Current understanding of prostate cancer

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Prostate cancer is a large and ever evolving area of research. Over £22 million was spent on prostate cancer research in the UK alone last year. When funding research it is important to have a strong knowledge of the field as it stands: where there is potential for making a real difference to patients and how money can be spent most wisely to achieve this. This literature review aims to summarise the key areas of prostate cancer research at this moment in time. It spans basic research into the biology of the disease, drugs and treatments that are in development and evaluation of the current standard of care treatments. Diagnosis, genetics and patient stratification are also discussed in detail. The end result is a broad overview of the field; where it has come from; the research that is currently being produced; and, where it might go in the future. The ultimate aim of all research is to gain knowledge that will translate into tangible outcomes for patients: increasing survival, reducing mortality and improving quality of life. By summarising our current knowledge attention can be focused in the most critical and promising areas of research.

INTRODUCTION

Globally there were over 1.2 million cases of prostate cancer diagnosed in 2018 and over 350,000 deaths from the disease[25]. Prostate cancer accounts for 23% of male cancer diagnoses in the UK[26]. It is also the second most common cause of cancer death in the UK, after lung cancer[26]. In many cases, prostate cancer is a slow growing (indolent) disease. As such, survival rates in the UK are high, with 85% of men surviving for 5 years after diagnosis[12]. Many men die *with* prostate cancer, due to co-morbidities, rather than *of* prostate cancer. Prostate cancer-specific mortality at 10 years post-diagnosis is as low as 5% for clinically localised disease but increases to 66% for advanced disease with at least 1 metastasis[78]. It therefore follows that preventing or finding a treatment for advanced prostate cancer will have the largest impact on prostate-cancer specific mortality. It also highlights the need to consider the quality of life of men living with cancer, as many men will live a significant amount of time with the disease.

DIAGNOSIS

Diagnosing prostate cancer is challenging. Current methods lead to both under-diagnosis of clinically significant disease and over-diagnoses of indolent disease that may never produce symptoms[45]. As many of the treatment options for prostate cancer carry significant side-effects (e.g. incontinence and erectile dysfunction), it is important to develop diagnostic tools that can

distinguish clinically significant disease (requiring treatment) from indolent disease (that can be monitored by active surveillance but does not require significant intervention).

Currently, men are likely to be diagnosed either by abnormal serum PSA levels or abnormal digital rectal examination (DRE) both of which will be followed up by transrectal ultrasonography (TRUS)-guided biopsy to confirm disease[81].

Cancer can undergo both grading and staging at diagnosis and during treatment to assess disease progression. Grading is an assessment of how much the cancer resembles normal tissue (low-grade cancer) or cancerous tissue (high-grade cancer). In prostate cancer the Gleason grading system is used. The cancer is given two grades based on the two most common histological appearances of the cells, across all the tissue samples from biopsy (up to 18 samples), and the two scores are added together. This produces a range of scores between 6 (least aggressive) and 10 (most aggressive). These grades can then be further classified into grade groups[73].

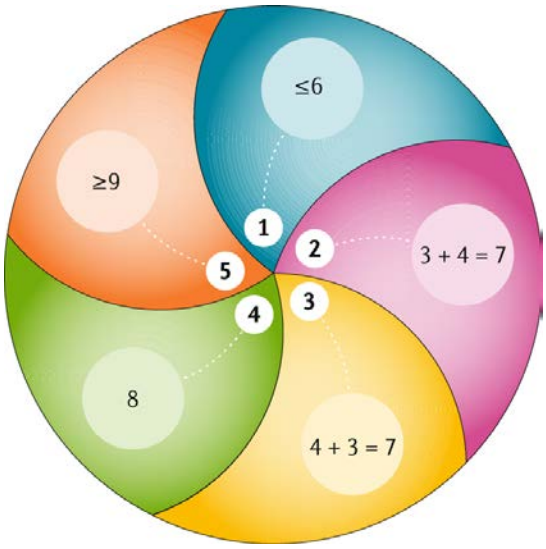


Figure 1. Visual representation of Gleason scores (outer numbers) and Gleason grade groups (inner numbers). Taken from Sathianathen, et al., (2018)[73].

Staging, on the other hand, describes how far the cancer has spread.

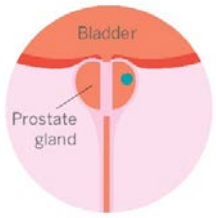
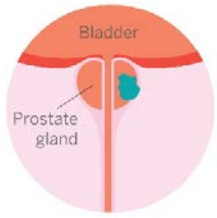
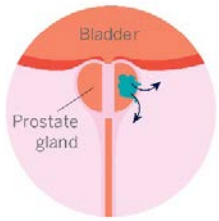
Stage	Description	Diagram
T1	Early prostate cancer that can only be seen under the microscope	
T2	Early prostate cancer that can be felt by rectal examination	
T3	Locally advanced prostate cancer that may cause urinary problems	
T4	Late prostate cancer probably with secondary tumours or metastases.	

Figure 2. Table illustrating features of cancer at different stages (National Cancer Institute, 2015)[54].

The lymph nodes around the prostate can either be positive (N1) or negative (N0) for cancer. If the cancer has metastasised further than the lymph nodes it is denoted M1 while non-metastatic disease is M0[54].
This section outlines the tools available for the diagnosis of prostate cancer, how they are currently used, and their potential role in the future of prostate cancer diagnosis.

Digital rectal examination

This is a procedure in which a doctor or a specialist inserts their index finger into the back passage to feel the prostate through the rectum wall. If abnormalities of the prostate, such as lumps, are felt this triggers subsequent biopsy[55].

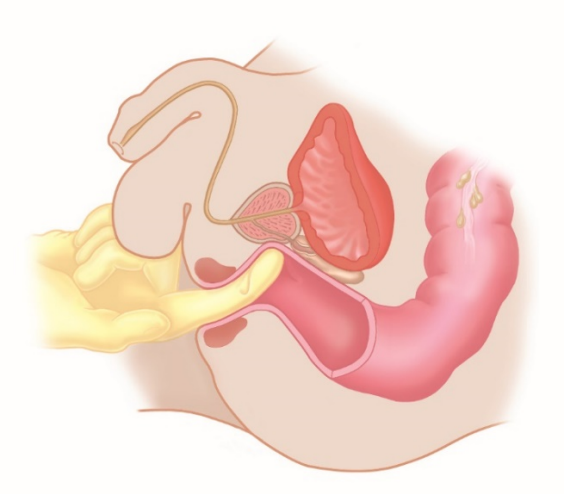


Figure 3. Diagram showing the process of digital rectal examination (DRE).

PSA testing

Prostate-specific antigen (PSA) is produced by the prostate and can leak into the blood. The amount of PSA in the blood can be measured from a blood test and reported as serum PSA in μg L^{-1}. Elevated levels of PSA can indicate prostate cancer, however, can also be indicative of other benign conditions such as benign prostatic hyperplasia (BPH) or prostatitis (inflammation of the prostate)[71]. 1 in 4 men with elevated serum PSA (4-10 μg L^{-1}) will go on to be diagnosed with prostate cancer[20]. However, up to 15% of men with prostate cancer have normal PSA levels[56] and so PSA testing will lead to a false-negative in these cases. This illustrates the limitations of serum PSA testing: its relatively poor specificity and sensitivity. PSA screening reduces a man's chance of dying from prostate cancer but also results in the unnecessary treatment of many men.

The European Randomized Study of Screening for Prostate Cancer (ERSPC[77]) and Prostate, Lung, Colorectal and Ovarian Cancer Screening (PLCO[5, 62]) trials analysed population-based PSA screening for prostate cancer. These studies indicated minimal and no significant decrease, respectively, in mortality between groups that were PSA tested and those that were not. ERSPC later reported that PSA screening prevents 3 cases of metastatic disease and 1 case of cancer-specific mortality per 1,000 men[77]. Additionally, the introduction of active surveillance to monitor low-risk disease without intervention has somewhat reduced the problem of over-treatment. The proportion of men diagnosed with localised disease that underwent radical treatment within the first year of diagnosis dropped from 12% in 2014/15 to 8% in 2015/16[58]. This suggests a reduction in overtreatment and increased utilisation of active surveillance protocols. As such, while population-based screening may not be indicated, individualised PSA testing should not be discounted as a diagnostic tool.

In the UK there is no national screening programme for prostate cancer. The NHS instead uses an informed choice programme, called prostate cancer risk management. This provides men over 50 who ask their GP about PSA testing with information including the pros and cons of PSA testing, such as the possibility of a false positive causing unnecessary, invasive follow-up

tests. The aim is to enable them to make an informed choice about whether they want to take the PSA test or not. A free-at-point-of-access PSA test is available on the NHS to men aged over 50 who choose to have one[56]. Similarly, in America, the U.S. Preventive Services Task Force[89] updated their guidelines on PSA testing in 2018. Their recommendation is that in men aged 55-69 the decision to take a PSA test is an individual one and that clinicians should provide information on the benefits and potential harms to help individuals make a decision. USPSTF recommend against PSA testing in men over 70 years old.

TRUS-guided biopsy

TRUS-biopsies are currently the only way of confirming cancer in the prostate. An ultrasound probe is inserted into the back passage to image the prostate. A needle is then inserted along this probe and between 8 and 10 biopsy samples are taken from the prostate, guided by the ultrasound image[11]. The needle may be inserted via the perineum (transperineal biopsy) or the rectum (transrectal biopsy). After the procedure almost all patients will experience blood in the urine, semen or bowel. 10-50% will experience discomfort in the area of the biopsy for a few days after the operation. There are also rarer but more serious side-effects like septicaemia, which occurs in around 2% of patients. TRUS-guided biopsies may fail to detect significant cancer, for example if the cancerous area of the prostate is not sampled. This failure occurs in between 1 in 10 and 1 in 50 (2% - 10%) patients[11]. These can then be used to confirm the presence of cancer and for cancer grading (as described above).

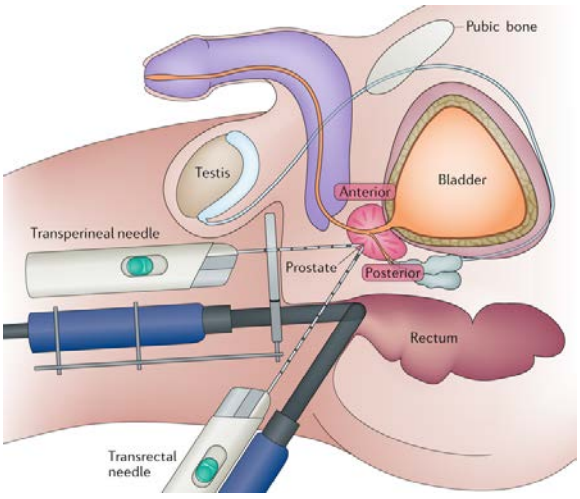


Figure 4. Diagram showing both the transrectal and transperineal approaches for TRUS-guided biopsy. Taken from Maio, M. & Hansen, A. (2017).

Multiparametric MRI

Multi-parametric magnetic resonance imaging (mpMRI) is a newly emerging imaging technology that has shown potential as a diagnostic tool for prostate cancer. mpMRI describes a series of imaging techniques that use MRI – the excitation and subsequent relaxation of protons – to produce a range of different images of the tumour.

The European Association of Urology suggest mpMRI has two main clinical uses (1) as a triage test to reduce unnecessary biopsy, and (2) to improve the detection of clinically significant disease by enabling targeted biopsy[81]. A U.K. study (PROMIS[3]) found that mpMRI-guided biopsy identifies up to 18% more clinically significant cancers and leads to a 5% reduction in the diagnosis of clinically insignificant disease compared to traditional TRUS-guided biopsy[3]. The use of mpMRI as triage before biopsy allowed up to 27% of patients to avoid primary biopsy[3]. Other potential uses include imaging before a repeat biopsy when clinical suspicion of disease persists after initial negative biopsy and to trigger biopsy in men undergoing active surveillance.

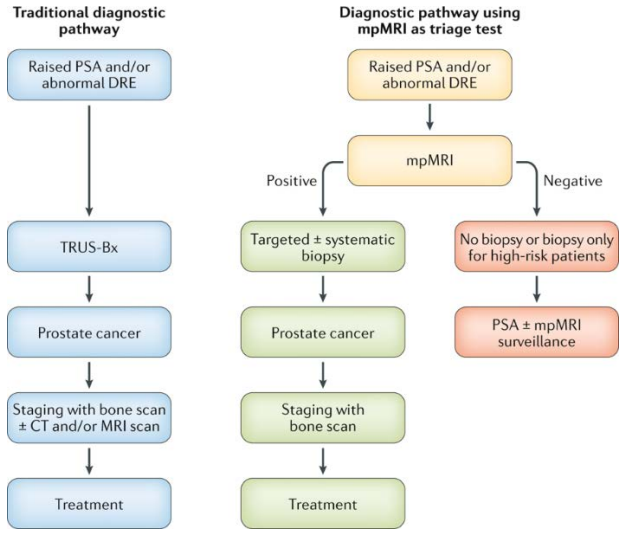


Figure 5. Proposed new diagnostic pathway in which mpMRI is used to triage patients before biopsy. Taken from Stabile et al., 2019)[81].

Based on the outcomes from PROMIS and other similar trials, NICE 2019 guidelines on prostate cancer diagnosis recommended that all men have an mpMRI scan before biopsy[57]. Only 51% of NHS areas routinely performed mpMRI before biopsy in 2016 and it is estimated that only one third of eligible men had an mpMRI scan before biopsy[63]. However, the number of men receiving mpMRI during their diagnosis pathway increased from 44% in 2014/15 to 51% in 2016/17[58]. There was also a shift over this period towards pre-biopsy scans as opposed to post-biopsy scans.

There are still issues to be addressed in the use of mpMRI. For example, the accuracy of the test is highly dependent on the skill of the radiologist and inter-observer variability can reduce reproducibility. The Prostate Imaging Reporting and Data System (PI-RADS) was introduced (version 1 in 2012 and version 2 in 2015) in an effort to standardise reporting[81]. This reporting system produces high specificity (0.89) and selectivity (0.73) diagnosis. There are also questions over the best methods to guide biopsy with mpMRI and the best entry point for the needle (transrectal or transperineal)[81]. Cost-effectiveness may also be a concern although one study suggested that the high initial costs are mitigated in the long-term due to fewer false positives, better tumour staging reducing costs associated with overtreatment and fewer diagnoses of late-stage disease[16].

PSMA-guided PET imaging

Prostate specific membrane antigen (PSMA) is a cell surface enzyme that is highly expressed in prostate cancer cells. PSMA-positron emission tomography (PET) imaging can be used as a diagnostic and staging tool in prostate cancer. Antibodies or small molecules that bind to PSMA are conjugated to radionuclides to allow targeted imaging of the tumour and identification of any metastases[68]. PSMA is expressed by the vast majority of prostate cancer cells; one study found PSMA expression in all benign epithelium and primary cancers, and most lymph node metastases[82]. The degree of expression correlates with a number of metrics for tumour aggressiveness, including Gleason score, likelihood of metastasis, and the development of castration resistance. High PSMA levels are associated with a significant increase in PSA recurrence[61].

PSMA-PET imaging can be used to identify locally recurrent cancer and metastatic cancer that could not previously be visualised. Proof-of-concept demonstrating PSMA-PET ability to detect metastatic disease was established by ProstaScint (an indium-111 radiolabelled monoclonal antibody). The intrinsic limitations of this imaging agent, such as its long biodistribution and its targeting of an intracellular PSMA epitope, mean it has since been replaced by more modern alternatives[68]. Modern radiotracers include ^{89}Zr and ^{68}Ga conjugated to either J591 PSMA antibody or small molecule inhibitors of PSMA. Recently, single-photon emitting radionuclides, such as technetium-99, have also been used. First in human data shows that technetium-99-labeled small molecule PSMA inhibitors showed promise in detecting metastases earlier than conventional bone scanning[91].

Biomarkers

PSA is an example of a biomarker for prostate cancer that can be detected in a liquid biopsy (blood test). However, as discussed above, PSA lacks both specificity and sensitivity as a test for prostate cancer. The search for new and more accurate diagnostic and prognostic biomarkers for prostate cancer is an active area of research. Biomarkers may be genetic signatures, RNAs or proteins and can be detected by different methods. The following is a brief summary of biomarkers that are currently in clinical use or that are being studied in the lab for potential use.

Prostate cancer antigen 3 (PCA3) is a long non-coding RNA that is highly expressed in prostate cancer but is not expressed by normal prostate tissue. A PCA3 score can be obtained from post-DRE urine samples to aid in prognostic predictions and therefore inform treatment choice[71]. 11 clinical studies have been performed to assess PCA3 accuracy in men referred for biopsy due to abnormal test results (elevated PSA, abnormal DRE etc.)[7]. The PCA3 test has lower sensitivity than PSA but higher specificity. False negatives for the PCA3 test were low (10-26%). PCA3 accuracy ranged from 64-84%. This makes PCA3 a promising compliment to PSA testing to increase overall accuracy, particularly in the case of patients with elevated PSA but at least one previous negative biopsy[7]. Furthermore, unlike serum PSA, PCA3 score is independent of prostate volume and therefore less likely to trigger unnecessary biopsy in patients with benign prostate hyperplasia[7]. Some recent studies have also suggested a possible prognostic role for PCA3, finding correlation between PCA3 score and tumour volume or Gleason score. PCA3 is therefore a useful biomarker for avoiding unnecessary, invasive biopsies and also has potential alone, or in combination with other biomarkers, to be predictive of prognostic outcomes[30]. PCA3 is approved for clinical use in the U.S. However, it is not routinely available on the NHS so private healthcare facilities are the only place PCA3 testing is available in the UK.

TMPRSS2-ERG gene fusions occur in around 50% of prostate cancer cases[71]. As with PCA3, this genetic aberration can be detected in post-DRE urine sediments[71]. As a diagnostic test, the detection of TMPRSS2-ERG gene fusions has high specificity but low sensitivity (few false positives but many false negatives). This test is not currently in clinical use and, due to its low sensitivity, is not useful on its own. However, it has potential to be used in conjunction with other non-invasive tests to reduce unnecessary biopsy[71]. The prognostic value of TMPRSS2-ERG is controversial, with some reports of it being associated with poor clinical outcomes (aggressive disease, metastasis and mortality) and others finding no evidence of such an association[71]. Other genetic aberrations (e.g. PTEN loss, BRCA1/2 mutation) are likely to have similar diagnostic value to TMPRSS2-ERG gene fusions with high specificity but not occurring in every case and therefore having low sensitivity. However, they may carry significant value in stratifying patients into distinct subgroups of cancer that respond differentially to treatments (see: genetics).

Circulating tumour cells (CTCs) can be detected in the blood of some prostate cancer patients. The number of CTCs correlates with poor prognosis in cases of castration-resistant cancer[71]. Useful information can be extracted from CTCs, including identifying genetic alterations such as those described above (PTEN loss, TMPRSS2-ERG gene fusions, BRCA1/2 mutation). The current laboratory techniques for detecting CTCs in patient blood samples are expensive, time-consuming and lack sensitivity. Currently only about 50% of prostate cancer patients have detectable levels of CTCs in their blood and so there is potential for improving detection methods to increase the number of patients in which CTCs, and the potential diagnostic and prognostic information they carry, could be detected in a larger proportion of patients. There is a need for optimisation and standardisation of CTC detection methodology and identification of appropriate biomarkers that can be derived from them[59]. Large cohort clinical studies are needed before information derived from CTCs can be used for diagnosis in the clinic.

Prostate cancer progression can also cause altered microRNA (miRNA) expression. A novel mechanism for detecting these expression changes is in exosomes. Exosomes are small extracellular vesicles that can be detected in blood, semen or urine. As well as altered miRNA expression both TMPRSS2-ERG and PCA3 are detectable in exosomes[71]. The appeal of using exosomes and CTCs for diagnosis is that they can be detected in urine and blood tests and therefore represent an additional non-invasive test that can be used to reduce the incidence of unnecessary, invasive biopsies.

Cancer grading and staging are aimed at assessing the risk and aggressiveness of cancer. The biomarkers discussed here can also contribute to this process. The current process of assessing patients and dividing them into, for example, low, intermediate, or high-risk groups, uses a combination of prognostic indicators such as PSA level and Gleason score. The disease classification can then be used to inform treatment choice. However, the current division of patients into groups is flawed and can result in both overtreatment (due either to lack of trust in categorisation procedures or incorrectly categorised intermediate or high-risk disease) and a lack of treatment where it may have been beneficial (incorrect categorisation of low-risk disease)[45]. As a result, any biomarkers that could improve the assessment of cancer aggressiveness due to their prognostic value could be clinically useful. In the case of genetic biomarkers, stratification of cancer into distinct molecular subtypes has the potential to facilitate the use of personalised medicine to target the specific molecular aberration present in an individual's cancer.

GENETICS AND SIGNALING

The genetic makeup and resulting signalling pathways that make up a prostate cancer tumour determine its characteristics. This includes how aggressive the disease is and which treatments the tumour is likely to respond to. The genetic makeup of a single tumour may be heterogeneous and metastases may differ in their profile to the primary tumour. This section details the most common genetic aberrations associated with prostate cancer. It also covers aspects of disease biology relating to signalling such as androgen dependence, the development of castration resistance and the progression to metastases.

PTEN

Phosphate and tensin homologue (PTEN) mutations are estimated to be present in about 20% of primary prostate tumours and up to 50% of mCRPC cases[46]. 49% of the 150 mCRPC cases in one study harboured a mutation at some point in the PTEN-PI3K-AKT pathway including: biallelic loss of PTEN; mutations, amplifications or activating fusions in PIK3CA or activating mutations in AKT1A[67].PTEN loss may have potential as a genomic biomarker for aggressive prostate cancer[46]. In order for it to be used in the process of diagnosis and choosing from treatment options, tumour tissue and/or blood liquid assays need to be validated for the detection of PTEN mutations. It may be predictive of treatment responses and informative in assessing men for suitability for active surveillance protocols. PTEN loss is associated with increased risk of the cancer being upgraded in a subsequent biopsy or radical prostatectomy (e.g. increased number of positive cores), discontinuation of active surveillance and adverse histopathological features at prostatectomy[46].

PTEN mutations lead to activation of the PI3K-AKT-mTOR signalling pathway. In normal cells PTEN catalyses the conversion of PIP3 to PIP2. As such, it antagonises the activity of PI3K enzymes which catalyse the reverse reaction thereby inhibiting the activity of AKT and downstream mTOR. Loss of PTEN leads to accumulation of PIP3, activation of AKT and modulation of mTOR activity. This can have downstream effects on the regulation of apoptosis and the cell cycle, cell proliferation and differentiation, metabolism and cell invasion[46]. PTEN loss is thought to be a critical step in the progression of some cancers from hormone-naïve to castration resistant disease. Activation of the PI3K-AKT-mTOR, caused by PTEN loss or other genetic aberrations, is associated with adverse oncological outcomes[46].

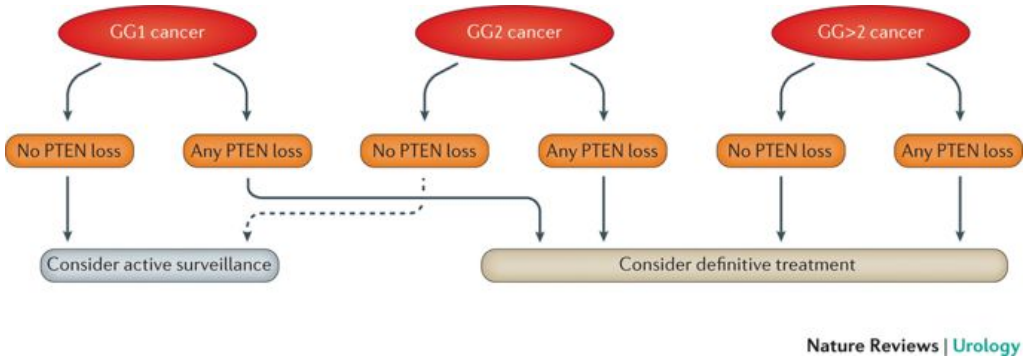


Figure 6. Flow chart illustrating the proposed use of PTEN statuas and Gleason grade group system to inform treatment choice. Taken from Lotan, T. et al., (2018)[46].

Another example of a gene that can be mutated in this pathway is Pik3ca - which encodes a subunit of the PI3K enzyme. In mice, mutations in this gene alone cause invasive prostate carcinoma and in cooperation with PTEN loss causes de novo CRPC (as opposed to slowly acquired CRPC in single mutants[60]. These mutations, as with PTEN loss, hyper activate the PI3k pathway. This has many downstream consequences, for example the activation of mTORC2, which phosphorylates and inactivates the metastasis suppressing N-Myc-downregulated gene 1. This knowledge has led to the generation of a novel, clinically relevant mouse model for prostate cancer. In these mice Pik3ca harbours a heterozygous activating mutation in prostate epithelial cells which results in prostate carcinoma[60].

Pik3ca mutation occurs in only around 4% of prostate cancer cases, but, copy number gain or amplification of this gene appears in as many as 62% of cases, often (39%) in conjunction with PTEN loss[60]. Single Pik3ca mutants develop locally invasive prostate carcinoma. PTEN loss alone causes early onset of invasive carcinoma and significantly greater tumour burden and heterogeneity. Although they are part of the same signalling pathway, mutations in these genes are not functionally redundant. PTEN loss in conjunction with Pik3ca mutation promotes tumorigenesis and facilitates metastasis, as well as predisposing the individual to CRPC. Mouse models with concomitant PTEN and Pik3ca mutations rapidly progress to castration-resistant prostate cancer and de novo resistance to ADT[60].

DNA repair pathways

Genomic aberrations in DNA damage repair pathways appear in 20-30% of advanced mCRPC cases[15] some of which (8%) are germline mutations and so are heritable[67]. Patients with genetic aberrations in different genes represent separate subgroups of patients who may benefit from particular treatments targeted to the specific genetic aberration in their tumour. A challenge for genomic stratification of prostate cancer is intrapatient heterogeneity[15]. In this scenario the genetic signature of cancer cells may differ between tumours in the same patient or even within the same tumour. This is likely to be especially common in cancers with DNA damage repair mutations which lead to genomic instability[15].

In normal cells, DNA damage is detected at specific checkpoints in the cell cycle. When damage is identified the cell cycle is halted so the DNA can be repaired. If the repair is unsuccessful the cell enters a state of quiescence or undergoes apoptosis. Damage to different genes causes the loss of different types of DNA repair processes. One of the most common mutations in prostate cancer is in BRCA2. This gene is involved in homologous recombination, a mechanism for repairing double-stranded DNA breaks. When this mechanism is no longer available double-stranded breaks are repaired by non-homologous end joining – an error prone method that's use results in genome instability[15].

WNT signaling

WNT proteins have roles during development in regulating self-renewal, cell proliferation, migration and differentiation. WNT proteins stabilise β-catenin. The accumulation of β-catenin in the cytoplasm and/or nucleus of the cell is an indication of dysregulation of the WNT signalling pathway[53]. Many prostate tumours have this phenotype; in one study 18% of tumours analysed harboured mutations in the WNT signalling pathway[67].

WNT associated aberrations include: activating mutations in CTNNB1 (the β-catenin gene), inactivating mutations in APC and AXIN1 (proteins involved in β-catenin break down) and aberrations in RNF43, ZNRF3 and RSPO2. These changes are more common in CRPC than hormone-naïve cancer. WNT signalling in the tumour microenvironment appears to be involved in the development of resistance to ADT. For example, therapy induced DNA damage activates WNT signalling and promotes therapy resistance[53].

Androgen dependence

Androgen dependence was first identified by Huggins and Hodges in 1941[36]. They showed that castration by bilateral orchiectomy reduced the levels of acid phosphatase present in the serum of many prostate cancer patients. Inhibiting or otherwise decreasing androgen function led to a decrease in prostatic acid phosphatase (an enzyme produced by the prostate that can leak into the urine) levels to around that of controls. From this they concluded that prostate cancer malignancy is influenced by male hormones[36].

Androgens act via the androgen receptor (AR). When androgens bind to this receptor in the cytoplasm it can translocate to the nucleus where it binds to sections of DNA called androgen response elements (AREs). AREs influence the transcription of some genes, collectively referred to as androgen-regulated genes. The ultimate effect of AR activation in prostate cancer cells is cell proliferation and the inhibition of apoptosis[84].

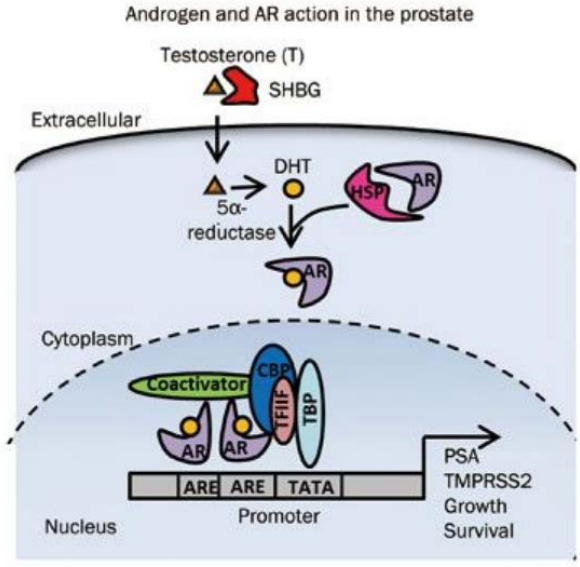


Figure 7. Diagram illustrating the action of the androgen receptor: the binding of dihydrotestosterone (testosterone metabolite) in the cytoplasm, translocation to the nucleus and action at androgen response elements in the genome. This increases the expression of genes such as PSA and TMPRSS2 and, ultimately, promotes cell growth and survival. Taken from Tan, M. et al., (2015)[84].

As prostate cancer is reliant on the AR for growth, targeting this receptor or otherwise reducing androgen function is an effective initial treatment for prostate cancer. This treatment technique

is called androgen deprivation therapy (ADT). ADT is a mainstay of prostate cancer treatment, particularly in cases of advanced disease (when the disease has spread outside the prostate). However, its use is limited as cancer will ultimately develop resistance to these treatments and continue to grow despite ongoing treatment. This disease state is referred to as castration-resistant prostate cancer (CRPC, mCRPC if accompanied by metastases).

Castration resistance

It is now generally thought that resistance to ADT is not due to androgen independence but rather is due to residual androgens continuing to activate the AR-regulated genes. This may be by activation of AR variants or through signalling from other receptors stimulating typically AR-regulated genes[95]. AR overexpression is sufficient to drive castration-resistance[95]. Point mutations (single changes to the amino acid sequence) in the ligand-binding domain (LBD) of the AR are present in 15-20% of CRPC cases; however, if the occurrence of AR gene amplification (an increase in the number of copies of the gene) is included this number jumps to over 60% of cases[95]. Mutations in the LBD of the AR can cause AR antagonists (e.g. flutamide) to become agonists. If this is the case then halting ADT can cause a temporary clinical improvement in patients with these mutations, known as anti-androgen withdrawal syndrome[95].

One mechanism for the development of resistance to both first- and second-generation ADT is the expression of splice variants of the androgen receptor (AR). All androgen receptor variants (ARVs) are truncated, meaning they lack a LBD but retain the N-terminal transactivation domain and DNA binding domains. This, in theory, results in a constitutively active receptor, although whether this is the case with all ARVs is under debate, as is their larger role in prostate cancer and castration resistance[95].

ARVs are expressed in normal prostate tissue. The expression of both AR and ARVs increases in response to ADT (with expression of AR remaining relatively greater than that of ARVs). In one prostate cancer cell line, derived from another cell initially hormone sensitive line (CWR22) but propagated after castration-induced regression[80], AR-V7 expression is high and this cell line is resistant to enzalutamide. Small interfering RNA (siRNA) knock-down of AR-V7 recovers sensitivity to enzalutamide in this cell line. However, AR-V7 expression is also observed in other cell lines (e.g. LNCaP) that are hormone-sensitive. However, forced AR-V7 expression in ADT-sensitive, ARV negative cell line, LNCaP, promotes tumour growth but is not sufficient to confer enzalutamide resistance to these cells[95]. Pre-clinical models of prostate cancer are limited in their ability to mimic human cancer and more studies in men are needed to establish the role of ARVs in castration-resistance.

Early evidence supports the role of ARVs in the development of castration resistance. A number of different ARVs have been characterised including AR-V1, AR-V3 and AR-V9. However, AR-V7 is the most extensively studied due to its frequent expression in CRPC and the availability of a variant specific antibody. AR-V7 expression correlates with resistance to abiraterone and enzalutamide. This variant of the AR lacks the ligand-binding domain, the direct and indirect target of enzalutamide and abiraterone respectively. Men in whom the expression of AR-V7 mRNA is detectable in circulating tumour cells shower lower PSA responses, shorter PSA-progression free survival (PFS), shorter radiographic PFS and reduced overall survival on abiraterone or enzalutamide than men with no detectable AR-V7 mRNA[6]. In fact, this study observed no appreciable benefit of enzalutamide or abiraterone in any patient with detectable AR-V7 mRNA[6].

It is also possible that androgens bypass the AR, activating the same signalling cascade via another receptor, such as the glucocorticoid receptor (GR)[95]. The prostate cancer cell line LREX' displays acquired enzalutamide resistance and is reliant on GR for enzalutamide-resistant growth. The GR is presumed to be able to regulate a subset of AR-regulated genes thereby promoting castration resistance[95]. Paradoxically, glucocorticoids (GR agonists) benefit some CRPC patients. This is due to the inhibition of ACTH secretion. ACTH stimulates the adrenal glands to release androgens and so inhibiting its secretion inhibits the production of adrenal androgens. However, in cancers with high GR expression glucocorticoids are likely to have a negative clinical effect. The progesterone receptor could also play a similar role to the GR. High expression of the progesterone receptor is associated with disease recurrence[95].

A small subset of prostate cancers and some cells within heterogeneous tumours express little to no AR. These cells often have a neuroendocrine phenotype. It is unclear whether these cells arise through the differentiation of cancerous cells to a neuroendocrine phenotype, possibly due to selection pressure from treatment, or directly from normal neuroendocrine cells in the prostate. Neuroendocrine cancers like these are associated with the loss of RB1 and TP53 and are aggressive, castration resistant cancers[95].

Metastasis

The 5-year overall survival rate for localised prostate cancer is approximately 100%. However, in prostate cancer cases with distant metastases 5-year survival drops to only 31%[23]. About 80% of men who die from prostate cancer will have bone metastases[23]. These statistics illustrate the high lethal potential of metastatic prostate cancer. Bone, as well as the lymph nodes adjacent to the prostate, are the most common sites of prostate cancer metastasis. Why bone is such a common site of metastases is not yet fully understood. The Paget model proposes that tumour cells metastasise only to organs well suited for the tumour's growth. According to this model, bone is likely to have a microenvironment that is favourable to the growth of prostate cancer cells, and evidence for this has emerged[23].

The first stage in metastasis is for tumour cells to break off from the primary tumour and enter the circulation[23]. This is thought to occur through a process called epithelial-to-mesenchymal transition (EMT). During this process cells lose the junctions that bind them to other cells. One way in which this occurs is by a change in the type of cadherin expressed by the cell[23]. E-cadherin proteins are important in the formation of cell-cell junctions in epithelial cells. Loss of E-cadherin expression can lead to reduced cell-cell adhesion and promote the detachment of cells from the primary tumour[23].

It has been shown that prostate cancer cells express the gene ALPL which encodes the protein alkaline phosphatase (ALP)[18]. ALP is an osteoblastic enzyme and expression in prostate cancer cells, along with other bone-related markers (e.g. RANKL) contributes to osteomimicry by prostate cancer cells. This similar expression profile may partially explain why prostate cancer cells so commonly metastasise to bone. Tumour-derived ALPL expression is associated with EMT markers and high levels of expression are associated with a significant decrease in disease free survival. It has been shown that ALPL knockdown in prostate cancer cell lines increases cell death, promotes mesenchymal-to-epithelial transition (the reverse of EMT) and reduces cell migration[18].

Although ADT monotherapy is initially effective, it often results in relapse of more malignant, castration-resistant disease within 1-2 years of starting therapy[51]. It has been hypothesised that

changes within prostate cancer cells caused by ADT promote the selection of more malignant tumour cells. ADT initially causes prolonged hypoxia which slows tumour growth. However, this period of hypoxia is followed by angiogenesis and the accompanying expression of EMT associated genes[51]. This provides a possible explanation for why ADT is often followed by the relapse of more malignant disease. It could also explain why the addition of docetaxel to ADT - likely increasing the cell death that occurs in the initial hypoxic stage of ADT treatment and decreasing the likelihood of recurrence - is associated with increased survival[51].

Prostate cancer cells bind preferentially to bone marrow endothelial cells compared to other types of endothelial cells. There is also chemo-attraction between prostate cancer cells and bone. There is thought to be complex interactions between tumour cells and bone, including bi-directional microenvironment interactions (involving, for example, integrins)[23]. Tumour growth affects bone remodelling which in turn promotes further tumour growth. As described above, prostate cancer cells express many genes/proteins that are markers for bone cells. Tumour cells can cause osteoblastic lesions by secreting osteoblastic factors such as insulin growth factor (IGF) which promote osteoblast activity and is associated with cancer cell proliferation and chemotaxis[23]. Prostate cancer cells can also activate osteoclasts through the secretion of cytokines such as RANKL. In this way the normal process of bone turnover is disrupted and the likelihood of skeletal related events (SREs: radiation to the bone, surgery to bone, fractures or compression of the spinal cord) is increased[23].

While the process of prostate cancer cell metastasis to bone is not fully understood, it is clear that it plays a significant role in disease mortality. Metastasis to bone can cause bone pain and significantly reduce quality of life. Treating men to prevent bone metastasis and reduce the likelihood of skeletal related events can improve survival and quality of life.

TREATMENT OPTIONS FOR LOCALIZED DISEASE

Radical prostatectomy

Radical prostatectomy (RP) is the surgical removal of the entire prostate gland. This can cure localised prostate cancer by removing all of the cancerous cells, however, it also carries a high risk of side-effects including erectile dysfunction and incontinence. Two clinical trials show no difference in overall survival between patients undergoing surgery vs active surveillance (PIVOT[96]) or surgery vs active surveillance or radiotherapy (ProtecT[28]) for localised prostate cancer. However, disease progression is lower in men treated with RP than those in the active surveillance group. The number needed to treat (NNT), which describes the number of people treated in order to avoid 1 unfavourable outcome (in this case cancer-specific mortality), can be as high as 42 for the use of radical prostatectomy in the treatment of intermediate risk prostate cancer[1]. This means that 42 men undergo RP, and are at risk of experiencing side-effects, for every one life saved by RP. Overtreatment such as this is costly both financially and in terms of quality of life for men who undergo unnecessary treatment. This highlights the need for improved patient stratification to identify those with high-risk disease – in which case NNT for RP drops dramatically to 13[1]– who are likely to benefit from prostatectomy and exclude men with low-risk disease who are unlikely to need such radical intervention.

The two most common side-effects of radical prostatectomy are erectile dysfunction and urinary incontinence. The likelihood of these effects depend on a number of factors including pre-operative risk factors and if it was possible to spare nerves during surgery. It is estimated that an additional 7.9 men treated with RP as opposed to active surveillance will result in one extra

man experiencing urinary incontinence[21]. For every 2.7 men treated with RP as opposed to active surveillance 1 additional man will experience erectile dysfunction[21]. 1 in 7 men undergoing RP will experience erectile dysfunction[21]. RP also leads to loss of ejaculation leaving men infertile; it is possible to store sperm before treatment for later use for IVF, and men should be informed of this before undergoing treatment. As such, surgery carries a high-risk of long-term side-effects that will likely impact on quality of life, for example through impacts on men's sex life and relationships with partners.

Radical radiotherapy and brachytherapy

Radiotherapy (often referred to as external beam radiotherapy, EBRT) can be used to cure localised prostate cancer or to slow the progression of more advanced cancer. However, the likelihood of recurrence after EBRT treatment is 1 in 3. The ProtecT trial[28] found reduced rates of disease progression for patients who underwent radiotherapy compared with active surveillance but this did not translate into an overall survival benefit. As with RP, radiotherapy is associated with urinary incontinence and erectile dysfunction. However, the occurrence of these side effects is lower than for RP, for example only 42% of patients reporting erectile dysfunction 6 months after surgery (compared to 88% of RP patients)[29].

Brachytherapy is a type of radiotherapy, but instead of an external radiation source, radioactive beads are implanted into the prostate to irradiate the tumour from within. It can be given alone or in combination with EBRT and/or ADT and does not exclude the possibility of future surgery, although subsequent surgery is rare. It comes in two different forms: low dose rate (LDR) permanent source implantation or high dose rate (HDR) temporary source implantation. Both of these treatment are appropriate first-line monotherapies for low and medium risk disease[98]. Brachytherapy has comparable outcomes to other treatment options and is associated with better quality of life compared to those who undergo surgery[98]. It can also be used in combination with external beam radiotherapy (EBRT) to treat intermediate and high risk disease. Both types of brachytherapy have comparable outcomes but each has their own set of advantages. For example, HDR-BT has low operator dependence and typically produces fewer irritative symptoms while LDR-BT has lower initial equipment costs and can be completed in a single implant[98].

Brachytherapy may also be applicable in some cases of biochemical recurrence after initial treatment with EBRT. However, its application is limited ideally to cases of local disease only, with no evidence of metastases, where more than 4.5 years has elapsed since initial radiotherapy and there were no toxic effects associated with the earlier treatment[98].

Active surveillance

Active surveillance (AS) is the process of monitoring prostate cancer without making any radical intervention to treat the cancer. This is possible in some low-risk cases of prostate cancer because prostate cancer is often indolent (slow growing) and may not produce any symptoms for a long time. The introduction of PSA testing caused an increase in the incidence of prostate cancer[73] with many men experiencing no symptoms at the time of diagnosis. While early detection is extremely valuable in cases of aggressive cancer, it can have detrimental effects in cases of indolent disease as it can lead to treatment that may ultimately be unnecessary. Active surveillance avoids the harm associated with overtreatment, avoiding the severe side-effects - including incontinence and erectile dysfunction – that can be caused by radical prostate cancer treatments.

It is estimated that almost half of patients diagnosed with prostate cancer are eligible for active surveillance programmes at the time of their diagnosis[43]. This is because their cancer has favourable prognostic indicators such as low Gleason score and low serum PSA levels. The clinical trials PIVOT and ProtecT report the outcomes of patients treated with active surveillance compared to radical prostatectomy (PIVOT[96]) or compared to radical prostatectomy or radiotherapy (ProtecT[28]). While radical treatment reduced rates of disease progression, neither study found a significant difference in overall survival between radical treatment interventions and active surveillance. The interpretation of results from the PIVOT trial are limited due to the older demographic of patients enrolled in this trial. However, AS may be more beneficial in older patients likely to have more co-morbidities and lower life-expectancy than their younger counterparts and so if their disease is asymptomatic it is more likely to remain that way until death, usually from causes other than prostate cancer.

There are still some hurdles to be overcome in the use of active surveillance. A consensus is needed on how best to select patients that are suitable for AS from those at risk of more aggressive, metastatic disease who require immediate treatment intervention. At present there is no common criteria used to select patients for active surveillance. Criteria for inclusion can include: a tumour stage of T1c or T2; serum PSA levels < 10 ng ml^{-1}; Gleason score ≤6; and, 2 or fewer cancer positive core biopsy samples and/or a maximum of 50% of tumour material per core sample[43]. This process would benefit from the identification of molecular biomarkers that can be used to stratify patients into different subtypes, and in particular to identify those patients most at risk of metastatic disease (and therefore not suitable for AS). Several possible genetic markers are being investigated (e.g. BRCA2). Genetic markers are likely to be the best tool for risk assessment but research is also looking to validate blood based assays (e.g. CAV-1, MYC, CTCs) that could provide genetic or similar information with a minimally invasive test[43].

Movember's Global Action Plan Prostate Cancer Active Surveillance Initiative (GAP3) database is the largest global prostate cancer active surveillance database. Data from GAP3 indicates that around 43% of men drop out of active surveillance protocols in the first five years[92]. This is mainly due to signs of disease progression triggering treatment intervention, although a small proportion progressed to treatment intervention despite no signs of disease progression[92]. This highlights the need for improved patient stratification to identify men whose cancer is likely to progress as needing treatment at the time of diagnosis. Men progressing to treatment despite no signs of disease progression suggests anxiety or lack of trust in current stratification protocols (on the part of the clinician and/or the patient). This group is likely to be reduced by more robust stratification procedures and may also benefit from mental health support for men on active surveillance.

There is also a need for further investigation into the best monitoring programmes that are minimally disruptive to the patient but sufficient to identify disease progression[43]. Many tools can be used for this including regular PSA testing and DRE as well as the potential use of more novel tools such as mpMRI (see: diagnosis).

TREATMENT OPTIONS FOR METASTATIC PROSTATE CANCER

Androgen deprivation therapy

The synthesis and action of androgens has been a key target in the treatment of prostate cancer since androgen dependence was discovered. Hormone therapy is used to treat locally

advanced prostate cancer and in combination to treat advanced disease. The aim is to deprive the tumour of androgens (hence, androgen deprivation therapy, ADT) and therefore slow or halt the progression of the cancer. This may control the cancer but it is not curative. Hormone therapy can take the form of orchiectomy (removal of the testes), drugs that block the release of androgens (e.g. goserelin (Zoladex), degarelix (Firmagon)) or drugs that interfere with the action of androgens (e.g. bicalutamide (Casodex))[64]. Primary ADT causes erectile dysfunction in as many as 85% of patients and is also associated with systemic side effects, such as osteoporosis[21].

Furthermore, most patients will eventually develop resistance to these traditional hormone therapy agents. This takes different amounts of time for different people but always eventually results in what is termed castration-resistant prostate cancer (CRPC). Second generation ADT drugs have been developed to treat castration-resistant disease: abiraterone (Zytiga), an inhibitor of CYP17A1, an enzyme involved in androgen production; and enzalutamide (Xtandi), an antagonist of the AR. These drugs can be effective in patients who have developed resistance to other types of hormone therapy. Enzalutamide showed improved overall survival in patients with mCRPC who had previously been treated with docetaxel (AFFIRM[74]) and in minimally symptomatic or asymptomatic mCRPC that had not been previously treated with chemotherapy (PREVAIL[10]). Abiraterone has also been shown to prolong overall survival in both of these settings[14, 69]. The addition of abiraterone to traditional ADT along with prednisone has shown improved overall survival and radiographic progression free survival in patients with newly diagnosed, high risk metastatic castration-sensitive cancer (LATITUDE[22]). This treatment combination has also shown improved overall survival and failure-free survival in men with locally advanced or metastatic prostate cancer (STAMPEDE[37]).

These drugs have clinical use as treatments for metastatic CRPC. However, as with traditional ADT, their use is limited by the development of resistance to these agents. This limitation in our ability to treat mCRPC is the focus of much research in the field: to improve our understanding of how resistance develops and to develop new treatments that circumvent these resistance mechanisms (see: androgen resistance).

Chemotherapy

Chemotherapy uses cytotoxic drugs to kill cancerous cells. These drugs often target rapidly dividing cells, for example by acting at various points during cell division. This means they are effective against cancer cells but can also kill dividing healthy cells, such as hair, bone marrow and epithelial cells leading to, sometimes severe, side-effects. The most common chemotherapy drug used in the treatment of prostate cancer is docetaxel (Taxotere). It brings about cytotoxic effects by binding to microtubules and blocking depolymerisation, thus arresting the cell in metaphase of mitosis, preventing further cell division and inhibiting cancerous growth[85]. In 2015, the clinical trial CHAARTED[44] showed that the addition of docetaxel to ADT improved overall survival in patients with high-volume, hormone-naïve metastatic prostate cancer compared to ADT alone[44], STAMPEDE[37] also confirmed that addition of docetaxel to the standard of care (first-line hormone therapy) increased overall survival, although this benefit was accompanied by an increased likelihood of adverse events (e.g. neutropenia, endocrine disorder, gastrointestinal disorder)[37]. It has also been shown that the addition of a course of docetaxel chemotherapy at the beginning of ADT produces longer survival for patients with metastatic prostate cancer[83].

Focal therapy

An emerging option for the treatment of local disease are focal therapies. Focal therapy is the term used for highly focused, high energy treatments that target only the cancerous area in the prostate. Focal therapy should leave healthy cells intact and there is evidence that these treatments have reduced risk of side-effects[3].

Treatment	Explanation	Stage	
High-intensity focused ultrasound	Using highly-focused ultrasound to heat the specific tumour area causing tumour necrosis.	Available in the UK only as part of a clinical trial.	Biochemical disease free survival at 2 years 83% for low risk disease[35].
Cryotherapy	Using extreme cold to disrupt cell membrane causing necrosis and vascular thrombosis.	Available in the UK only as part of a clinical trial.	75% of low risk patients had negative biopsies at 3.7 year follow up. Continence and potency observed in 100% and 86% of patients respectively[90].
Targeted radiotherapy	Radiation delivered directly to tumour site.	Brachytherapy available (see above) PSMA-guided radiotherapeutics in clinical trials (see below).	Trials for delivery mechanisms ongoing (see below).

Figure 8. The emerging types of focal therapy for prostate cancer (Eggener et al., 2007)[19].

Focal therapy for oligometastatic disease can be PSMA-guided, delivering the agent directly to its target: prostate cancer cells. Oligometastatic disease is used to describe a disease state between local cancer and widespread metastatic disease. Focal treatment of this type of cancer may have long-term benefits and may even be curative. PSMA-PET scanning shows promise as a tool for improving the staging of (oligo)metastatic disease and therefore enabling the empirical use of focal therapy to delay the use of systemic therapy[68]. Delaying systemic therapy in favour of focal therapy reduces the risk of side-effects and delays the development of castration-resistant disease meaning men can be treated effectively for longer. PSMA-targeted radiotherapeutics have also shown potential to treat metastatic castration resistant prostate cancer (mCRPC). One such agent, 177Lu-PSMA-617, is at various trial stages including a phase III trial for treating mCRPC in combination with standard of care treatments (VISION[86]) and a phase I trial in combination with immunotherapy agent pembrolizumab[87].

Immunotherapy

This type of treatment aims to harness the power of the immune system to treat cancer. Immunotherapies have produced very promising results in other forms of cancer and as such

there has been much interest in immunotherapy for prostate cancer. However, many trials have had disappointing results, likely due to the immunosuppressive microenvironment of prostate cancer tumours[48]. However, prostate cancer survival is correlated with the types and distribution of infiltrating lymphocytes. TGF-β levels are correlated with high Gleason score and higher pathologic tumour stage[66]. These points indicate that immunotherapy has potential for being an effective treatment for prostate cancer. Prostate cancer is also an attractive target for immunotherapy in other ways: prostate cancer cells express many tumour specific antigens and its generally slow growth allows time for immunotherapy to have an effect[48, 94].

At present, there is only one approved immunotherapy for prostate cancer: Sipuleucel-T (Provenge). This was the first vaccine approved as a cancer treatment. Sipuleucel-T is an autologous vaccine meaning it is highly personalised and is made using cells from the patient. T-cells are taken from the patient and incubated with prostatic acid phosphatase (PAP, a protein expressed on prostate cancer cells) before being reinfused into the patient to produce a PAP-specific T-cell response[48]. Phase III clinical trials showed that it has efficacy in treating mCRPC compared to placebo with an approximately 4 month improvement in median survival[41]. Despite this demonstrated efficacy, Sipuleucel-T is not widely used due to the high level resources required for its use and a lack of cost-effectiveness[32]. Sipuleucel-T is currently licensed in the U.S. was approved in Europe but has since been withdrawn for commercial reasons. This also makes it a less favoured treatment option as many clinicians have no or very limited experience of prescribing Sipuleucel-T.

Other immunotherapy agents are in development for the treatment of prostate cancer. For example, PROSTVAC-VF, a viral vector vaccine, showed promising results for treating minimally symptomatic mCRPC prolonging overall survival in phase II studies. However, one phase III study was prematurely ended due to futility (no benefit to overall survival indicated)[27] although there are others with results still pending[88]. Another area of interest is immune checkpoint inhibitors. Ipilimumab, an antibody that blocks CTLA-4 receptors, preventing immune suppression, showed benefit over placebo for progression free survival although no benefit to overall survival was observed. Analysis indicates a greater benefit for patients with better prognostic indicators providing yet more evidence for the need to accurately stratify prostate cancer patients by disease risk.

Targeting specific genetic aberrations

PTEN-targeted therapies are in development for the treatment of prostate cancer. PI3K-AKT-mTOR pathway inhibitors showed initial promise but have limited clinical efficacy as single agents for treating CRPC among unselected patients. Clinical efficacy may be achievable with AKT inhibitors in patients selected based on evidence of PTEN loss. Pan-PI3K inhibitors are toxic due to their off-target effects. Isoform specific inhibitors may mitigate these toxic effects while maintaining clinical benefit and therefore could have a role in prostate cancer treatment[46]. The recent discovery of PTEN-long, an alternative form of PTEN that is cell membrane permeable, secreted by cells and can enter other cells, has sparked investigations into the therapeutic potential of this compound[33]. PTEN-long antagonises PI3K signalling and induces tumour cell death in vitro and in vivo. Further studies are needed to establish the clinical potential of exogenous PTEN-long[33].

One possible treatment for prostate cancer that has shown promise in the treatment of other cancers is poly(ADP) ribose polymerase (PARP) inhibitors. PARP enzymes are involved in transcriptional regulation and the detection and recruitment of other proteins to sites of DNA

damage. Inhibition of PARP enzymes prevents the repair of single stranded DNA breaks. These single-stranded breaks progress to double-stranded breaks. In cells lacking HR (e.g. BRCA2 mutants) these double stranded breaks lead to mutation and are often lethal[15]. PARP protein accumulation at replication forks may also trigger apoptosis and therefore have a direct cytotoxic effect[15]. PARP inhibitors may be effective in combination with ADT. ADT causes increased expression of PARP proteins and so inhibition of these proteins may cause synthetic lethality (when the simultaneous dysfunction of two genes is lethal where either deficiency alone would not be)[9]. This has been demonstrated in preclinical models but further study is needed to establish whether it will translate into an effective treatment for men.

Olaparib (Lynparza) is an example of a PARP inhibitor; it has achieved some positive results in clinical trials. In a small phase II trial partial responses were achieved in about half of patients carrying BRCA2 mutations. Previous treatment with platinum based chemotherapy seemed to correlate with a lack of response and so resistance to these agents may confer some cross-resistance to PARP inhibitors. TOPARP-A, a phase II study of Olaparib in treating metastatic prostate cancer in unselected patients, showed that Olaparib had antitumour activity strongly associated with mutations in DNA damage repair genes. This included responses in all 7 patients with BRCA2 loss and responses in patients with aberrations in other DDR genes (e.g. ATM)[49]. Following this success TOPARP-B investigated Olaparib in the treatment of a pre-selected group of metastatic prostate cancer patients with mutations in DDR genes. In this scenario Olaparib was found to improve progression free survival in heavily pre-treated patients (who had progressed on ADT and were post-docetaxel treatment). BRCA1/2 aberrations were most sensitive but responses were also achieved in patients with other DDR aberrations[50]. Combination trials are also being evaluated, for example DNA damaging agents are likely to have complementary activity to PARP inhibitors and their synergistic action is likely to produce greater efficacy than either treatment alone.

Agents targeting WNT signalling are in the early stages of trials to treat a number of cancers. In the future, these agents may well prove useful in selected prostate cancer cases. There are a range of points in the pathway that can be targeted. Porcupine – a membrane-bound-O-acetyltransferase that is essential for WNT protein secretion – inhibitors can prevent WNT secretion[53]. Porcupine inhibitors have been shown to be effective against tumours in mice. LGK974, an orally available inhibitor, entered phase I trials in patients with RNF43, ZNRF3 or RSPO2 fusions. The early data indicates a tolerable safety profile and potential antitumour activity[39]. The WNT receptor WNT-5a can be antagonised by antibodies which has been shown to reduce metastasis of gastric cancer cells in vivo[31]. A phase Ia study of OMP-54F28, a decoy WNT receptor, in patients with advanced solid tumours find it to be both safe and efficacious with 2 of the 26 patients maintaining stable disease for more than 6 months[40]. Phase Ib studies are now underway in ovarian, pancreatic and hepatic cancer. This drug or others with similar mechanisms may be applicable to selected prostate cancer cases but clinical trials are needed to assess the efficacy of such drugs in prostate cancer. FZD7, a frequently upregulated WNT receptor in cancer, has also been targeted with antibodies and decoy receptors[53]. These agents produce reduced cancer stem cell (CSC) frequency and induce cell differentiation to less tumorigenic cell types that are more susceptible to conventional chemotherapy.

Other potential targets in this pathway include inhibiting signal transduction or promoting β-catenin degradation with the use of PARP inhibitors to stabilise the destruction complex, thereby inhibiting WNT signalling[53]. Some endogenous WNT antagonists are epigenetically silenced in cancer. Therefore, drugs aimed at inhibiting epigenetic machinery, such as histone deacetylase (HDAC) inhibitors or DNA methyltransferase (DNMT) inhibitors may also indirectly

reactivate WNT signalling. By reactivating silenced genes (e.g. SFRP1) inhibition of growth and initiation of apoptosis may be achieved in cancerous cells[53]. These, as with all other agents targeting WNT signalling, require further testing in preclinical prostate cancer models and in selected prostate cancer patients to ascertain their safety and efficacy.

Our increasing knowledge of the disease and in particular the development of castration resistance also provides opportunities for novel drug development. To target ARVs (as well as AR), the aim is to develop drugs that bind to the N-terminal transactivation domain (NTD) or the DNA binding domain (DBD) rather than the LBD which is lacking in these variants. Small molecule inhibitors that bind to these domains of the protein are already under investigation. Small molecule inhibitors of both the DBD[13] and NTD[4] show promise in CRPC preclinical models. It could also be possible to prevent resistance caused by AR overexpression with drugs that promote degradation of AR (AR degraders). One such AR degrader produced PSA declines in 13% of CRPC patients in early clinical trials. However, the trial was halted due to gastrointestinal toxicities[95].

Bone targeted agents

Bone is a frequent site of prostate cancer metastasis and SREs can cause significant pain, reducing quality of life. There are currently two treatments available to delay the occurrence of SREs: zoledronic acid (Zometa) - a bisphosphonate to strengthen bone; or Denosumab (Prolia) - a monoclonal antibody targeting RANKL to reduce osteoblastic breakdown of bone. Both of these treatments delay the occurrence of SREs[24, 70 79] .

Due to the association between metastasis and mortality, there is also some interest in more novel treatments targeting bone. Studies have revealed an association between the Src family of kinases (SFK) and prostate cancer progression. As such, inhibitors of these kinases have been investigated as potential treatments. One such inhibitor is Dasatinib (Sprycel), which has entered several clinical trials for mCRPC. In a phase II trial, Dasatinib as a single agent showed favourable safety and efficacy with 44% of patients experiencing no disease progression over the trial period[97]. However, in phase III trials in combination with docetaxel[8] and abiraterone[17], Dasatinib showed no benefit compared to placebo when used in combination with docetaxel and no benefit when used in combination with abiraterone compared to abiraterone alone. The abiraterone trial acknowledges it had a limited cohort and that positive trends were observed in some patients that did not reach statistical significance[17]. Further study into this combination may therefore be warranted.

The SRC signalling pathway has been shown to be enriched in BRCA2-altered tumours. Early evidence from studies in prostate cancer cell lines harbouring BRCA2 aberrations suggests that the combined use of SRC inhibitors and PARP inhibitors is more effective at inhibiting cell growth than either treatment alone[42]. This suggests this combination has great potential for testing in clinical trials.

CONCLUSION

There are many promising developments occurring in the field of prostate cancer. This is an exciting and critical stage to be participating in research. While treatment for local disease can be curative, the current treatments for advanced disease can control disease only for a limited time. Much research is focussed on finding ways to prevent or treat castration-resistant advanced disease. This will have the greatest impact on prostate cancer mortality. Other areas

are also evolving to fit with our increasing knowledge of this disease. The increasing availability of better imaging and the emerging biomarkers for prostate cancer are likely to transform how the disease is diagnosed and reduce overtreatment. Improved patient stratification based on the prognostic significance of biomarkers will enable more informed decision making when selecting the treatment that is right for the individual. Many emerging treatments are effective in only a subset of patients with specific genetic aberrations in their tumours. This likely means that the future of treating prostate cancer lies in personalise medicine, where each patient's treatment is tailored to treat his specific tumour.

References

1. Abdollah, F. et al., (2012). 'Survival benefit of radical prostatectomy in patients with localised prostate cancer: estimations of the number needed to treat according to tumour and patient characteristics.' *The Journal of Urology,* vol. 188, issue 1, pp 73-83.
2. Ahmed, H. et al., (2012). 'Focal therapy for localised unifocal and multifocal prostate cancer: a prospective development study.' *The Lancet Oncology,* vol. 13, issue 6, pp 622-632.
3. Ahmed, H. et al., (2017). 'Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study.' *The Lancet,* vol. 389, issues 10,071, pp 815-822.
4. Andersen, R. et al., (2015). 'Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor.' *Cancer Cell,* vol. 17, issue 6, pp 535-546.
5. Andriole, G. et al., (2009). 'Mortality results from a randomized prostate-cancer screening trial.' *The New England Journal of Medicine,* vol. 360, pp 1310–1319.
6. Antonarakis, E. S. et al., (2014). 'AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer.' *The New England Journal of Medicine,* vol. 371. Pp 1028-1038.
7. Apurich, M. et al., (2011). 'Contemporary role of prostate cancer antigen 3 in the management of prostate cancer.' *European Urology,* vol. 60, issue 5, pp 1045-1054.
8. Araujo, J. et al., (2013). 'Docetaxel and dasatinib or placebo in men with metastatic castration-resistant prostate cancer (READY): a randomised, double-blind phase 3 trial.' *The Lancet Oncology,* vol. 14, issue 13, pp 1307-1316.
9. Asim, M. et al., (2017). 'Synthetic lethality between androgen receptor signalling and the PARP pathway in prostate cancer.' *Nature Communications,* vol. 8, article number 374.
10. Beer, T. et al., (2014). 'Enzalutamide in metastatic prostate cancer before chemotherapy.' *The New England Journal of Medicine,* vol. 371, pp 424-433.
11. British Association of Urological surgeons (BAUS, 2017). *Transrectal ultrasound-guided biopsies of the prostate gland.* [PDF]. Available at: https://www.baus.org.uk/_userfiles/pages/files/Patients/Leaflets/TRUSP%20biopsies.pdf . Accessed on: 21/08/19.
12. Cancer Research UK (CRUK, n.d.). *Prostate cancer statistics.* [Website]. Available at: https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/prostate-cancer#heading-Two. Accessed on: 22/08/19.
13. Dalal, K. et al., (2014). 'Selectively targeting the DNA-binding domain of the androgen receptor as a prospective therapy for prostate cancer.' *Journal of Biological Chemistry,* vol. 289, issue 38, pp 26417-26429.
14. De Bono, J. S. et al., (2011). 'Abiraterone and increased survival in metastatic prostate cancer.' *The New England Journal of Medicine,* vol. 364, pp 1995-2005.

15. Mateo, J. et al., (2016). ‘DNA repair in prostate cancer: biology and clinical implications.’ *European Urology,* vol. 71, pp 417-425.
16. De Rooij, N. et al., (2014). ‘Cost-effectiveness of magnetic resonance imaging and MR-guided targeted biopsy versus systematic transrectal ultrasound-guided biopsy in diagnosing prostate cancer: A modelling study from a health care perspective.’ *European Urology,* vol. 66, issue 3, pp 430-436.
17. Dorff, T. et al., (2019). ‘Randomised phase II trial of abiraterone alone or with Dasatinib in men with metastatic castration-resistant prostate cancer (mCRPC).’ *Clinical Genitourinary Cancer,* vol. 17, issue 4, pp 241-247.
18. Edwards, C. et al., (2017). ‘Tumour-derived alkaline phosphatase regulates tumour growth, epithelial plasticity and disease-free survival in metastatic prostate cancer.’ *British Journal of Cancer,* vol. 116, pp227-236.
19. Eggener, S. et al., (2007). ‘Focal therapy for localised prostate cancer: a critical appraisal of rationale and modalities.’ *The Journal of Urology,* vol. 178, issue 6, pp 2260-2267.
20. Elster, N. (2019). *Your quick guide to PSA tests.* [Website] Available at: https://pcrc.alchemy.digital/psa-tests-guide/. Accessed on: 12/08/19.
21. Fenton, J. et al., (2018). *Prostate-specific antigen-based screening for prostate cancer: a systematic evidence review for the U.S. Preventive Services Task Force.* [Book] Rockville, MD: Agency for Healthcare Research and Quality. Available at: https://www.ncbi.nlm.nih.gov/books/NBK518890/. Accessed on: 22/08/19.
22. Fizazi, K. (2019). ‘Abiraterone plus prednisone in patients with newly diagnosed high-risk metastatic castration-sensitive prostate cancer (LATITUDE): final overall survival analysis of a randomised, double blind, phase 3 trial.’ *The Lancet Oncology,* vol. 20, issue 5, pp 686-700.
23. Jin, JK. et al., (2011). ‘Steps in prostate cancer progression that lead to bone metastasis.’ *International Journal of Cancer,* vol. 128, issue 11, pp 2545-2561.
24. Gillessen, S. et al., (2015). ‘Management of patients with advanced prostate cancer: recommendations of the St Gallen advanced prostate cancer consensus conference (APCCC) 2015.’ *Annals of Oncology,* vol. 26, pp 1589-1604.
25. Global Cancer Observatory, International Agency for Research on Cancer, WHO (GLOBOCAN, 2018[a]). *Prostate cancer fact sheet.* [PDF]. Available at: https://gco.iarc.fr/today/fact-sheets-cancers. Accessed on: 12/07/2019
26. Global Cancer Observatory, International Agency for Research on Cancer, WHO (GLOBOCAN, 2018[b]). ‘*United Kingdom fact sheet.’* [PDF file]. Available at: http://gco.iarc.fr/today/fact-sheets-populations. Accessed on: 12/07/2019
27. Gulley, J. et al., (2019). ‘Phase III trial of PROSTVAC in asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer.’ *Journal of Clinical Oncology,* vol. 37, issue 13, pp 1051-1061.
28. Hamdy, F. et al., (2016). ’10-year outcomes after monitoring, surgery, or radiotherapy for localised prostate cancer.’ *The New England Journal of Medicine,* vol. 374, pp 1415-1424.
29. Heijnsdijk, E. et al., (2012). ‘Quality-of-life effects of prostate-specific antigen screening.’ *The New England Journal of Medicine,* vol. 367, pp 595-605.
30. Hessels, D. & Schalken, J. (2009). ‘The use of PCA3 in the diagnosis of prostate cancer.’ *Nature Reviews Urology,* vol. 6, pp 255-261.
31. Hideaki, H. et al., (2012). ‘An anti-Wnt5a antibody suppresses metastasis of gastric cancer cells in vivo by inhibiting receptor-mediated endocytosis.’ *Molecular Cancer Therapeutics,* vol. 11, issue 2, pp 298-307.

32. Holko, P. & Kawalec, P. (2014). ‘Economic evaluation of Sipuleucel-T immunotherapy in castration-resistant prostate cancer.’ *Expert Review of Anticancer Therapy,* vol. 14. Issue 1, pp 63-73.
33. Hopkins, B. et al., (2013). ‘A secreted PTEN phosphatase that enters cells to alter signalling and survival.’ *Science,* vol. 341, issue 6144, pp 399-402.
34. Hopkins, B. et al., (2014). ‘PTEN function: the long and the short of it.’ *Trends in Biochemical Sciences,* vol. 39, issue 4, pp 183-190.
35. Horie, S. et al., (2008). ‘Focal therapy with high-intensity-focused ultrasound in the treatment of localised prostate cancer.’ *Japanese Journal of Clinical Oncology,* vol. 38, issue 3, pp 192-199.
36. Huggins, C. & Hodges, C. (1941). ‘The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate.’ Vol. 1, issue 4, pp 293-297.
37. James, N. et al., (2016). ‘Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial.’ *The Lancet,* vol. 387, pp 1163-1177.
38. James, N. et al., (2017). ‘Abiraterone for prostate cancer not previously treated with hormone therapy.’ *The New England Journal of Medicine,* vol. 377, pp 338-351.
39. Janku, F. et al., (2015). ‘Phase I study of WNT974, a first-in-class Porcupine inhibitor, in advanced solid tumours.’ [Abstract]. *AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics*, abstract C45.
40. Jimeno, A. et al., (2017). ‘A first-in-human phase 1 study of anticancer stem cell agent OMP-54F28 (FZD8-Fc), decoy receptor for WNT ligands in patients with advanced solid tumours.’ *Clinical Cancer Research,* vol. 23, issue 24, pp 7490-7497.
41. Kantoff, P. et al., (2010). ‘Sipuleucel-T immunotherapy for castration-resistant prostate cancer.’ *The New England Journal of Medicine,* vol. 363, pp 411-422.
42. Khan, N. et al., (2019). ‘Combination treatment of PARP and SRC inhibitors in BRCA2 mutated prostate cancer.’ [Abstract]. *AACR annual meeting proceedings 2019,* vol. 79, issue 13, abstract number 283.
43. Kim, J. et al., (2019). ‘Active surveillance for prostate and thyroid cancers: evolution in clinical paradigms and lessons learned.’ *Nature Reviews Clinical Oncology,* vol. 16, pp 168-184.
44. Kyriakopoulos, C. et al., (2018). ‘Chemohormonal therapy in metastatic hormone-sensitive prostate cancer: long-term survval analysis of the randomised phase 3 E3805 CHAARTED trial.’ *Journal of Clinical Oncology,* vol. 36, issue 11, pp 1080-1087.
45. Loeb, S. et al., (2014). ‘Overdiagnosis and overtreatment of prostate cancer.’ *European Urology,* vo. 65, issue 6, pp 1046-1055.
46. Jamaspishvili, T. et al., (2018). ‘Clinical implications of PTEN loss in prostate cancer.’ *Nature Reviews Urology,* vol. 15, pp 222-234.
47. Luo, J. et al., (2018). ‘Role of the androgen receptor variants in prostate cancer: report from the 2017 mission androgen receptor variants meeting.’ *European Urology,* vol. 73, issue 5, pp 715-723.
48. Maio, M. & Hansen, A. (2017). ‘A comprehensive review of immunotherapies in prostate cancer.’ *Critical Reviews in Oncology/Hematology,* vol. 113, pp 292-303.
49. Mateo, J. et al., (2015). ‘DNA-repair defects and Olaparib in metastatic prostate cancer.’ *The New England Journal of Medicine,* vol. 373, pp 1697-1708.
50. Mateo, J. et al., (2019). ‘TOPARP-B: a phase II randomised trial of the poly(ADP)-ribose polymerase (PARP) inhibitor Olaparib for metastatic castration resistant prostate

cancers (mCRPC) with DNA damage repair (DDR) alterations.' *Journal of Clinical Oncology,* vol. 37, issue 15, pp 5005.
51. McKenna, D. et al., (2016). 'Androgen deprivation in LNCaP prostate tumour xenografts induces vascular changes and hypoxic stress, resulting in promotion of epithelial-to-mesenchymal transition.' *British Journal of Cancer,* vol. 114, pp 659-668.
52. Mottet, N. et al., (2018). *EAU – ESTRO – SEUR –SIOG guidelines on prostate cancer.* [PDF] Available at: https://uroweb.org/guideline/prostate-cancer/. Accessed on: 30/07/19.
53. Murillo-Garzon, V. & Kypta, R. (2017). 'WNT signalling in prostate cancer.' *Nature Reviews Urology,* vol. 14, pp 683-696.
54. National Cancer Institute (2015). *Cancer Staging.* [Website]. Available at: https://www.cancer.gov/about-cancer/diagnosis-staging/staging#targetText=The%20TNM%20Staging%20System,widely%20used%20cancer%20staging%20system.&targetText=The%20N%20refers%20to%20the,other%20parts%20of%20the%20body. Accessed on: 29/08/19.
55. National Cancer Institute (n.d.). *NCI Dictionary of Cancer Terms: digital rectal examination.* [Website]. Available at: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/digital-rectal-examination. Accessed on: 29/08/19.
56. National Health Service (2018). *PSA testing.* [Website] Available at: https://www.nhs.uk/conditions/prostate-cancer/psa-testing/. Accessed on: 30/07/19.
57. National Institute for Health and Care Excellence (2019). *Prostate cancer: diagnosis and management.* [Website]. Accessed on: 13/08/19.
58. National Prostate Cancer Audit (2019). *Annual report 2018.* [PDF]. Available at: https://www.npca.org.uk/reports/npca-annual-report-2018/. Accessed on: 21/08/19.
59. Paterlini-Brechot, P. & Benali, N. (2007). 'Circulating tumour cells (CTC) detection: clinical impact and future directions.' *Cancer letters,* vol. 253, issue 2, pp 180-204.
60. Pearson, H. et al., (2018). 'Identification of Pik3ca mutation as a genetic driver of prostate cancer that cooperates with *Pten* loss to accelerate progression and castration-resistant growth.' *Cancer Discovery,* vol. 8, issue 6, pp 764-779.
61. Perner, S. et al., (2007). 'Prostate-specific membrane antigen expression as a predictor of prostate cancer progression.' *Human Pathology,* vol. 38, issue 5, pp 696-701.
62. Pinsky, P. et al., (2017). 'Extended mortality results for prostate cancer screening in the PLCO trial with median follow-up of 15 years.' *Cancer,* vol. 123, issue 4, pp 592-599.
63. Prostate cancer UK (2017). *Prostate Cancer UK mpMRI before biopsy Freedom of Information request data.* [Website]. Available at: https://public.tableau.com/profile/ali.cooper#!/vizhome/mpMRIFOIpublicdashboard-ProstateCancerUK_0/FullresultsStory. Accessed on: 21/08/19.multiparametric
64. Prostate Cancer UK (PCUK, n.d.). *Hormone therapy.* [Website]. Available at: https://prostatecanceruk.org/prostate-information/treatments/hormone-therapy. Accessed on: 29/08/19.
65. Public Health England (2016). *PSA testing and prostate cancer: advice for well men aged 50 and over.* [PDF] Available at: https:/%/www.gov.uk/government/publications/prostate-specific-antigen-testing-description-in-brief. Accessed on: 12/08/19.
66. Reis et al., (2011). 'Tgf-β1 expression as a biomarker of poor prognosis in prostate cancer.' *Clinics,* vol. 66, issue 7.
67. Robinson, D. et al., (2015). 'Integrative clinical genomics of advanced prostate cancer.' *Cell,* vol. 161, pp 1215-1228.

68. Rowe, S. et al., (2016). ‘PET imaging of prostate-specific membrane antigen in prostate cancer: current state of the art and future challenges.’ *Prostate cancer and prostatic diseases,* vol. 19, pp 223-230.
69. Ryan, C. et al., (2013). ‘Abiraterone in metastatic prostate cancer without previous chemotherapy.’ *The New England Journal of Medicine,* vol. 368, pp 138-148.
70. Saad, F. et al., (2004). ‘Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer.’ *Journal of the National Cancer Institute,* vol. 95, issue 11, pp 879-882.
71. Saini, S. (2016). ‘PSA and beyond: alternative prostate cancer biomarkers.’ *Cellular Oncology,* vol. 39, issue 2, pp 97-106.
72. Sartor, O. & de Bono, J. S. (2018). ‘Metastatic prostate cancer.’ *The New England Journal of Medicine,* vol. 378, pp 645-657.
73. Sathianathen, N. et al., (2018). ‘Landmarks in prostate cancer.’ *Nature Reviews Urology,* vol. 15, pp 627-642.
74. Scher, H. et al., (2012). ‘Increased survival with enzalutamide in prostate cancer after chemotherapy.’ *The New England Journal of Medicine,* vol. 367, pp 1187-1197.
75. Schroder, F. et al., (2009). ‘Screening and prostate-cancer mortality in a randomized European study.’ *The New England Journal of Medicine,* vol. 360, pp 1320–1328.
76. Schroder, F. et al., (2012). ‘Screening for prostate cancer decreases the risk of developing metastatic disease: findings from the European Randomized Study of Screening for Prostate Cancer (ERSPC).’ *European Urology,* vol. 62, pp 745–752.
77. Schroder, F. et al., (2014). ‘Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up.’ *Lancet,* vol. 384, pp 2027–2035.
78. Shukla et al., (2015). ‘Evaluation of the current prostate cancer staging system based on cancer-specific mortality in the surveillance epidemiology and end results database.’ *Clinical genitourinary cancer,* vol. 13, issue 1, pp 17-21.
79. Smith, M. et al., (2012). ‘Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial.’ *The Lancet,* vol. 379, issue, 9810, pp 39-46.
80. Sramkoski, D. et al., (1999). ‘A new human prostate carcinoma cell line, 22Rv1.’ *In Vitro cellular & developmental biology, Animal,* vol. 35, issue 7, pp 403-409.
81. Stabile, A. et al., (2019). ‘Multiparametric MRI for prostate cancer diagnosis: current status and future directions.’ *Nature Reviews Urology.*
82. Sweat, S. et al., (1998). ‘Prostate-specific membrane antigen expression is greatest in prostate adenocarcinoma and lymph node metastases.’ *Urology,* vol. 52, vol. 4, pp 637-640.
83. Sweeney, C. (2015). ‘Chemohormonal therapy in metastatic hormone-sensitive prostate cancer.’ *The New England Journal of Medicine,* vol. 373, pp737-746.
84. Tan, M. et al., (2015). ‘Androgen receptor: structure, role in prostate cancer and drug discovery.’ *Act Pharmacologica Sinica,* vol. 36, pp 3-23.
85. Thirumaran, R. et al., (2007). *Cancer immunotherapy: Chapter 7 – Cytotoxic chemotherapy in clinical treatment of cancer.* Elsevier Inc: Amsterdam, Netherlands.
86. U.S. National Library of Medicine: ClinicalTrials.gov (2018). Identifier: NCT03511664. ‘Study of 177Lu-PSMA-617 in Metastatic Castrate-Resistant Prostate Cancer (VISION).’ Available at: https://clinicaltrials.gov/ct2/show/NCT03511664. Accessed on: 21/08/19.
87. U.S. National Library of Medicine: ClinicalTrials.gov (2019). Identifier: NCT03805594. ‘177Lu-PSMA-617 and Pembrolizumab in Treating Patients with Metastatic Castration-Resistant Prostate Cancer.’ Available at: https://clinicaltrials.gov/ct2/show/NCT03805594. Accessed on: 21/08/19.

88. U.S. National Library of Medicine: ClinicalTrials.gov (2019). Identifier: NCT01322490. 'A randomised, double-blind, phase 3 efficacy trial of PROSTVAC-V/F +/- GM-CSF in men with asymptomatic or minimally symptomatic metastatic castrate-resistant prostate cancer (Prospect).' Available at: https://clinicaltrials.gov/ct2/show/study/NCT01322490. Accessed on: 02/08/19.
89. U.S. Preventive Services Task Force (2018). 'Screening for prostate cancer US preventive services task force recommendation statement.' *Journal of the American Medical Association,* vol. 319, pp 1902-1913.
90. Ukimura, O. et al., (2012). 'Focal cryotherapy for clinically unilateral, low-intermediate risk prostate cancer in 73 men with a median follow-up of 3.7 years.' *European Urology,* vol. 62, issue 1, pp 55-63.
91. Vallabhajosula, S. et al., (2014). '^{99m}Tc-Labeled Small-Molecule Inhibitors of Prostate-Specific Membrane Antigen: Pharmacokinetics and Biodistribution Studies in Healthy Subjects and Patients with Metastatic Prostate Cancer.' *The Journal of Nuclear Medicine,* vol. 55, issue 11, pp 1791-1798.
92. Van Hemelrijck, M. et al., (2019). 'Reasoning for discontinuing active surveillance: assessment of 21 centres in 12 countries in the Movember GAP3 consortium.' *European Urology,* vol. 75, issue 3, pp 523-531.
93. Vlaeminck-Guillem, V. et al., (2010). 'Urinary prostate cancer 3 test: toward the age of reason?' *Urology,* vol. 75, issue 2, pp 447-453.
94. Wang, X. et al., (2005). 'Autoantibody signatures in prostate cancer.' *The New England Journal of Medicine,* vol. 353, pp 1224-1235.
95. Watson, P. et al., (2015). 'Emerging mechanisms of resistance to androgen receptor inhibitors in prostate cancer.' *Nature Reviews Cancer,* vol. 15, pp 701-711.
96. Wilt, T. et al., (2012). 'Radical prostatectomy versus observation for localised prostate cancer.' *The New England Journal of Medicine,* vol. 367, pp 203-213.
97. Yu, E. et al., (2011). 'Once-daily Dasatinib: expansion of phase II study evaluating safety and efficacy of Dasatinib in patients with metastatic castration-resistant prostate cancer.' *Urology,* vol. 77, issue 5, pp 1166-1171.
98. Zaorsky, N. et al., (2017). 'The evolution of brachytherapy for prostate cancer.' *Nature Reviews Urology,* vol. 14, pp 415-439.