REVIEW

Signalling pathways in prostate carcinogenesis: potentials for molecular-targeted therapy

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ABSTRACT

Prostate cancer represents a major health issue and its incidence is rising globally. In developed countries, prostate cancer is the most frequently diagnosed cancer and the second most common cause of death from cancer in men. Androgen deprivation reduces tumour activity in approx. 80% of patients with advanced disease, but most tumours relapse within 2 years to an incurable hormone-resistant state. Even for patients with early disease at the time of diagnosis, a proportion of patients will unfortunately develop relapsed disease following radical therapy. Treatment options for patients with hormone-resistant prostate cancer are very limited and, even with toxic therapy, such as docetaxel, the life expectancy is only improved by a median of 2 months. Advances in molecular oncology have identified key signalling pathways that are considered to be driving events in prostate carcinogenesis. The activation of multiple signalling pathways increases further the possibility of cross-talk among ‘linear’ signalling cascades. Hence signalling networks that may incorporate distinct pathways in prostate cancer, particularly in hormone-resistant disease, are increasingly appreciated in drug development programmes. With the development of potent small-molecule inhibitors capable of specifically suppressing the activities of individual ‘linear’ cascades, it may be that, by combining these agents as guided by the molecular signature of prostate cancer, a more efficient therapeutic regime may be developed. Therefore the present review focuses on evidence of abnormal signalling in prostate cancer and the potential of these targets in drug development, and incorporates key findings of relevant clinical trials to date.

Key words: androgen, carcinogenesis, hormone, prostate cancer, receptor tyrosine kinase.

Abbreviations: ADT, androgen-deprivation therapy; AR, androgen receptor; BAP, bone alkaline phosphatase; BPH, benign prostatic hypertrophy; CML, chronic myeloid leukaemia; CYP, cytochrome P; DRE, digital rectal examinations; EBRT, external beam radiotherapy; EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular-signal-regulated kinase; ET, endothelin; FGF, fibroblast growth factor; FGFR, FGF receptor; GnRH, gonadotrophin-releasing hormone; HDAC, histone deacetylase; HDT, hormone-deprivation therapy; HER, human EGFR; HRPC, hormone-resistant prostate cancer; HSP, heat-shock protein; IFN, interferon; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IGF-1R, type I IGF receptor; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MDM2, murine double minute 2; AS-MDM2, antisense MDM2; MEK, MAPK/ERK kinase; mTOR, mammalian target of rapamycin; mTORC1, mTOR–raptor (regulatory associated protein of mTOR) complex; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; Pd, Philadelphia; PI3K, phosphoinositide 3-kinase; PSA, prostate specific antigen; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RCC, renal cell carcinoma; RRP, retropubic radical prostatectomy; RTK, receptor tyrosine kinase; SCLC, small cell lung cancer; NSCLC, non-SCLC; SFK, Src family kinase; SRD5, steroid 5α-reductase; TKI, tyrosine kinase inhibitor; TSG, tumour-suppressor gene; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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Table 1  Treatment options for prostate cancer
T, tumour staging; N, lymph node staging; M, metastases.

<table>
<thead>
<tr>
<th>Disease stage</th>
<th>Treatment option</th>
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<tbody>
<tr>
<td>Early stage (or organ-confined disease T1/2N0M0)</td>
<td>Watchful waiting: suitable for patients with low-grade small-volume disease and life expectancy &lt;10 years. Treat if a rise in PSA.</td>
</tr>
<tr>
<td>Conservative management</td>
<td>Active surveillance: if disease is considered clinically insignificant and at low risk of progression. Involves 3 monthly PSA check and DRE. Repeat biopsy at 12 months.</td>
</tr>
<tr>
<td>Radical treatment</td>
<td>Radiotherapy: EBRT or interstitial brachytherapy</td>
</tr>
<tr>
<td>Locally advanced (T1N1M0)</td>
<td>Radical prostatectomy: RRP, laparoscopic or robotic-assisted prostatectomy</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>Other experimental methods yet to be validated in formal randomized controlled trials include cryotherapy and HIFU (high-intensity focused ultrasound) therapy</td>
</tr>
<tr>
<td>HRPC</td>
<td>ADT: GnRH analogue and/or anti-androgen</td>
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<tr>
<td></td>
<td>Radiotherapy + ADT</td>
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<tr>
<td></td>
<td>Subcapsular orchidectomy</td>
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<td></td>
<td>ADT: Second- or third-line hormonal manipulation: discontinue steroidal or non-steroidal hormones or addition of anti-androgen, oestrogen, glucocorticoid or enzymatic inhibitor of adrenal androgen synthesis</td>
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<tr>
<td></td>
<td>If metastatic disease, consider chemotherapy (docetaxel and prednisolone)</td>
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CLINICAL MANAGEMENT OF PROSTATE CANCER

Prostate cancer is the most commonly diagnosed cancer and the second commonest cause of cancer related death in men in the Western world [1]. The incidence of prostate cancer increases with age and over 70% of patients with prostate cancer are over the age of 65 years [2]. With an aging society, it is therefore inevitable that prostate cancer will become an increasing health burden in years to come.

Treatment options for men diagnosed with prostate cancer depend on a number of factors, including patient performance status, disease status (tumour grade and stage) and social factors (Table 1). Prostate cancer diagnosed at an early stage (or when organ-confined) are potentially curable and various options are available for these patients. Watchful waiting may be considered if the patient has low-grade small-volume disease and a life expectancy of <10 years. Follow-up for these patients will focus on serial serum PSA (prostate specific antigen) measurements and, if a significant absolute rise (2 ng/ml) is detected, the patient can be considered for intervention such as medical treatment [3].

Active surveillance is offered to patients who are found to have prostate cancer which is thought to be clinically insignificant and at low risk of progression. Active surveillance involves PSA measurements and DRE (digital rectal examinations) every 3 months with repeat prostate biopsies to re-stage the cancer every 12–24 months or if significant changes are found in the PSA level or DRE. If these show that the cancer is progressing, then treatment with curative intent will be recommended, in most cases being either surgery or radiotherapy; however, the optimal protocol for active surveillance remains to be validated in a prospective trial.

Radical treatment is offered for patients who have localized disease and good life expectancy. Surgical options include RRP (retropubic radical prostatectomy), with laparoscopic or robotic-assisted approaches gaining popularity in recent years. Whether these novel techniques will translate into a better outcome await formal assessment. Conformal EBRT (external beam radiotherapy) or brachytherapy represent radiation-based curative options for patients with early disease.

Androgens are the primary regulators of prostate cancer cell growth and differentiation and prostate cancer is often androgen-dependent, with the majority regressing following initial androgen-ablation treatment. Current medical therapy for patients diagnosed with prostate cancer includes anti-androgens and GnRH (gonadotrophin-releasing hormone) analogues. Anti-androgens block the effect of androgens directly on target cells by inhibiting their binding to the AR (androgen receptor). GnRH analogues work at the level of the pituitary with continued administration producing down-regulation of GnRH receptors thereby reducing the release of gonadotrophins, which leads to inhibition of androgen production. Medical therapy is a treatment option either alone or in addition to radiotherapy in patients with locally advanced disease. Hormone deprivation is the treatment of choice in patients with metastatic disease, most often in the form of medical therapy but some patients may be offered a subcapsular orchidectomy.
which has the advantage of achieving rapid androgen ablation.

Unfortunately, approx. 20% of patients do not have a favourable response and, even among the responders, there is an 80% risk of relapse at a median period of 24 months following hormone manipulation. Patients are diagnosed with HRPC (hormone-resistant prostate cancer) when they have evidence of a rising PSA (PSA ≥ 2 ng/ml above the nadir) [3]. Treatment for these patients is limited. Secondary (and tertiary) hormonal manipulations, such as discontinuation of steroidal or non-steroidal hormones or the addition of anti-androgens, oestrogens, glucocorticoids or enzymatic inhibitors of the adrenal androgen synthesis pathway, may produce a transient biochemical response. In addition, patients with clinically localized disease receiving radical treatment have a significant failure rate over a 5–10 year period of follow-up (reported rates of 23% post-aa and 63% post-EBRT) [4].

Docetaxel has been licensed for use in combination with corticosteroid therapy in men with metastatic HRPC following the results of two phase III trials [5,6]. The mean survival benefit in these studies only measured 2 and 2.5 months respectively, and the timing of this treatment remains controversial. Trials are now focusing on improving the efficacy of docetaxel by combining it with novel biological agents. In addition, there is now an increasing interest in testing the efficacy of novel agents in hormone-naïve disease, which may result in a better overall response and outcome. The STAMPEDE (Systemic Therapy in Advancing or Metastatic Prostate cancer: Evaluation of Drug Efficacy) study, a five-arm randomized-controlled trial, is one example, aimed to recruit patients with high-risk prostate cancer. This large multi-centre trial examines the efficacy of combining androgen-ablation therapy with a number of agents including docetaxel, zoledronic acid (bisphosphonate) and celecoxib [a COX-2 (cyclo-oxygenase-2) inhibitor] [7].

### ABERRANT SIGNALLING PATHWAYS INVOLVED IN PROSTATE CARCINOGENESIS

Abnormal signalling is thought to mediate many of the tumorigenic activities involved in cancer development and progression; discoveries in this field offer potential targets for new drug development. The progression of epithelial prostate cells from a normal differentiated state, in which proliferation and apoptosis are tightly balanced, to a malignant state involves a combination of events resulting in the activation of oncogenes in addition to the loss of TSGs (tumour-suppressor genes), which critically control aspects of the hallmarks/phenotypes of cancer (Table 2) [8]. Many signalling pathways have been found to be important in prostate carcinogenesis and, in recent years, targeted therapy has emerged as a key focus for prostate cancer research (see the list of relevant current trials in Table 3).

RTKs (receptor tyrosine kinases) for growth factors are essential for the transduction of extracellular signals to their cytoplasmic effectors. RTKs activate several pathways controlling cell proliferation and differentiation as well as migration and apoptosis. In normal cells, the activity of RTKs is tightly regulated; however, in cancer, constitutive activation of RTKs is essential for maintaining the malignant phenotype.

TSGs critically regulate the cell cycle, apoptosis, DNA repair, senescence and angiogenesis. Deranged TSG function in carcinogenesis can result from two distinct mechanisms. The function of a TSG can be impaired by (i) mutation or deletion abnormalities, or (ii) binding to a regulatory protein which can either inhibit the function or impair the stability of the target TSG (see examples in the section p53 and prostate cancer below). TSGs such as p53 and PTEN (phosphatase and tensin homologue deleted on chromosome 10) are important in prostate carcinogenesis.

### AR SIGNALLING

The AR regulates prostate organogenesis as well as the development and progression of prostate cancer. Androgen deprivation leads to apoptosis in a proportion of prostate cancer cells and those which do survive arrest in the G1-phase of the cell cycle [9]. However, as prostate cancer progresses, cells evolve and develop mechanisms to survive in an androgen-depleted environment. This progression is recognized to involve an active AR, and various mechanisms in which this altered signalling is implicated in the transition to hormone (or castrate) resistance have been described [10].

Reactivation (or the continued activation) of the AR and AR-responsive pathways allow tumours to develop a hormone-independent phenotype through altered

### Table 2 Hallmarks of cancer and the role of novel agents in prostate cancer

<table>
<thead>
<tr>
<th>Hallmark of cancer</th>
<th>Example of targeted therapy</th>
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<tbody>
<tr>
<td>Self-sufficiency in growth factors</td>
<td>Erlotinib (EGFR inhibitor)</td>
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<tr>
<td>Evading apoptosis</td>
<td>YM155 (survivin inhibitor)</td>
</tr>
<tr>
<td>Tissue invasion and metastasis</td>
<td>Dasatinib (Src inhibitor)</td>
</tr>
<tr>
<td>Sustained angiogenesis</td>
<td>Bevacizumab (VEGF inhibitor)</td>
</tr>
<tr>
<td>Increased cell metabolism</td>
<td>CCI-779 (mTOR inhibitor)</td>
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<tr>
<td>Target</td>
<td>Agent</td>
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<td>---------------------</td>
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<tr>
<td>PI3K pathway</td>
<td>Temsirolimus (CCI-779)</td>
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<td></td>
<td>Everolimus (RAD001) +</td>
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<tr>
<td></td>
<td>docetaxel in metastatic HRPC</td>
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<td></td>
<td>Deforolimus (AP23573)</td>
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<tr>
<td>Src family kinase</td>
<td>Dasatinib</td>
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<tr>
<td></td>
<td>+ docetaxel in metastatic HRPC</td>
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<tr>
<td>EGFR</td>
<td>AZD-0530</td>
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<tr>
<td></td>
<td>+ docetaxel or mixantrone in</td>
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<tr>
<td></td>
<td>HRPC</td>
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<tr>
<td></td>
<td>Erlotinib</td>
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<tr>
<td>EGF and VEGFR</td>
<td>Dasatinib and bevacizumab</td>
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<td>EGFR and HER2</td>
<td>Lapatinib</td>
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<td>IGF-1R</td>
<td>CP-751,871</td>
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<td>A12</td>
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<td>HSP27</td>
<td>OGX-427</td>
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<tr>
<td>HSP90</td>
<td>17-AAG</td>
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<tr>
<td>VEGFR</td>
<td>Bevacizumab</td>
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<td></td>
<td>+ docetaxel in HRPC</td>
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<tr>
<td>Angiogenesis</td>
<td>Thalidomide</td>
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<tr>
<td>ETα receptor</td>
<td>Atrasentan</td>
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<td></td>
<td>YM155</td>
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<tr>
<td>Androgen receptor</td>
<td>MDV-3100</td>
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<td></td>
<td>BMS-641988</td>
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<tr>
<td>CYP17</td>
<td>Abiraterone acetate</td>
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<tr>
<td>Sce reductase</td>
<td>Dutasteride</td>
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<td>(SRD5A1 and SRD5A2)</td>
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AR sensitivity, AR amplification and AR mutations [11,12]. Mutations in the AR can lead to activation by non-androgenic steroid molecules and anti-androgens. This may explain why 10–30% of patients who develop resistant cancer following treatment with anti-androgens may have a paradoxical fall in PSA levels when the particular anti-androgen is discontinued [13].

Circulating serum androgen levels are not completely eliminated with HDT (hormone-deprivation therapy). Although serum testosterone levels are significantly reduced, serum levels of adrenal androgens remain unaffected. Intraprostatic androgens are also reduced sufficiently with HDT to induce a response in untreated prostate cancer cells. It is interesting to note that HRPC tumours have increased endogenous synthesis of androgens [14], along with an up-regulation of the enzymes required for steroidogenesis [15,16]. Hence tumour cells, particularly in castrate-resistant disease, with their increased intracrine androgenic production, may be responsible for tumour progression despite low serum androgen levels.

Growth factors and their signalling cascades, such as HER2 (human EGFR [EGF (epidermal growth factor) receptor] 2), IGF-1 (insulin-like growth factor-1) and EGF, can also activate the AR via the PI3K (phosphoinositide 3-kinase)/Akt and MAPK (mitogen-activated protein kinase) pathways and reduce or negate the need for ligand binding (Figure 1). Overexpression of these co-activators in prostate cancer leads to indirect activation of the AR, and many agents currently in trials for use in HRPC target these signalling pathways.

In order for HRPC to establish, prostate cancer cells must also overcome the apoptotic effects of androgen depletion. The induction of AR-independent pathways, including RTK-mediated networks, allows prostate cancer cells to survive via the up-regulation of anti-apoptotic proteins such as survivin and Bcl-2.

Genetic alterations are important in the metastatic progression of tumour cells. In addition to these changes, alterations in the tumour microenvironment are required to allow local growth and invasion as well as distant metastasis to develop. It has been suggested that cells within the stroma secrete different growth factors, extracellular matrices, metalloproteinases and/or angiogenic molecules that are responsible for driving prostate cancer cells into a tumorigenic and invasive phenotype. It is now thought that a more efficacious method of treatment may
In HRPC, AR remains functional and is thought to contribute significantly to cancer progression. A number of mechanisms of AR activation in an androgen-depleted environment have been described (see text for further details) and cross-talk from RTK signalling pathways plays an important role in HRPC. The symbol signifies the development and evaluation of key inhibitors in clinical trials, including PI3K inhibitors (CCI-779 and RAD001), EGFR inhibitors (erlotinib, gefitinib and cetuximab), an EGFR and HER2 dual inhibitor (lapatinib), IGF-1R inhibitors (A12 and CP-751,871), AR inhibitors (MDV-3100 and BMS-641988), a 5α-reductase inhibitor (dutasteride), an apoptosis/survivin inhibitor (YM155), and a CYP17 inhibitor (abiraterone; to reduce adrenal and intra-tumour androgen biogenesis). See Table 2 and the text for details. Abbreviations: DHT, dihydrotestosterone; PIP2, PtdIns(4,5)P2; PIP3, PtdIns(3,4,5)P3.

**MOLECULAR MECHANISMS OF TARGETED THERAPY**

Different mechanisms have been used to target molecular signalling in cancer, with inhibition of RTK signalling offering the most success to date. Currently, two classes of compounds are commonly used to inhibit RTK activation: small-molecule TKIs (tyrosine kinase inhibitors) and mAbs (monoclonal antibodies). Although both inhibit RTK signalling, they have distinct targeted epitopes and mechanisms of action [17]. TKIs can translocate through plasma membranes and interact with the cytoplasmic domain of cell-surface receptors and intracellular signalling molecules. They competitively bind to the ATP-binding site in the catalytic domain of the receptor, inhibiting autophosphorylation and the activation of intracellular signal transducers.

In contrast, mAbs can only act on molecules that are expressed on the cell surface or secreted, as they are unable to pass through the cell membrane. Different ways mAbs inhibit RTK signalling are suggested which can be separated further into direct and indirect mechanisms. Direct action includes the following: blocking the function of target signalling molecules or receptors, stimulating function which results in apoptosis, and targeting function by conjugating mAbs with toxins, radioisotopes or cytokines. The indirect action described involves the binding of immunoglobulins to the surface of the cells mediating complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, both of which eventually lead to cancer cell death.

TKIs tend to offer the most efficient method of targeted therapy as they block the kinase activity of the targeted receptor, significantly inhibiting the activation of downstream signalling. Depending on the selectivity
of the candidate compounds, they may act as multi-target agents as they are prone to bind to different RTKs due to the structure of the ATP-binding pocket being highly conserved within the tyrosine kinase family. In contrast, mAbs tend to be specific inhibitors and have been shown to only offer modest antitumour activity when used alone, with more significant effects being observed when combined with chemotherapy.

In addition to TKIs and mAbs, other types of targeted therapy have also been used successfully in pre-clinical studies. Antisense oligonucleotides target specific sequences in the mRNA of interest, implicated to be a causative factor for carcinogenesis, thus inhibiting its expression and protein translation. Antisense oligonucleotides are currently in clinical trials as anticancer agents; however, to date none have been approved for use.

MOLECULAR-TARGETED THERAPY: EXAMPLES OF RECENT SUCCESS IN OTHER TUMOUR TYPES

BCR-ABL tyrosine kinase in CML (chronic myeloid leukaemia)

CML is a myeloproliferative disease which is characterized by the expansion of a clone of haemopoietic cells that carry the Ph (Philadelphia) chromosome. The Ph chromosome is due to a reciprocal translocation between the long arms of chromosomes 9 and 22. This translocation results in a novel fusion gene BCR-ABL, which encodes a constitutively active protein tyrosine kinase. The treatment of CML has been revolutionized since the discovery of a relatively specific inhibitor of the BCR-ABL tyrosine kinase, imatinib (Glivec). Treatment with this small-molecule inhibitor in patients diagnosed with chronic-phase CML results in high rates of complete cytogenetic remission (>87%) and molecular remissions with low or undetectable amounts of BCR-ABL transcripts [18]. Imatinib is now established as standard therapy for this patient group taking over from its predecessor IFN-α (interferon-α). Results from a 5-year follow-up study have been published confirming durable responses in patients with chronic-phase CML [19]. That study followed patients who had been initially treated with imatinib and then were either continued on treatment with this TKI or given IFN-α and cytarabine. Patients treated with imatinib had high rates of cytogenetic response and the estimated overall survival at 60 months was 89% compared with previous studies of IFN-α plus cytarabine, with survival rates of approx. 65% [20].

Approx. 10% of patients treated with imatinib will subsequently develop resistance, and between 50 and 90% of these cases are associated with mutations in the kinase domain of BCR-ABL [21]. Overexpression of Src-related kinases has also been implicated in treatment resistance, which may explain the efficacy of some second-generation BCR-ABL inhibitors, such as nilotinib and dasatinib, in imatinib-relapsed disease [22,23]. Novel therapies continue to be developed as advances in the molecular understanding of disease progression in CML emerge.

Trastuzumab (herceptin) in breast cancer

Another example of successful targeted therapy is HER2-mediated therapy in breast cancer. HERs, namely HER1–4, are a group of four transmembrane tyrosine kinase receptors that normally regulate cell growth and survival. HER2 gene amplification and protein overexpression is found in 20–30% of invasive breast cancers [24]. HER2-positive breast cancer patients in general have decreased overall survival and differential responses to standard chemotherapeutic and hormonal regimes [25].

Trastuzumab (herceptin) is a mAb to the HER2 ectoderm and has been shown to significantly improve the outcome for HER2-positive breast cancer patients. It acts by binding to the extracellular juxtamembrane domain of HER2, resulting in inhibition of proliferation and reduced survival of HER2-dependent tumours. Trastuzumab has been shown to significantly improve patient outcome in HER2-positive breast cancer of both early stage and metastatic stage [26,27]. Trastuzumab is recommended as a treatment for women with early-stage HER2-positive breast cancer following surgery, chemotherapy (neoadjuvant or adjuvant) and radiotherapy [28]. It is also recommended that all patients who have HER2-positive advanced breast cancer be considered for treatment with trastuzumab either as monotherapy (if metastatic and had previous chemotherapy) or in combination with chemotherapy and/or hormonal agents [29,30].

TARGET SIGNALLING IN PROSTATE CANCER

Despite the success of targeted therapy in other tumour types and the improved understanding of abnormal signalling activities in prostate carcinogenesis, none of the novel agents studied so far have shown adequate efficacy to justify their routine use in prostate cancer. Research and drug development programmes therefore continue to strive for a better understanding of the signalling network involved and in order to develop a more efficacious treatment regime.

PI3K pathway

The PI3K pathway has been shown to regulate multiple cellular events in prostate cancer. PI3K activation results in the catalytic conversion of PtdIns(4,5)P2 into PtdIns(3,4,5)P3 which, in turn, activates Akt. mTOR (mammalian target of rapamycin) is a serine/threonine kinase that regulates cell growth and is involved in tumorigenesis. Akt phosphorylates and activates mTOR
to enhance cell growth. PTEN is a TSG that negatively regulates the PI3K pathway. In prostate cancer, loss of PTEN and/or Akt activation is coupled with a high Gleason score (towards an undifferentiated phenotype), an advanced clinical stage and poorer prognosis [31,32]. The PI3K pathway is also associated with hormone-resistance and chemotherapeutic insensitivity. High levels of phospho-Akt immunostaining have been shown to be predictive of biochemical recurrence, and phospho-Akt-1 expression has been suggested to be an independent prognostic marker of biochemical recurrence-free survival in a subgroup analysis of patients with Gleason scores of 6 and 7 (n = 488; P = 0.0012) [31]. In vitro studies have shown that PTEN loss is associated with increased resistance to both doxorubicin and paclitaxel, and treatment with a PI3K inhibitor reverses this chemoresistance in prostate cancer cells [33].

Inhibition of PI3K signalling has been studied in vitro using two small-molecule inhibitors that have been available for some time, namely wortmannin and LY24002. Both of these have demonstrated antitumour effects in prostate cancer cell lines [34]; however, both have a relatively broad spectrum of activity inhibiting other kinases related to PI3K such as ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia mutated- and Rad3-related). There is a large family of PI3Ks, including the four class I lipid kinase isoforms p110α, p110β, p110δ and p110γ. Each of these isoforms is thought to have an individual role in cell behaviour. Of note, p110α protein has been found to be overexpressed and mutated in a number of solid tumours, including prostate cancer [35]. Targeting the p110α isoform in cancer is therefore an attractive strategy in drug development for prostate cancer. PI-103 inhibits the PI3K pathway at multiple sites including the p110α isoform as well as mTORC1 [mTOR–raptor (regulatory associated protein of mTOR) complex 1] and mTORC2. This dual p110α/mTOR inhibitor has been shown in PC3 cells to reduce proliferation and invasion and has significant antitumour activity in xenograft tumour models [36]. Formal published results on the use of PI-103 in clinical trial are awaited.

Inhibition of Akt is another important strategy for drug development. A number of small-molecule inhibitors, including A-443654, Akt-1-I and Akt-1-2, have been tested in vitro and in pre-clinical in vivo models for their antitumour effects with promising findings [37]. Future investigations of these agents in the clinic will inform us of their potential as novel therapies.

PTEN inactivation results in deregulated signalling through the mTOR pathway. mTOR is the target of the antibiotic rapamycin, which is used as an immunosuppressant following renal transplantation. At present there are three rapamycin derivatives in development: temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573). Both CCI-779 and RAD001 have been shown to have beneficial effects in vivo. Transgenic mice with activated Akt or PTEN deficiencies have decreased tumour growth when treated with CCI-779 [38]. Similarly, RAD001 has been shown to reverse prostate neoplastic phenotypes in mice expressing human Akt [39]. AP23573 has been shown to have promising antitumour activity in sarcoma and selected haematological malignancies [39a]. A phase II trial using CCI-779 for patients with HRPC has recently finished recruiting; however, to date no results are available. Both RAD001 and AP23573 and are currently being assessed in phase II clinical studies.

**SFK (Src family kinase)**

SFKs are a group of non-receptor protein tyrosine kinases which are involved in tumour adhesion, motility, invasion and angiogenesis. SFK members Src and Lyn are highly expressed in prostate cancer cell lines as well as in the majority of prostate cancer specimens [40,41]. Src signalling is involved in the androgen-induced proliferation of prostate cancer cells, and it has been suggested that Src is involved in the transition to androgen-independent growth [42]. Bone metastases occur in the majority of prostate cancer patients with advanced disease. Src inhibition in vivo has shown reduced morbidity, lethality and incidence of bone metastases in breast cancer mouse models [43]. Therefore a small-molecule inhibitor targeting Src may have the therapeutic advantage in prostate cancer of minimizing morbidity associated with bone metastases and possibly improving survival outcome.

Dasatinib is a SFK/Abl inhibitor and has in vitro activity in prostate cancer cells. Proliferation, invasion and migration have been shown to be reduced when DU145 cells were pre-treated with dasatinib [40]. A significant reduction in tumour growth and metastases have also been demonstrated when dasatinib was used in prostate cancer xenograft models. Phase II studies are currently ongoing to assess dasatinib in HRPC as well as combination therapy with docetaxel in metastatic disease. Results from phase II trials in imatinib-resistant CML with dasatinib have shown minimal toxicity thus reducing concerns that Src inhibition may suppress multiple pathways and be associated with high levels of adverse effects [44,45].

AZD-0530, another Src inhibitor, has been shown to inhibit the growth of prostate cell lines and suppress migration in PC3 and DU145 cells [46]. It also suppressed the growth and metastasis of androgen-independent LNCaP cells in vivo [47]. Studies in healthy volunteers have found only mild side effects with AZD-0530, and a phase II trial in HRPC has recently started. Finally bosutinib, another Src/Abl inhibitor, has in vitro and in vivo activity in models of CML [48], colon cancer [49] and breast cancer [50]; no published results are available for prostate cancer to date.
ErbB receptor family

The ErbB family of RTKs include EGFR (ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). Both EGFR and HER2 have critical roles in cell growth, differentiation and the motility of normal and cancer cells through the activation of downstream signalling pathways such as the MAPK and PI3K/Akt pathways. EGFR is highly expressed in primary prostate cancer and associated metastases [51]. Overexpression of EGFR is associated with poor prognosis [52] and the transition of androgen-sensitive disease to androgen independence [53].

Current anti-EGFR therapies include both TKIs and mAbs. Small-molecule TKIs have been the most successful method to date in EGFR targeting, with erlotinib (Tarceva) approved for use in the treatment of pancreatic cancer and NSCLC [non-SCLC (small cell lung cancer)] after failure of at least one prior chemotherapy regimen [54,55]. Progression-free survival in NSCLC, however, is only improved by 2 months with a reported response rate of 8.9 % [55]. Methods have been investigated to improve patient selection with both EGFR mutation and an increase in EGFR copy number recognized as biomarkers for favourable response to erlotinib in NSCLC [56,57]. Erlotinib has also been studied in HRPC in combination with docetaxel. Results of a phase II study demonstrated no beneficial anticancer activity with erlotinib added to docetaxel monotherapy [58]. However, the pre-treatment EGFR status was not available. Further studies of erlotinib in non-metastatic prostate cancer and chemotherapy naive disease are ongoing. EGFR mutation and amplification of the EGFR gene have been shown to occur frequently in advanced prostate cancer; however, unlike NSCLC, there are currently no stratified studies to include either EGFR mutation or gene amplification to improve patient selection for treatment with anti-EGFR therapy [59]. Erlotinib combined with bevacizumab [a VEGF (vascular endothelial growth factor) inhibitor] has been shown in NSCLC to increase progression-free survival and this combination regime is currently being tested in prostate cancer patients following radical prostatectomy [60].

Gefitinib (Iressa) is another orally active EGFR TKI which has been shown to have antiproliferative activity in prostate cancer cell lines [61]. EGFR suppression with gefinitib results in significant growth inhibition in PC3 xenografts [62], and has been shown to reduce the incidence of prostate cancer metastasis in nude mice [63]. Phase I findings of gefitinib monotherapy in a range of solid tumours have shown promising antitumour activity [64]; however, phase II results in non-metastatic HRPC reported no positive response [65,66]. EGFR status was assessed in a subset of patients where moderate-to-strong staining was observed in 12 out of 16 cases [65]. There was no correlation between EGFR expression and PSA decline or time to progression, suggesting that, in prostate cancer, EGFR overexpression is not indicative of a response to gefitinib.

PD168393 also selectively inhibits EGFR and it has been shown to sensitize prostate cancer cells to the cytotoxic activity of paclitaxel [67]. There is currently no in vivo or clinical evidence of the effect of this small-molecule inhibitor in prostate cancer.

A number of anti-EGFR mAbs have been introduced over recent years, with cetuximab being approved for use in patients with colorectal cancer refractory or intolerant to irinotecan and in patients with squamous cell carcinoma of the head and neck [68,69]. Phase I results of panitumumab, another EGFR mAb, in advanced solid tumours has shown that treatment was well-tolerated, but patients with prostate cancer had a very limited response [70]. Patients with HRPC are currently being recruited for phase II trials of cetuximab treatment combined with either docetaxel or mitoxantrone. Taken together, although EGFR inhibitors, namely erlotinib, gefitinib and PD168393, all target the kinase domain of EGFR and have encouraging results to support their clinical use in a number of tumour types, it is disappointing that similar efficacy has not been seen in prostate cancer. Many factors may contribute to this (see the considerations in the Conclusions section). The lack of stratification among patients according to their EGFR status (namely expression level, mutation and amplification), as an indicator of the significance of abnormal EGFR function as a driving event in prostate carcinogenesis, may partly explain the negative results in a number of trials. Future studies should focus on the targeted assessment of these novel agents in prostate cancer sufferers with defined genetic (or epi-genetic) lesions involving EGFR.

Amplification of the HER2 gene and/or overexpression of the HER2 protein occurs in 20–30 % of breast cancer patients and is associated with an unfavourable outcome [24,25]. In contrast, the impact of HER2 in prostate cancer is much less clear, with HER2 overexpression and/or amplification being identified much less frequently in prostate cancer [71]. There is also no strong consensus regarding its impact in clinical outcome. This may account for the negative findings in a phase II study using trastuzumab (herceptin) in HRPC [72], which did not support a phase III trial of herceptin.

HER2 is an orphan receptor and functions as a co-receptor. Previous studies have shown that HER2/HER3 dimerization and activation may stimulate AR-mediated signalling in an androgen-depleted environment [73]. Agents that may target HER2 dimerization signalling include pertuzumab and lapatinib. Pertuzumab is a mAb which inhibits HER2 dimerization with other HER family members, including EGFR, HER3 and HER4 [74]. Unfortunately, a phase II trial of pertuzumab in HRPC showed no PSA response, although the treatment was well-tolerated [75]. A phase I study of docetaxal and pertuzumab in solid tumours shows promising results in...
FGFR [FGF (fibroblast growth factor) receptor] family

FGFs, including FGF1, FGF2, FGF6, FGF8 and FGF17, are all expressed at increased levels in prostate cancer [80–85]. FGFs signal through activation of FGFR1–4, which leads to downstream signalling through multiple pathways, including the MAPK and PI3K pathways. FGFR4 is overexpressed in prostate cancer with a strong expression associated with high-grade disease and decreased survival [86]. FGFR1 and FGFR2 are also overexpressed in prostate cancer when compared with BPH (benign prostatic hypertrophy); however, FGFR2 has no correlation with tumour grade or stage [87]. Recent transgenic models validate further the role of FGFR signalling as key to prostate carcinogenesis [87a].

Over recent years, work has focussed on the selective targeting of the receptors involved in FGF signalling as a novel therapeutic approach in prostate cancer. FGFR inhibition using siRNA (small interfering RNA) to target FGFR4 in prostate cancer cells shows suppression of proliferation and invasion [87]. SU5402 potently blocks FGFR1 tyrosine kinase activity while weakly inhibiting PDGF [PDGF (platelet-derived growth factor) receptor] function. In vivo, SU5402 has been shown to decrease xenograft tumour growth and suppress PSA and promotrilysin expression in a prostate cancer model [88]. To date there are no published results of an FGFR inhibitor in clinical trials.

IGF-1R (type I IGF receptor)

IGF-1R is activated by IGF-I or IGF-II. The IGF-1R is crucial in maintaining the malignant phenotype, with evidence showing its role in proliferation, angiogenesis and apoptosis. IGF-1R activation is ligand-dependent and once activated IGF-1R recruits and phosphorylates adaptor proteins, which serve as docking sites for other signalling molecules. This results in the activation of intracellular signalling pathways, including PI3K and ERK1/2 (extracellular-signal-regulated kinase 1/2) of the MAPK pathway.

IGF-1R is significantly up-regulated in prostate cancer when compared with benign prostatic epithelium [89], and in vitro studies have shown that IGF-1R overexpression is associated with androgen-independent, anti-apoptotic and pro-mitotic signalling, processes which drive prostate cancer disease progression [90].

Several approaches have been used to inhibit IGF-1R signalling via a reduction or neutralization of circulating IGF-I or by inhibition of IGF-1R activation. At present, the most promising method is to use antibodies against IGF-1R. The human antibody A12 has been used in prostate xenograft tumours to study the beneficial effects of blocking IGF-1R signalling following castration [91]. IGF-1R inhibition enhanced the effect of castration and prolonged tumour-specific survival. Combination treatment was also associated with a decrease in AR signalling and nuclear AR localization. These results suggest that IGF-1R inhibition in conjunction with androgen ablation enhances the inhibition of signalling through the AR, which remains important even in HRPC. IGF-1R inhibition in xenografts has also been shown to potentiate the activity of cytotoxics [92]. A12 and CP-751,871, another anti-IGF-1R mAb, are currently in phase II trials to test their efficacy in combination with docetaxel and prednisone in the treatment of HRPC.

Both IGF-I and IGFBP-3 (IGF-binding protein-3) have been reported to be associated with an increased risk of prostate cancer. Conflicting evidence for both biomarkers has been published, however, with recent evidence showing no observed association for IGFBP-3 [93,94].

HSPs (heat-shock proteins)

HSPs are cellular chaperones involved in the regulation and stabilization of a number of key signal transduction proteins including MAPK, Akt and SFKs [95].

It has been suggested that HSP27 (27 kDa HSP) may be at the centre of many pathways involved in the
regulation of the response of a cell to treatment-induced stress, and targeting it may lead to silencing of multiple survival pathways. Apoptosis resistance is associated with an increased expression of multiple HSPs, and small HSPs such as HSP27 have been found to be important chaperones which protect cancer cells against apoptosis [96]. Cytotoxic treatments, such as chemotherapy and radiotherapy, have a negative effect on cells by inducing apoptosis. HSP27 expression is low or absent in hormone-naive prostate cancer, with increasing levels demonstrated in tumours once treatment is commenced. In HRPC, HSP27 is uniformly overexpressed and it is thought that this molecular chaperone is important in the progression of prostate cancer from hormone-sensitive to hormone-resistant [97]. The development of hormone resistance is thought to be attributed to decreased apoptotic rates, rather than an increase in proliferation [98].

The antisense oligonucleotide OGX-427, which targets HSP27, has been shown to inhibit HSP27 expression and synergizes with androgen ablation and chemotherapy in prostate cancer xenograft models [96]. In vitro studies have also been published showing that down-regulation of HSP27 radiosensitizes prostate cancer cells [99]. A phase I clinical trial of the OGX-427 either alone or with docetaxel is currently recruiting patients.

HSP90 (90 kDa HSP) is a key regulator of ligand-independent nuclear localization and activation of AR in androgen-refractory prostate cancer cells [100]. HER2, Raf-1 and Akt are also regulated by HSP90. Geldanamycin and its derivative 17-AAG are anasmycins that interfere with the action of HSP90, leading to the degradation of HSP client proteins. Both have been shown to have antitumour effects in prostate cancer cells. Despite its potential use as an effective cancer treatment, geldanamycin has several major drawbacks as a drug candidate including hepatotoxicity. 17-AAG, however, has a more favourable toxicity profile. Using an in vitro prostate cancer model, 17-AAG inhibited tumour growth as well as sensitizing tumour responses to taxol treatment [101].

Phase I results have recently been published of 17-AAG in patients with advanced cancer of which 18 of the 54 patients had HRPC [102]. No partial or complete responses were observed, with only one HRPC patient demonstrating a 25 % decline in PSA with treatment. 17-AAG may, however, be more beneficial in enhancing the effect of cytotoxic therapy rather than as a monotherapy. Phase I and II trials are therefore currently recruiting patients for treatment in combination with docetaxel and other cytotoxics.

**Anti-angiogenic agents**

Inhibition of angiogenesis has emerged as a promising therapeutic target for a number of solid tumours. Anti-angiogenic agents can reduce intratumoural interstitial pressure and increase drug delivery of anticancer agents. This mode of anticancer therapy has been successful recently in the treatment of RCC (renal cell carcinoma) [103,104]. Sorafenib is an orally active multi-kinase inhibitor which appears to have an anti-angiogenic effect in RCC due to its inhibitory effect toward VEGFRs (VEGF receptors) and their targets. Increased production of VEGF is implicated in the progression of clear-cell RCC [103]. Sorafenib has been licensed for maintenance treatment of metastatic RCC following the positive result in the trial in advanced clear-cell RCC [104].

Overexpression of VEGF and its receptors are associated with the progression of prostate cancer [105]. VEGF-R2 inhibition in orthotopic prostate cancer models reduced tumorigenicity and metastases, supporting the potential use of anti-VEGF agents in prostate cancer [106]. Results of phase II trials using sorafenib in HRPC, however, have shown minimal effects on PSA response or radiographical appearance of bone metastases [107–109].

Bevacizumab is a humanized murine mAb to VEGF which has been shown to provide clinical benefit in colorectal cancer, NSCLC and breast cancer. Combining bevacizumab with 5-fluorouracil inhibits angiogenesis and tumour growth in mouse prostate cancer models [110]. Currently bevacizumab is in phase II studies for use in high-risk cases in combination with medical or radiotherapy. A phase III trial is also recruiting patients with HRPC to assess the effect of bevacizumab with docetaxel.

PDGF is involved in autocrine stimulation of tumour cells, regulation of stromal fibroblasts as well as tumour angiogenesis [111]. PDGF is an RTK that has two subunits, α and β. Upon PDGF binding, these subunits either homo- or hetero-dimerize. Immunohistochemistry has shown PDGFαR and PDGFβR to be expressed in 88 % of primary prostate tumours and in 80 % of bone marrow metastases [112]. Inhibition of this signalling pathway appears to be an attractive target in prostate cancer; however, an initial phase II trial using the PDGF receptor inhibitor SU101 had minimal effects in HRPC [112].

Imatinib is an inhibitor of PDGFR signalling as well as the BCR-ABL tyrosine kinase. A number of phase II studies have been done using imatinib in prostate cancer patients who have a biochemical relapse following radical (radiotherapy or prostatectomy) treatment. As a single agent, imatinib has limited biochemical activity with a significant incidence of grade 3 and 4 toxicity leading, in some cases, to early trial closure [113–115]. There has been a suggestion that this PDGFR inhibitor may have a more beneficial role if used in combination with taxane-based chemotherapy. Pre-clinical models have demonstrated synergistic effects of imatinib and paclitaxel when used in mouse models of prostate cancer bone metastases [116]. Recent published results of a phase II trial combining imatinib and docetaxel in HRPC patients with bony metastases showed no therapeutic benefit despite confirmation of effective phospho-PDGFR inhibition [117]. An osteolytic model of bone metastases was used...
in the pre-clinical study as opposed to the osteosclerotic lesions typically seen in human prostate cancer, which may contribute to the discrepancy in efficacy observed in this pre-clinical model and clinical trials. High levels of grade 3 toxicities were also observed in this phase II trial of imatinib and docetaxel and it has been recommended that further studies of this combination should not be pursued.

Other agents with anti-angiogenic properties that may be of clinical benefit in HRPC include thalidomide and its analogues. Thalidomide has multiple mechanisms of action including immunomodulatory effects on the tumour microenvironment [118]. Prostate cancer progression and metastasis has been suggested to be mediated by stromal–epithelial interactions, which could be targeted by thalidomide. Thalidomide is also known to have anti-angiogenic properties from results of pre-clinical studies [119]. Phase I and II studies have shown promising results when thalidomide was used in HRPC patients as well as in studies using combination therapy with docetaxel [120–122]. These results support the need for additional studies of thalidomide in HRPC either alone or as combination therapy, and current studies are focussing in particular on the potential effect of thalidomide on bone metastases.

**ET (endothelin) axis**

The ET axis comprises of the three peptides ET-1, ET-2 and ET-3 and their receptors ET\(_A\) and ET\(_B\). Most of the activities of ET-1 are mediated via the ET\(_A\) receptor. ET-1 has important roles in a host of biological functions, including cellular proliferation, apoptosis and angiogenesis. It also stimulates osteoblast proliferation, leading to osteoblastic bone metastases which is typical of prostate cancer [123].

In metastatic HRPC, ET receptors are overexpressed and higher levels of ET are associated with progressive disease [124]. Atrasentan is a selective ET\(_A\) receptor antagonist that inhibits or reverses the downstream effects of ET-1. Phase I and II studies of atrasentan have provided encouraging results when used in men with metastatic HRPC [125,126]. However, a phase III trial comparing atrasentan with placebo in patients with metastatic HRPC did not show any significant delay in disease progression [127]. BAP (bone alkaline phosphatase) was measured as a biomarker of disease progression, and increases from baseline to final BAP were significantly lower in the patients treated with atrasentan. This suggests that atrasentan may have targeted activity in the bone microenvironment and that using this ET\(_A\) receptor antagonist may potentially prevent bone metastases formation or slow the onset of skeletal-related events in HRPC patients. A phase III trial studying the possible synergistic effect of atrasentan and docetaxel is currently recruiting patients with HRPC who have bone metastases.

**Anti-apoptotic proteins**

Accelerated or dysregulated proliferation is well-recognized as a major causative factor in tumour development and progression. In addition to this, defective apoptosis (programmed cell death) has been highlighted as a key factor in carcinogenesis. Disruption of these anti-apoptotic signals through selective therapeutic targeting could offer a novel strategy for drug-development programmes and a number of potential targets are currently being reviewed. Survivin is a proto-oncogene which is a member of the inhibitor of apoptosis family and has been associated with phenotypically aggressive prostate cancer and androgen resistance [128,129]. Inhibition of this pathway would aim to lower the anti-apoptotic threshold in cancer cells. YM155 is a novel small-molecule inhibitor of survivin that induces apoptosis in prostate cancer cell lines and regression of tumour growth in HRPC xenografts [130]. Phase II trials are currently under way with YM155 and docetaxel in HRPC.

It has recently been shown that IGF-I/mTOR signalling increases levels of survivin in prostate cancer cells [131]. This suggests that suppression of IGF-I/Akt/mTOR signalling may be beneficial to lower an anti-apoptotic threshold maintained by survivin in aggressive prostate cancer.

Bcl-2 is another anti-apoptotic regulatory protein which is associated with poor therapeutic response and poor clinical outcome in SCLC. There is also strong in vitro evidence to show that Bcl-2 suppression in SCLC is associated with enhanced chemosensitivity. Disappointingly, a phase II study in patients with advanced SCLC treated with carboplatin and etoposide ± G3139 (or oblimersen, a Bcl-2 antisense oligonucleotide) had no added effects with the addition of oblimersen; there was in fact a potential negative impact on survival [132]. Of note, oblimersen combined with chemotherapy has been shown to improve survival in melanoma patients [133]. To explain the negative results in SCLC, two possible explanations are: (i) despite promising data from in vitro and in vivo model systems, Bcl-2 overexpression does not play a critical role in clinical SCLC, which would argue for more relevant in vivo model systems; and (ii) oblimersen is not suppressing Bcl-2 at a sufficient level for it to enhance chemotherapeutic sensitivity. Tumour biopsy following oblimersen treatment would enable formal assessment of the target status, an important consideration for future trial design. Finally, it may also be possible that off-target effects, such as immunostimulatory effects, may be responsible for the effects observed in melanoma but not in SCLC.

Bcl-2 is overexpressed in HRPC [134], and its inhibition results in delayed development of hormone-resistance and enhanced effects of chemotherapy in mouse models of prostate cancer [135]. A phase II study of docetaxel with oblimersen showed no additional benefit in overall survival and PSA response rates.
to docetaxel monotherapy in HRPC [136]. Protein expression of Bcl-2 was analysed in peripheral blood mononuclear cells pre- and post-treatment in order to assess the pharmacodynamics of oblimersen treatment. No correlations between Bcl-2 levels and response rates or survival were observed. However, once again, an intratumoural biomarker, namely Bcl-2 level, was not ascertained. Currently, there are no trials recruiting prostate cancer patients for treatment with oblimersen.

AR signalling

Targeting the reactivation of AR signalling in HRPC is currently the focus of many drug development programmes and clinical trials. Anti-androgens currently approved for use in prostate cancer (bicalutamide, flutamide and cyproterone acetate) have limited use in hormone-resistant disease and all have been observed to convert into agonists in progressive disease [137]. A number of novel anti-androgens are currently being introduced which have significantly higher affinity than bicalutamide for the AR. Both novel anti-androgens MDV-3100 and BMS-647566 are currently in phase I clinical trials in HRPC.

HRPC progression may be due to residual serum androgens as well as up-regulated intracrine androgen synthesis from the tumour cells. Therefore methods to lower androgen levels further are under investigation. Ketaconazole is a synthetic antifungal agent which is currently used in some centres for patients with HRPC due to its action as a potent inhibitor of CYP450 (cytochrome P450)-dependent adrenal and testicular androgen production. Studies have shown that ketaconazole has a modest activity in HRPC; however, its use is associated with a rise in adrenal androgen levels at the time of progression [138,139].

CYP17 is a microsomal enzyme that catalyses two key steroid reactions in both adrenal and tumour intracrine androgen biosynthesis involving 17 α-hydroxylase and CYP17 α-reductase SRD5A1 and SRD5A2. The type 2 enzyme has been identified as the dominant type in benign prostate tissue, and finasteride, a specific type 2 inhibitor, is approved for use in BPH. It has been suggested previously that finasteride may prevent or delay the development of prostate cancer [142]. The Prostate Cancer Prevention Trial studied men who were prescribed either finasteride or placebo for 7 years, and, although a 24.8% reduction in the prevalence of prostate cancer in men on finasteride was observed, treatment with this 5α-reductase inhibitor was associated with a significant increase in high-grade disease [142]. Dutasteride (a dual inhibitor of SRD5A1 and SRD5A2) is currently being evaluated as a chemopreventive agent in prostate cancer, as SRD5A1 has been shown to be up-regulated in progressive prostate cancer [143]. Dutasteride has also been shown to inhibit in vivo tumour growth when combined with castration in androgen-responsive xenograft models [144]. In addition, phase II and III trials are currently recruiting patients with prostate cancer of various stages for assessment of treatment with dutasteride alone or in combination with ADT (androgen-deprivation therapy).

TARGETING TSGs

In addition to inhibiting oncogenic signalling, drug development programmes are also targeting TSGs through either activation or induced expression as an alternative approach for advanced prostate cancer therapy.

p53 and prostate cancer

Although the frequency of p53 mutations in early prostate cancer is low, heterozygous loss-of-function mutations are often observed in advanced disease [145]. Furthermore, p53 turnover is maintained by the E3 ubiquitin ligase MDM2 (murine double minute 2) which binds to the C-terminus of p53 and targets it for degradation. Overexpression of this p53 regulator has been observed in several cancer types including prostate cancer [146]. Inhibition of the interaction between MDM2 and p53 allows reactivation of p53, and this is currently a promising anticancer strategy. Small-molecule MDM2 inhibitors, such as nutlin-3, have shown promising antitumour activity in LNCaP xenograft models (wild-type p53) [147]. Evidence suggests that p53 signalling is also important in androgen signalling, with wild-type p53 overexpression being associated with decreased androgen function [148]. In vitro treatment with nutlin-3 has been shown to have a suppressive effect on androgen signalling [149]. AS-MDM2 (antisense MDM2) oligonucleotides are another method of targeting the interaction between MDM2 and p53. Treatment with AS-MDM2 enhanced the in vitro efficacy of radiotherapy and chemotherapy in prostate cancer cells [150]. In vivo, AS-MDM2 sensitizes androgen-sensitive xenografts to ADT [151]. The MAPK and PI3K pathways are also involved in p53 regulation. p53 can activate the Raf/MEK (MAPK/ERK kinase)/ERK pathway and ERK can stabilize p53 by phosphorylation in cervical cancer cells [152]. On the other hand, Akt phosphorylates MDM2, enhancing its
activity and destabilizing p53 [153]. The p53, PI3K and MAPK pathways are connected functionally and targeting these signalling systems either in isolation or together may synergize further the effects of specific (conventional) therapies in prostate cancer. Therefore it is important to incorporate relevant robust patient selection and accurate target validation in the design of future trials to facilitate the assessment of the value of such therapies.

**Epigenetics in prostate cancer**

Epigenetic changes encompass a number of reversible cellular events, including DNA methylation and histone modifications, which can modulate gene expression and alter tumour phenotypes. A number of genes are hypermethylated and silenced in prostate cancer such as GSTP1 (glutathione transferase p1), commonly hypermethylated in prostate cancers (>90%) [154]. DNA methylation levels can be altered by chemical inhibition of DNA methyltransferase enzymes. However, to date, no demethylating agent has shown a significant response in solid tumours [155,156].

Modification of the surrounding histones in which the DNA is packaged is another important epigenetic mechanism involved in carcinogenesis. The expression of HDACs (histone deacetylases) is frequently up-regulated in prostate cancer with increased expression being associated with hormone-refractory disease [157]. HDAC inhibitors induce growth arrest and apoptosis in vivo as well as regulating angiogenic and immune functions [158]. A limited response however has been observed with HDAC inhibitors in solid tumours [159]. Although an increase in histone acetylation was observed in the peripheral blood mononuclear cells of treated patients, the histone acetylation status in the target organ was not assayed. Overall, HDAC inhibition alone does not appear to be effective as a cancer therapy [159].

**CONCLUSIONS**

Current clinical trials with novel biological agents frequently allocate patients to treatment regimes without prior analysis of gene expression and/or genotype. High-throughput gene expression profiling can be used to assess gene expression signatures from individual tumours. Such methods of molecular profiling would offer an opportunity to link the oncogenic process with potential therapeutic strategies, which may offer a more advantageous clinical outcome for the patient [160]. Such a global approach will hopefully shed light on how individual signalling cascades interact in the context of a network to drive prostate carcinogenesis: this will have important implications in drug development.

Another crucial aspect of drug development is the availability of biomarker(s) for a number of purposes, including their use in risk assessment, diagnosis, prognostic stratification and therapy monitoring. Although a detailed discussion of biomarkers is outside the remit of the present review, it is relevant to highlight the following requirements for drug development: (i) a robust method for in vivo assessment of quantifiable effects on the target of interest by the candidate agents is important, (ii) patient selection and stratification as guided by biologically relevant biomarker(s) will provide a strong rationale for the design of future clinical trials, and (iii) clinical effects of the novel treatment on prostate cancer growth and progression will be assessed by the appropriate (surrogate) end points. Studies using many of the novel agents discussed have attempted to identify biomarkers of target inhibition or downstream signalling to guide patient selection and follow-up, for example phospho-EGFR and phospho-MAPK with EGFR inhibitors. Repeat tissue sampling, however, is not always feasible particularly in advanced disease; more acceptable methods of sampling such as serum or urine would be preferable. Hence there is an urgent need for validated urine or serum markers to evaluate the status of targets of interest as well as the tumour as a whole. Serum PSA is currently used as a biomarker in prostate cancer. Its increasing use in recent years has facilitated early diagnosis of prostate cancer. PSA measurement has a number of drawbacks as it can also be elevated due to BPH or prostatitis, leading to a significant number of false-positive results. There is a need for more specific biomarkers for prostate cancer, which can be used alone or in conjunction with PSA. Alternative biomarkers (serum and urine) are currently in trial; however, to date no specific targeted test for screening or patient follow-up has been identified [161].

Epigenetic changes have also been studied as both diagnostic and prognostic tools in prostate cancer. It is apparent that genetically identical tumours have differing phenotypes due to altered epigenetic arrangements and analysis of methylation changes or histone modifications in individual tumours has the potential to allow clinicians to predict patient prognosis and direct targeted therapy. Targeted therapy in haematological cancers has proved more successful than in solid tumours. Haematological malignacies arise from specific genetic mutations, offering single molecular candidates for drug targeting. In contrast, solid tumours tend to be heterozygous involving multiple pathways. Targeting a single molecule or pathway therefore may not be sufficient to significantly influence the malignant phenotype, as signalling through the other pathways may compensate for the effects of the single target affected by treatment. Different pharmacological strategies have therefore been pursued to inhibit multiple pathways or multiple steps within the same pathway for use in advanced solid tumours. Multi-targeted agents or a combination of single-targeted drugs may maximize effective target inhibition and have a complementary impact on downstream signalling.
Sorafenib is a multi-targeted TKI which is approved for clinical use in RCC. Sorafenib blocks RTK signalling (VEGFR, PDGFR, c-Kit and b-RAF) and inhibits downstream signalling through the MAPK pathway, preventing tumour growth by anti-angiogenic, anti-proliferative and/or pro-apoptotic effects. Other novel multi-target inhibitors, which have been developed, include sunitinib, imatinib and lapatinib. Multi-targeted TKIs avoid drug-drug interactions and better compliance may be achieved with administration of a single compound. On the other hand, combining specific TKIs may increase treatment efficacy with the exact titration for each agent allowing optimal target inhibition. However, drug–drug interactions may potentially lead to altered responses.

Another method, which may increase antitumour activity, is to combine different classes of inhibitors, for example a mAb and a TKI against the same single target. In vitro and in vivo evidence has shown that combining mAb therapy and a TKI to target different molecular domains of the same receptor can potentiate cellular toxicity due to non-overlapping mechanisms of action and partially overcome acquired resistance to any single inhibitor. A number of trials are currently studying the potential effects of combination regimes of targeted therapies with docetaxel. Docetaxel improves survival in patients with hormone-refractory disease by a mean of only 2.5 months. Minimum survival improvements in this patient group would therefore convey significant results compared with docetaxel monotherapy, which is currently the only effective treatment option available.

In summary, current treatment options for prostate cancer remain unsatisfactory and better management options, particularly for HRPC patients, are required. Understanding the signalling pathways involved in prostate carcinogenesis has lead to the development of a number of potential new drugs with many reaching clinical trial. To date none of the targeted therapies have shown adequate efficacy for routine usage. The discrepancy between pre-clinical and clinical findings may be a reflection of the fundamental differences between the currently available pre-clinical (usually murine) models and clinical cancer. Development of a relevant prostate cancer transgenic mouse driven by validated molecular lesions may provide a more meaningful model for clinical prostate cancer and may allow a more clinically relevant assessment of novel anticancer treatments prior to clinical trial. Several promising targets and agents are continuing to emerge and it is imperative that multi-disciplinary teams incorporating urological surgeons, oncologists and laboratory scientists are involved in bringing these novel treatments to clinical trial.

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