Current management of prostate cancer: dilemmas and trials

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ABSTRACT. The past decade has witnessed significant advances in our understanding of the biology of prostate cancer. Androgen ablation/androgen receptor inhibition remains as the mainstay of treatment for advanced prostate cancer. Our understanding of the biology of prostate cancer has increased exponentially owing to advances in molecular biology. With this knowledge many intriguing issues have come to light, which clinicians and scientists alike strive to answer. These include why prostate cancer is so common, what drives the development of prostate cancer at a molecular level, why prostate cancer appears refractory to many families of cytotoxic chemotherapeutics, and why prostate cancer preferentially metastasizes to bone. Two clinical forms of prostate cancer have been identified: indolent organ confined disease, which elderly men often die of, and aggressive metastatic disease. A method of distinguishing between these two forms of the disease at an organ-confined stage remains elusive. Understanding the mechanisms of castrate resistance is a further issue of clinical importance. New trials of treatments, including molecular agents that target prostate cancer from a range of angles, have been instituted over the past 10–15 years. We can look at these trials not only as a chance to investigate the effectiveness of new treatments but also as an opportunity to further understand the complex biology of this disease.

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Understanding the biology of cancer is the key to successfully treating it. In 1942 Huggins and Hodges [1] first recognised that prostate cancer is androgen-dependent. Therapeutically harnessing this biological feature, in the form of androgen ablation therapy, improves overall survival (OS) and has been in clinical use since the 1960s [2].

The rising incidence of prostate cancer over the past 50 years can be explained by increasing longevity and the development of prostate-specific antigen (PSA) testing [3]. This epidemiological trend is reflective of the fact that PSA testing has unmasked disease, which would otherwise be clinically silent disease. Prostate cancer is extremely common, affecting 80% of males by the age of 80 [3]; this is so common that it could be considered an inevitable consequence of aging. It is unknown why prostatic epithelium demonstrates age-related dysplastic change [4]. Most epithelial cancers are more common with advancing age, but often there is a link to cumulative carcinogen exposure (for example, non-melanoma skin cancers and lung cancer) [5–7]. There is a recognised clinical subtype of breast cancer with biological parallels to prostate cancer, being age associated, relatively slow growing and hormone sensitive, but it is not as common as prostate cancer [3, 8, 9].

A further pathophysiological observation is that prostate cancers are heterogeneous, both within individuals and across the affected population [10]. Tumour heterogeneity would be expected if prostate cancers arise owing to the accumulation of random genetic hits over time. However, the high incidence of prostate cancer

suggests a unifying factor or pathway—a carcinogenic driving force, or susceptibility that is prevalent, age related and prostate specific, yet gives rise to molecularly heterogeneous tumours. Why prostate cancers are so common is an interesting and unexplained phenomenon.

Treatment of prostate cancer has focused predominantly on inhibition of androgen receptor (AR) activation. As the treatment of cancer has shifted towards molecular targeted therapy, new trials of treatments approaching prostate cancer from a range of angles have been instituted over the past 5–10 years. We can look at these trials not only as a chance to investigate the effectiveness of new treatments, but as an opportunity to further understand the complex biology of this disease.

Can we exploit the molecular changes seen in prostate cancer to develop effective therapy?

After the AR the earliest identified molecular targets of clinical interest in prostate cancer were PSA and prostatic acid phosphatase (PAP). Both PSA and PAP are expressed by normal prostatic epithelial cells. They are of interest as they are predominantly prostate-specific proteins and continue to be expressed by prostate cancers. Both PAP and PSA have been used as serum and immunohistochemical markers of prostate cancer [11].

Expression of PAP has been detected in more than 95% of primary prostate cancers. While its use as a serum biomarker has largely been superseded by PSA, it still plays a role in diagnosis of prostate cancer as an immunohistochemical marker. Recently, renewed interest in PAP has been spurred by the development of the

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novel immunotherapeutic agent sipuleucel-T. This treatment is an infusion of activated autologous mononuclear cells, which have been exposed *ex vivo* to a granulocyte monocyte colony stimulating factor (GM-CSF)/PAP fusion protein.

Three Phase III randomised placebo-controlled trials have been carried out in patients with metastatic castrate-refractory prostate cancer (CRPC). Pooled results of two small Phase III trials including 147 patients in total demonstrated a median survival of 23.3 *vs* 18.9 months in favour of the sipuleucel-T arm [12]. This translates to a significant 33% reduction in risk of death [hazard ratio (HR) 1.5, $p=0.011$]. A non-significant 21% reduction in risk of disease progression (HR 1.26, $p=0.111$) and a low PSA response rate of 4.8% were seen. They were followed by the IMPACT trial, which enrolled 512 patients randomised 2:1 to treatment or placebo [13]. Again a statistically significant 23% reduction in risk of death from any cause was seen (HR 0.77, $p=0.04$). However, this benefit failed to be reflected in other end point evaluations, with a median time to progression (TTP) of 14.6 *vs* 14.4 weeks ($p=0.63$) and only 8/311 treated patients showing a PSA response.

PROSTVAC is a vaccination-based immunotherapy, which stimulates production of host anti-PSA antibody by delivering doses of recombinant PSA-expressing Vaccinia virus along with immune stimulatory co-factors. In a Phase II study, patients with CRPC were randomised to receive vaccination or placebo [14]. Median OS was 25.1 months in the treatment arm *vs* 16.6 months in the placebo arm (HR 0.56, $p=0.0061$). Again, no difference in progression-free survival (PFS) was seen between the two arms (3.8 *vs* 3.7 months, HR 0.88, $p=0.6$). Similar incongruous PFS and OS results were witnessed in a trial of the immunotherapeutic agent ipilimumab in malignant melanoma [15]. PFS may not be a suitable end point assessment of the impact of immunotherapy. The effects of therapy may not be evident in short-term analyses as it requires a longer period for an effective anti-cancer immune response to develop. The discordance between PSA response and OS in these trials raises questions about the use of PSA response as a surrogate end point in trials of immunotherapeutic agents.

Normal prostate epithelium has unique features and these features can be exploited clinically. By understanding the biology of prostate carcinogenesis we have been able to identify therapeutic targets. Prostate cancers commonly possess chromosomal rearrangements, and in this context *TMPRSS2-ETS* (transmembrane proteinase serine 2–E26 family of transcription factors) fusions are of particular interest. Microarray and histological studies have estimated that they can be found in 50–70% of prostate cancers [16]. Translocation of the *TMPRSS2*

promoter brings *ETS* genes under androgen regulation, which consequently results in their increased expression. An association between *TMPRSS2-ETS* translocations and poor prognosis has been recognised [17–19]. A correlation between the presence of *TMPRSS2-ETS* translocations and increasing Gleason grade has been observed, and it also appears to be present more frequently in metastatic disease [18, 20]. Studies in prostatic intraepithelial neoplasia (PIN) have demonstrated low levels of *TMPRSS2/ETS* rearrangements, suggesting that they may not be initiating events in prostatic carcinogenesis but develop later in the process [21]. Further studies have identified a link between PTEN (phosphatase and tensin homologue) deletion and *TMPRSS2/ERG* (transmembrane protease serine 2: *ETS*-regulated gene) rearrangements, suggesting a co-operative mechanism, with both defects required to drive tumorigenesis in mouse models [22]. PTEN deletions lead to AKT (serine/threonine protein kinase) and ultimately PI3K (phosphoinositide-3-kinase) activation. Studies suggest that approximately 25% of prostate cancers possess both *TMPRSS2/ERG* rearrangement and PTEN deletion, and this subset may benefit from AKT/PI3K/mTOR (mammalian target of rapamycin) targeted therapy.

The growth factor receptors ErbB1 and ErbB2 are both expressed by prostate epithelial cells. In fact their expression has been shown to increase with progression of disease [23, 24]. Preclinical trials investigating activity of the ErbB1 inhibitor gefitinib were promising [25], but failed to translate to clinical response in CRPC. In two Phase II trials, no patient achieved PSA decline [26, 27]. In one trial 87% (35/40) of patients had PSA stabilisation [26]. Likewise, studies with the ErbB1 inhibitor erlotinib failed to produce PSA responses [28, 29]. Phase II trials using the multityrosine-kinase inhibitor sorafenib have shown a significant proportion of patients achieving disease stabilisation [30–32]. Interestingly, discordance was seen between radiological response and PSA response, suggesting that PSA is not an accurate biomarker of response to treatment in tyrosine-kinase inhibitor (TKI) trials [31].

As yet, the molecular events that define prostate cancer are unknown. The search for molecular targets and targeted therapies is ongoing. The question of the applicability of PSA as a surrogate survival end point in both immunotherapy and targeted therapy trials is doubtful because of the mechanism of actions of these agents and the biology of PSA production in advanced disease.

Prognostic biomarkers

A further interesting and unresolved dilemma is the clinical behaviour of prostate cancers. The majority of

Table 1. D'Amico classification

Risk	PSA (ng ml ⁻¹)	Gleason grade	Stage	Relative risk of prostate-specific mortality	
				Post prostatectomy	Post radiotherapy
Low	<10	≤6	T1c–T2a	1.0	1.0
Intermediate	10–20	7	T2b	4.9	5.6
High	>20	8–10	T2b	14.2	14.3

PSA, prostate-specific antigen.

prostate cancers behave in an indolent manner, classically described as disease that males die *with* and not *of*. However, a proportion of prostate cancers exhibit an aggressive phenotype, which metastasises widely and is fatal [33]. The molecular changes that lead to these phenotypic differences are unclear. As a result it is at present a challenge for clinicians to reliably predict the prognosis of patients who present with localised disease. Clinicians must avoid overdiagnosing and treating a population of predominantly elderly patients, while striving to diagnose and effectively treat potentially fatal cancers.

A range of prognostic tools are currently available to clinicians, the majority of which are based on PSA level, pathological/radiological stage and Gleason grade [34–36]. One of the most straightforward is the D'Amico classification [35], which stratifies prostate cancer as a low-, intermediate- or high-risk disease based solely on PSA level, Gleason grade and clinical stage (Table 1).

This classification has been tested in a cohort of 7618 patients and correlates with recurrence risk following surgery or radiotherapy for localised disease. Other models in use include the Kattan post-operative nomogram, which includes additional post-operative variables, and Partin tables [34, 36]. These valuable tools are validated and provide the best available evidence to aid clinicians and patients when making treatment decisions.

In an attempt to improve on existing prognostic models, molecular signatures have been generated based on microarray gene expression analysis. Retrospective analyses of patterns of gene expression in primary tumours have been performed in an attempt to identify a predictive/prognostic gene signature [37–40]. There is little or no overlap between the genes that make up these prognostic gene signatures. The 70-gene signature produced by Yu et al [39] was more accurate in predicting aggressive behaviour than Gleason score. By contrast, other signatures fail to provide significant benefit in terms of sensitivity and specificity, above existing clinical models. It is a topic of debate as to whether the molecular changes that define behaviour of localised tumours are present at a localised stage, or whether they accumulate in an evolutionary fashion as disease progresses. If molecular changes accumulate as disease progresses, it might explain why molecular signatures derived from gene expression patterns at time of diagnosis of localised disease fail to predict risk of disease progression.

As well as seeking to define prognostic signatures, array analysis has been deployed to better understand the molecular biology of prostate cancer. Analysis of gene expression from samples representative of the spectrum of disease progression from normal epithelium to PIN, localised disease and metastatic disease, and of samples with increasing Gleason grade have been performed. These studies show that normal tissue and tumour exhibit distinguishable patterns of gene expression [39, 41–45]. However, changes appear to be more marked between primary tumour and metastatic disease than between primary tumour and normal tissue [39]. There is also differential gene expression evident with advancing Gleason grade [43]. Individual genes have been identified in independent arrays as being linked to metastatic disease (*e.g.* hepsin, AMACR) [46].

Based on the example of breast cancer and lymphoma [47–49], unsupervised hierarchical clustering has attempted to identify subtypes of prostate cancer [39, 41]. In one study three subsets were identified that showed an association with outcome; however, this observation is not robust enough to translate to a clinically applicable subclassification of prostate cancer. Overall, for microarray analysis of prostate cancer the heterogeneous nature of the disease means that large sample numbers would be required to generate meaningful clinically applicable data.

Circulating tumour cells (CTCs) may prove to be the bridge between molecular and pathological prognostic classification of prostate cancer. Prostate epithelial cells can be detected in the circulation in normal circumstances and a release of epithelial cells is commonly seen after surgical manipulation of the prostate. However, normal epithelial cells are rapidly cleared from the circulation [50]. Tumour cells can be distinguished from normal epithelial cells based on expression of a unique pattern of surface markers (CD45– cytokeratin+) [51]. While CTCs are reasonably rare events, techniques including immunoprecipitation and reverse-transcription polymerase chain reaction (RT-PCR) have been optimised to effectively isolate them [52].

CTC levels have been demonstrated to correlate with outcome in a number of trials and can also act as a marker of response to treatment [53–56]. A cut-off CTC count of 5/7.5 ml of blood has been identified as being associated with survival advantage in response to treatment [55]. CTC counts outperformed PSA in predicting survival in patients with metastatic prostate cancer on chemotherapy [56]. CTCs have an added advantage as they provide a substrate for further genetic analysis of tumours. It is possible to use extracted CTCs to perform molecular biomarker testing—for example, AR mutation analysis, *TMPRSS2/ERG* expression, AR amplification and so on [57].

The results of recent clinical trials, several of which are highlighted throughout this review, have raised questions regarding the utility of PSA as a marker of response to therapy, particularly as a surrogate for OS [58–60]. In this setting CTCs may provide an equivalent, or perhaps superior, alternative. As a result CTC assessment is being included in ongoing Phase III trials, which will allow further validation of their usefulness.

Treatment of castrate-refractory prostate cancer

First-line hormonal therapy for prostate cancer targets the androgen/androgen receptor axis suppressing androgen stimulated growth. This is achieved by either inducing castrate testosterone levels via surgical or medical castration (LHRHa; luteinizing hormone releasing hormone agonists), or by directly inhibiting the AR (anti-androgens). More than 90% of patients respond to first-line hormonal therapy, and this translates to an OS advantage [2, 61]. Inevitably, over on average 12–18 months, however, prostate cancers progress in the face of castrate testosterone levels. Multiple mechanisms are responsible for the development of CRPC and much remains to be learnt about the molecular biology of this

Figure 1. Mechanisms of castrate resistant disease. AA, anti-androgen; And, androgen; AR, androgen receptor; CoA, coactivator; CoR, corepressor; DHT, dihydrotestosterone; Mut AR, mutant androgen receptor.

stage of the disease [62, 63]. Proposed mechanisms include overexpression of the AR, AR mutations, AR activation by cross-signalling from alternative pathways, ligand-independent AR activation, altered steroidogenic enzymatic pathways leading to increased local androgen production and altered AR co-activator/corepressor interactions (Figure 1).

Regardless of the mechanism it is clear that the AR continues to be expressed in CRPC and rising PSA levels provide evidence that AR signalling remains active [64, 65]. Medical or surgical castration reduces serum androgen levels to $<50 \text{ ng dl}^{-1}$; however, androgens continue to be synthesised by the adrenals [66]. A study by Harper et al in 1974 [67] demonstrated that production of androgens by the adrenals alone can maintain serum androgen levels at one-fifth of pre-castrate levels. It has also been observed that serum androgen levels do not correlate with intraprostatic androgen levels, which, while reduced by castration, persist at levels capable of activating the AR [68]. The combination of persistent intraprostatic androgen and continued expression of a functional AR suggests that therapies capable of inducing a more profound inhibition of androgen may produce a therapeutic response in the setting of castrate resistance [69, 70]. Based on this concept, novel therapeutics have been developed, including inhibitors of adrenal androgen synthesis and next-generation AR inhibitors.

Inhibitors of adrenal androgen synthesis

There is a long history of clinical experience using steroids in advanced prostate cancer. Not only do they palliate the common symptoms of fatigue, anorexia and bone pain but via feedback inhibition they decrease adrenal androgen synthesis. The evidence supporting

their use is based on the placebo arms of multiple drug trials. Prednisolone produces PSA response rates of 9–33% [71, 72]. Hydrocortisone similarly has a response rate of ~20% as second-line monotherapy [73]. Dexamethasone, as a more potent corticosteroid, consequently has demonstrated a greater effect, with PSA response rates of 28–62% [74–76].

The most common use of ketoconazole is as an anti-fungal, but at high doses it is an inhibitor of the cytochrome P450 enzymes 11β -hydroxylase, 17α -hydroxylase and $C17,20$ lyase (Figure 2). Single-institution trials have demonstrated response rates of 55–64% as second-line therapy [77, 78]. At doses required to inhibit androgen synthesis, ketoconazole produces significant side effects, including hepatotoxicity, gastrointestinal (GI) toxicity and adrenal insufficiency. The CALGB 9583 trial was a randomised Phase III trial that compared anti-androgen withdrawal to withdrawal plus ketoconazole [79]. No difference in OS between the two arms was evident, but there was an 82% crossover rate to the ketoconazole arm. A 27% response rate was seen, which is lower than in previous trials, but for this group of patients median survival was 41 months compared with 13 months for those who failed to demonstrate a PSA response. Side effects led to a 20% discontinuation rate. Trials are currently ongoing examining ketoconazole in combination with other P450 inhibitors (e.g. hydrocortisone) and in combination with docetaxel, because of a favourable pharmacokinetic interaction producing increased serum levels of docetaxel.

Clinical experience of responses using inhibitors of adrenal androgens combined with increasing pre-clinical evidence of the potential of androgen inhibition in the setting of castrate-refractory disease prompted the search for novel inhibitors of adrenal androgen synthesis. Abiraterone acetate is a cytochrome P17 inhibitor that

Figure 2. Adrenal steroidogenesis.

blocks adrenal androgen production (Figure 2). It is 10–30 times more potent than ketoconazole. Its significant side effects of hypokalaemia, fluid retention and hypertension are due to secondary hyperaldosteronism, which can be ameliorated by concomitant administration of low-dose prednisolone. Two Phase II trials were carried out in patients with metastatic castrate-refractory disease who had received prior chemotherapy, and in some cases prior second-line hormonal therapy. They demonstrated PSA response rates of 43–51% [80, 81]. A subset analysis of patients pre-treated with ketoconazole demonstrated a shorter TTP suggestive of cross-resistance.

Two Phase III trials were instituted on the basis of positive Phase II results. One, in chemotherapy-naïve patients, has accrued over 1000 patients, with results pending. The second, in patients who have had prior chemotherapy, has recently reported [82]. 1195 patients with castrate-refractory metastatic prostate cancer, who had progressed following chemotherapy but were ketoconazole-naïve, were randomised to 2:1 to receive abiraterone acetate 1000 mg daily plus prednisolone or placebo plus prednisolone. Median OS was 14.8 months in the treatment arm *vs* 10.9 months in the placebo arm (HR 0.65, $p < 0.001$). All secondary end points were also positive with a PSA response rate of 29% *vs* 6% ($p < 0.001$), and a radiological response rate of 14% *vs* 3% ($p < 0.001$). Time to PSA progression (10.2 *vs* 6.6 months) and time to radiological progression (5.6 *vs* 3.6 months) were also improved. Time to skeletal-related event (SRE) was 9.9 *vs* 4.9 months, and patients on the treatment arm had

consistently improved pain scores. A highly specific steroidogenic cytochrome P450 c17 (CYP17) inhibitor, TAK-700, is currently in Phase I/II clinical trials and has shown a PSA response rate of 74% [83].

Antiandrogens have proven survival benefit in first line therapy of advanced prostate cancer both as monotherapy and in combination with medical or surgical castration [2]. Observational studies of patient cohorts, recording PSA decline and clinical improvement in response to antiandrogen withdrawal, were first published in the early 1990s. The phenomenon was predominantly associated with flutamide use. It was discovered to be due to mutation of the AR, leading to flutamide producing an agonist effect [84]. A greater than 50% decrease in PSA was observed in 11–30% of tumours in response to antiandrogen withdrawal [79, 85]. A similar withdrawal response has been demonstrated in association with both bicalutamide and nilutamide [71]. The effect is short lived, the median response duration ranging from 3.5 to 5 months.

MDV 3100 is a second-generation antiandrogen. It is a specific, competitive inhibitor of the AR with 8 times greater affinity for the AR than bicalutamide [86]. The drug was engineered using the non-steroidal AR agonist RU59063 as a structural base. It has shown promise in Phase I/II clinical trials, producing over 50% PSA response in 43% of patients (13/30) [87]. Two Phase III trials—AFFIRM in patients who have had prior docetaxel and PREVAIL in chemotherapy-naïve patients—are under way. The clinical success of second-generation

antiandrogens and adrenal androgen inhibitors raises the possibility that further responses may be possible using combination treatment mimicking combined androgen blockade (CAB) in the first-line setting. These treatments may also have potential in the first-line setting; however, the requirement for concomitant glucocorticoids with CYP17 inhibitors may be an issue here.

The androgen receptor is part of a family of steroid receptor proteins that share significant structural homology and include receptors for oestradiol, progesterone and vitamin D. Pre-clinical evidence pointed towards a role for calcitriol as a treatment for prostate cancer. Immunohistochemical staining of primary tumour samples demonstrated vitamin D receptor expression. Higher levels of vitamin D receptor expression correlate with lower PSA at diagnosis, localised disease, lower Gleason grade and lower incidence of lethal disease [88]. Also, calcitriol treatment was shown to inhibit tumour growth and development of metastases in the Dunning mouse model of prostate cancer [89, 90].

On the basis of preclinical evidence the ASCENT trial was commenced, combining weekly docetaxel plus high-dose 1,25 dihydroxyvitamin D $45\mu\text{g}$ compared with docetaxel plus placebo [91]. 250 patients with CRPC were enrolled with PSA response as the primary end point. Response rates were 63% in the treatment arm *vs* 52% in placebo arm ($p=0.07$). HR for death was 0.67 ($p=0.04$). Unfortunately, the follow-up trial, ASCENT II, was halted early as assessment of the primary end point, OS, demonstrated a higher rate of deaths in the treatment arm [92]. A small single-centre trial has shown a PSA response rate of 20% with low-dose oral ergocalciferol $25\mu\text{g}$ od [93]. The pre-clinical and early-phase trial data as well as case report series support a role for calcitriol as a treatment for CRPC. However the ideal dosing strategy has not been defined. The vitamin D receptor may also be of interest as a marker identifying a less aggressive subtype of prostate cancer.

Chemosensitivity in advanced disease

Two Phase III randomised clinical trials (TAX327 and SWOG 9916) published in 2004 redefined the standard of care in terms of chemotherapy for CRPC [94, 95]. Up to this point the combination of mitoxanthrone/prednisolone had been proven effective as a palliative treatment, improving patients' pain score, but had failed to demonstrate a survival advantage over prednisolone alone [96]. The results of TAX327 and SWOG 9916 identified docetaxel as the first cytotoxic agent to achieve a survival advantage in the treatment of CRPC.

Prostate cancer has long been considered to be a chemorefractory tumour [97]. In the 1980s and 1990s numerous drugs were tested in Phase II trials, including cisplatin, 5-FU, methotrexate, doxorubicin, etoposide and vinblastine, covering a broad spectrum of cytotoxic mechanistic approaches. They failed to produce response rates above 10% [97]. While a number of drugs (e.g. mitoxanthrone and vinorelbine) did demonstrate encouraging response rates in Phase II trials, this failed to translate to a survival benefit [73, 96, 98]. There were two explanations: either prostate cancer was inherently chemorefractory or the particular cytotoxics tested had

mechanisms of action that were ineffective in prostate cancer.

Docetaxel, a member of the taxane family, acts to stabilise microtubules and prevent tubulin depolymerisation, which ultimately leads to G2/M arrest and apoptosis. In TAX327 docetaxel 75mg m^{-2} plus prednisolone increased median survival from 16.5 to 18.9 months ($p=0.009$) [94]. This was accompanied by an increase in pain response rate from 22% to 35% ($p=0.01$), and in quality of life response rate from 13% to 22% ($p=0.009$). Similar response rates were seen in the SWOG 9916 trial; however, here a regimen of docetaxel 60mg m^{-2} plus estramustaine 280mg m^{-2} was compared with mitoxanthrone/prednisolone [95]. Median survival was increased from 15.6 to 17.5 months ($p=0.02$). From these results it can be concluded that docetaxel provides a survival advantage above mitoxanthrone/prednisolone in CRPC. The addition of estramustaine increased toxicity with no additional survival benefit, making docetaxel/prednisolone the first-line therapy of choice for men fit for chemotherapy.

Cabazitaxel is also a member of the taxane family and hence has a similar mechanism of action. It has shown promising activity in pre-clinical studies, being capable of producing a complete response in DU145 xenograft tumours. A Phase III clinical trial in CRPC was initiated comparing cabazitaxel with mitoxanthrone in patients who had failed to respond to/progressed after at least two cycles of docetaxel [99]. 755 patients were randomised to receive either mitoxanthrone 12mg m^{-2} plus prednisolone, or cabazitaxel 25mg m^{-2} plus prednisolone. Cabazitaxel produced a response rate of 14.4% *vs* 4.4% for mitoxanthrone. This translated to a significant improvement in median PFS (2.8 *vs* 1.4 months, HR 0.74, $p<0.0001$) and an improvement in median OS (15.1 *vs* 12.7 months, HR 0.7, $p<0.0001$). Relatively high rates of neutropenia were seen in this trial, which may be because of the use of higher doses of cabazitaxel than had previously been tested in Phase II trials in breast cancer. Despite this, cabazitaxel appears to be a promising agent in CRPC, and future head-to-head trials with docetaxel in the first-line setting will be interesting. The fact that it retains activity in docetaxel refractory patients suggests minimal cross-resistance and would allow for sequential use.

Another potentially promising new agent is ixabepilone. It is an epithelone, a family of microtubule-stabilising drugs that cause G2/M arrest and apoptosis. Their mechanism of action differs from taxanes in the mode of interaction with microtubules. Ixabepilone has shown promise in pre-clinical trials. It is active in mouse xenograft models of taxane resistance, including cell lines that overexpress multiple drug resistance and possess β -tubulin mutations [100]. Phase II clinical trials have been conducted in chemotherapy-naïve and docetaxel-refractory patient populations [101, 102]. Patients with CRPC who had received no prior chemotherapy were randomised to receive ixabepilone 35mg m^{-2} plus oral estramustaine 280mg or ixabepilone alone. PSA response rates ($>50\%$ decrease) were seen in 69% (31/45) in the combination arm and 48% (21/44) in the single agent arm. Median PFS was 5.2 *vs* 4.4 months [101]. Thus, both alone and in combination, ixabepilone produced significant responses. In the second-line setting in patients with docetaxel-refractory disease, ixabepilone remains capable

of inducing a response rate of 17% as monotherapy and of 43% in combination with mitoxantrone [100].

Satraplatin is a novel platinum agent. Like other family members it intercalates deoxyribonucleic acid (DNA), creating intra- and interstrand cross-links, which ultimately lead to G2/M arrest and apoptosis. In pre-clinical testing satraplatin showed activity against the prostate cancer cell lines LNCaP, PC3 and DU145 [103]. Interestingly, it was also active in multidrug-resistant cell lines, including the breast cancer line NCI-Adr-Res, which is resistant to doxorubicin, taxol and docetaxel.

A Phase II study was performed by the European Organisation for Research and Treatment of Cancer in the first-line setting [72]. Here 50 patients were randomised to satraplatin 100 mg m⁻² plus prednisolone *vs* prednisolone alone. PFS was significantly prolonged in the satraplatin arm 5.2 *vs* 2.5 months ($p=0.023$). A >50% PSA decline was witnessed in 33.3% of the satraplatin-treated patients *vs* 8.7% of the prednisolone-only arm. However this failed to produce a significant OS advantage (14.9 *vs* 11.9 months, p =not significant). On the basis of these results, however, a Phase III trial SPARC (Satraplatin and Prednisone Against Refractory Cancer) was established [104]. Here 950 patients who had progressed after at least two cycles of chemotherapy were randomised 2:1 to receive satraplatin 80 mg m⁻² or placebo. Median PFS was increased by 11.1 *vs* 9.7 weeks ($p=0.001$). Improvement was also seen in PSA response rates (25.4% *vs* 12.4%, $p<0.001$) and time to pain progression (66.1 *vs* 22.3 weeks, $p<0.001$). However, again, this failed to translate to a significant improvement in OS (61.3 *vs* 61.4 weeks, p =not significant).

An explanation for the discrepancy between PFS and OS data may lie in the effect of satraplatin on PSA and the use of PSA as a marker of progression. It has been demonstrated in LNCaP cells that satraplatin treatment can inhibit PSA protein production. Messenger ribonucleic acid expression levels were unchanged and the population of cells involved were not killed by the treatment. This suggests that an *in vivo* PSA reduction may not represent tumour cytotoxicity. This would explain why a PSA response and PFS response (determined by PSA) could be witnessed without having an impact on OS.

Hormone-refractory prostate cancer has been considered chemorefractory. The reason for this could be a generalised mechanism, such as poor drug penetration due to the vasculature of prostate cancers, or high rates of expression of drug efflux pumps. However the encouraging results of recent trials with docetaxel, cabazitaxel and ixabepilone suggests that by choosing a mechanistically appropriate drug, responses can be achieved. It is of interest that the central family of drugs that do produce responses all target microtubules and not DNA replication. A large proportion of prostate cancers are relatively slow-growing compared with other tumour types, which may explain this fact. Inducing apoptosis via microtubules is thus a more effective way of producing tumour kill than inhibiting DNA replication.

Mechanism and treatment of bone metastases

By far the most common site (often the sole site) of metastases in prostate cancer is to bone [50, 105]. Up to

80–90% of patients with CRPC have bony disease, and often the majority of symptoms and complications experienced by men with prostate cancer result from bony disease. In addition, osteoporosis secondary to long-term antiandrogen therapy can exacerbate the problem. Efforts to better understand the biology of this process aim to develop treatments specifically targeting the symptoms of bony disease. The ultimate aim is to identify treatments that prevent the development of bone metastases.

The mechanism by which prostate cancer preferentially selects bone as a site of metastases is as yet incompletely understood. Metastasis of any solid tumour can only occur as a result of multiple co-operative defects in normal cellular function (Figure 3). It is likely, given the heterogeneity of prostate cancers, the multistep nature of the metastatic process and the fact that prostate cancers can also metastasise to other sites, that this process is achieved by a variety of mechanisms. However, based on what is already understood a number of therapeutic targets have been identified.

An early step in the development of metastases is a change in adhesion molecule expression. In prostate cancer epithelial cells commonly switch from E- to N-cadherin expression, and demonstrate decreased β -catenin expression. This is accompanied by altered patterns of integrin expression. This leads to loss of cell–cell and cell–stroma interactions between tumour cells and prostatic connective tissue. Prostate cancer's predilection for bone may be due to this altered pattern of cell surface markers, leading to increased affinity of malignant epithelial cells for bone marrow endothelial cells, or due to local production of chemoattractants. However, the pattern of cell surface markers expressed normally by prostate epithelial cells allows those cells shed into the circulation in normal circumstances to bind endothelial selectins and integrins and to be rapidly cleared from circulation. Therefore the homing, locking and docking function of malignant epithelial cells may work as normal. The development of viable metastatic foci depends upon the interaction between malignant prostate epithelial cells and the bone microenvironment, which provides a permissive environment, allowing metastatic cells to thrive. The search for novel therapeutics has focused on this interaction.

Prostate cancer metastases are osteoblastic, with increased bony deposition giving them a sclerotic appearance. There is, however, increased osteoclast activity evidenced by the fact that both bone-specific alkaline phosphatase (a marker of bone formation) and urinary N-telopeptide (uNTP; a marker of bone collagen breakdown) are both elevated in patients with osteoblastic metastatic disease. Growth factor release and cell signalling pathway interactions between tumour cells and osteoblasts/osteoclasts are responsible for the alterations in bone physiology that give rise to metastatic foci. A symbiotic relationship develops with tumour cells and osteoblasts. In normal bone a balance is maintained between osteoclast and osteoblast function. A number of pathways are involved in this process, namely *Src* (an oncogene), Wnt (a signalling pathway) and RANK (receptor activator of nuclear factor κ B) signalling.

Two classes of agents have been developed to specifically inhibit osteoclast activity in bone. Bisphosphonates bind to the surface of bone and prevent osteoclast-mediated osteolysis. Zoledronic acid (ZA) is the most

Figure 3. Mechanism of bone metastasis. 1. Primary prostate cancer—loss of cell–cell and cell–stroma interactions and cellular dedifferentiation. 2. Spread within circulation (blood/lymphatic)—cells breach basement membrane via gained ability to physically breakdown local connective tissue combined with increased motility. MMP, matrix metalloprotein; TIMP, tissue inhibitor of matrix metalloprotein. 3. Distant seeding—cells must develop the ability to anchor to distant endothelium, migrate into distant tissue and once there thrive by being receptive to growth signals from locally produced growth factors. IGF, insulin-like growth factor; IL-1, interleukin-1; PDGF, platelet-derived growth factor; PTHrP, parathyroid hormone related peptide; RANKL, receptor activator of nuclear factor- κ B ligand; TGF β , transforming growth factor β ; TNF α , tumour necrosis factor α .

potent inhibitor of osteoclast activity among the range of bisphosphonates currently available. ZA can decrease levels of uNTP by 70–80%, whereas pamidronate reduces levels by 50% [106]. RANK is a member of the tumour necrosis factor receptor superfamily and is expressed by osteoclasts. RANK is activated by RANK ligand, which is expressed by bone marrow stromal cells and osteoblasts. Denosumab is a monoclonal antibody that binds and inactivates RANK ligand, and hence inhibits osteoclast activation.

A number of trials in prostate cancer, breast cancer and multiple myeloma have demonstrated that zoledronic acid can reduce the incidence of SREs in patients with established bony disease [106–108]. This effect is particular for ZA, as trials using clodronate and pamidronate failed to demonstrate benefit [109, 110]. This can be accounted for by the significantly increased ability of ZA to inhibit osteoclast activity over either clodronate or pamidronate [106]. ZA was approved by the Food and Drug Administration in the USA in this setting, but may now be replaced by denosumab as a recent trial has demonstrated that denosumab has an

enhanced effect over ZA [111]. Denosumab decreased the incidence of SRE by 18% over ZA (HR 0.82, $p=0.004$). Also, the time to development of first SRE was delayed by 3.6 months ($p=0.0002$).

Bisphosphonates have been proven to increase bone mineral density in patients on long-term androgen deprivation therapy [112]. This includes alendronate, pamidronate and neridronate, as well as zoledronic acid. Another option is the use of selective oestrogen receptor modulators raloxifene and toremifene [112]. Even more positively, two recent studies using denosumab and toremifene have demonstrated for the first time a reduction in the pathological fracture rate [113, 114].

Osteoclast-targeted therapy can thus reduce the rate of SRE, but no evidence exists that it can prevent the development of bone metastases in prostate cancer or impact OS. Endothelin-1 (ET-1) is produced by normal prostate epithelium and production is increased in prostate cancer. ET-1 has a mitogenic, pro-angiogenic and antiapoptotic action on prostate cancer cells. ET-1, via the Eta receptor, also acts in the bone microenvironment.

It increases osteoblast activity and decreases osteoclast activity. Several compounds have been developed to inhibit the ET-1 endothelin A receptor (ET_AR) axis, but the two most advanced are atrasentan and zibotentan. Both are ET_AR antagonists.

Despite promising Phase II data suggesting atrasentan may prolong time to disease progression, a Phase III trial of atrasentan monotherapy in CRPC failed to produce similar results [115, 116]. Trials of atrasentan in combination with docetaxel are in their infancy. A small Phase II trial was completed, which failed to show PSA responses in line with single-agent docetaxel therapy. A larger Phase III trial (SWOG S0421) is under way. Zibotentan, a similar agent, which is more specific for ET_AR, has been trialled as monotherapy for CRPC in a Phase II double-blind randomised control [117]. The results of this trial failed to show improvement in TTP or radiological response, yet a significant improvement in OS was seen. This again raises the question of the use of surrogate end points in the design of clinical trials with agents with novel mechanisms of action. A Phase III trial in combination with docetaxel is under way.

Src signalling has been identified as a drugable target pathway in prostate cancer. The pathway is involved in the transduction of signals relating to growth, proliferation, adhesion, angiogenesis, motility and survival of prostate cancer cells. The pathway demonstrates enhanced activity in prostate cancer cells. This activation increases with progressive disease and correlates with poor prognosis [118]. *Src* signalling appears important in non-ligand-dependent AR signalling in CRPC. *Src* signalling is also pivotal in the regulation of osteoblast/osteoclast activation, negatively regulating osteoblasts and positively regulating osteoclasts. *Src* inhibition has been shown to reduce proliferation, adhesion and invasion of prostate cancer cell lines and osteoclast activity.

A number of *Src* inhibitors have entered into clinical trial in prostate cancer, the two most advanced being dasatinib and saracatinib. Dasatinib is a pan-TKI inhibiting *Src*, platelet-derived growth factor receptor, *abl* and *kit* (oncogenes) signalling, whereas saracatinib is *Src* family kinase-specific. Data from two Phase II studies using dasatinib monotherapy in patients with HRPC failed to demonstrate a difference in PFS [119]. What was shown was a significant fall in levels of the markers of bone turnover bone-specific alkaline phosphatase and urinary uNTP. Similarly, a Phase II trial of saracatinib monotherapy in CRPC patients failed to show a difference in PFS compared with placebo [120]. A combination trial of dasatinib and docetaxel is underway (CA180-227).

Conclusion

Understanding the molecular biology of a tumour is the key to treating it. The last decade has witnessed significant advances in our understanding of the biology of prostate cancer, which has successfully translated to new therapeutic options. What was previously termed androgen-independent prostate cancer is now described as castrate-refractory prostate cancer. Recognition of the significant contribution of adrenal androgens in the face of castrate serum testosterone levels, combined with

persistent AR activity in advanced disease, has led to the development of new therapeutic modes of inhibition of the androgen/AR axis.

Pioneering therapeutic strategies are being applied to the treatment of prostate cancer. Targeting the mechanism of metastatic spread of a tumour is one such novel approach. Endothelin inhibitors targeting metastatic spread of prostate cancer to bone have proved successful. Sipeucel-T use in prostate cancer is the first cancer immunotherapy to progress successfully through clinical trials and achieve FDA approval. Prostate cancer had long been considered chemorefractory. The discovery of taxanes has reversed this misconception. It appears that specifically targeting microtubules is an effective strategy in treating prostate cancer. This has highlighted the importance of mechanism of action in the choice of conventional cytotoxics.

A constant theme when examining clinical trials of novel therapies in prostate cancer is the use of intermediate and surrogate end points. The use of surrogate end points speeds up the process of drug development. With increasing use of sequential therapies and longer survival, the interpretation of OS as a trial end point becomes more complicated. In prostate cancer, PSA progression has provided an intermediate end point. However, the accuracy and applicability of PSA as a surrogate end point has come into question [59, 60]. As noted throughout this article, numerous novel therapies have produced OS effects in trials but failed to impact PSA progression [12, 31, 72]. It has been widely recognised that while most patients demonstrate a rising PSA with progressive disease, over shorter time periods such as those assessed in trials, a lack of concordance with radiological response is common. Both PSA and bone scans can worsen before they improve in response to therapy. In addition, given the heterogeneity of prostate cancer, a mixed response at different sites may be achieved. Treatments (for example, immunotherapy) may achieve their effect over a longer time scale; therefore, intermediate end points fail to detect true therapeutic effect. Certain therapies, by their nature, may have no effect on tumour mass, such as bone-targeted therapies designed to prevent SREs. Alternatively, treatments may be predominantly cytostatic, halting progression (e.g. TKIs). Individual drugs may affect biomarker expression directly and, as a result, changes in biomarker levels do not represent tumour response (e.g. satraplatin and PSA). Finally, it should be borne in mind that PSA expression is androgen-regulated, and hence its expression is a marker of AR activity. Its use as a surrogate end point may therefore only be applicable to trials of agents targeting the androgen/AR axis. Circulating tumour cells may provide an additional or alternative option, as they not only act as a marker of disease progression but can also be used as a substrate for molecular biomarker testing. It would appear that biomarker and end-point selection in clinical trials needs to be individualised based on the biology of the treatment in question.

The molecular biology of prostate cancer is complex and poses many questions, such as why prostate cancer is so common, what drives the development of prostate cancer and why it preferentially metastasises to bone. Perhaps the most clinically relevant issue is how to differentiate indolent from aggressive disease. Overall, however, recent advances in therapy provide cause for optimism.

Urological oncologists are now faced with a new (and rather more pleasant) dilemma: how best to sequence/combine the range of treatment options for prostate cancer that are now available, where once there were few.

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