# Drug discovery in advanced prostate cancer: translating biology into therapy

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## Abstract

Castration-resistant prostate cancer (CRPC) is associated with a poor prognosis and poses considerable therapeutic challenges. Recent genetic and technological advances have provided insights into prostate cancer biology and have enabled the identification of novel drug targets and potent molecularly targeted therapeutics for this disease. In this article, we review recent advances in prostate cancer target identification for drug discovery and discuss their promise and associated challenges. We review the evolving therapeutic landscape of CRPC and discuss issues associated with precision medicine as well as challenges encountered with immunotherapy for this disease. Finally, we envision the future management of CRPC, highlighting the use of circulating biomarkers and modern clinical trial designs.

Key points

- Castration-resistant prostate cancer (CRPC) is associated with a poor prognosis and poses considerable therapeutic challenges.
- Recent genetic and technological advances have provided insights into prostate cancer biology and enabled the identification of novel drug targets and potent molecularly targeted therapeutics for the disease.
- Promising targets in CRPC include the androgen receptor and its variants, key signalling pathways such as phosphoinositide 3-kinase (PI3K)–AKT and WNT signalling, and DNA repair defects.
- The therapeutic landscape of CRPC is evolving, with an increased focus on research into tumour heterogeneity, immuno-oncology, minimally invasive circulating tissue biomarkers, and modern clinical trial designs.
- The use of state-of-the-art, high-throughput, genomic platforms enabling patient stratification will permit optimization of the development of current and future drugs for CRPC.

## Introduction

Prostate cancer is the most common malignancy in men and a leading cause of cancer-related male mortality[1]. Although potentially curable when confined to the prostate, ~20% of patients will present with metastatic disease, and others develop disease progression despite local therapies such as surgery or radiotherapy[2].

In 1941, Charles Huggins first reported the beneficial effects of systemic androgen ablation in patients with advanced prostate cancer. This finding ultimately led to our understanding that blockade of the androgen receptor (AR) in prostate cancer represents an effective antitumour strategy. The current initial treatment of metastatic prostate cancer remains androgen-deprivation therapy (ADT), either through surgical castration or through medical castration with anti-androgens or luteinizing hormone-releasing hormone (LHRH) agonists or antagonists. Although ADT leads to remissions lasting ~2–3 years, the disease inevitably progresses to castration-resistant prostate cancer (CRPC), which is associated with a poor prognosis and poses considerable therapeutic challenges.

Recent advances in high-throughput, genome-wide profiling technologies, such as next-generation sequencing and RNA interference screening, have provided considerable insights into prostate cancer biology and have enabled the identification of specific dependencies and

vulnerabilities that can be exploited as novel drug targets[3]. Furthermore, technologies such as structure-based design have enabled the discovery and development of potent molecularly targeted therapeutics for these identified targets. It is envisioned that these advances will enable personalized treatments of patients with advanced prostate cancer — a possibility that is especially relevant because of the high intra- and inter-patient heterogeneity that exists in this disease[4,5].

In this Review, we summarize the different genomic techniques currently used to identify targets in CRPC. We focus on the most promising targets for prostate cancer drug discovery, including AR and its variants, on which many advanced and treatment-resistant tumours depend for growth and survival. We also highlight key signalling pathways as potential sources of targets, including phosphoinositide 3-kinase (PI3K)–AKT and WNT signalling, and DNA repair defects, as well as other emerging targets. We then discuss the evolving CRPC therapeutic landscape and explore issues associated with precision medicine. Finally, we envision the future care of patients with CRPC, which might involve the increased use of circulating biomarkers and modern clinical trial designs for improved outcomes.

## Genomic technologies

In 2010, Taylor and co-workers reported a comprehensive approach to defining the genomic and transcriptomic profile of prostate cancer, which involved the analysis of more than 200 tumours[3]. They mapped the prostate cancer oncogenome using arrays for measuring copy number and gene expression, as well as complete exon resequencing for identifying specific mutations[3]. This process identified distinct genomic alterations in the 138 evaluated genes. For example, the nuclear receptor co-activator *NCOA2* was implicated as an oncogene in 11% of tumours, and the transmembrane protease serine 2 (*TMPRSS2*)–*ERG* gene fusion was associated with a deletion at chromosome 3p14. This latter finding, when combined with expression data and focal deletion patterns, implicated forkhead box protein P1 (FOXP1), RING1 and YY1 binding protein (RYBP) and SHQ1 as potential cooperative tumour suppressors[3]. Subsequent analysis of copy number data revealed the functional relevance of these alterations in driving prostate cancer. Similar studies have used whole-genome sequencing (WGS)[6,7] and, more recently, exome capture-based next-generation sequencing (NGS)[8,9,10] to further map the prostate cancer genomic landscape. The characterization of CRPC by other methods, including RNA sequencing (RNA-seq)[11] and metabolomics[12], has also contributed to this body of knowledge. In this section, we provide an overview of the contribution provided by each of these respective technologies to drug discovery in CRPC.

### *Whole-genome and whole-exome sequencing.*

Advances in NGS using massively parallel sequencing have increased throughput, enabling the detection of rare mosaic variants or mutations, at reduced cost. Thousands to millions of sequencing reactions can be performed in parallel, and cloning and template amplification of the sequenced DNA fragments can be fully automated or rendered unnecessary[13]. As a result, comprehensive tumour assessments with whole-exome sequencing (WES) and WGS are becoming increasingly widely available[14]. A recent WES study[9] confirmed genomic aberrations in multiple commonly affected genes believed to be critically important in CRPC. These genes include those encoding the tumour suppressor p53 (TP53), AR, phosphatase and tensin homologue (PTEN), breast cancer type 2 susceptibility protein (BRCA2), serine-protein kinase ataxia telangiectasia mutated (ATM), catenin-β1, retinoblastoma-associated protein (RB1), zinc finger homeobox protein 3 and APC. In addition, genes with as yet

unclear roles in prostate cancer were identified. These genes include those encoding mixed lineage leukaemia protein 2 (MLL2), olfactory receptor 5L1 and cyclin-dependent kinase 12 — although the lattermost may be a key DNA repair protein[9]. Loss of TP53 and/or RB1 function has since been implicated in conferring resistance to the next-generation AR inhibitor enzalutamide (Xtandi; Astellas Pharma), possibly by priming prostate cancer cells to differentiate into more primitive, undifferentiated, small-cell cell types[15]. Conversely, aberrations of *RB1* in CRPC cell lines are associated with sensitivity to mitotic inhibitors, indicating that taxanes and other tubulin-binding drugs have therapeutic potential in *RB1*-variant tumours[16].

In a separate study[6], complete sequencing of seven prostate cancer genomes and their paired non-cancer prostate tissue counterparts identified cancer-associated disruptions not previously implicated in prostate cancer. The analysis identified chromodomain-helicase-DNA-binding protein 1 (*CHD1*), cell adhesion molecule 2, *PTEN* and membrane-associated guanylate kinase inverted 2 (*MAGI2*; a PTEN-interacting protein) as having a role in prostate cancer[6]. Recently, Stand Up To Cancer and the Prostate Cancer Foundation sponsored an international initiative for prostate cancer research, involving exome and transcriptome analyses of archived and fresh CRPC tumour biopsies[17]. This initiative provides a crucial step towards elucidating genomic variation patterns associated with prostate cancer and identifying novel actionable targets.

*Targeted DNA sequencing.*

Targeted DNA sequencing is a pragmatic, high-throughput and inexpensive method for identifying putative oncogenic aberrations of genes such as *AR*, *BRCA2*, *ATM*, CHK2 checkpoint homologue (*CHEK2*), PI3K catalytic isoform p110α (*PIK3CA*), *KRAS* and *BRAF*[3,18]. Specific therapeutic strategies can then be developed on the basis of such data to target actionable genomic aberrations[3]. Recently, a novel NGS-based platform was applied to formalin-fixed paraffin- embedded tumour biopsy tissue to identify genomic alterations in known cancer-related genes[19]. The analysis identified *AR* alterations, the *TMPRSS2–ERG* fusion, *PTEN* loss, *TP53* mutations, *RB1* loss, *MYC* gain, *PIK3CA* mutation, *BRCA2* loss and *ATM* mutations as particularly associated with prostate cancer aggressiveness[19]. This technology is particularly relevant clinically, as tissue biopsies are commonly processed using formalin-fixed paraffin-embedded methods[20].

Copy number alterations, including focal high-level amplification in genes such as *AR* and *FOXA1*, as well as deletion of *PTEN* and *CHD1*, have been identified in prostate cancer using comparative genomic hybridization array (aCGH)[9,18,21]. These recurrent, high-level gains or losses can indicate potential cancer drivers, such as *AR* amplification in prostate cancer[22]. In addition to *AR*, several clinical trials are evaluating whether loss of the tumour suppressor gene *PTEN* (as determined using fluorescent *in situ* hybridization (FISH) or immunohistochemistry) is a driver of CRPC by pharmacologically targeting downstream substrates along the PI3K–AKT signalling pathway[23,24,25,26,27,28]. However, early clinical data suggest that targeting the PI3K–AKT pathway at a single point may not be sufficient for antitumour activity owing to pathway feedback loops and signalling crosstalk; therapeutic strategies concurrently targeting AR and PI3K–AKT signalling may have superior antitumour activity[29]. In addition to aCGH, other genomic hybridization techniques, such as FISH, can isolate the position of genes to confirm their involvement in rearrangements, such as in the fusion genes *TMPRSS2–ETV1* and *TMPRSS2–ERG*[30].

*RNA-seq.*

RNA-seq uses NGS to characterize the RNA transcriptome, detecting expression of non-coding RNA, gene fusions, somatic mutations and alternatively spliced forms[11]. Gene expression analyses can assess *AR* expression in CRPC and the continued dependence of CRPC cells on AR signalling[31,32,33]. RNA-seq has also demonstrated that increased expression of *FOXA1*, a cofactor known to interact directly with AR[34], is associated with increased AR activity and cell growth in an androgen-depleted context. The resultant increased AR activity in this setting could cause resistance to castration, suggesting that FOXA1 could be a potential target for recurrent disease[35]. Furthermore, RNA-seq studies have identified many constitutively active splice variants of *AR*, which represent another mechanism of resistance of CRPC and thus another potential target in CRPC[36,37]. Notably, such targets would not have become evident through genomic analysis alone. The presence of these splice variants is associated with acquired resistance to the steroid 17α-hydroxylase (also known as CYP17A1) inhibitor abiraterone (Zytiga; Janssen Biotech) and the next-generation AR inhibitor enzalutamide[38,39]. Moreover, worse prognosis and refractoriness to enzalutamide after abiraterone as well as to abiraterone after enzalutamide are associated with these splice variants[38,39]. By contrast, two studies (in small groups of patients) have indicated that these splice variants do not associate with taxane resistance[40,41]. Targeting the amino terminus of the AR splice variant products, potentially using peptidomimetics that inhibit AR interactions with other proteins, is now a major area of interest for CRPC drug discovery[42].

Long non-coding RNAs (lncRNAs) are not transcribed into proteins and can be expressed at very low levels, rendering them difficult to detect even by RNA-seq; however, lncRNAs seem to be important in prostate cancer[43]. For example, RNA-seq of prostate cancer xenografts identified a lncRNA called *PCAT18* that appears to be prostate cancer-specific. Moreover, *in vitro*, LNCaP and C4-2 cells exhibited decreased cellular proliferation, migration and invasion upon *PCAT18* silencing with small interfering RNA (siRNA), suggesting that this lncRNA could be a useful target for inhibiting metastatic prostate cancer[43]. Two other lncRNAs, *PCANR1* and *PCANR2*, which regulate prostate cancer cell growth, were identified using integrative analysis of prostate cancer lncRNA expression profiles and clinical outcomes to predict potential drivers of progression[44]. Thus, *PCANR1* and *PCANR2* are also promising therapeutic targets. High expression of the lncRNA *SChLAP1* was also recently identified as a prognostic biomarker for metastatic disease progression of prostate cancer, supporting further investigation into its role as a potential biomarker for treatment intensification in aggressive prostate cancer[45].

Paired-end massively parallel transcriptome sequencing, which uses unique short sequences at the 5′ and 3′ ends of a DNA fragment, was used to search for potentially druggable driver gene fusions in *ETS*-rearrangement-negative prostate cancers. This approach identified two gene fusions involving *RAF* (*SLC45A3–BRAF* and *ESRP1–RAF1*) that may be responsive to RAF kinase inhibitors or MAPK/ERK kinase (MEK) inhibition[46]. RNA-seq has also revealed various alternative-splicing events, such as exon skipping in α-methylacyl-CoA racemase (*AMACR*), intron retention in *KLK3* (the gene encoding prostate-specific antigen (PSA)) and alternative 5′ and 3′ splice sites[47], which, together with their encoded proteins, may serve as potential novel therapeutic targets.

*Other RNA-based technologies.*

Short hairpin RNA (shRNA) and siRNA analyses and cancer outlier profile analyses (COPAs) have been used to unravel pathways involved in prostate cancer and to provide a basis on which to develop new drugs for CRPC.

siRNA silencing of the expression of *MLL*, which acts as an epigenetic transcription factor, inhibits AR signalling *in vitro*[9]. siRNAs have also been used in mouse prostate epithelial cells to knock down the expression of the chromatin remodeller CHD1 to model a *CHD1* deletion that is associated with wild-type speckle-type POZ protein (SPOP) in *ETS* gene family fusion-negative prostate cancers[9,48,49]. This *Chd1* knockdown resulted in increased invasiveness and proliferation in the mouse prostate epithelial cells[49]. In addition, siRNAs targeting *PCANR1* or *PCANR2* inhibited growth in the androgen-dependent prostate cancer cell line LNCaP-abl[44]. siRNA knockdown of the epigenetic regulator enhancer of zeste homologue 2 (*EZH2*) in LNCaP-abl cells demonstrated a role of this factor as a co-activator of AR and other transcription factors[50]. This function of EZH2 is independent of its main role in silencing gene expression via its histone methyltransferase activity, and suggests a potential utility for combination therapy against EZH2 and AR[50].

An shRNA library approach characterized by Fellman and Lowe[51] was used to identify specific shRNAs that synergize with decitabine, a DNA methyltransferase inhibitor, to promote cell death in human prostate cancer cells[52]. One such synergizing shRNA was specific for Aurora A kinase (*AURKA*), indicating a potential application of combining silencing gene expression and therapeutic agents, such as decitabine, in treating prostate cancer. Epigenetic regulators — including DNA methylation pathway mediators, histone-modifying enzymes, chromatin-remodelling factors and structural chromosomal proteins — have a key role in cancer (reviewed in Ref. 53). The discovery of genomic aberrations in genes encoding these regulators has led to the evaluation of specific anticancer therapies such as bromodomain and extraterminal (BET) inhibitors.

Bromodomains are protein domains that recognize monoacetylated lysine residues, such as those on the N-terminal tails of histones, and are required for protein–histone association and chromatin remodelling. The bromodomain-containing protein ATPase family AAA domain-containing protein 2 (ANCCA; also known as ATAD2) has been identified as a potential therapeutic target owing to its androgen-mediated ability to stimulate expression of EZH2, which is a subunit of the polycomb repressive complex 2 (PRC2)[54]. PRC2 targets genomic regions for epigenetic silencing and has histone methyltransferase activity. Targeting the N-terminal bromodomain of bromodomain-containing protein 4 (BRD4) with small-molecule BET inhibitors, such as JQ1 or I-BET762, in AR-signalling-competent CRPC cells led to antiproliferative effects and reduced cancer cell survival[55,56]. BRD4 interacts with the N-terminal domain of AR; consequently, bromodomain inhibitors can disrupt AR recruitment to target genes, AR-mediated transcription and oncogenic AR-mediated induction of *TMPRSS2–ERG* transcription[55]. Furthermore, these inhibitors act downstream of AR, which may explain why BET inhibition has demonstrated greater efficacy in CRPC xenograft mouse models than the AR antagonist enzalutamide[55]. Phase I studies of BET inhibitors are currently ongoing[57,58].

Another RNA-based process to identify targets of interest is COPA. This technique can identify outlier gene expression profiles in prostate cancer based on the median and absolute deviation of gene expression profiles in benign prostate tissue[30]. COPA can identify

translocations in which the promoter region from one gene is translocated to the intact coding region of an oncogene, thereby upregulating the expression of the latter. By applying COPA to the Oncomine database[59] — a compendium of cancer microarray gene expression profiles — *TMPRSS2–ETS* rearrangements and high-level *AR* amplification were identifed[30]. Outlier meta-analysis (meta-COPA) of seven prostate cancer-profiling studies subsequently revealed transcriptional signatures that could distinguish between *ETS*-rearrangement-positive and *ETS*-rearrangement-negative tumours, and found the serine protease inhibitor Kazal-type 1 (*SPINK1*) to be specifically upregulated in *ETS*-rearrangement-negative cancers[60]. *SPINK1* knockdown attenuated invasion *in vitro* and may therefore be a useful target in *ETS*-rearrangement-negative prostate cancer[60].

*Metabolomic technologies.*

In the same way that analyses of the cancer transcriptome has revealed targets not evident in the genome, it now seems likely that profiling of the cancer metabolome may also be informative[12]. One study used gene expression data from Oncomine Concept Maps[61] to direct an analysis of biochemical pathways that are enriched in CRPC[62]. Mass spectrometry analysis and metabolic phenotyping identified 19 metabolites whose levels were altered in CRPC compared with androgen-dependent prostate cancer[62]. These metabolites mapped to a network of pathways that describe increased UDP glucuronosyltransferase (UGT) activity[62,63]. UGT activity in androgen-sensitive tissues is thought to be important for modulating the activity of androgens[63]. Although this pathway has previously been reported to be a potential predictor of treatment failure or disease recurrence[62], it may also represent a potential source of targets for drug discovery in CRPC.

## Targets for drug discovery

Rather than constituting a single disease, prostate cancer comprises multiple distinct and well-defined molecular subtypes that have different driver and passenger genomic alterations (Table 1). A recent study of the genomic landscape of metastatic CRPC has demonstrated that ~90% of prostate cancers harbour genomic aberrations that are potentially clinically actionable[17]. Using chemogenomic annotation and druggability assessment of key genetic aberrations, we find that CRPC is associated with a highly druggable network with multiple novel potential targets. A better understanding of these crucial molecular alterations will facilitate the translation of the most promising targets into disease molecular stratification and clinically relevant predictive biomarkers that will help ensure correct treatment allocation for a significant number of patients with CRPC.

In this section, we focus on four main aspects of CRPC biology that we believe are the most promising and tractable from a drug discovery perspective: the AR, the PI3K–AKT pathway, WNT signalling and DNA repair defects (Fig. 1). Importantly, all are targets or pathways that may be modulated by existing drugs or investigational compounds (Table 2).

*The AR.*

*AR* is the most commonly altered gene in CRPC, with mutations occurring in nearly two-thirds of cases[17]. Aberrations in *AR* that lead to the reactivation of AR signalling in CRPC include mutations, gene amplification or overexpression, and the expression of constitutively active *AR* splice variants[64,65]. The AR is a nuclear steroid hormone receptor that contains a central DNA-binding domain (DBD), a ligand-binding domain (LBD), a hinge region and a

large N-terminal domain (NTD)[66]. AR-LBD mutants can be promiscuously activated by anti-androgens (which activate, for example, AR-T878A and AR-W741C mutants)[67,68], glucocorticoids (which activate AR-L702H)[69], and adrenal androgens and progesterones (which activate AR-V715M)[70]. Mutations can also result in conformational changes to the AR; for example, the T878A mutation alters the stereochemistry of the LBD[71]. AR splice variants lacking the LBD[72] can be constitutively active and result in the expression of AR-target genes[37,73]. The AR splice variant 7 (arguably the most commonly expressed splice variant in prostate cancer) has been implicated in the development of CRPC and to resistance to ADT, enzalutamide and abiraterone[74].

Since the discovery that CRPC commonly remains AR-driven, there has been renewed interest in dissecting the specific underlying mechanisms of resistance[75,76] and in developing novel AR-targeted therapies that can overcome such resistance[77]. Hormonal therapies currently available for CRPC treatment target either hormone production or prevent hormone-mediated activation of AR by blocking the LBD[78]. AR-LBD inhibition can be achieved through AR antagonists that now include the potent next-generation compounds enzalutamide and ARN-509. These compounds have high AR affinity and demonstrate *in vitro* and *in vivo* activity in bicalutamide-resistant models of prostate cancer with *AR* overexpression and mutant AR[79,80]. Inhibitors of androgen biosynthesis (and potentially also of AR), such as abiraterone and galeterone[81], also decrease AR signalling. Recent evidence showed that abiraterone is converted in CRPC to the more active metabolite $\Delta^4$-abiraterone (D4A), which blocks multiple steroidogenic enzymes and directly inhibits the AR, providing an additional mechanism of action for the clinical activity of abiraterone[82].

The development of new inhibitors that act through non-LBD interfaces is an unmet clinical need. A recent study demonstrated that AR splice variant dimerization is key to AR splice variant signalling and involves a dimerization site near the DBD; targeting this interaction may therefore be of pharmacological utility in the future[83]. Targeting the AR NTD could provide an alternative way to block the receptor, with the advantage of bypassing resistance due to AR splice variant expression or *AR* mutations. However, this approach has been challenging because the NTD exhibits high flexibility and intrinsic disorder in solution, which together have prevented resolution of the NTD crystalline structure, and thus hampered virtual docking and other drug discovery strategies.

One small-molecule inhibitor of the AR NTD, EPI-001, has undergone preclinical investigation[83]. The compound was identified from a library of marine sponge extracts that were screened for inhibitory activity against both ligand-dependent and ligand-independent activation of the AR by blocking the transactivation of the AR NTD by forskolin, interleukin-6 (IL-6) or androgen[83]. EPI-001 was shown to covalently bind to the activation function 1 (AF-1) region of the NTD to block interactions of proteins with the AR, and to reduce the transcriptional activity of both full-length and AR splice variants[83]. Importantly, EPI-001 reduced androgen-induced proliferation and growth of CRPC xenografts expressing AR splice variants without significant toxic effects[83,84]. Concerns have been raised, however, that this compound has broad thiol-alkylating activity and has multiple mechanisms of action, including modulation of peroxisome proliferator-activated receptor-$\gamma$[85]. EPI-506, a derivative of EPI-001, has recently entered a phase I/II trial in patients with CRPC whose disease has progressed after prior enzalutamide or abiraterone therapy[86].

A surface exposed pocket on the AR DBD has also recently been proposed as an alternative site for AR inhibition. Small molecules designed to selectively bind to this pocket effectively

block transcriptional activity of full-length and spliced AR forms[87]. As discussed above, inhibiting the bromodomain BRD4, which interacts with the AR NTD, is another potential strategy to target AR-meditated gene transcription in CRPC[55]. Another potential target in CRPC is the heat shock protein 70 (HSP70) co-chaperone B cell lymphoma 2 (BCL-2)-associated athanogene 1 (BAG1), which stimulates AR activity (and regulates other steroid receptors)[88].

A novel strategy of interest has been the use of HSP90 inhibitors to target wild-type and mutant AR[89,90]. HSP90 is a chaperone protein that binds AR (as well as other proteins important in mediating prostate cancer progression), and maintains full-length AR in a high-affinity ligand-binding conformation. Inhibition of HSP90 results in the degradation of full-length AR and a suppression of AR signalling[91,92]. HSP90 inhibitors also result in depletion of AR splice variant 7 in *in vitro* and *in vivo* models[93]. A phase I study demonstrated preliminary antitumour responses in a range of advanced solid tumours, including CRPC, with the HSP90 inhibitor alvespimycin[94]. However, other phase I and phase II studies of HSP90 inhibitors have been generally disappointing, demonstrating poor patient tolerability and only modest antitumour activity[95,96,97]. Nonetheless, we envision that AR chaperone inhibitors that can potently and continuously suppress full-length AR and truncated AR splice variant 7 could have important antitumour activity in CRPC.

Other strategies that do not target the AR NTD directly have also been explored in CRPC. For example, the calcium-dependent proteinase calpain has been shown to cleave the AR into an androgen-independent isoform[98]. Studies identified a calpain cleavage site in the hinge region of the AR, indicating that calpain-mediated proteolytic cleavage could represent a possible mechanism of post-transcriptional loss of the AR LBD, and that calpain inhibition could be potentially explored as a therapeutic strategy in CRPC[98]. Another strategy involves bypassing resistance due to AR splice variants by enhancing AR degradation with compounds such as ASC-J9 (also known as dimethylcurcumin), which targets AR splice variant 7 (Ref. 99). Blocking deubiquitylating enzymes, such as ubiquitin carboxy-terminal hydrolase 12, that are key to maintaining AR deubiquitylation and stability may also be a potential therapeutic target in CRPC[100,101].

As AR signalling pathways undergo modulation through complex protein–protein interactions, targeting intracellular networks downstream of AR activation could be a potential strategy for inhibiting aberrant AR activation and androgen-independent tumour growth[102]. One such potential target is the PTEN–AKT–FOXO1 axis. FOXO1 binds to the transcription activation unit 5 motif in the AR NTD in the nucleus and inhibits transcriptional activity of AR splice variants in prostate cancer cells *in vitro*[103,104]. In prostate cancer cells lacking PTEN, activated AKT phosphorylates FOXO1, resulting in its nuclear exclusion. This process facilitates the interaction of AR with co-activators, thereby favouring androgen-independent activation of the AR. In the future, it is likely that improved genomic analysis will identify additional androgen-independent isoforms of AR, potentially revealing other potential drug targets in CRPC[105].

*The PI3K–AKT signalling pathway.*

A well-established and druggable set of alterations in prostate cancer has been identified in the PI3K–AKT signalling pathway. PI3K pathway alterations in CRPC include *PTEN* loss, abnormalities in the genes encoding inositol polyphosphate 4-phosphatase type II and PH domain leucine-rich repeat-containing protein phosphatases, and *PIK3CA* aberrations[3]. Loss-

of-function mutations or deletions in *PTEN* are commonly observed in advanced prostate cancer (with loss of heterozygosity at the *PTEN* locus present in up to 60% of patients)[9,18,106]. NGS studies have also identified complex rearrangements that disrupt both *PTEN* and the gene encoding its interacting protein MAGI2 (Ref. 6). *Pten* loss in mouse models leads to precursor prostate cancer lesions, and these mice develop invasive carcinomas when such features are combined with other alterations such as aberrations in *Erg*, *Tp53* and *Myc*[107,108,109].

Recent data also indicate that *PTEN* loss induces cellular senescence. However, infiltration of myeloid- derived suppressor cells can block this senescence. Specifically, *Pten*-null prostate tumours in mice are infiltrated by a population of $CD11b^{+}$, glucocorticoid receptor 1-positive myeloid cells that protect a population of proliferating tumour cells from senescence, thus sustaining tumour growth. These myeloid-derived suppressor cells appear to infiltrate the prostate along a chemokine–chemokine receptor axis involving CXC chemokine receptor (CXCR2), and release IL-1 receptor antagonist, which inhibits senescence and drives proliferation[110]. These findings identify a novel network that is established by innate immunity and that controls senescence and tumour growth, suggesting that targeting innate immunity may provide a novel therapeutic opportunity for treating prostate cancers lacking *PTEN*.

Critically, reciprocal feedback regulation between the PI3K–AKT pathway and AR signalling has been demonstrated in prostate cancers lacking *PTEN*, supporting the need for combinatorial antitumour strategies for such tumours[28]. In tumours lacking *PTEN*, PI3Kα activity is suppressed, and PI3K signalling is instead driven by PI3Kβ[111]. However, PI3Kβ inhibition only transiently inhibits AKT–mechanistic target of rapamycin (mTOR) signalling because it relieves feedback inhibition on upstream substrates and thus causes activation of PI3Kα and a rebound in downstream signalling. Therefore, combined PI3Kα and PI3Kβ inhibition may be required to effectively block the PI3K–AKT axis in *PTEN*-lacking tumours[111]. Several PI3K–AKT pathway inhibitors are currently under clinical investigation in CRPC, as single agents and/or in combination with the approved drugs enzalutamide and abiraterone (Table 2).

*WNT signalling.*

RNA-seq recently identified WNT–β-catenin signalling as a novel, functionally important pathway for androgen-independent prostate cancer progression[9,112]. Aberrations that result in WNT pathway activation, such as loss of function of the adenomatous polyposis coli protein (APC) and mutually exclusive mutations in the gene encoding β-catenin, have been identified in metastatic CRPC[9,112]. Moreover, uncommon WNT pathway-activating mutations, including *RNF43* mutations and amplification or rearrangements of R-spondin family members (including *RSPO2* and *RSPO3*) have been identified in metastatic CRPC[9,112]. Overall, there is potential for targeting WNT signalling in the subset of CRPCs with activation of this pathway.

Multiple targeted strategies have been pursued to achieve this goal, including the inhibition of porcupine (PORCN), a membrane-bound *O*-acyltransferase enzyme required for WNT secretion, or of tankyrases (members of the poly(ADP-ribose) polymerase (PARP) family of proteins)[113,114,115]. Preliminary data from a phase I clinical trial of the first-in-class PORCN inhibitor WNT974 in patients with advanced solid tumours demonstrated a manageable safety profile, with side effects including dysgeusia, gastrointestinal symptoms, fatigue,

asthenia and hypercalcaemia[116]. The phase I expansion cohort will restrict accrual to patients with cancers harbouring molecular aberrations that portend WNT ligand dependence, such as *RNF43* mutations and *RSPO* fusions.

*DNA repair defects.*

Genomic aberrations of DNA defect repair genes have been reported in both CRPC and high-risk localized disease. The most commonly aberrant genes are *BRCA2* and *ATM*, and other genes involved in homologous recombination (HR) DNA repair, nucleotide excision repair (NER) and mismatch repair (MMR). Aberrations in genes involved in HR occur in up to 30% of CRPC cases[17,19,117] and have important implications for antitumour treatment because HR defects in cancer cells may be exploited by anticancer therapeutics such as platinum therapy and PARP inhibitors through synthetic lethal approaches. Platinum drugs have been repeatedly reported to have a 20–30% response rate in advanced prostate cancer, but, until recently, no studies have pursued biomarkers predictive of the antitumour activity of such drugs. Other DNA repair defects, such as aberrations in genes involved in NER, may sensitize tumour cells to platinum therapy but not PARP inhibition[118].

Antitumour activity has been reported in a phase II clinical trial of the PARP inhibitor olaparib (Lynparza; AstraZeneca), with ~33% of evaluable patients with metastatic CRPC showing an antitumour response[119]. Importantly, most patients in this trial experiencing clinical benefit had tumours harbouring defects in HR DNA repair genes[119]. Data from other studies also suggest that PARP is required for ETS and AR function, suggesting that PARP inhibitors may have broader antitumour activity[120,121]. Multiple trials are now investigating PARP inhibition, either as a single agent or in combination with abiraterone, in men with CRPC who have sporadic or germline *BRCA1* or *BRCA2* mutations (Table 2). It is important to note that recent studies have surprisingly reported that 8–15% of metastatic prostate cancers have germline defects in actionable DNA repair genes; these findings will have an impact on the clinical care of men with advanced prostate cancer and will provide support for the routine germline testing of selected genes such as *BRCA2* and *ATM* in this patient population[17,122].

In addition, abnormalities in genes involved in MMR and potentially other defects in DNA repair can also increase tumour mutational load, which can sensitize cancers to immunotherapy with multiple therapeutics targeting the programmed cell death 1 (PD1)–PD1 ligand 1 (PDL1) axis or cytotoxic T lymphocyte-associated antigen 4 (CTLA4)[123,124]. So far, immune checkpoint- targeting treatments have shown limited evidence of antitumour activity in CRPC, with infrequent but impressive responses. Patient selection approaches in CRPC for immune-checkpoint targeting have yet to be pursued[125].

ETS *gene rearrangements.*

The ETS family of transcription factors (including ERG, Friend leukaemia integration 1 transcription factor (FLI1), ETV1 and ETV6) have important oncogenic roles in many prostate cancers[126]. Approximately 50% of prostate cancers harbour *ETS* rearrangements, most frequent of which are *ERG* rearrangements, and these are usually under the control of an androgen-regulated promoter element, most frequently *TMPRSS2* (Ref. 30). *ERG* overexpression increases cell invasion, induces cell proliferation and *AR* expression[127]. Overexpression of ETS factors induces prostatic intraepithelial neoplasia in genetically engineered mouse models[128] and, when combined with increased AR signalling or *Pten* loss,

leads to the development of invasive prostate carcinomas in such mice[108,129,130]. Depletion of ETS factors *in vitro* reduces tumour cell motility and invasiveness, and decreases tumour growth *in vivo*. Inhibition of *ETS* oncogene signalling is a promising therapeutic strategy, with drug discovery efforts under way.

Transcription factors have generally been considered undruggable targets; however, new strategies to modulate their transcriptional activity show promise[131]. These strategies include disrupting crucial protein–protein or DNA–protein interactions and restricting binding at the epigenetic level by modulating chromatin accessibility[132]. For example, dithiophene diamidine compounds can interfere with the ERG–DNA binding interaction[132]; DB1255, the most active of such compounds, interacts specifically with DNA at ERG-binding sites, inhibiting ERG–DNA complex formation[132]. Another strategy is the specific targeting of the *TMPRSS2–ERG* gene fusion junctions using liposomal nanovectors containing an optimized siRNA that causes sequence-specific silencing of gene expression of the two most common *TMPRSS2–ERG* fusion gene mRNA junctional isoforms (type III and type VI)[133]. Alternatively, targeting downstream effectors of *TMPRSS2–ERG* gene fusion or other proteins upregulated in *ERG*-positive cancers, such as the phospholipase 2 group VII (PLA2G7)[134,135], has also been reported. *PLA2G7* mRNA expression correlates with *ERG* expression in prostate cancer specimens, and *PLA2G7* silencing by siRNA sensitized *ERG*-rearrangement-positive and *PLA2G7*-positive VCaP cells to oxidative stress, reducing cell viability[134,136].

Drugs that exhibit antitumour activity in other malignancies by blocking additional specific pathways may also be evaluated for potential use in prostate cancers[137]. For example, the small molecule YK-4-279 inhibits the oncogenic FLI1 in Ewing sarcoma breakpoint region 1 protein (*EWS*)–*FLI1*-rearranged Ewing sarcoma[137]. YK-4-279 blocks the binding of RNA helicase A to *EWS–FLI1*, induces apoptosis in Ewing sarcoma cells and reduces orthotopic xenograft growth. Similar to FLI1, ERG and ETV1 are also ETS transcription factors, and YK-4-279 has been shown to inhibit ERG- and ETV1-mediated transcription in *ETV1*-fusion-positive LNCaP cells and in *ERG*-fusion-positive VCaP prostate cancer cells[138]. These results suggest that YK-4-279 could also have antitumour activity in prostate cancer[138].

*The MAPK signalling pathway.*

Arguably less common in prostate cancer, but nevertheless still clinically relevant and potentially targetable, is oncogenic activation of RAS–RAF–MEK signalling. Such activation includes uncommon (1–2%) recurrent *BRAF* and *RAF1* rearrangements as well as rare mutations of these genes and other aberrations of genes activating this pathway, including *HRAS*, sprouty-related, EVH1 domain-containing protein 1 (*SPRED*), *SPROUTY*, fibroblast growth factor (*FGF*) and FGF receptor (*FGFR*)[46]. As ETS proteins are downstream effectors of RAS–RAF–MEK–extracellular signal-regulated kinase (ERK) signalling, resistance to AR blockade in *ETS*-rearranged prostate cancer has been postulated to involve RAS–RAF–MEK signalling. Activation of the MAPK pathway could also activate ETS signalling in some *ETS*-rearrangement-negative tumours. Evidence for this comes from a *Kras*-mutated, *Pten*-deleted genetically engineered mouse model that exhibited high ETV4 expression owing to co-activation of PI3K and RAS signalling[139]. Studies of RAS–RAF–MEK inhibitors in CRPC are now needed to understand which subtypes of these cancers are driven by MEK, to further elucidate the importance of this pathway in CRPC.

*AURKA.*

AURKA inhibition is another possible strategy for targeting a specific type of prostate cancer known as neuroendocrine prostate cancer (NEPC). NEPC is an aggressive subtype of prostate cancer that can arise *de novo* or with castration resistance. NEPC frequently metastasizes to visceral organs, responds only transiently to chemotherapy and has a poor prognosis. *ERG* fusions have been reported in ~50% of NEPC cases[140], suggesting that NEPC is clonally derived from adenocarcinoma and distinct from small-cell carcinomas. The cell cycle kinase AURKA and the transcription factor NMYC (encoded by *MYCN*) may cooperate to induce neuroendocrine differentiation in prostate cancer[141]. Co-amplification of *AURKA* and *MYCN* has been reported in NEPCs[141,142]; thus, AURKA inhibition may have antitumour activity against NEPC[141] (Table 1). Meanwhile, AURKA inhibition has been shown to induce MYCN degradation in neuroblastoma models with antitumour activity[143].

## The evolving therapeutic landscape

The therapeutic landscape of CRPC is rapidly evolving. Several large phase III trials have now demonstrated survival benefit in patients with CRPC. For example, TAX327 [144] tested the effects of the taxane chemotherapy docetaxel, and the COU-AA-301 [145] and COU-AA-302 [146] trials evaluated the CYP17 inhibitor abiraterone in the post- and pre-docetaxel settings, respectively. The AFFIRM[147] and PREVAIL[148] trials investigated the next-generation AR antagonist enzalutamide in the post- and pre-docetaxel settings, respectively, whereas the TROPIC[149] trial assessed the effects of cabazitaxel post-docetaxel. The ALSYMPCA[150] trial explored the alpha radiation-emitter radium-223-based radiopharmaceutical alpharadin (Xofigo; Bayer) in patients with bone-only metastases, and the IMPACT[151] trial studied the effects of the autologous active cellular immunotherapy sipuleucel-T (Provenge; Dendreon) (Fig. 2). Most of these therapies were developed concurrently over a relatively short time period and have been approved by the US Food and Drug Administration (FDA). Furthermore, the recent CHAARTED trial showed that docetaxel combined with ADT, when initiated for hormone-sensitive disease, improves cancer control and confers improved overall survival compared with ADT alone[152]. These data are supported by the STAMPEDE trial, which demonstrated improved survival with docetaxel in this patient population[153].

There is an urgent need to define the optimal sequence of use of these agents to maximize patient benefit[154]. Such optimization should ideally be guided by scientific rationale through molecular subclassification of CRPC[5] (Fig. 2). However, the absence of robust surrogate measures of survival and the lack of predictive biomarkers makes acquiring data for the sequential use of these agents challenging[155]. Other important factors that will influence the therapeutic landscape of CRPC include tumour heterogeneity and the potential application of novel approaches in immuno-oncology.

*Tumour heterogeneity.*

Treating prostate cancer is complicated by intra- and inter-patient tumour heterogeneity owing to the transcriptomic and proteomic diversity caused by the many varied genomic aberrations[6,19]. Although specific genetic aberrations are uncommon, different genomic lesions frequently converge on specific cellular functions and pathways[156]. Efforts are being made to identify the underlying mechanisms that drive each individual cancer and to develop analytically validated biomarkers that predict tumour response to treatments. NGS with high-

throughput characterization of the genome and transcriptome is a practical way of identifying specific targetable aberrations[9], making precision medicine deliverable for patients with prostate cancer.

Overall, it is now possible to subdivide CRPCs into those with *AR* aberrations, *ETS* gene rearrangements, *PTEN* loss and/or DNA repair defects (Fig. 2) and to match these subtypes with appropriate antitumour therapies. With the increased application of NGS and validation studies, the list of putative predictive biomarkers is rapidly growly. Molecular analyses of tumours from 'exceptional responders' can provide novel insights into underlying mechanisms of response to a particular drug, leading to the identification of predictive biomarkers[157].

*Immuno-oncology.*

Intra-patient heterogeneity may be most prevalent in prostate cancers with DNA repair defects; some of these cancers have high mutational load, and studies of this patient subpopulation with immune checkpoint-targeting drugs is now indicated. There is a strong body of evidence supporting a role for immunotherapy in prostate cancer. Prostate cancer can be immunogenic, and histopathology samples have demonstrated infiltrating lymphocytes (including $CD4^+$ cells, $CD8^+$ cells and natural killer (NK) cells) and antigen-presenting cells (including dendritic cells and macrophages). The infiltrating $CD4^+$ lymphocytes include regulatory T cells ($T_{reg}$ cells), which inhibit the immune response, suppressing and downregulating the induction and proliferation of antigen-specific effector T cells[158]. Clinically, prostate tumours with higher counts of $T_{reg}$ cells have a worse prognosis[159,160]. By contrast, infiltrating NK cells play a major part in the antitumour response, and are associated with better prognosis[161]. Prostate cancer can evade the immune system by downregulating antigen presentation, escaping cytotoxic T cells, producing immunosuppressive cytokines (such as transforming growth factor-β) and recruiting $T_{reg}$ cells[162]. Activating the immune system against prostate cancer tumour-associated antigens, such as PSA, prostatic acid phosphatase (PAP), prostate-specific membrane antigen, prostate stem cell antigen and mucin 1, may assist the immune system in overcoming these evasive processes.

One example of an immunotherapy that is FDA-approved in CRPC is sipuleucel-T, a dendritic cell-based immunotherapy in which peripheral blood mononuclear cells are collected from the patient, enriched and incubated with PAP fusion protein PA2024 plus granulocyte–macrophage colony-stimulating factor (GM-CSF) for antigen-presenting cell activation and antigen processing, then infused back into the patient. Sipuleucel-T is well tolerated and improves overall survival by 4 months compared with placebo[151]. Benefit in overall survival is reported to correlate with numbers and activation of antigen-presenting cells after treatment, as well as with a post-baseline peripheral immune response to PA2024 or PAP[163].

Novel approaches in immuno-oncology, such as immune-checkpoint inhibitors, have now transformed the therapeutic landscape in patients with advanced melanoma and in patients with non-small-cell lung cancer[164,165,166]. Such approaches have been explored in CRPC in nonselected patients. Ipilimumab (Yervoy; Bristol-Myers Squibb) is a human immunoglobulin G1 monoclonal antibody that binds to and blocks CTLA4. CTLA4, a key negative regulator of T cell responses, is constitutively expressed on $T_{reg}$ cells and mediates their immunosuppressive effect[167]. Early-phase studies in CRPC with ipilimumab showed a number of patients with a >50% decline in levels of serum PSA and one complete response

according to the RECIST (Response Evaluation Criteria in Solid Tumors) guidelines. However, in a phase III trial against placebo, there was no significant effect of ipilimumab in the overall survival primary end point, although the drug did show signs of antitumour activity[168,169,170].

Multiple other immunotherapies are currently being evaluated in CRPC, including the PD1 inhibitor pembrolizumab (Keytruda; Merck & Co.), which has recently demonstrated activity in other cancers including MMR-defective cancers[124,171,172]. Optimal patient selection, treatment timing and sequence of immunotherapies remain under investigation, as does their application in combination immunotherapy strategies, in which they may act synergistically in CRPC.

PROSTVAC-VF immunotherapy comprises two recombinant viral vectors encoding transgenes encoding PSA and three immune co-stimulatory molecules: B7.1 (also known as CD80), intercellular adhesion molecule 1 (ICAM1) and lymphocyte function-associated molecule 3 (LFA3). PROSTVAC-VF is initially delivered in a vaccinia-based vector, followed by six immune boosts in a fowlpox-based vector, with each dose given in conjunction with granulocyte–macrophage colony- stimulating factor (GM-CSF). A phase II study comparing PROSTVAC-VF to control empty vectors showed an 8.5-month improvement in median overall survival in men with minimally symptomatic CRPC[173]; a phase III study is currently under way[174].

## Outlook

These are exciting times in the management of CRPC, with a collection of antitumour agents now available for use. There are several lessons to be learnt from other cancers that are beginning to be managed successfully in a bona fide personalized medicine framework[175]. There now needs to be clear prioritization of tumour drivers for therapeutic targeting with consideration of the complex cancer clonal and subclonal structures involved, including the study of evolving subclonal dynamics during treatment[176]. Molecular stratification of patient groups will clearly be key to successful drug development. Moreover, strategies to identify potential escape mechanisms will be important to direct the optimal sequential application of drugs. This approach may involve the molecular characterization of sequential tumour biopsies or circulating plasma DNA or circulating tumour cells (CTCs) in therapeutic clinical trials[175] to deliver precision medicine for patients with CRPC (Fig. 2).

### *Minimally invasive and tissue biomarkers in CRPC.*

Although tumour biopsies remain the gold standard for CRPC tissue sampling, issues concerning accessibility and the ease of safe rebiopsy remain challenging and rate-limiting. In marrow-infiltrating CRPC, bone biopsies have been assessed for AR and CYP17 expression using immunohistochemistry[177]. In addition, assessments of *AR* copy number using polymerase chain reaction, *TMPRSS2–ERG* status using FISH, and testosterone levels using mass spectrometry, as well as exome and transcriptome studies, have been performed[177]. Extensive research has also now been undertaken with several non-invasive strategies, including the use of CTCs[178,179], plasma DNA[180,181,182], urine[183] and exosomes[184,185] for predictive biomarker analyses.

Although most studies of CTCs in CRPC have focused on CTC enumeration in the past, the spotlight has now switched towards the molecular characterization of CTCs, using techniques

such as multicolour FISH and NGS[186]. CTCs also have the potential to serve as intermediate end point biomarkers, and may accurately reflect clinical benefit. This finding can facilitate earlier 'go–no-go' drug development decisions to be made on treatment efficacy, thus potentially reducing costs and accelerating drug approval. Other potential applications for CTC assessments include the study of treatment effects *ex vivo* through the generation of primary cell cultures from CTCs or through CTC-derived xenografts. In one study, prostate cancer biopsy specimens and CTCs were cultured in a 3D organoid system that retained the histological and molecular features of the patient specimen[187]. WES of the specimen was then carried out to identify genetic variability and aberrations[187]. The first seven fully characterized organoid lines were shown to recapitulate the molecular diversity of prostate cancer subtypes, including *TMPRSS2–ERG* fusion, *SPOP* mutation, *SPINK1* overexpression and *CHD1* loss. Such an approach may potentially enable the development of a wide range of patient-derived prostate cancer lines amenable to genetic and pharmacological testing[187].

In the future, it is likely that whole-genomic, transcriptomic and proteomic single-cell analyses will become more feasible and cost-effective, enabling the serial monitoring of molecular changes of individual CTCs to antitumour treatments in real time. Genomic profiling of individual CTCs, cell-free DNA and tissue biopsies may be used to investigate tumour heterogeneity and to monitor clonal evolution during treatment (for example, to track the development of resistant clones associated with disease progression)[175,188,189]. Indeed, it is now possible to perform whole-genome analysis of cell-free DNA[190]. Such studies can reveal gene copy-number aberrations, *AR* amplification and *AR* mutations that are associated with resistant disease[190]. Recent studies have also developed and validated prognostically useful gene expression signatures of CRPC that are linked to inflammatory and immune responses; it has been postulated that mRNA expression profiles may also be acquired from CTCs and tumour-released exosomes[191,192,193]. Such signatures could be used as predictive or pharmacodynamic biomarkers for immunotherapy treatments.

*Modern clinical trial designs.*

Future trials must take into account the histopathological and genotypic characteristics of CRPC and assess the impact of intra- and inter-patient heterogeneity on therapeutic outcomes. Potential strategies may include adaptive and umbrella trials that assess putative predictive biomarkers to deliver precision medicine. These trials can be challenging to perform and involve navigating through complex regulatory hurdles, gaining access to multiple drugs from different sources and off-label use of novel agents or approved drugs — all potential pitfalls[194].

Ultimately, despite advances in our understanding of prostate cancer and of potential therapeutic targets, there continues to be a high attrition rate in drug development for CRPC. For example, a phase III trial of cabozantinib (Cabometyx/Cometriq; Exelixis) was terminated early owing to inefficacy, despite demonstrating single-agent antitumour activity[195]. Clinical trials with novel and rationally based designs may reduce drug development failures by selecting patients predicted to respond. An example of such an approach is the phase II TOPARP study, which was based on the concept of synthetic lethality, whereby exploitable DNA repair defects exist in CRPC, as with other tumour types such as ovarian and breast cancers[196,197]. In this trial, the PARP inhibitor olaparib was initially used in nonselected patients with metastatic CRPC before a preplanned analysis identified a biomarker-defined sensitive subgroup, followed by a prospective validation

cohort. Such novel trial designs will become increasingly important, particularly as our understanding of the inter-patient heterogeneity of CRPC reveals its underlying complexity.

Future clinical trials must also evaluate intratumour heterogeneity and clonal evolution within a patient, collecting multiple tumour biopsies, cell-free DNA, exosomes, CTCs and urinary tumour DNA. Another strategic approach involves rapid autopsy programmes when patients succumb despite drug treatment. Together, these strategies provide opportunities to obtain tumour tissue that would otherwise not be amenable to biopsy, to allow a retrospective construction of the genomic landscape and interactions with the microenvironment.

## Conclusions

We have entered a time of rapid change and progress in prostate cancer medicine. Importantly, modern technological advances have also provided us with major new insights into CRPC biology. Several effective new drugs have been approved over the past few years and there are now multiple novel investigational agents undergoing evaluation, as well as new targets being exploited in drug discovery that could provide further benefit to patients with CRPC. Tackling issues of drug resistance to different antitumour agents, as well as the exploration of immuno- oncology strategies in CRPC, will continue to be critical challenges. In addition, rational combinations — either concomitantly or sequentially — to overcome resistance will be important, alongside the longitudinal genomic profiling of circulating plasma DNA to support adaptive drug administration. Importantly, because patients with CRPC are now regularly living more than 5–10 years with this disease, we need to continue to focus not only on how long patients live but also on how well they are living, to maximize quality of life. We envision that the use of state-of-the-art, high-throughput, genomic platforms that enable patient stratification will facilitate the optimal use of current and future drugs. This may be further enhanced through rational antitumour treatment strategies, with novel clinical trial designs that implement the 'pharmacological audit trail', which provides a rational framework for assessing the risk of failure of the development of a new agent, driving further improvements in patient care[175,198].

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TABLES and FIGURES

## Table 1: Potential therapeutic targets in CRPC

| Target | Possible treatments | Comments |
|---|---|---|
| EZH2 | EZH2 inhibitors, HDAC inhibitors or microencapsulated DIM (BR-DIM) | • *In vitro* inhibition of HDAC attenuates EZH2 activity[228]<br>• BR-DIM upregulates let-7 and downregulates EZH2 expression in cell culture[229] |
| PD1 | PD1 or PI3K inhibitors | • PDL1 is highly expressed in some patients with CRPC[230]<br>• PDL1 expression correlates with PI3K activation and PDL1 immunoresistance is attenuated by PI3K pathway inhibitors in cell culture[231] |
| RAS–RAF–MEK–ERK pathway | MAPK pathway inhibitors | • Trials ongoing |
| AR | LHRH agonists or antagonists; AR antagonists; inhibitors of androgen synthesis, AR LBD, BRD4, AR NTD, HSP90 or calpain; or ASC-J9 | • Novel agents targeting AR splice variants need evaluation |
| PI3K–AKT signalling | PI3K pathway inhibitors | • Combination studies with PI3K pathway inhibitors are ongoing[25,232–236] |
| ATM | ATM or ATR inhibitors | • Profound synthetic lethal interaction between ATR and the ATM–p53 pathway in cells treated with DNA-damaging agents suggest utility of ATR inhibitors in cancers with aberrant ATM[237]<br>• SNPs in *ATM* are associated with prostate cancer aggressiveness[238] |
| *TMPRSS2–ETS* fusions (e.g. *–ERG*, *–ETV1* or *–FLI1*) | ERG pathway inhibitor (e.g. WP1130, DB1255 or UK-4-279), PARP inhibitors[120] or drugs disrupting transcription factor–DNA or co-factor interactions | • The USP9X inhibitor WP1130 induces ERG degradation and impaired downstream gene expression in cell cultures and mouse models[239]<br>• DB1255 reduces ERG transcriptional activity[132]<br>• UK-4-279 decreases downstream mRNA and protein expression in *ETV1*- and *ERG*-fusion-positive prostate cancer cells[138] |
| DNA repair defects | PARP inhibitors or immune checkpoint inhibitors | • PARP inhibitors are effective against *BRCA1* or *BRCA2* mutations[240–242]<br>• Trials are ongoing for immune checkpoint inhibitors |
| SPINK1 | EGFR inhibitors | • siRNA knockdown of overexpressed *SPINK1* in *ETS*-rearrangement-negative prostate cancer cell culture attenuates invasion[60]<br>• Cetuximab attenuates growth of prostate cancer xenografts with *Spink1* overexpression in mice[243] |
| CHD1 | Unknown | • *CHD1* loss is a unique driver of aggressive prostate cancer[244–246] |
| SPOP | Unknown | • SPOP-mutant prostate cancers show a distinct pattern of genomic alterations[10] |
| AURKA or NMYC | AURKA inhibitors | • *AURKA* and *MYCN* are often (40%) co-amplified in neuroendocrine prostate cancers[141]<br>• AURKA inhibitors have demonstrated activity in neuroendocrine prostate cancers (*in vitro*)[141] and in *MYCN*-amplified tumours (*in vivo*)[143] |
| RB1 | Mitotic inhibitors[16] | • RB1 loss of function is implicated in resistance to enzalutamide[15] |
| WNT | Porcupine inhibitors or tankyrase inhibitors | • Loss of APC function activates the WNT pathway and causes prostate cancer in mice[247] |
| MYC | BET inhibitors | • BET inhibition reduces *MYC* expression in prostate cancer cell lines and in a patient-derived model, with inhibition of cell growth and tumour burden *in vivo*[248] |

## Table 2: Investigational medicinal products in CRPC

| Drug names (lead company) | Mode of action | Efficacy | Most recent reference or trial number |
|---|---|---|---|
| ARN-509 (Johnson & Johnson) | Inhibits AR nuclear translocation and DNA binding | Reduction (≥50%) in PSA in ~47% patients at 12 weeks | • Phase I[249]<br>• Phase III ongoing[250] |
| Cabometyx, Cometriq or cabozantinib (Exelis) | Small molecule inhibitor of FLT3, VEGFR1, VEGFR2, VEGFR3, c-Met, KIT (also known as cKIT), TIE1, TIE2, RET, TRKB and AXL | COMET-1: median OS was 11.0 months for drug versus 9.8 months for prednisolone | • Phase III[251]<br>• Combination studies ongoing[252] |
| Custirsen or OGX-011 (Teva Pharmaceuticals) | Antisense oligodeoxynucleotide that inhibits TRPM2 (also known as clusterin) | SYNERGY: median OS of drug plus docetaxel plus prednisone was 17 months, versus 14 months with docetaxel plus prednisone | • Phase III[253]<br>• Combination studies ongoing[254] |
| DCVAC/PCa (SOTIO Group) | Dendritic cell vaccine against tumour antigens | Prolonged PSA-doubling time by ~3.4 times | • Relapsed prostate cancer[255]<br>• Phase III ongoing[256] |
| Lutrate (GP Pharm) | Inhibits pituitary gland secretion of gonadotropins | Suppressed testosterone production in 97% of patients at 1 month | • Phase III[257]<br>• 3- and 6-month formulations are under development |
| Orteronel (Takeda) | Inhibits CYP17A1 and AR | Study terminated owing to lack of efficacy versus placebo | • Phase III[258]<br>• Maintenance studies suspended[259] |
| ProstAtak or AdV-tk (Advantagene) | Adenoviral vector expressing herpes simplex virus thymidine kinase gene, plus synthetic acyclic guanosine analogue | Reduced (20%) absolute risk in recurrence for early-stage prostate cancer | • Phase I[260]<br>• Phase III combination with radiotherapy ongoing[261] |
| Prostvac or PSA-TRICOM (Bavarian Nordic) | Vaccine vectors that infect antigen-presenting cells and generate proteins for immune activation | Improved median survival by ~8.5 months and led to declines in PSA (38%) and rate of PSA increase (47%) | • Phase II[173]<br>• Phase III ongoing[222] |
| Sprycel or dasatinib (Bristol-Myers Squibb) | Inhibits SRC family protein tyrosine kinases and BCR–ABL fusion protein | No survival benefit in combination with docetaxel | • Phase III combination with docetaxel[262] |
| TASQ (Active Biotech AB) | Inhibits angiogenesis by targeting MRP14 | Improved PFS by 4 months versus placebo in metastatic CRPC | • Phase II[263]<br>• Phase III completed[264] |
| Yervoy or ipilimumab (Bristol-Myers Squibb) | CTLA4-specific antibody | Median OS was 11.2 months with ipilimumab and 10.0 months with placebo | • Phase III[170] |
| DI17E6 or EMD525797 (Merck & Co.) | Inhibits $\alpha_v$ subunit of human integrins | Radiographic SD at ≥18 weeks in 69% of patients | • Phase II[265] |
| Ozarelix (Spectrum Pharmaceuticals) | LHRH antagonist | Suppressed testosterone to castration levels in hormone-dependent prostate cancer; reduced PSA by ≥50% in 97% of patients | • Preclinical[266]<br>• Phase II completed[267] |
| ATL101 (ATLAB Pharma) | Targets PSMA | Dose-dependent PSA decline of ≥50% in 10–27% of patients; CTC decline at 4–6 weeks in 64% patients | • Phase II[268] |
| BIND-014 (BIND Therapeutics) | Polymeric nanoparticles targeting PSMA and containing docetaxel | Phase I: PR in one patient with prostate cancer | • Phase I[269]<br>• Phase II completed[270] |
| Capesaris or GTx-758 (GTx) | Non-steroidal selective ER1α agonist | PSA decline of ≥30% in 36% of patients | • Phase II[271] |
| Quinacrine or CBLC102 (Cleveland BioLabs) | NF-κB transcription inhibition, p53 transcription induction (restoring p53-dependent apoptotic pathways and tumour cell apoptosis) | Preclinical studies showed synergy with paclitaxel; phase II data showed 1 of 31 patients had PR with quinacrine alone | • Preclinical[272]<br>• Phase II completed[273] |
| EPO906 or patupilone (Novartis) | Induces tubulin polymerization and stabilizes microtubules | PSA decline of ≥50% in 13–47% of patients; measurable PR in 24% of patients | • Phase II[274] |
| G-202 or thapsigargin prodrug (GenSpera) | Targets PSMA and SERCA pump | Preclinical studies show ~50% regression in mouse xenograft model | • Preclinical[275]<br>• Phase II (withdrawn)[276] |
| GVAX (Aduro Biotech) | Vaccine that expresses GM-CSF and stimulates the immune response | Stable PSA in 19% patients; ≥50% decline in PSA in 1 patient; median OS of 35 months in high-dose group | • Phase I/II[277] |
| IRX4204 (Io Therapeutics) | RXR agonist | PSA decline of ≥50% in 13% of patients | • Phase II[278] |

**Figure 1: The cellular biology of prostate cancer.**
The complex underlying cellular biology and signalling cascades associated with castration-resistant prostate cancer (CRPC) are illustrated. Several of the molecules depicted — for example, poly(ADP-ribose) polymerase (PARP), the androgen receptor (AR) and molecules in the phosphoinositide 3-kinase (PI3K)–AKT pathway — have been implicated as possible drug targets in CRPC. 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; APC, adenomatous polyposis coli protein; ARE, androgen response element; AR-SV, AR splice variant; BAD, BCL-2-associated agonist of cell death; BCL-2, B cell lymphoma 2; BCL-X, BCL-2-like protein 1; BET, bromodomain and extraterminal; BRD, bromodomain-containing protein; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DNMT, DNA methyltransferase; DSH, dishevelled; eIF4E, eukaryotic translation initiation factor 4E; EZH2, enhancer of zeste homologue 2; FKHR, forkhead; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GSK3β, glycogen synthase kinase 3β; HDAC, histone deacetylase; HIF1α, hypoxia-inducible factor 1α; HSP, heat shock protein; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; IRS1, insulin receptor substrate 1; JMJD2, Jumonji domain-containing protein 2A; LRP, lipoprotein receptor-related protein; LSD1, lysine-specific histone demethylase 1A; MAPK, mitogen-activated protein kinase; MDM2, double minute 2 protein; MEK, MAPK/ERK kinase; mTORC, mechanistic target of rapamycin complex; NF-κB, nuclear factor-κB; p70S6K, p70 ribosomal S6 kinase; PDK1, 3-phosphoinositide-dependent protein kinase 1; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; $PIP_3$, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue; RHEB, Ras homologue enriched in brain; RTK, receptor tyrosine kinases; S6RP, S6 ribosomal protein; SUZ12, suppressor of zeste 12; *TMPRSS2*, transmembrane protease serine 2; TSC, tuberous sclerosis.

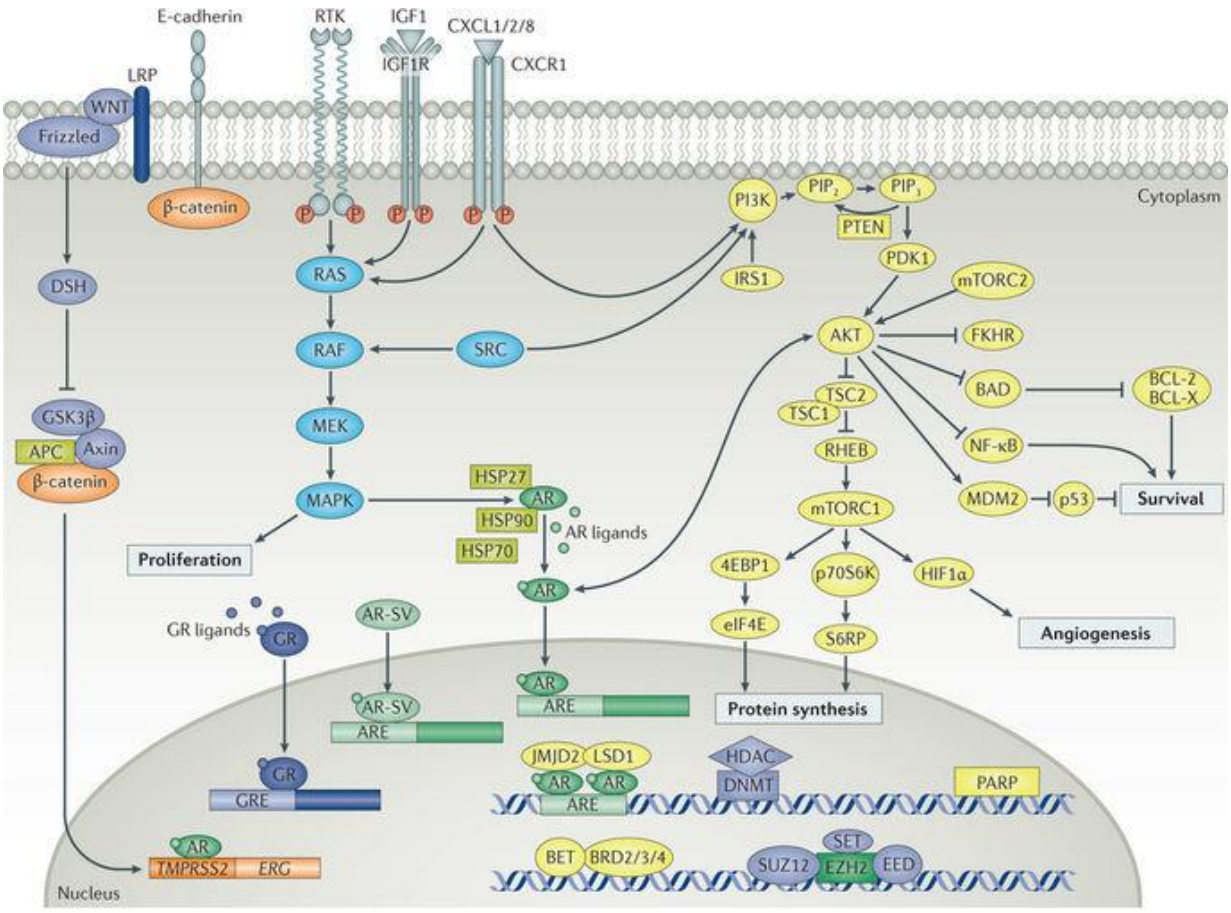

**Figure 2: CRPC treatment in the present and in the future.**
Until 2010, the gold standard treatment for castration- resistant prostate cancer (CRPC) was docetaxel chemotherapy. However, the recent US approval of abiraterone, enzalutamide, cabazitaxel, alpharadin (also known as radium-223) and sipuleucel-T now provides a range of agents for patients with CRPC (part **a**). In the future (part **b**), this landscape will inevitably change substantially, as various factors, including patient factors, resistance to prior therapies and local drug availability, determine the sequence of delivery of drugs. In addition, efforts will be made to molecularly stratify patients to matched targeted therapies. For example, tumours can be characterized using tumour-targeted next-generation sequencing or other exome, transcriptome or whole-genome analyses to identify DNA repair defects and to determine mutational load. Determination of truncal clonal and subclonal aberrations, androgen receptor (AR) signalling status and AR splice variant expression (for example, using immunohistochemical analysis or RNA *in situ* hybridization), and evaluation of the tumour stroma and infiltrated immune cells such as $CD8^+$ cells and myeloid derived suppressor cells (MDSCs) should also inform treatment strategies. The decision for treatment transition should incorporate standard clinicopathological measures, novel biomarkers (such as circulating tumour cells (CTCs) and circulating plasma DNA) and patient-derived xenografts and organoid cultures. CSF1R, macrophage colony-stimulating factor 1 receptor; CXCR, CXC chemokine receptor; mTORC, mechanistic target of rapamycin complex; PARP, poly(ADP-ribose) polymerase; PI3K, phosphoinositide 3-kinase; TAZ, transcriptional co-activator with PDZ-binding motif; YAP1, Yes-associated protein 1.