAK-700,

Executive summary

- Despite the use of population-wide prostate-specific antigen screening in the USA, approximately one third of patients eventually develop metastatic prostate cancer after definitive local treatment.
- Conventional systematic androgen deprivation therapy is largely effective, but with proliferation of resistant clones of resistant cells under selective pressure, as well as accumulation of molecular changes, castration resistance eventually develops.
- Multiple mechanisms of resistance to androgen deprivation have recently been identified, including: intratumor androgen production/conversion/sequestration, persistent serum androgens via adrenal production, AR gene amplification, AR mutation, increased expression of AR coactivators, AR splice variants and epigenetic AR modifications.
- With advances in the understanding of castration resistance, several novel agents targeting specific mechanisms are now in clinical trial testing for the treatment of metastatic castration-resistant prostate cancer.
- Abiraterone, an irreversible oral inhibitor of the steroidal enzyme CYP17, has demonstrated overall survival benefit in a randomized, Phase III (COU-AA-201) clinical trial in the treatment of metastatic castration-resistant prostate cancer which had previously been treated with docetaxel.
- Regimens combining novel hormonal therapies may change the outlook for this difficult-to-treat disease and are being explored in recently launched clinical trials.

Apoptone and TOK-001, have been developed and are already demonstrating promising antitumor activity in early clinical trials. The optimal combinations and sequencing of these novel therapies will be the focus of clinical research in the near future.

Financial & competing interests disclosure

William K Oh is a consultant for Medivation, Amgen, Centocor, Sanofi-Aventis and Bellicum, and has received funding from Millenium. Matthew D Galsky is a consultant for Pfizer, Bristol-Myers Squibb, GlaxoSmithKline and Foundation Medicine. He has received funding from Viatar and Celgene. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

1 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J. Clin.* 59, 225–249 (2009).

2 Ryan CJ, Elkin EP, Small EJ, Duchane J, Carroll P. Reduced incidence of bony metastasis at initial prostate cancer diagnosis: data from CaPSURE. *Urol. Oncol.* 24, 396–402 (2006).

3 Zincke H, Oesterling JE, Blute ML, Bergstralh EJ, Myers RP, Barrett DM. Long-term (15 years) results after radical prostatectomy for clinically localized (stage T2c or lower) prostate cancer. *J. Urol.* 152, 1850–1857 (1994).

4 Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *CA Cancer J. Clin.* 22, 232–240 (1972).

5 Walsh PC, DeWeese TL, Eisenberger MA. A structured debate: immediate versus deferred androgen suppression in prostate cancer-evidence for deferred treatment. *J. Urol.* 166, 508–515; discussion 15–16 (2001).

6 Cereceda LE, Flechon A, Droz JP. Management of vertebral metastases in prostate cancer: a retrospective analysis in 119 patients. *Clin. Prostate Cancer* 2, 34–40 (2003).

7 Edwards J, Bartlett JM. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer. Part 2: androgen-receptor cofactors and bypass pathways. *BJU Int.* 95, 1327–1335 (2005).

8 Nishiyama T, Hashimoto Y, Takahashi K. The influence of androgen deprivation therapy on dihydrotestosterone levels in the prostatic tissue of patients with prostate cancer. *Clin. Cancer Res.* 10, 7121–7126 (2004).

9 Fung KM, Samara EN, Wong C *et al.* Increased expression of type 2 3α-hydroxysteroid dehydrogenase/type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) and its relationship with androgen receptor in prostate carcinoma. *Endocr. Relat. Cancer* 13, 169–180 (2006).

10 Titus MA, Schell MJ, Lih FB, Tomer KB, Mohler JL. Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin. Cancer Res.* 11, 4653–4657 (2005).

11 Stanbrough M, Bubley GJ, Ross K *et al.* Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res.* 66, 2815–2825 (2006).

12 Small EJ, Halabi S, Dawson NA *et al.* Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a Phase III trial (CALGB 9583). *J. Clin. Oncol.* 22, 1025–1033 (2004).

13 Ryan CJ, Halabi S, Ou SS, Vogelzang NJ, Kantoff P, Small EJ. Adrenal androgen levels as predictors of outcome in prostate cancer patients treated with ketoconazole plus antiandrogen withdrawal: results from a cancer and leukemia group B study. *Clin. Cancer Res.* 13, 2030–2037 (2007).

14 Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat. Rev. Cancer* 1, 34–45 (2001).

15 Koivisto P, Kononen J, Palmberg C *et al.* Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res.* 57, 314–319 (1997).

16 Gregory CW, Johnson RT Jr, Mohler JL, French FS, Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. *Cancer Res.* 61, 2892–2898 (2001).

17 Labrie F, Dupont A, Belanger A *et al.* Treatment of prostate cancer with gonadotropin-releasing hormone agonists. *Endocr. Rev.* 7, 67–74 (1986).

18 Culig Z, Klocker H, Eberle J *et al.* DNA sequence of the androgen receptor in prostatic tumor cell lines and tissue specimens assessed by means of the polymerase chain reaction. *Prostate* 22, 11–22 (1993).

19 Koivisto P, Kolmer M, Visakorpi T, Kallioniemi OP. Androgen receptor gene and hormonal therapy failure of prostate cancer. *Am. J. Pathol.* 152, 1–9 (1998).

20 Fenton MA, Shuster TD, Fertig AM *et al.* Functional characterization of mutant androgen receptors from androgen-independent prostate cancer. *Clin. Cancer Res.* 3, 1383–1388 (1997).

21 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.* 81, 242–251 (1999).

22 Scher HI, Kelly WK. Flutamide withdrawal syndrome: its impact on clinical trials in hormone-refractory prostate cancer. *J. Clin. Oncol.* 11, 1566–1572 (1993).

23 Zhao XY, Malloy PJ, Krishnan AV *et al.* Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. *Nat. Med.* 6, 703–706 (2000).

24 Kelly WK. Endocrine withdrawal syndrome and its relevance to the management of hormone refractory prostate cancer. *Eur. Urol.* 34(Suppl. 3), 18–23 (1998).

25 Rocchi P, So A, Kojima S *et al.* Heat shock protein 27 increases after androgen ablation and plays a cytoprotective role in hormone-refractory prostate cancer. *Cancer Res.* 64, 6595–6602 (2004).

26 Rocchi P, Beraldi E, Ettinger S *et al.* Increased Hsp27 after androgen ablation facilitates androgen-independent progression in prostate cancer via signal transducers and activators of transcription 3-mediated suppression of apoptosis. *Cancer Res.* 65, 11083–11093 (2005).

27 Craft N, Shostak Y, Carey M, Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. *Nat. Med.* 5, 280–285 (1999).

28 Raffo AJ, Perlman H, Chen MW, Day ML, Streitman JS, Buttyan R. Overexpression of bcl-2 protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. *Cancer Res.* 55, 4438–4445 (1995).

29 Miyake H, Nelson C, Rennie PS, Gleave ME. Overexpression of insulin-like growth factor binding protein-5 helps accelerate progression to androgen-independence in the human prostate LNCaP tumor model through activation of phosphatidylinositol 3′-kinase pathway. *Endocrinology* 141, 2257–2265 (2000).

30 Wang L, Hsu CL, Chang C. Androgen receptor corepressors: an overview. *Prostate* 63, 117–130 (2005).

31 Holter E, Kotaja N, Makela S *et al.* Inhibition of androgen receptor (AR) function by the reproductive orphan nuclear receptor DAX-1. *Mol. Endocrinol.* 16, 515–528 (2002).

32 Loy CJ, Sim KS, Yong EL. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. *Proc. Natl Acad. Sci. USA* 100, 4562–4567 (2003).

33 Gross M, Liu B, Tan J, French FS, Carey M, Shuai K. Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. *Oncogene* 20, 3880–3887 (2001).

34 Hu R, Dunn TA, Wei S *et al.* Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res.* 69, 16–22 (2009).

35 Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res.* 68, 5469–5477 (2008).

36 Watson PA, Chen YF, Balbas MD *et al.* Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. *Proc. Natl Acad. Sci. USA* 107, 16759–16765 (2010).

37 Perry AS, Watson RW, Lawler M, Hollywood D. The epigenome as a therapeutic target in prostate cancer. *Nat. Rev. Urol.* 7, 668–680 (2010).

38 Wang LG, Ossowski L, Ferrari AC. Androgen receptor level controlled by a suppressor complex lost in an androgen-independent prostate cancer cell line. *Oncogene* 23, 5175–5184 (2004).

39 Karpf AR, Bai S, James SR, Mohler JL, Wilson EM. Increased expression of androgen receptor coregulator MAGE-11 in prostate cancer by DNA hypomethylation and cyclic AMP. *Mol. Cancer Res.* 7, 523–535 (2009).

40 Nightingale KP, O'Neill LP, Turner BM. Histone modifications: signalling receptors and potential elements of a heritable epigenetic code. *Curr. Opin Genet. Dev.* 16, 125–136 (2006).

41 Roth SY, Denu JM, Allis CD. Histone acetyltransferases. *Annu. Rev. Biochem.* 70, 81–120 (2001).

42 Thiagalingam S, Cheng KH, Lee HJ, Mineva N, Thiagalingam A, Ponte JF. Histone deacetylases: unique players in shaping the epigenetic histone code. *Ann. NY Acad. Sci.* 983, 84–100 (2003).

43 Sonpavde G, Aparicio AM, Zhan F *et al.* Azacitidine favorably modulates PSA kinetics correlating with plasma DNA LINE-1 hypomethylation in men with chemonaive castration-resistant prostate cancer. *Urol. Oncol.* (2009) (Epub ahead of print).

44 Attard G, Reid AH, Yap TA *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.* 26, 4563–4571 (2008).

45 Ryan CJ, Smith MR, Fong L *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.* 28, 1481–1488 (2010).

46 Attard G, Reid AH, 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.* 27, 3742–3748 (2009).

47 Danila DC, Morris MJ, de Bono JS *et al.* Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. *J. Clin. Oncol.* 28, 1496–1501 (2010).

48 Scher HI, Logothetis C, Molina A *et al.* Abiraterone acetate (AA) plus low dose prednisone (P) improves overall survival (OS) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) who have progressed after docetaxel-based chemotherapy (chemo): results of COU-AA-301, a randomized, double-blind, placebo-controlled Phase III study. Presented at: ESMO, Milan, Italy, 11 October 2010.

49 Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res.* 61, 3550–3555 (2001).

50 Chen CD, Welsbie DS, Tran C *et al.* Molecular determinants of resistance to antiandrogen therapy. *Nat. Med.* 10, 33–39 (2004).

51 Tran C, Ouk S, Clegg NJ *et al.* Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 324, 787–790 (2009).

52 Scher HI, Beer TM, Higano CS *et al.* Antitumour activity of MDV3100 in castration-resistant prostate cancer: a Phase 1–2 study. *Lancet* 375, 1437–1446 (2010).

53 Dreicer R, MacVicar GR, MacLean D, Zhang T, Stadler WM. Safety, pharmacokinetics, and efficacy of TAK-700 in castration-resistant, metastatic prostate cancer: a Phase I/II, open-label study. Presented at: *2010 ASCO Genitourinary Cancers Symposium.* San Francisco, CA, USA, 5–8 March 2010.

54 Ahlem C, Kennedy M, Page T *et al.* 17α-alkynyl 3α, 17β-androstanediol non-clinical and clinical pharmacology, pharmacokinetics and metabolism. *Invest. New Drugs* (2010) (Epub ahead of print).

55 Montgomery B, Ryan C, Corey E *et al.* Results of preclinical and clinical Phase I/II open-label dose-ranging trial with HE3235, a synthetic adrenal hormone, in castrate-resistant prostate cancer. Presented at: *AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics.* Boston, MA, USA 2009.

56 Morris CJR, Stickney DR, Frincke JM, Reading CL, Scher HI. HE3235, a synthetic adrenal hormone, in patients with castration-resistant prostate cancer (CRPC): clinical Phase I/II trial results. Presented at: *2010 ASCO Genitourinary Cancers Symposium.* San Francisco, CA, USA, 5–8 March 2010.

57 Harbor BioSciences I. Harbor BioSciences Apoptone® for prostate cancer shows a partial overall response-new positive Phase I/IIa data. Weber R (Ed.). Harbor BioSciences, Inc., San Diego, CA, USA (2010).

58 No authors listed. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. Prostate Cancer Trialists' Collaborative Group. *Lancet* 355, 1491–1498 (2000).

59 Akaza H, Hinotsu S, Usami M *et al.* Combined androgen blockade with bicalutamide for advanced prostate cancer: long-term follow-up of a Phase 3, double-blind, randomized study for survival. *Cancer* 115, 3437–3445 (2009).

60 Taplin ME, Regan MM, Ko YJ *et al.* Phase II study of androgen synthesis inhibition with ketoconazole, hydrocortisone, and dutasteride in asymptomatic castration-resistant prostate cancer. *Clin. Cancer Res.* 15, 7099–7105 (2009).

61 Hussain A, Dawson N, Amin P *et al.* Docetaxel followed by hormone therapy in men experiencing increasing prostate-specific antigen after primary local treatments for prostate cancer. *J. Clin. Oncol.* 23, 2789–2796 (2005).

62 Figg WD, Woo S, Zhu W *et al.* A Phase I clinical study of high dose ketoconazole plus weekly docetaxel for metastatic castration-resistant prostate cancer. *J. Urol.*183, 2219–2212 (2010).

■ Websites

101 Montgomery RB. ARMOR1: Phase 1/2, open label, dose-escalation, selected dose comparison trial of TOK-001 for the treatment of chemotherapy naive castration-resistant prostate cancer. http://clinicaltrials.gov/ct2/show/NCT00959959?term=tok+001&rank=1

102 Neo-adjuvant abiraterone + luteinizing hormone-releasing hormone (LHRH) versus LHRH in prostate cancer. Cougar Biotechnology, Inc., 2010. http://clinicaltrials.gov/ct2/show/NCT01088529?term=abiraterone&rank=9

103 ClinicalTrials.gov: COU-AA-301. http://clinicaltrials.gov/ct2/show/NCT00638690?term=cou+301&rank=1

104 ClinicalTrials.gov: COU-AA-302. http://clinicaltrials.gov/ct2/show/NCT00887198?term=cou+302&rank=1

105 ClinicalTrials.gov: AFFIRM. http://clinicaltrials.gov/ct2/show/NCT00974311?term=affirm&rank=1

106 ClinicalTrials.gov: PREVAIL. http://clinicaltrials.gov/ct2/show/NCT01212991?term=prevail+AND+mdv&rank=1

107 ClinicalTrials.gov: NCT01193244. http://clinicaltrials.gov/ct2/show/NCT01193244?term=orteronel&rank=2

108 ClinicalTrials.gov: NCT01193257. http://clinicaltrials.gov/ct2/show/NCT01193257?term=orteronel&rank=1