The genetic landscapes of urological cancers and their clinical implications in the era of high-throughput genome analysis

Alexander Light*,†, Aamir Ahmed‡, Prokar Dasgupta§ and Oussama Elhage§

*Department of Surgery, Cambridge University Hospitals NHS Foundation Trust, University of Cambridge, Cambridge, †Bedford Hospital NHS Trust, Bedford Hospital, Bedford, ‡Centre for Stem Cell and Regenerative Medicine, King’s College London, and §Department of Urology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK

Objective
With the advent of high-throughput genome analysis, we are increasingly able to sequence and hence understand the pathogenic processes underlying individual cancers. Recently, consortiums such as The Cancer Genome Atlas (TCGA) have performed large-scale projects to this end, providing significant amounts of information regarding the genetic landscapes of several cancers.

Patients and Methods
We performed a narrative review of studies from the TCGA and other major studies. We aimed to summarise data exploring the clinical implications of specific genetic alterations, both prognostically and therapeutically, in four major urological cancers. These were renal cell carcinoma, muscle-invasive bladder cancer/carcinoma, prostate cancer, and testicular germ cell tumours.

Results
With these four urological cancers, great strides have been made in the molecular characterisation of tumours. In particular, recent studies have focussed on identifying molecular subtypes of tumours with characteristic genetic alterations and differing prognoses. Other prognostic alterations have also recently been identified, including those pertaining to epigenetics and microRNAs. In regard to treatment, numerous options are emerging for patients with these cancers such as including immune checkpoint inhibition, epigenetic-based treatments, and agents targeting MAPK, PI3K, and DNA repair pathways. There are a multitude of trials underway investigating the effects of these novel agents, the results of which are eagerly awaited.

Conclusions
As medicine chases the era of personalised care, it is becoming increasingly important to provide individualised prognoses for patients. Understanding how specific genetic alterations affects prognosis is key for this. It will also be crucial to provide highly targeted treatments against the specific genetics of a patient’s tumour. With work performed by the TCGA and other large consortiums, these aims are gradually being achieved. Our review provides a succinct overview of this exciting field that may underpin personalised medicine in urological oncology.

Keywords
urological cancer, renal cell carcinoma, muscle-invasive bladder cancer, prostate cancer, testicular germ cell tumours, genomics

Introduction
With further understanding of disease pathophysiology and the development of a wide range of therapeutic agents, we are constantly chasing the goal of personalized medicine. This is the use of information regarding a particular patient’s genetics, exact disease profile, and other environmental variables to form management plans highly targeted towards that patient with the aim of a superior clinical outcome.

Although the true realization of personalized medicine is distant, a highly desirable area of application is oncology. Malignant tumours are notoriously heterogeneous, naturally making a personalized treatment regimen especially important. Given the genetic basis of cancer, the advent of high-throughput genome analysis techniques has helped us better understand the precise genetic faults underlying tumours. Consortiums such as The Cancer Genome Atlas (TCGA) have sought to sequence genomes of cancerous tissue from thousands of different samples. TCGA projects...
compare malignant tissue specimens with matched normal tissue controls, employing techniques such as whole-exome sequencing, reverse-phase protein arrays, RNA sequencing, microRNA (miRNA) sequencing, DNA methylation, and single-nucleotide polymorphism (SNP) arrays to analyse samples. Identification of abnormalities is then often coupled with survival analyses to confer clinical implications. Started in 2005, TCGA currently lists data from 11 000 patients on 33 different tumours.

With the comprehensive identification of important tumoural alterations, research has started to characterize the role of these alterations in tumourigenesis. This information in turn helps to identify potential drug targets, an exciting field that has seen multiple novel tumour-specific treatments emerge, some of which have entered mainstream use. Assessing the precise mutational burdens of tumours may also be important for determining prognosis as well as diagnosing cancer. Ultimately, we are slowly piecing together our understanding of tumourigenesis and developing more and more targeted treatments.

The present review aims to synthesize data primarily from TCGA but also other major sources regarding the identification and clinical significance of key alterations in four major urological cancers. These are renal cell carcinoma (RCC), muscle-invasive bladder cancer/carcinoma (MIBC), prostate cancer, and testicular germ cell tumours (TGCTs).

Renal Cell Carcinoma

The three major histological subtypes of RCC are clear-cell RCC (ccRCC; ~ 75%), papillary RCC (pRCC; 15–20%) and chromophobe RCC (chRCC; ~ 5%) [1]. In the most recent TCGA paper in 2018, 843 tumour specimens were analysed, including 488 ccRCC, 274 pRCC, and 81 chRCC, each with matched normal tissue [2]. Single tumour samples from patients had DNA and RNA sent for whole-exome sequencing as well as copy number, DNA methylation, and RNA sequencing. Table 1 lists key alterations for each subtype with their prognostic and therapeutic implications.

Clear-Cell RCC

The TCGA analysis identified nine significantly mutated genes in ccRCC [2]. ccRCC was also associated with loss of chromosomes 3p and gain of 5q. Overall, ccRCC had the second worst prognosis of all RCC subtypes, behind only CpG island methylator phenotype (CIMP) RCC, discussed below.

von Hippel-Lindau

An instrumental, early event in ccRCC tumourigenesis is bi-allelic inactivation of the von Hippel-Lindau (VHL) gene on chromosome 3p25. VHL regulates hypoxia-inducible factor (HIF) protein, which itself regulates hypoxia pathways, with important roles in tumour angiogenesis, cell migration, proliferation and permeability. VHL mutation was observed in 55.1% of ccRCC tumours in the TCGA paper, and in 72.6% in a similar analysis of 106 ccRCC tumours by the TRACERx Renal consortium [1,2].

Only one study has explicitly studied the association between VHL status and targeted therapy, showing no association between VHL status and response rate to vascular endothelial growth factor (VEGF) inhibitors [3]. A recent systematic review of three large phase III randomized controlled trials (RCTs) using VEGF inhibitors as adjunctive therapy confirmed no benefit regarding disease-free or overall survival for surgically managed localized RCC [4]. Several trials of VEGF inhibitors are ongoing, including two phase III trials (Table 2). Tyrosine kinase inhibitors (TKIs) targeting HIF-2 also have shown promise in early studies and could emerge as a prominent treatment option (Table 2) [5,6].

Chromosome 3p

Although bi-allelic VHL inactivation is considered a hallmark of ccRCC pathogenesis, the importance of chromosome 3p in ccRCC extends far beyond this gene. Located at 3p21 are PBRM1, BAP1 and SETD2, genes encoding histone and chromatin regulators. These are all frequently mutated in ccRCC, at rates of 38.0%, 11.0% and 13.2%, respectively [2]. Loss of expression of these genes highly correlates with each other, with the TRACERx Renal study observing loss of chromosome 3p in 95.3% of tumours [2].

SETD2 encodes a histone methyltransferase, with roles in maintaining genome integrity. SETD2 mutations lead to increased hypermethylation, itself associated with higher-stage ccRCC and poorer survival [2,7]. Along with SETD2, hypermethylation of two WNT pathway regulatory genes, SFRP1 and DKK1, was also significantly correlated with worse survival. Hypermethylation could be targeted with demethylating agents; however, several phase I and II studies of various agents have shown only limited benefit [8]. Three-phase II trials are in process (Table 2).

BAP1 encodes the histone deubiquitinating enzyme BRCA1-associated protein and promotes chromosomal stability. BAP1 mutations correlate with poorer survival in ccRCC [2], and this has been confirmed in both patients with low-risk and those with metastatic disease in several other studies [7,9-11].

PBRM1 encodes the BAF180 subunit of the SWI/SNF chromatin remodelling complex, with inactivation demonstrated to promote ccRCC cell proliferation and migration [12]. In a retrospective analysis of two independent cohorts of patients with sporadic ccRCC (145 and 327 patients), overall survival for patients with PBRM1-mutant
tumours was improved compared to patients with BAP1-mutant tumours in both cohorts (hazard ratios of 2.7 and 2.8, respectively); however, patients with tumours harbouring dual mutations had the worst overall survival [11].

BAP1 and PBRM1 mutations have been studied in clinical trials. A recent phase II trial compared everolimus, an mTOR inhibitor, followed by sunitinib, a VEGF-receptor TKI, vs the opposite sequence in patients with metastatic RCC [13]. In the everolimus arm, BAP1-mutant tumours had much shorter progression-free survival vs wild-type tumours (4.9 vs 10.5 months). PBRM1-mutant tumours showed much longer progression-free survival vs wild-type tumours in the everolimus arm (12.8 vs 5.5 months), but no difference in the sunitinib arm. Another metastatic ccRCC study found no

| Subtype | Altered gene, signature, or subgroup | Prognostic implication | Therapeutic implication |
|---------|------------------------------------|------------------------|------------------------|
| ccRCC   | VHL                                | No association [2]     | VEGF inhibitors provide no benefit [3,4]. TKIs against HIF-2 under study |
|         | PBRM1                              | Greater survival [11] or no benefit [2,7,10] | Everolimus followed by sunitinib shows benefit, but sunitinib followed by everolimus does not [13]. VEGF inhibitors have shown both benefit and no effect [14,15]. Anti-PD-1 monotherapy has shown benefit [16] |
|         | BAP1                               | Reduced survival [2,7,9-11] | Everolimus followed by sunitinib worsens survival, but sunitinib followed by everolimus does not [13] |
|         | SETD2                              | Reduced survival [2,7]  | Demethylating agents have shown limited benefit [8]. Further trials in progress |
|         | CDKN2A, TP53                        | Reduced survival [2]    |                        |
|         | Pyruvate dehydrogenate complex genes | Reduced survival [2]    |                        |
|         | AMPK                               | Reduced survival [2]    |                        |
|         | Ribose sugar metabolism genes      | Reduced survival [2]    |                        |
|         | PDCD1 (PD-1)                       | Reduced survival [17]   |                        |
|         | CD247 (PD-L1)                      | Greater survival [17]   |                        |
|         | Th2 signature                      | Reduced survival [2]    |                        |
|         | Th17 signature                     | Greater survival [2]    |                        |
|         | Sarcomatoid tumours (TP53, BAP1, ARID1A mutations, loss of heterozygosity affecting 1p, 9, 10, 14, 17p, 18, 22) | Reduced survival compared to other ccRCC [23] |                        |
| pRCC    | MET                                | Reduced survival (both type 1 and 2). Also 100% associated with CIMP tumours, with the worst survival of any RCC subtype [2] | Avelumab/pembrolizumab plus axitinib shows significantly longer PFS and ORR [18,19] |
|         | CDKN2A                             | Reduced survival [2]    | Anti-MET TKIs crizotinib, savolitinib, and foretinib have shown good ORR [27-29] |
|         | PBRM1                              | Reduced survival [2]    |                        |
|         | TP53                               | Reduced survival [2]    |                        |
|         | Ribose sugar metabolism genes      | Reduced survival [2]    |                        |
|         | Increased hypermethylation          | Reduced survival [2]    |                        |
|         | Th2 signature                      | Reduced survival [2]    |                        |
|         | CIMP tumours (low mutation rate, 100% CIMP hypermethylation, reduced FH expression, increased expression of cell cycle, hypoxic response, and ribose sugar metabolism genes, and Th2 signature) | Earlier age of presentation. Reduced survival, the worst of any RCC subtype [2,30] |                        |
| chRCC   | PTEN                               | Reduced survival [2]    |                        |
|         | CDKN2A                             | Reduced survival [2]    |                        |
|         | Increased hypermethylation          | Reduced survival [2]    |                        |
|         | SFRP1                              | Reduced survival [2]    |                        |
|         | DKK1                               | Reduced survival [2]    |                        |
|         | Th2 signature                      | Reduced survival [2]    |                        |
|         | Metabolically divergent tumours (reduced expression of genes for the Krebs cycle, electron transport chain, AMPK pathway, ribose synthesis pathway, increased Th2 signature) | Reduced survival, and reduced compared to other chRCC [2] |                        |
|         |                                    |                        |                        |

ccRCC, clear-cell RCC; chRCC, chromophobe RCC; CIMP, CpG island methylator phenotype; FH, fumarate hydratase; HIF, hypoxia-inducible factor; ORR, overall response rate; pRCC, papillary RCC; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.
Table 2 Ongoing targeted therapy trials in RCC on the basis of known genetic alterations.

| Drug class          | Trial no., name | Phase | Drug                          | Combination agent | Comparator | Estimated enrolment | Tumour                  | Estimated completion | Preliminary results                                                                 |
|---------------------|-----------------|-------|-------------------------------|-------------------|------------|---------------------|------------------------|----------------------|------------------------------------------------------------------------------------|
| VEGF inhibitors     | NCT01865747, METEOR | III   | Caborzantinib                 | Everolimus         | 658        | Metastatic ccRCC    | June 2019              | Longer PFS and OS in Caborzantinib arm vs Everolimus arm (HR 0.58; \( P = 0.0001 \); HR 0.66, \( P = 0.0003 \)). Serious adverse events in 39.58% (Caborzantinib) vs 43.17% (Everolimus) |
|                     | NCT02627963     | III   | Tivozanib hydrochloride       | Sorafenib         | 350        | Metastatic ccRCC    | Dec. 2019              |                                                                   |
|                     | NCT03103066     | II    | PT2385                        |                   | 4          | Non-metastatic von Hippel-Lindau disease-associated ccRCC | Sept. 2022             |                                                                   |
|                     | NCT02974738     | I     | PT2977                        |                   | 125        | Advanced/metastatic solid tumour, including RCC ccRCC   | June 2020              |                                                                   |
|                     | NCT03634540     | II    | PT2977                        | Caborzantinib     | 118        | Advanced/metastatic ccRCC                               | Sept. 2022             |                                                                   |
|                     | NCT03401788     | II    | PT2977                        |                   | 50         | Non-metastatic von Hippel-Lindau disease-associated RCC ccRCC | March 2023             |                                                                   |
| Epigenetic therapy | NCT03308396     | Ib/Ib| Guadecitabine                 | Darvalumab        | 58         | Various, including metastatic/ unresectable kidney cancer related to HLRCC | Dec. 2020              |                                                                   |
|                     | NCT03165721     | II    | Guadecitabine                 |                   | 70         | Various, including RCC                                   | Dec. 2021              |                                                                   |
| CDK4/6 inhibitor    | NCT02619253     | I/Ib | Vorinostat                    | Pembrolizumab     | 57         | Progressive advanced/metastatic UC                       | May 2020               |                                                                   |
|                     | NCT02788201     | II    | Various, including vorinostat |                   | 20         | Various, including RCC                                   | July 2021              |                                                                   |
|                     | NCT0305889, PICKRCC | I     | Abemaciclib                   | Sunitinib         | 22         | Metastatic ccRCC                                         | Nov. 2022              |                                                                   |
|                     | NCT02465060, The MATCH Screening Trial | II | Various, including palbociclib, pending next-generation sequence results |                   | 6452        | Various, including advanced RCC                          | June 2022              |                                                                   |
| Drug class       | Trial no., name                  | Phase | Drug                  | Combination agent                        | Comparator | Estimated enrolment | Tumour                      | Estimated completion | Preliminary results                                                                 |
|------------------|----------------------------------|-------|-----------------------|------------------------------------------|------------|--------------------|-----------------------------|---------------------|-------------------------------------------------------------------------------------|
| Immunotherapy    | NCT02420821, Immotion151         | III   | Atezolizumab          | Bevacizumab                              | Sunitinib  | 915                | Advanced/metastatic ccRCC    | Dec. 2021           | Longer PFS in all subgroups with intervention (HR 0.74, \( P < 0.001 \) in PD-L1 positive patients. HR 0.83, \( P = 0.0254 \) in ITT population). No difference in OS (HR 0.81, \( P = 0.0895 \)) |
|                  | NCT03024996, IMmotion010        | III   | Atezolizumab          | Placebo                                  | Sunitinib  | 778                | Non-metastatic RCC           | April 2024          |                                                                                     |
|                  | NCT03288532, RAMPART             | III   | Durvalumab            | Tremelimumab, Durvalumab monotherapy, or active monitoring | Placebo    | 1750               | Non-metastatic RCC           | Dec. 2037           |                                                                                     |
|                  | NCT02811861, CLEAR               | III   | Lenvatinib            | Everolimus or pembrolizumab              | Sunitinib  | 1050               | Advanced ccRCC               | Feb. 2021           |                                                                                     |
|                  | NCT03141177, CheckMate 9ER      | III   | Nivolumab             | Cabozantinib                             | Sunitinib  | 630                | Advanced/metastatic ccRCC    | April 2023          |                                                                                     |
|                  | NCT03050013, PROSPER RCC         | III   | Nivolumab             | Ipilimumab                               | Placebo    | 800                | ccRCC at high risk of relapse| July 2023           |                                                                                     |
|                  | NCT03592472, RENA VIV            | III   | Nivolumab             | Radical/partial nephrectomy              | Sunitinib  | 805                | Non-metastatic RCC           | Nov. 2023           |                                                                                     |
|                  | NCT03260894, KEYNOTE-679/ ECHO-302 | III   | Pembrolizumab         | Epacadostat                             | Sunitinib or pembrolizumab                | 129        | Advanced/metastatic ccRCC    | June 2020           |                                                                                     |
|                  | NCT03142334, MK-3475-56/ KEYNOTE-564 | III   | Pembrolizumab         | Placebo                                  | Sunitinib  | 950                | Advanced ccRCC               | Dec. 2025           |                                                                                     |
| MET kinase       | NCT02019693, INC280              | III   | Various, including crizotinib | Sunitinib                              | 22        | pRCC               | Various, including RCC       | Jan. 2021           |                                                                                     |
| inhibitor        | NCT02788201                      | II    | Various, including crizotinib | Sunitinib                              | 20        | Unresectable/advanced/metastatic pRCC with MET mutation | Aug. 2019 |
|                  | NCT02081596, CALYPSO             | II    | Savolitinib           | Sunitinib or cabozantinib               | 60        | Advanced/metastatic pRCC    | Jan. 2021          |                                                                                     |
|                  | NCT02465060, The MATCH Screening Trial | III   | Various, including crizotinib, pending next-generation sequence results | Sunitinib or cabozantinib               | 180       | Advanced/metastatic ccRCC or pRCC | Sept. 2019 |
|                  |                                 | II    | Savolitinib or crizotinib, or savolitinib plus MED47.36 | MED47.36, or tremelimumab plus MED47.36  | 195       | Advanced/metastatic ccRCC or pRCC | June 2022          |                                                                                     |

ccRCC, clear cell renal cell carcinoma; HLRCC, hereditary leiomyomatosis and RCC; HR, hazard ratio; ITT, intention to treat; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; pRCC, papillary renal cell carcinoma; RCC, renal cell carcinoma; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor. Where available, preliminary results have been listed. Only phase III immunotherapy trials are shown. Phase III trials are set in bold text.
association between PBRM1 mutation and response to sunitinib [14]. In contrast, in a third study, PBRM1 mutations were enriched amongst responders to anti-VEGF therapy [15]. Interestingly, a fourth study identified that bi-allelic PBRM1 loss-of-function tumours treated with anti-PD-1 monotherapy were associated with significantly increased clinical benefit [16]. Although much further work is required, studies like these place the role of certain mutations into the context of specific treatment, which in the future may permit personalized prognostic and therapeutic strategies.

Other Implicated Genes

Mutation, hypermethylation or deletion of CDKN2A was seen in 16.2%, and was the only alteration to be associated with poorer survival across the RCC cohort and within each major histological subtype [2]. CDKN2A regulates CDK4/6, key for the G1-S transition of the cell cycle. Therefore, CDK4/6 inhibitors may be a future therapeutic option, and have shown benefit in early trials in other cancers. Current trials are ongoing (Table 2).

An important driver gene across many cancers, TP53, was mutated in 2.6% and was associated with decreased survival in ccRCC [2]. MTOR and PTEN, important components of the PI3K/AKT pathway, were mutated in 6.7% and 4.5%, respectively, and had no association with survival. In regard to metabolic genes, expression of pyruvate dehydrogenase complex (PDC) activation genes was generally low in ccRCC. However, lower expression of PDC activation genes, AMPK, and increased expression of ribose sugar metabolism genes were all independently associated with greater stage and reduced survival. Targeting these pathways may become an important treatment option.

Immunogenetics

Immune gene expression signatures have been previously shown to be increased in ccRCC relative to other subtypes, including PDCD1 (PD-1) and CD247 (PD-L1), and this was confirmed in the most recent TCGA analysis [2,17]. An increased Th2 signature expression was frequently observed and associated with poor survival within each subtype. In contrast, increased Th17 signature expression was associated with improved survival. A previous analysis showed that PDCD1 expression correlated with poorer survival, but that CD247 expression correlated with improved survival [17].

The results of two large phase III immunotherapy RCTs have recently been published. In previously untreated PD-L1-positive advanced RCC, avelumab plus axitinib compared to sunitinib showed a much longer progression-free survival (13.8 vs 7.2 months) and greater objective response rate (55.2% vs 25.5%) [18]. In previously untreated advanced RCC, pembrolizumab plus axitinib compared to sunitinib demonstrated better 12-month survival (89.9% vs 78.3%), progression-free survival (15.1 vs 11.1 months), and objective response rate (59.3% vs 35.7%) [19]. An exciting area of cancer research, numerous phase III trials are underway with promising preliminary results (Table 2) [20]. Study of immunotherapy, however, should also focus on adverse events, particularly those that are immune-related. In the avelumab plus axitinib trial, 38.2% of the intervention arm experienced immune-related adverse events, primarily thyroid disorders (24.7%), with 9.0% of intervention arm participants experiencing grade 3 or worse adverse events [18]. In the pembrolizumab plus axitinib trial, 62.9% of the intervention arm had grade 3 or worse adverse events related to the treatment, particularly hepatotoxicity and thyroid disorders [19]. Given the increasing mainstream use of immunotherapy in urological cancers, it is increasingly important for urologists and other healthcare professionals to recognize and appropriately manage adverse effects when they arise [21].

Sarcomatous Change

Sarcomatous change occurs in 1–8% of RCC tumours, usually affecting ccRCC [22]. Sarcomatoid ccRCC produces larger, more invasive tumours that carry a much worse prognosis [23]. Although their pathogenesis is not well understood, these tumours do possess unique mutations, for example, TP53. In one study involving exome sequencing of 21 matched normal-carcinomatous-sarcomatoid specimens, sarcomatoid and carcinomatous elements within single tumours shared a mean of 41.7% somatic single-nucleotide variants, suggesting both elements derive from a common mutated cell of origin [24]. Bi-allelic PT53 mutations were present in 32% of sarcomatoid elements, but absent in carcinomatous elements. Bi-allelic mutations of known driver genes BAP1 and ARID1A mutations were also found to be sarcomatoid-specific. Last, loss of heterozygosity unique to sarcomatoid elements was repeatedly observed on chromosomes 1p (containing ARID1A), 9, 10, 14, 17p (containing TP53), 18 and 22. These results suggest that sarcomatoid elements obtain further, specific driver mutations that drive dedifferentiation and ultimately sarcomatous change.

Treatment for sarcomatous RCC is difficult. However, a 2009 study demonstrated a partial response in 19% of patients with sarcomatoid metastatic RCC to anti-VEGF therapy [25]. More recently, a phase II trial demonstrated that sunitinib with gemcitabine achieves an overall response rate of 26% in sarcomatoid metastatic RCC [26].

Papillary RCC

Papillary RCC is further categorized into types 1 and 2. Type 1 tumours are often multifocal and associated with hereditary pRCC, carrying the best survival outcomes along with chRCC.
[2]. They are associated with gains of chromosomes 7 and 17. Conversely, type 2 tumours are more heterogeneous and associated with hereditary leiomyomatosis and RCC (HLRCC), and slightly worse prognosis. pRCC carries the highest mutation rate of RCC subtypes, and TCGA identified nine significantly mutated genes for it [2].

Commonly Altered Genes

Unlike ccRCC, VHL mutation does not play a major role, mutated in just 1.1% [2]. Instead, MET (8.3%) and FAT1 (6.0%), are most commonly mutated. CDKN2A alterations were also seen in 5.0% and 18.6% of type 1 and type 2 pRCC, respectively, and were associated with poorer survival in both. PBRM1, SETD2 and BAP1 mutations occurred in 4.5%, 6.4% and 5.6%, respectively. Of these, PBRM1 was associated with decreased survival in type 1 PRCC. TP53 mutation (1.5%), as with ccRCC, was also associated with poorer survival. PTEN mutation (3.4%) had no survival association. Similar to ccRCC, increased expression of ribose sugar metabolism genes correlated with decreased survival in pRCC overall, with greater expression seen in type 2 over type 1.

Of the above, MET has received the most attention therapeutically. In a phase II trial of crizotinib, a MET TKI, in patients with or without MET-mutant advanced or metastatic type 1 pRCC, the overall response rate was 50% in the MET-mutant cohort [27]. Also showing high response rates and tolerability in phase II trials are savolitinib and foretinib [28,29]. There are currently multiple trials underway investigating anti-MET agents (Table 2).

CpG Island Methylator Phenotype Tumours

Through similar methods to their more recent analysis, the 2016 TCGA analysis identified a CIMP subtype of type 2 pRCC [30]. These tumours carried a significantly earlier age of presentation, together with the worst survival outcomes of any RCC subtype [2]. Although they possess the lowest mutation rate of any subtype, CDKN2A is hypermethylated in 100%, and there is decreased fumarate hydratase (FH) mRNA, and increased expression of cell cycle progression and hypoxic response genes. Likely owing to the loss of FH expression, CIMP tumours displayed the greatest expression of ribose metabolism genes. A highly metabolically active tumour may explain its aggressiveness; increased expression of these metabolic genes was associated with poor survival in pRCC, similar to ccRCC. CIMP tumours also showed, along with ccRCC, a significantly increased immune gene signature expression, including the Th2 signature. CIMP tumours may therefore be targetable with checkpoint inhibition therapy, although there are no studies investigating this. Further work is certainly needed to investigate targeted treatment for this rare, newly identified patient cohort with currently dire prognosis.

Hypermethylation

Increased hypermethylation was also associated with PBRM1 or SETD2 mutations in type 2 pRCC. Similar to ccRCC, increased hypermethylation in pRCC is also associated with advanced disease and poorer prognosis, with or without including CIMP in the analysis. Also, as with ccRCC, SFRP1 and DKK1 hypermethylation correlated with poorer survival [2]. Future trials investigating demethylating agents should stratify patients by histology, mutation and methylation status to determine how effective these therapies are according to these important parameters.

Chromophobe RCC

Chromophobe RCC carries a significantly lower mutation rate compared to ccRCC and pRCC, with TCGA identifying just two significantly mutated genes [2]. These were TP53 (31.1%) and PTEN (8.1%). Unlike other subtypes, PTEN but not TP53 mutation was associated with poorer survival in chRCC. chRCC is also associated with whole chromosomal deletions of 1, 2, 6, 10, 13 and 17. Furthermore, CDKN2A alterations (19.8%), as with other subtypes, were associated with poorer survival [2].

Metabolically Divergent Tumours

In the 2018 TCGA analysis, a unique chRCC subset with a distinct metabolic gene signature was identified for the first time, termed metabolically divergent chRCC [2]. Relative to other chRCC tumours, these had low expression of Krebs cycle, electron transport chain genes, and AMPK pathway genes, but increased expression of ribose synthesis genes. They also had very high Th2 gene signature scores, were all stage III or IV, lacked the aneuploidy typically associated with chRCC, and conferred significantly worse survival compared to other chRCC. Further investigation into characterizing and treating these tumours will aid these patients who currently have poor prognosis.

Diagnostic Biomarkers

Aside from prognostic and therapeutic implications, it is important to consider the diagnostic implications of identified genetic alterations. Ideally, it would be possible to identify products in blood or urine produced by tumours
corresponding to said alterations. Unfortunately, work in this area is limited, with many candidate biomarkers, such as NGAL and KIM-1, showing low sensitivity and specificity [31]. The most promising candidates at present are AQP1 and PLIN2, both of which are elevated in the urine of patients with ccRCC and those with pRCC. A recent study of 57 patients with small renal masses undergoing partial nephrectomy demonstrated that using measurements of both yielded 100% sensitivity and specificity for diagnosis of ccRCC or pRCC [32]. Research into miRNA urinary biomarkers is also promising but preliminary [33].

**Summary**

The further characterization of classic genetic alterations, as well as the identification of more novel alterations naturally lends itself to a great need for new research identifying the clinical implications of alterations.

In regard to prognostic implications, we currently know of several markers of poor prognosis (Table 1). These frequently observed alterations to chromosome 3p are important. In ccRCC, hypermethylation of SETD2, as well as other WNT-pathway genes SFRP1 and DKK1, associate with poorer survival, as do mutations to PBRM1 and BAP1. Hypermethylation of SFRP1 and DKK1, and PBRM1 mutation also confers a poor prognosis in pRCC. CDKN2A is also implicated. Poorer survival was seen with CDKN2A alterations in all RCC subtypes, and hypermethylation of this gene was seen in 100% of CIMP tumours, a set of tumours that is associated with the worst survival in all patients with RCC studied. Furthermore, mutations to the guardian of the genome TP53 have been shown to confer poorer prognosis in ccRCC, pRCC, although not chRCC. TP53 mutations are also enriched in tumours that have undergone sarcomatous change, associated with greater size and aggression. In addition, gene alterations reflecting increased ribose sugar metabolism were associated with worse survival in ccRCC, pRCC, including CIMP tumours where expression was greatest, and metabolically divergent chRCC. Last, immunogenetic markers are key for prognosis too. Increased Th2 expression is associated with poorer survival in ccRCC, and is frequently observed in deadly CIMP tumours and metabolically divergent chRCC.

Genetic alterations, in addition to having prognostic significance, can by extension confer therapeutic significance (Table 1). Therapeutically, immunotherapy is certainly emerging, with significant improvements in survival and objective response rate in high-profile phase III trials. Anti-MET TKIs are also receiving significant attention. However, other novel therapies have been less impressive, including VEGF inhibitors. The identification of tumourigenic alterations has produced some plausible drug targets and production of agents, for example, anti-HIF-2 TKIs, CDK4/6 inhibitors, or demethylating agents. However, it will be many years before any of these treatments enter mainstream use and it is likely many will fail to show benefit. Now that key alterations are being better characterized, it will be prudent for future trials to measure specifically how patients with certain alterations respond to targeted therapy, thereby allowing more personalized treatment regimens. Indeed, the presence of an alteration or degree of gene signature expression does not directly correlate with response to targeted therapy.

**Carcinoma Invading Bladder Muscle**

Approximately 25% of bladder cancer, primarily urothelial carcinoma (UC), presents as MIBC, with much poorer prognosis compared to non-muscle-invasive disease. TCGA has published two analyses of chemotherapy-naïve MIBC, the first involving 131 tumours in 2014, and the second involving 412 tumours in 2017, both with the same numbers of matched normal samples [34,35].

**Commonly Altered Genes**

Table 3 lists key alterations with their prognostic and therapeutic implications. MIBC has a high somatic mutation rate, similar to that of non-small-cell lung cancer and melanoma [34]. This is primarily attributable to APOBEC-mediated mutagenesis, an early event in bladder cancer tumourigenesis, owing to high APOBEC3A and APOBEC3B expression [35]. Interestingly, despite the high mutational burden, tumours with high APOBEC-signature mutagenesis showed a promising 75% 5-year survival rate. This may be attributable to a heightened host immune reaction to the greater mutational burden, thus suppressing tumour growth.

The 2014 analysis identified 32 significantly mutated genes, many of which had not been previously identified as being significant [34]. The 2017 analysis subsequently identified a further 58 significantly mutated genes [35]. A total of 48% of tumours had TP53 mutations, which were mutually exclusive with MDM2 alterations. A further 6% and 19% of tumours showed mutually exclusive amplification or overexpression of MDM2, resulting in functional TP53 inactivation in 73%. RB1 mutations were observed in 17%, and these were mutually exclusive with CDKN2A deletions (22%). Also common were alterations to KMT2D (28%), KDM6A (26%), ARID1A (25%), PIK3CA (22%), EP300 (16%), FGFR3 (14%), CREBBP (13%), HER2 (12%), TSC1 (10%), as well as E2F3 amplification in 12%.

**Epidermal Growth Factor Receptor Family**

HER2 and epidermal growth factor receptor (EGFR) have important roles in bladder cancer proliferation, and hence have been targeted in several trials. A Phase II trial of
chemotherapy plus trastuzumab showed a 70% response rate in 109 patients with advanced UC, of whom 52.3% had HER2 overexpression or amplification [36]. However, cardiac toxicity was higher than expected. In contrast, when used as maintenance therapy after toxicity was higher than expected. In contrast, when used as 

Table 3 Known genetic alterations in carcinoma invading bladder muscle that affect prognosis, and/or have known or proposed therapeutic utility.

| Subtype                        | Altered gene, signature, or subgroup                                                                 | Prognostic implication                                                                 | Therapeutic implication                                                                 |
|--------------------------------|-------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Overall                        | *APOBEC3A/B*<br>HER2, and the EGFR family                                                      | Greater survival [34]                                                                     | Mixed results in trials using anti-HER2/EGFR family agents [35-38]. Concern regarding adverse events for cetuximab [37]. Trials are underway                                                                 |
| Luminal-papillary MIBC         | *PIK3CA*                                                                                       | Best prognosis for any MIBC subtype [34]                                                | Poor response to immunotherapy [43]. Targeted FGFR3 inhibitors have shown early but promising results [44-50]. Trials of anti-EGFR agents are underway |
| Luminal-infiltrated MIBC       | *EP300*                                                                                       | Intermediate prognosis, similar to basal-squamous and luminal tumours [34]               | Neoadjuvant chemotherapy is ineffective and may worsen survival [42]. Immunotherapy trials vary, with benefit in one [43], and no effect in others [52]. Numerous trials are ongoing |
| Luminal-subtype MIBC           | Increased expression of *UPK2*, *UPK1A*, *KRT20*, *SNX31*                                     | Intermediate prognosis, similar to basal-squamous and luminal tumours [34]               | Not yet clear                                                                                                                                    |
| Basal-squamous MIBC            | Increased expression of *CD44*, *KRT5*, *KRT6A*, *KRT14*, *TGMI*, *DSC3*, *PI3*, *CIS* genes. Frequent *TP53* mutations, loss of SHH signalling. Greatest expression of *PD-L1* and *CTLA4* of any subtype | Intermediate prognosis, similar to basal-squamous and luminal tumours [34]. Poorer prognosis compared to luminal tumours in one study [42] | More sensitive than luminal tumours to neoadjuvant chemotherapy [42]. Possibly immunotherapy, but trials need to focus specifically on these tumours to confirm |
| Neuronal MIBC                  | Increased expression of *MS11*, *PLEKHG4B*, neuronal differentiation genes, neuronal development genes, neuroendocrine markers. Mutations in both *TP53* and *RB1*, or in *TP53* with *E2F3* amplification in 50% | Poorest survival of any subtype [34]                                                     | Not yet clear. Possibly etoposide-cisplatin neoadjuvant chemotherapy, or somatostatin analogues but further research and trials are required |

CIS, carcinoma in situ; EGFR, epidermal growth factor receptor; MIBC, carcinoma invading bladder muscle; UC, urothelial carcinoma.

Targeting other members of the EGFR family has produced mixed results. In a phase II trial, the anti-EGFR cetuximab showed no benefit in advanced UC when added to chemotherapy, but instead was associated with an increase in grade 3–5 adverse outcomes [38]. More encouragingly, in a phase II trial of 23 patients with platinum-resistant metastatic UC, the pan-HER inhibitor afatinib achieved the primary endpoint of 3-month progression-free survival in 21.7% [39]. Notably, all responders had *HER2* or *ErbB3* mutations, whereas no wild-type *HER* participants achieved the primary endpoint. A subsequent phase II single-arm trial of afatinib has commenced for patients with advanced UC with *EGFR*/HER2/HER3 alterations (Table 4).

### PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway has been targeted without much success in MIBC. In a phase II study of 45 patients with metastatic UC previously treated with chemotherapy,
| Drug class                  | Trial no., name                              | Phase | Drug                      | Combination agent | Comparator | Estimated enrolment | Tumour                                      | Estimated completion | Preliminary results |
|---------------------------|---------------------------------------------|-------|---------------------------|-------------------|------------|--------------------|---------------------------------------------|---------------------|----------------------|
| HER2/EGFR family inhibitor| NCT02780687, LUX-Bladder 1                   | II    | Afatinib                  |                   |            | 80                 | Recurrent/metastatic platinum-refractory UC with HER2 or ERBB3 mutation, HER2 amplification, or EGFR amplification | March 2020          |                      |
|                           | NCT02465060, The MATCH Screening Trial      | II    | Various, including afatinib, pertuzumab, and trastuzumab, pending next-generation sequence results |                   |            | 6452               | Various, including bladder cancer           | June 2022           |                      |
| mTOR inhibitor            | NCT03047213                                  | II    | Sapanisertib              |                   |            | 209                | Advanced/metastatic UC with TSC1 or TSC2 mutation | June 2020           |                      |
|                           | NCT02465060, The MATCH Screening Trial      | II    | Various, including sapanisertib, pending next-generation sequence results |                   |            | 6452               | Various, including bladder cancer           | June 2022           |                      |
| Epigenetic therapy        | NCT02546661, BISCAY                         | Ib    | Various, including oxiurib | Durvalumab         |            | 30                 | Metastatic MIBC                              | March 2020          |                      |
|                           | NCT02783300                                  | I     | Various, including temsirolimus | GSK3326595        |            | 317                | Various, including bladder cancer           | July 2021           |                      |
| FGFR family inhibitor     | NCT01976741                                  | I     | Rogaratinib               |                   |            | 168                | Various, including refractory, advanced, or bladder UC | Sept. 2019          |                      |
|                           | NCT02546661, BISCAY                         | Ib/II | Rogaratinib, Atezolizumab | Placebo           |            | 210                | Advanced/metastatic cisplatin-ineligible UC with high FGFR1 or FGFR3 expression | July 2022           |                      |
|                           | NCT02465060, The MATCH Screening Trial      | II    | Various, including AZD4547 | Durvalumab         |            | 196                | Metastatic FGFR3-mutant or FGFR fusion MIBC | March 2020          |                      |
|                           | NCT02401542, FIERCE-21                      | I/II  | Vofatamab, Docetaxel      | Placebo plus docetaxel |         | 300                | Advanced/metastatic refractory/relapsed FGFR3-altered UC | Dec. 2021           |                      |
|                           | NCT03123055, FIERCE-22                      | Ib/II | Vofatamab                 | Pembrolizumab     |            | 74                 | Advanced/metastatic UC                       | Sept. 2022          |                      |
## Table 4 (continued)

| Drug class     | Trial no., name                  | Phase | Drug       | Combination agent                                                                 | Comparator                                      | Estimated enrolment | Tumour                   | Estimated completion | Preliminary results                  |
|----------------|----------------------------------|-------|------------|-------------------------------------------------------------------------------------|------------------------------------------------|--------------------|------------------------|----------------------|---------------------------------------|
| Immunotherapy  | NCT02807636, IMvigor130           | III   | Atezolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 1200               | Advanced/metastatic UC | Nov. 2020             |                                       |
|                | NCT03775265                      | III   | Atezolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 475                | Advanced/metastatic UC | June 2025             |                                       |
|                | NCT02516241                      | III   | Durvalumab  | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 1200               | Metastatic UC          | Sept. 2019            |                                       |
|                | NCT03080988, CheckMate901       | III   | Nivolumab   | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 475                | Unresectable/metastatic UC | Dec. 2022            |                                       |
|                | NCT02285436, KEYNOTE-045        | III   | Pembrolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 542                | Refractory UC          | March 2020            | Longer OS in pembrolizumab arm (HR 0.73, \(P = 0.00224\)), including strongly PD-L1 positive tumours (HR 0.57, \(P = 0.00483\)). No difference in PFS (HR 0.98, \(P = 0.41648\)), including strongly PD-L1 positive tumours (HR 0.89, \(P = 0.23958\).) |
|                | NCT02853305, KEYNOTE-361        | III   | Pembrolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 990                | Advanced/unresectable/metastatic UC | May 2020             |                                       |
|                | NCT03924895, KEYNOTE-866        | III   | Pembrolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 790                | MIBC                   | Jan. 2025             |                                       |
|                | NCT03244384, AMBASSADOR         | III   | Pembrolizumab | Platinum-based chemotherapy, Radiotherapy, physician’s choice chemotherapy          | Placebo plus platinum-based chemotherapy       | 739                | Non-metastatic MIBC   | June 2025             |                                       |

EGFR, epidermal growth factor receptor; HR, hazard ratio; MIBC, carcinoma invading bladder muscle; PFS, progression-free survival; TURBT, transurethral resection of bladder tumour; UC, urothelial carcinoma. Where available, preliminary results have been listed. Only phase III immunotherapy trials are shown. Phase III trials are set in bold text.
everolimus showed limited response [40]. However, one patient showed a durable response, and was later shown to have a TSC1 mutation [41]. TSC1 mutations are seen in 10% of patients with MIBC, so this could be a potential therapeutic biomarker for everolimus [35]. A phase II trial is currently investigating the novel mTOR inhibitor sapanisertib in patients with advanced or metastatic UC with a TSC1/2 mutation, as is the aforementioned MATCH Screening trial (Table 4).

Epigenetics

TCGA identified 157 epigenetically silenced genes [35]. EP300 and CREBBP, encoding histone acetylases, are commonly inactivated in bladder cancer; however, a phase II trial using the histone deacetylase inhibitor mocetinostat in platinum-refractory EP300/CREBBP-altered advanced UC recently reported significant toxicities, with insufficient response to warrant further investigation [42]. Current trials of other epigenetic drugs are ongoing (Table 4).

Molecular Subtypes

Although tailoring interventions to genetic defects is a vital concept, a parallel concept going forward is tumour molecular subtyping. Based on mRNA expression, TCGA noted five distinct molecular subtypes [35]. This built on previous work where various groups had identified similar but overlapping subtypes. The TCGA subtypes were luminal-papillary (35%), luminal-infiltrated (19%), luminal (6%), basal-squamous (35%), and neuronal (5%). In this case, the luminal and neuronal subtypes were newly discovered.

Luminal-Papillary Tumours

Luminal tumours can be further differentiated into luminal-papillary, luminal-infiltrated, and luminal subtypes. In luminal-papillary tumours, uroplakins UPK2 and UPK1A were highly expressed, as were markers of urothelial differentiation FOXA1, GATA3 and PPARG.

In 44%, FGFR3 was significantly mutated, amplified, overexpressed, or formed a fusion gene with TACC3. There was also preserved sonic-hedgehog (SHH) signalling and a low carcinoma in situ (CIS) signature. These tumours had a low risk of progression, were lower stage, and had the best prognosis of any subtype [35,43].

Immunotherapy with atezolizumab for these tumours shows the lowest response of any subtype [44]. TKIs against FGFR3 may, however, have more potential. Erdafitinib, a pan-FGFR inhibitor, demonstrated a 42% overall response rate and 80% disease control rate in 96 patients with metastatic or surgically unresectable FGFR3-mutant or FGFR2/3-fusion UC [45]. Importantly, an overall response rate of 70% was also observed in patients previously treated with immune checkpoint inhibitors, a group with poor prognosis and a high unmet need. The FGFR3-specific monoclonal antibody MFG187S produced long-term disease stability in five of 10 patients with advanced UC [46]. Fourth, another FGFR3-specific monoclonal antibody, vofatamab, demonstrated promising overall response rates and progression-free survival in advanced or metastatic UC with FGFR3 mutation or fusion [47]. Last, phase I studies for pan-FGFR inhibitors AZD4547, BGJ398, Debio 1347 and rogaratinib have shown promise [48–51]. Several phase I and II trials are currently underway for FGFR inhibition in FGFR3-altered tumours (Table 4).

Luminal-Infiltrated Subtype

The luminal-infiltrated tumours had a mesenchymal expression signature, with greater lymphocytic infiltration, and increased expression of the miR-200 family, smooth muscle and myofibroblast genes. Of these tumours, 80% had a chemoresistant phenotype, characterized by wild-type p53. In one study, neoadjuvant chemotherapy may have even shortened overall survival [43]. These tumours had an intermediate 5-year survival similar to that of basal-squamous and luminal subtypes [35].

These tumours also had moderately heightened immune gene expression, including PDCD1 (PD-1) and CD274 (PD-L1). In contrast to luminary-papillary tumours, a previous multicentre phase II trial of metastatic or unresectable bladder cancer demonstrated that this subtype benefitted most from atezolizumab therapy owing to higher levels of PD-L1 [52]. Future immunotherapy trials in this cohort will help further characterize its role. Certainly, further work is required into identifying therapeutic biomarkers and what tumours will benefit most from immune checkpoint inhibitor. Higher levels of PD-L1 does not imply responsiveness to immune checkpoint inhibitor, and there have been inconsistent reports as to the utility of PD-L1 as a therapeutic biomarker. In the aforementioned atezolizumab trial, response was also observed in tumours with low or absent PD-L1-positive infiltrating immune cells [52]. Furthermore, in the multicentre phase II Imvigor210 trial, enrichment of PD-L1 in tumours did not correlate with response [53]. Third, high expression was not associated with response in the phase III KEYNOTE-045 trial either [54]. It has been suggested that inconsistencies with how PD-L1 status is measured may complicate the situation further. Numerous ongoing phase III trials may provide more information regarding therapeutic biomarkers and the response of certain subgroups, such as luminal-infiltrated tumours, to immunotherapy (Table 4).

Luminal Subtype

The luminal subtype tumours displayed the greatest expression of UPK2 and UPK1A, as well as KRT20 and SNX3I, genes highly expressed in terminally differentiated
urothelial umbrella cells. These tumours had similar prognosis to luminal-infiltrated and basal-squamous tumours [35]. Owing to its novel discovery, the best targeted treatment for this subtype is not yet clear.

**Basal-Squamous Tumours**

Basal-squamous tumours had high expression of basal and stem-like markers including CD44, KRT5, KRT6A and KRT14. They also had high expression of squamous differentiation markers including TGM1, DSC3 and PI3, increased expression of CIS genes, and loss of SHH signalling. These tumours had a significantly high rate of TP53 mutations and were also more common in women. The basal-squamous subtype also displayed the strongest immune expression signature, including PD-L1 and CTLA4. Owing to this, immunotherapy may be a rational therapeutic option, but this would need to be further investigated in this specific cohort.

Untreated basal-squamous tumours had a poorer prognosis compared to luminal tumours in one trial [43]. However, these tumours also demonstrated the most marked improvement in 3-year survival when treated with neoadjuvant chemotherapy, from 49.2% to 77.8%. This may be explained through heightened deregulation of the cell cycle owing to TP53 mutations.

**Neuronal Tumours**

Interestingly, the majority of these tumours displayed no histological features of neuroendocrine bladder tumours. These tumours had strong expression of neuronal differentiation and development genes, like MSI1 and PLEKHG4B, together with neuroendocrine markers. In 50% of samples there were mutations in both TP53 and RB1, or TP53 mutation and E2F3 amplification. Indeed, inactivation of TP53 and RB1 are characteristic of small-cell neuroendocrine tumours. Overall, 85% had alterations to the cell-cycle pathway.

Of all subtypes, neuronal tumours carried the poorest survival and work into identifying optimal treatment will be key. Given its high cell cycle gene expression, neuronal tumours could be targeted with etoposide-cisplatin neoadjuvant chemotherapy. Furthermore, the neuronal subtype may represent a pan-cancer class of tumour given its similarity of expression to other neuroendocrine cancers. Accordingly, therapeutic options beneficial for other neuroendocrine tumours may prove useful here, for example, somatostatin analogues.

**Diagnostic Biomarkers**

An area of unmet need in bladder cancer research is the discovery of a urinary biomarker for diagnosis and surveillance of malignancy with high sensitivity and specificity, and certainly one that outperforms urine cytology. Although many biomarkers are currently commercially available, utility remains limited. Proteomic-based, metabolomic-based and epigenetic-based urinary biomarkers are under investigation, including mutant FGFR3, methylated DNA, and miRNA [55]. This is an emerging area of work, and it is likely that a panel of alterations will produce the best results. For example, one recent pilot study of 16 patients performed next-generation sequencing on urinary cell-free DNA in patients with haematuria, identifying a five-gene panel (TERT, FGFR3, TP53, PIK3CA, KRAS) and a seven-gene panel (TERT, FGFR3, TP53, PIK3CA, KRAS, HRAS, ERBB2). Addition of age and gender to the model yielded excellent diagnostic accuracy (area under the curve [AUC] of 0.966 and 0.959, respectively) [56]. It will be also rewarding to see how the molecular characterization of MIBC is integrated into future biomarker research.

**Summary**

Unlike RCC where classification into subtypes is generally histological, previous research has managed to establish molecular subtypes in MIBC. Although various groups differ in their definitions of subtypes, these are nonetheless important for providing discrete prognostic and therapeutic information (Table 3). Here, on the basis of characteristic gene signatures, TCGA has classified MIBC into luminal-papillary, luminal-infiltrated, luminal, basal-squamous, and neuronal tumours. Each carries individual prognosis. Luminal-papillary tumours carry the best prognosis, neuronal tumours the worst. Meanwhile, luminal, luminal-infiltrated, and basal-squamous tumours confer an intermediate prognosis. As research continues, it will be important to further characterize these groups and yield more information regarding prognosis. Furthermore, it will be rewarding to see results translated into diagnostic biomarker research.

Therapeutically, immunotherapy is certainly due to enter mainstream practice, with many phase III trials in progress (Table 4). However, not all MIBC is immunosensitive and herein lies the importance of molecular subtyping (Table 3). Luminal-papillary tumours show a low response, whilst luminal-infiltrated tumours with heightened immune gene expression have a much greater response. The application of molecular subtyping to future trials is therefore key. Indeed, basal-squamous tumours may be highly susceptible to immunotherapy. In regard to other targeted therapies, anti-FGFR therapy is promising but at an early stage in tumours with altered or fused FGFR, typically luminal-papillary tumours. Treatment with anti-EGFR therapy has had mixed results, whilst trials involving mTOR inhibitors and epigenetic therapy are in preliminary stages. Of note, it will be vital to further characterise rare neuronal tumours, and whether
options such as neoadjuvant chemotherapy or somatostatin analogues are effective.

**Prostate Cancer**

Prostate cancer is the second most common cancer in men worldwide. Prostate adenocarcinoma is a highly heterogeneous disease, with large differences in clinical evolution. A significant proportion of men will have indolent disease that would never have been detected otherwise. In contrast, some men will develop aggressive metastatic disease with very poor prognosis. Currently, variables such as Gleason scoring, PSA level, and staging help in stratifying patients for the management of prostate cancer, but are less helpful with predicting prognosis. Molecular characterization of prostate cancer will aid better individual understanding of each patient’s disease.

**Commonly Altered Genes**

TCGA’s most recent analysis for prostate cancer from 2015 involved 333 primary prostate carcinomas with matched normal tissue samples [57]. Average follow-up duration was <2 years, however, which precluded survival analyses owing to the long clinical course of prostate cancer. Table 5 lists key alterations with their prognostic and therapeutic implications.

Prostate cancer is considered to be a cancer involving a low mutational burden, unlike other carcinomas not associated with a powerful exogenous mutagen. This was confirmed by TCGA, who observed 19 non-synonymous mutations per tumour genome (0.94 mutations/Mb). TCGA found 13 significantly mutated genes, seven of which had not been previously identified. These included PTEN (17%), SPOP (11%), TP53 (8%), ATM (6%), FOXA1 (4%), BRAF (2%), CDKN1B (1%) and AKT1 (1%). Interestingly, although several known BRAF-activating mutations were identified, the canonical V600E mutation was not. When compared with a cohort of 150 castration-resistant metastatic prostate cancer samples, there were significantly more alterations in TP53, RB1, as well as components of the PI3K, DNA repair, and androgen receptor (AR) pathways [57,58].

The most common abnormalities were actually copy-number alterations at chromosomal arm level, including recurrent gains of chromosomes 7 and 8q, and heterozygous losses of 8p, 13q, 16q and 18. The amplifications, of which 20 were identified, commonly spanned regions with known oncogenes including CCND1 (11q13.2, 2%), MYC (8q24.21, 8%), FGFR1 and WHSCIL1 (8p11.23, 8%). A total of 35 deletions were observed, which often spanned important tumour suppressors, including TP53 (17p13.1), CDKN1B (12p13.1) and MAP3K1 (5q11.2). Co-deletion of CDKN1B and MAP3K1 in E8S-intact tumours is associated with aggressive disease and significantly poorer survival [59].

The authors classified tumours by the frequency of somatic copy-number alterations into either mostly unaltered genomes (‘quiet’, 17%), intermediate level of alterations (50%), and high level of alterations (33%). These subtypes were similarly distributed between the molecular subtypes. As mentioned, survival analyses were not possible, but the latter had significantly higher Gleason scores and PSA levels.

**DNA Repair Pathways**

In all, 19% of tumours harboured alterations in DNA repair pathways. This included alterations to ATM, FANCD2, RAD51C, CDK12, BRCA1 and BRCA2. In addition, as mentioned, DNA repair gene alterations were more frequently seen in metastatic samples [57,58]. DNA repair pathways have been the subject of several phase II trials. In a non-randomized study of 50 patients with metastatic castration-resistant tumours, a 33% response rate was seen with olaparib [60]. However, on subgroup analysis, 88% of patients with a detectable alteration to a DNA repair pathway responded. Preliminary results from the first 85 patients of an ongoing trial involving rucaparib demonstrated PSA and radiographic responses in 48% and 45% of BRCA1/2-altered patients, respectively. Trial completion will inform us whether similar responses are observed in patients with defects in other repair pathways [61]. Results are also awaited from the TOPARP trial, investigating olaparib in patients with advanced, castration-resistant prostate cancer (Table 6). In addition to several trials stratifying patients by their DNA repair gene status, there are currently a wealth of ongoing phase II trials and one phase III trial investigating DNA repair-targeting drugs in prostate cancer (Table 6).

Genetic variants may also play a role in both radiation toxicity and radioresistance. A study of 802 men with radiotherapy-treated localized prostate cancer identified that two BRCA1 SNPs were significantly associated with progression to lethal disease over a 12-year follow-up period. The rs4473733 SNP was associated with a 35% mortality risk reduction, whilst the rs8176305 SNP was associated with a twofold increase in lethal prostate cancer post-radiotherapy [62]. Although the functionality of these SNPs is unknown, the role of BRCA1 in tumourigenesis suggests a causative link may be likely. Interestingly, a retrospective study of 1302 patients with local or locally advanced disease found germline BRCA mutations to be an independent factor for worse metastasis-free survival and cancer-specific survival [63]. This association held when mutation carriers and non-carriers treated with radiotherapy were compared; however, there was no association in those treated with surgery.

**PI3K/AKT/mTOR Pathway**

The PI3K/AKT/mTOR pathway was also the subject of several driver alterations, notably to PTEN, but also to
Increased alterations to the PI3K pathway genes were also observed in metastatic samples [58,59]. Loss of PTEN has previously been shown to confer reduced survival [64]. PTEN-deleted tumours become PIK3CB-dependent owing to feedback suppression of PIK3CA; therefore, loss of both genes may further elevate PI3K signalling, making it a potential therapeutic target [65]. The same study demonstrated that in vitro inhibition of PI3Kα and β signalling causes marked increases in AR activity. However, combined suppression of both PI3K isoforms and AR leads to significant tumour regression.

Unfortunately, clinical data for PI3K targeting have been less promising. A multicentre phase II trial of buparlisib, a pan-class I PI3K inhibitor, with or without enzalutamide in metastatic, castration-resistant tumours demonstrated limited activity [66]. Similarly, a phase II trial of the mTORC1/2 inhibitor MLN0128 showed poor response with dose-limiting toxicities [67]. mTORC inhibitors sirolimus, everolimus, temsirolimus and ridaforolimus have also shown disappointing results in several trials [68]. Despite underwhelming data from targeting this pathway, results are awaited from the phase III RCT of the AKT inhibitor ipatasertib in combination therapy for metastatic castration-resistant prostate cancer (ERG prostate cancer and ETV4 prostate cancer).

Table 5 Known genetic alterations in prostate cancer that affect prognosis, and/or have known or proposed therapeutic utility.

| Subtype | Altered gene, signature, or subgroup | Prognostic implication | Therapeutic implication |
|---------|-------------------------------------|------------------------|------------------------|
| Overall | CDKN1B plus MAP3K1 High level of somatic copy-number alterations DNA repair genes (ATM, FANCID2, RAD51C, CDK12, BRCAl and BRCAl2) | Reduced survival [59] Higher Gleason scores, higher PSA [57] | Good response to PARP inhibitors in phase II trials [60,61]. Dual inhibition with anti-androgens has shown mixed results in phase II trials [29,30]. A wealth of trials is ongoing, including one phase III trial | Poor responses to PI3K, mTORC inhibitors [66–68]. Several trials are underway | Sorafenib has shown some anti-tumour activity in phase II trials [70,71] |
|         | PI3K/AKT/mTOR pathway (PTEN, PIK3CA, PIK3CB, AKT1) MAPK pathway (BRAF, HRAS, RAC1, RRAS2) | Reduced survival with loss of PTEN [64] Improved survival with BRCAl rs4473733 post-radiotherapy for localized tumours [62] Reduced survival with BRCAl rs8176305 post-radiotherapy for localized tumours [62] Phosphorylated ERK1/2 in post-radical prostatectomy samples associated with biochemical recurrence [69] | SPOP mutations confer resistance to BET inhibitors [78]. DNA methyltransferases have shown modest benefit in small preliminary studies [81] Abiraterone and enzalutamide have shown significant PFS and mortality benefits in phase III trials [85–87]. Dual inhibition with PARP inhibitors have shown mixed results in phase II trials [91,92]. Trials are underway | Resistance to next-generation anti-androgen therapy [93] |
|         | Epigenetic alterations | | | |
| Androgen activity | | | | |
| Neuroendocrine transdifferentiation (loss of RB1 and TP53, MYCN amplification, epigenetic changes) SNP | Poorer survival [93] | | |
| SPOP prostate cancer | SPOP | Improved metastasis-free survival [76] | SPOP mutations confer resistance to BET inhibitors [78]. The anti-androgen abiraterone shows high response with or without CHD1 alterations [79] | |
| FOXA1 prostate cancer | FOXA1 | Reduced time to PSA recurrence post-radical prostatectomy [80] | |

PFS, progression-free survival; SNP, single-nucleotide polymorphism.

PIK3CA, PIK3CB and AKT1 [57].
| Drug class          | Trial no., name          | Phase | Drug        | Combination agent          | Comparator                      | Estimated enrolment | Tumour            | Estimated completion | Preliminary results                                                                 |
|---------------------|--------------------------|-------|-------------|-----------------------------|---------------------------------|--------------------|-------------------|---------------------|--------------------------------------------------------------------------------------------|
| DNA repair agent    | NCT02854436, Galahad     | II    | Niraparib   |                             |                                 | 301                 | mCRPC            | Feb. 2020           |                                                                              |
|                     | NCT01682772, TOPARP      | II    | Olaparib    |                             |                                 | 89                  | mCRPC            | Dec. 2019           |                                                                              |
|                     | NCT02893917              | II    | Olaparib    |                             | Olaparib plus cediranib        | 90                  | mCRPC            | Dec. 2019           |                                                                              |
|                     | NCT03263650              | II    | Olaparib    | Cabazitaxel plus carboplatin| Cabazitaxel plus carboplatin   | 96                  | mCRPC            | Oct. 2020           |                                                                              |
|                     | NCT03810105              | II    | Olaparib    |                             | Durvalumab                      | 32                  | Castration-sensitive, non-metastatic recurrent post-radical prostatectomy prostate cancer | Feb. 2021           |                                                                              |
|                     | NCT03432897, BrUOG 337   | II    | Olaparib    | Radical prostatectomy       |                                 | 13                  | Advanced prostate cancer with mutation in ≥1 DNA repair gene | Sept. 2021          |                                                                              |
|                     | NCT03012321, BRCAAway    | II    | Olaparib    | Olaparib                    | Abiraterone plus prednisone    | 70                  | mCRPC with mutation in ≥1 DNA repair gene | Jan. 2022           |                                                                              |
|                     | NCT03415995, TRIUMPH     | II    | Rucaparib   |                             |                                 | 30                  | Metastatic prostate cancer with mutation in ≥1 homologous recombination DNA repair gene | Nov. 2021           |                                                                              |
|                     | NCT02975934, TRITON3     | III   | Rucaparib   | Abiraterone acetate, enzalutamide, or docetaxel |                                 | 400                 | mCRPC with BRCA1/2 or ATM mutation | April 2022          |                                                                              |
|                     | NCT03148795, TALAPRO-1   | II    | Talazoparib |                             |                                 | 100                 | mCRPC with mutation in ≥1 DNA repair gene | March 2022          |                                                                              |
| Drug class | Trial no., name | Phase | Drug | Combination agent | Comparator | Estimated enrolment | Tumour | Estimated completion | Preliminary results |
|------------|----------------|-------|------|------------------|------------|---------------------|--------|---------------------|-------------------|
| Agent targeting PI3K pathway | NCT02525068 | II | AZD5363 | Enzalutamide | Enzalutamide | 136 | mCRPC | March 2020 | |
| Agent targeting PI3K pathway | NCT015363 | I | AZD5363 | Enzalutamide | | 36 | Prostate cancer with AKT mutation and resistance to enzalutamide | Oct. 2020 | |
| NCT03218826 | I | AZD8186 | Docetaxel | | | 58 | Metastatic prostate cancer with PTEN or PIK3CB mutation | April 2021 | |
| NCT01848285 | I | AZD8186 | Enzalutamide | | | 180 | Various, including advanced CRPC | Sept. 2019 | |
| NCT0135625 | I | AZD8186 | CC-115 | | | 118 | Various including advanced prostate cancer | Nov. 2019 | |
| NCT03618355 | Ib | Everolimus | Placebo | | | 24 | Prostate cancer | Aug. 2019 | |
| NCT03002338 | III | Everolimus | Placebo | | | 120 | Advanced CRPC | Dec. 2019 | |
| NCT01215096 | I | GSK2636771 | Enzalutamide | | | 64 | mCRPC with loss of PTEN | Dec. 2019 | |
| NCT01485861 | I/II | Ipatasertib | Abiraterone | | | 273 | Advanced/mCRPC | July 2019 | |
| NCT03175787, Ice-CAP | I/II | Ipatasertib | Atezolizumab | | | 51 | Various, including castration-resistant prostate cancer with PTEN loss | Nov. 2020 | |
| NCT03042001 | I | Ipatasertib | Rucaparib | | | 54 | Various, including advanced/mCRPC | Sept. 2021 | |
| NCT0307238, PATential150 | III | Ipatasertib | Abiraterone | | | 1100 | mCRPC with PTEN loss | Sept. 2023 | |
| NCT02407054 | II | LY3023414 | Enzalutamide | | | 23 | mCRPC | Nov. 2019 | |
| NCT02465060, The MATCH Screening Trial | II | Various, including BAY 80-6946, GSK2636771, and sapanisertib | | | | 6452 | Various, including prostate cancer | June 2022 | |
| Agent targeting MAPK pathway | NCT0360141 | II | Tomivosertib | | | 30 | Castration-resistant prostate cancer | April 2022 | |
| NCT02811242 | II | Trametinib | | | | 62 | mCRPC | Jan. 2021 | |
| NCT03878324 | I | Trametinib | | | | 52 | Various including mCRPC | Feb. 2021 | |
| NCT02465060, The MATCH Screening Trial | II | Various, including binimetinib, dabrafenib, and trametinib | | | | 6452 | Various, including prostate cancer | June 2022 | |
| Drug class                        | Trial no., name          | Phase | Drug          | Combination agent                        | Comparator                        | Estimated enrolment | Tumour      | Estimated completion | Preliminary results |
|----------------------------------|--------------------------|-------|---------------|------------------------------------------|-----------------------------------|---------------------|-------------|--------------------|---------------------|
| Anti-androgen agent with PARP inhibition | NCT03732820, Propel Study | III   | Olaparib      | Abiraterone                              | Abiraterone plus placebo          | 720                 | mCRPC       | Aug. 2022          | Longer PFS in the olaparib plus abiraterone arm vs placebo plus abiraterone (13.8 vs 8.2 months, no p value available) |
|                                  | NCT01972217              | II    | Olaparib      | Abiraterone                              | Abiraterone plus placebo          | 158                 | mCRPC       | Dec. 2019          |                     |
| Immunotherapy                    | NCT03012321, BRCAAway   | II    | Olaparib      | Abiraterone plus prednisone              | Abiraterone plus prednisone       | 70                  | mCRPC       | Jan. 2022          |                     |
|                                  | NCT03016312              | III   | Atezolizumab  | Enzalutamide                             | Enzalutamide                      | 771                 | mCRPC       | Nov. 2019          |                     |
|                                  | NCT00942331              | III   | Bevacizumab   | Gemcitabine, cisplatin                   | Placebo, gemcitabine plus cisplatin | 506                 | Metastatic prostate cancer | Nov. 2018          |                     |
|                                  | NCT03879122              | II/III| Nivolumab     | Androgen deprivation therapy plus docetaxel | Androgen deprivation therapy plus docetaxel, androgen deprivation therapy plus ipilimumab alternating with docetaxel and followed by nivolumab | 135                 | Metastatic hormone-sensitive prostate cancer | Dec. 2023          |                     |
|                                  | NCT04100018              | III   | Nivolumab     | Docetaxel plus prednisone                | Placebo, docetaxel, plus prednisone | 984                 | mCRPC       | May 2024           |                     |
|                                  | NCT04191096, KEYNOTE-991 | III   | Pembrolizumab | Enzalutamide plus androgen-deprivation therapy | Enzalutamide                      | 1232                | Metastatic hormone-sensitive prostate cancer | July 2026          |                     |
|                                  | NCT03834493, KEYNOTE-641 | III   | Pembrolizumab | Enzalutamide                             | Placebo plus enzalutamide         | 1200                | mCRPC       | April 2024         |                     |
|                                  | NCT03834506, KEYNOTE-921 | III   | Pembrolizumab | Docetaxel                                | Placebo plus docetaxel            | 1000                | Chemotherapy-naïve mCRPC | Feb. 2023          |                     |
|                                  | NCT03834519, KEFLYNK-010 | III   | Pembrolizumab | Olaparib                                 | Abiraterone plus prednisone or enzalutamide | 780                 | mCRPC       | Sept. 2022         |                     |
|                                  | NCT01436968              | III   | ProstAtak® (AdV-tk) | Valacyclovir plus radiation therapy with or without androgen deprivation therapy | Placebo plus valacyclovir plus radiation therapy with or without androgen deprivation therapy | 711                 | Localized prostate cancer | Dec. 2022          |                     |
|                                  | NCT03686683, PROVENT     | III   | Sipuleucel-T  |                                          | Active surveillance               | 450                 | Localized prostate adenocarcinoma with Gleason score <4 | May 2023           |                     |

mCRPC, metastatic castration-resistant prostate cancer; PFS, progression-free survival. Where available, preliminary results have been listed. Only phase III immunotherapy trials are shown. Phase III trials are set in bold text.
resistant prostate cancer (Table 6). Other trials investigating agents targeting this pathway, including several novel agents, are underway (Table 6).

MAPK Pathway

Defects in genes involved in the MAPK signalling pathway were found in 25% of tumours, including BRAF, HRAS, RAC1 and RRAS2 [57]. Detection of phosphorylated ERK1/2 in samples post-radical prostatectomy has previously been shown to be associated with biochemical recurrence [69]. The multi-kinase inhibitor sorafenib, which also inhibits Raf, has shown some anti-tumour activity in several phase II trials, both in combination and as monotherapy [70,71]. In addition, binimetinib, dabrafenib, tomivosertib and trametinib are currently being investigated (Table 6). However, in vitro studies have shown that inhibiting the PI3K signalling pathway leads to upregulation of the MAPK pathway [72]. Whilst this phenomenon may explain the disappointing results of targeting PI3K signalling, it emphasizes MAPK signalling as another target. Indeed, further trials are required to investigate dual inhibition of both pathways, a potentially very promising option.

Molecular Subtypes

Although previous studies have described the existence of different but overlapping molecular subtypes, TCGA aimed to unify these findings. A total of 74% of tumours were found to fall into one of seven subtypes as determined by oncogenic driver alterations.

ETS Family Subtypes: ERG, ETV1, ETV4 and FLI1 Fusions

Overall, 53% of all tumours were defined by fusions involving androgen-regulated promoters and the ETS transcription factor family, including ERG, ETV1, ETV4 and FLI1. The most common subtype was defined by fusions involving ERG (46%). Fusions involving ETV1, ETV4 and FLI1 accounted for 8%, 4% and 1%, respectively.

Accordingly, the most common alteration in prostate cancer has previously been identified as the TMPRSS2-ERG fusion, due to deletion of the region between these genes on 21q22.3. Meta-analysis has shown that this fusion gene does not significantly affect prognosis [73].

Aside from TMPRSS2, fusions were identified with other androgen-regulated genes such as SLC45A3 and NDRG1. Several tumours displayed overexpression of ETS genes, which were mutually exclusive with ETS fusions in ETV1, ETV4 and FLI1 tumours. This overexpression may have been attributable to epigenetics or cryptic translocations. Overexpression of ETV1, but not ETV4, is associated with reduced time to PSA recurrence post-radical prostatectomy [74]. In another study of Chinese patients, ETV4 overexpression conferred worse survival [75].

SPOP, FOXA1 and IDH1 Subtypes

SPOP-mutant tumours comprised 11% of tumours. These tumours were all mutually exclusive with ETS fusion gene tumours. These were associated with deletion of CHD1, and chromosomes 6q and 2q. The SPOP-mutant/CHD1-deleted tumours, in particular, were associated with greater DNA methylation, homogeneous gene expression signatures, and frequent overexpression of SPINK1. SPOP-mutant tumours were seen at a lower frequency in a separate cohort of metastatic tumours [57,58]. SPOP-mutant tumours have been shown to correlate with higher preoperative PSA levels, but better metastasis-free survival [76]. A canSAR knowledge base machine-learning analysis performed by one group identified that SPOP is a potentially druggable target, and concluded drug discovery efforts should focus on this protein as a future target [77].

BET inhibitors, shown in early studies to have promise in other cancers, have also shown little response in prostate cancer, with SPOP mutation demonstrated to confer BET inhibitor resistance [78]. SPOP also regulates AR activity, and SPOP-mutant tumours with or without CHD1 alterations have previously shown a higher response rate to abiraterone therapy [79].

FOXA1 tumours were defined by FOXA1 mutations, comprising 3% of tumours overall. High FOXA1 expression is strongly associated with early PSA recurrence post-radical prostatectomy [80]. The same canSAR analysis above also concluded FOXA1 to be a potentially druggable target [77].

IDH1-mutant tumours made up just 1% of tumours, and were not observed in the metastatic cohort [57,58]. These also had an early age of diagnosis. Further study is required to determine if novel drugs targeting IDH1 have a beneficial role for these tumours.

Unclassified Tumours

In all, 26% of tumours were not able to be classified, either because of abnormalities undetected to date, or because of additional alterations that occurred with subtype-defining alterations. These tumours, however, frequently displayed mutations of TP53, KDM6A and KMT2D, chromosome 6 and 16 deletion, and chromosome 8 (including MYC) and 11 (CCND1) amplification.

Epigenetics

A total of 164 genes that were epigenetically silenced were identified in the TCGA analysis [57]. Most of these genes are
frequently downregulated in metastatic disease. It was possible to classify tumours into one of four epigenetic clusters. Two-thirds of the ERG tumours had moderately higher levels of DNA methylation (cluster 3), whilst the vast majority of the remainder had almost twice as many hypermethylated loci (cluster 1). Cluster 1 methylation was almost exclusively seen with the ERG tumours. This suggests further classification of ERG tumours is possible, and it will be crucial for future work to identify the clinical implications of this. Aside from the ERG tumours, the other ETS family subtypes were found to have much more heterogeneous methylation, with tumours spread across each of the four epigenetic profiles. The SPOP and FOXA1 tumours primarily belonged to methylation cluster 2.

The IDH1 tumours all belonged to methylation cluster 2, and possessed the greatest levels of methylation and, accordingly, the greatest amount of gene suppression. Tumours in this group were associated with early-onset prostate cancer.

As mentioned, prostate cancers, possibly through SPOP mutations, are associated with resistance to BET inhibitors [78]. No trials of BET inhibitors are currently active. Targeting of DNA methyltransferases has only been examined in small preliminary clinical studies, showing modest benefit [81]. Classifying patients via methylation cluster or molecular subtype will help future trials determine which patient groups benefit most from epigenetic-targeted therapy.

In a recent genome-wide germline study of 589 patients with localized prostate cancers, one group identified and validated 1178 loci with altered methylation associated with malignant, but not normal, tissue [82]. Among these newly identified germline loci with malignant altered methylation were TCERG1L and AKT1, genes involved in known prostate tumourigenesis driver events, and demonstrated in this analysis to be predictive of aggressive disease [83,84].

**Androgen Activity**

Androgen activity is key in prostate cancer pathogenesis, and is central to the formation and subsequent overexpression of the majority of ETS fusions. Consequently, androgen deprivation therapy forms an important component of prostate cancer therapeutics. Despite this, it is less clear how individual tumours differ in terms of androgen sensitivity and dependence. The TCGA authors calculated an AR activity score from the expression pattern of 20 genes. Intriguingly, the ETS fusion genes had varying levels of AR transcriptional activity despite the fusion genes being under AR control. The SPOP and FOXA1 tumours actually had the greatest AR transcriptional activity. Comparison with metastatic castration-resistant samples also demonstrated increased AR activity in the metastatic cohort [57,58].

The anti-androgen abiraterone has shown significant mortality benefit when added to androgen deprivation therapy in phase III RCTs (38% mortality risk reduction, 55% progression-free survival risk reduction) [85]. The AR antagonist enzalutamide has also shown dramatic reductions in mortality and progression in phase III RCTs for both metastatic (81% progression-free survival risk reduction, 29% mortality risk reduction) and non-metastatic (71% risk reduction for mortality or metastasis) castration-resistant tumours [86,87]. In addition, oral AR antagonists apalutamide (72% risk reduction for mortality or metastasis, 55% progression-free survival risk reduction) and darolutamide (69% risk reduction for mortality or metastasis) have demonstrated excellent mortality benefits in phase III RCTs [88,89]. Whilst androgen deprivation therapy is a cornerstone of prostate cancer treatment, future trials that stratify patients by their molecular subtype or AR activity level may be key to determining which patients benefit the most.

Another promising aspect of AR targeting may be dual inhibition with PARP inhibitors. Several rationales have linked AR activity with DNA repair activity, including evidence that PARP-1 expression levels increase with exposure to anti-androgen therapy [90]. A phase II RCT of olaparib with abiraterone in 142 patients showed significantly improved progression-free survival compared to abiraterone alone (13.8 vs 8.2 months) [91]. In contrast, another phase II study showed no difference in progression-free survival when investigating abiraterone and prednisolone with or without veliparib, another PARP inhibitor (10.1 vs 11.0 months) [92]. This study also hypothesized a priori that ETS-fusion status may affect response, as ERG interacts with PARP1, and PARP1 is required for full ERG activity; however, no association was found. A phase III RCT is ongoing comparing olaparib plus abiraterone vs abiraterone and placebo (Table 6).

Another important consideration in treatment with androgen deprivation is the neuroendocrine phenotype [93]. Approximately 20–25% of tumours treated with next-generation anti-androgens become resistant, and have been shown to utilise lineage switching to become AR-independent cells with neuroendocrine characteristics. Normally, neuroendocrine cells comprise <2% of organ-confined disease [94]. These so-called neuroendocrine prostate cancers are very aggressive with high mortality. Although the mechanisms of this transdifferentiation is unclear, it is associated with loss of RB1, TP53 mutation or deletion, and MYCN amplification, which produces a state of lineage plasticity. Significant epigenetic changes driven by EZH2 and SOX2 then promote transdifferentiation into the neuroendocrine phenotype [95-98]. The combined loss of RB1 and TP53 occurs in approximately half of neuroendocrine tumours, but just 14% of prostate
adenocarcinoma [96]. Preclinical data suggest that inhibitors of EZH2 may block or reverse the neuroendocrine phenotype, restoring sensitivity to anti-androgens [98,99]. These inhibitors are being trialed in other solid tumours.

Single-Nucleotide Polymorphisms

Single-nucleotide polymorphisms have emerging roles in our understanding of prostate cancer. As discussed, two BRCA1 SNPs (rs4473733, rs8176305) are associated with altered survival post-radiotherapy for localized prostate cancer [62]. SNPs may also have a role in predicting radiation toxicity. Several SNPs have been identified that are significantly associated with development of specific adverse events through potential mechanisms. Erectile dysfunction has been linked to rs2268363 (FSHR, chromosome 2), rs11017104 (GLRX3, chromosome 10q26.3), and rs7245988 (NLRP11, chromosome 19q13.43) [10,101]. Urinary side effects have been linked to rs17599026 (KDM3B, chromosome 5q31.2), rs7720298 (DNAH5, chromosome 5p15.2), and rs17779457 (IFNk and MOB3B, chromosome 9p21.2) [12-104]. Last, rs7120482 and rs17630638 (SLC36A4, chromosome 11q14.3) are associated with rectal bleeding [15], whilst rs264663 (TANC1, chromosome 2q24.1) is associated with overall late toxicity [16]. These SNPs may find use in predictive models. A set of simulation studies identified, for low penetrance distribution models, that the addition of 78 SNPs to existing normal tissue complication probability models increases the AUC to 0.80 from 0.70 for predicting late effects. For moderate penetrance models, only 47 SNPs need to be added [17]. Validation of integrated models these in clinical studies will be important in developing the concept of personalized radiotherapy regimens.

Additionally, SNPs have been found to be associated with aggressive disease. rs11568818 on chromosome 1q11.2 was significantly associated with upgrade of Gleason scoring from grade 6 tumours in two cohorts treated either with surgery or active surveillance [18]. This locus lies just upstream from the transcription start site for the MMP7 gene, well known for its role in promoting local invasion and metastasis. In another study of patients undergoing active surveillance for low-grade tumours, the SNPs rs2735839 (KLK3 region on chromosome 19q13) and rs752822 (chromosome 5q32) together had a significant association with Gleason grade reclassification. KLK3 is a known prostate cancer susceptibility gene [19], whilst the rs752822 SNP lies within the CSNK1A1 gene that contributes to increased KLK3 expression through Wnt signalling.

Although several SNPs significantly associated with prostate cancer have been identified through genome-wide association studies, establishing causality has not yet been possible for many, and many plausible explanations remain speculative [110]. The use of SNPs in identifying prognosis and response to treatment is promising, but research is still very much at an early stage.

Immunotherapy

As has been discussed, immunotherapy represents a promising new avenue of treatment for many urological cancers [111]. However, progress using immunotherapy in prostate cancer is lacking, which is probably related to the relatively low mutational burden of prostate tumours. Sipuleucel-T is a therapeutic cancer vaccine and the only immunotherapeutic approved for the use of prostate cancer by the US Food and Drug Administration and has shown modest results, whilst other vaccines GVAX and PROSTVAC have shown minimal benefit [112]. Immunotherapy involving anti-CTLA4 therapy (ipilimumab) has been evaluated in two large phase III trials of metastatic castration-resistant prostate cancers, both showing no improvement in overall survival [113,114]. Furthermore, the use of the anti-PD-1 agent pembrolizumab in 23 patients with metastatic castration-resistant prostate cancer with PD-L1 expression ≥1% showed an objective response rate of 17.4%, reflecting four of 23 participants showing a partial response. Numerous trials involving single and combination immunotherapy are underway, however, to investigate new immunotherapeutic strategies. Phase III immunotherapy trials are detailed in Table 6.

Given the modest results of existing prostate cancer immunotherapy trials, the concept of patient selection may be particularly important. Patients with cancers harbouring mutations in DNA mismatch repair mechanisms are the hypothetical target of anti-PD-1 therapy, but these mutations are only seen at a rate of ~2% in advanced prostate cancer [58]. Nonetheless, one group reported that, in a study of four patients with metastatic castration-resistant prostate cancers with DNA mismatch repair mutations, nivolumab or pembrolizumab treatment caused a >50% reduction in PSA in two, and objective radiographic response in three [115]. In another study of 11 patients with prostate tumours with DNA mismatch repair mutation, anti-PD-1 or anti-PD-L1 therapy led to six patients experiencing a >50% reduction in PSA level [116]. A phase II single-arm trial administered combination durvalumab and olaparib to 17 patients with metastatic castration-resistant tumours with prior enzalutamide and/or abiraterone treatment [117]. Those with DNA damage repair mutations were more likely to respond. Another phase II trial also demonstrated greater response by patients with DNA damage repair mutations in metastatic prostate cancer when treated with nivolumab plus ipilimumab [118]. Furthermore, patients with CDK12 mutations may show similar sensitivity to immune checkpoint inhibitors, and a phase II trial is currently underway studying the effect of
combination immunotherapy in several metastatic cancers with CDK12 mutations, including metastatic castration-resistant prostate cancer (NCT03570619). Whilst very small, these retrospective studies and prospective trials do suggest significant potential for appropriate and successful use of immunotherapy application in prostate cancer harbouring faulty DNA repair mechanisms vs those without.

Aside from these options, emerging options, such as chimeric antigen T-cell receptor or cytokine-mediated therapies, are expected to contribute soon to the immunotherapeutic arsenal under study in prostate cancer [119,120].

**Diagnostic Biomarkers**

Testing using PSA for the diagnosis of prostate cancer is notorious for high false-positive and high false-negative rates. There exist several blood, urinary and tissue-based tests to support PSA testing [121]. Mi Prostate score is a urine-based test to predict presence of cancer at biopsy. It measures levels of PCA3, TMPRSS2-ERG and KLK3, and algorithmically combines results with serum PSA level. In its developmental and validation cohorts of 516 and 561 patients, respectively, the AUC value in predicting prostate cancer with Gleason \( \geq 7 \) was 0.77 [122]. The ExoDx Prostate (IntelliScore) assay measures urinary exosomes for levels of PCA3, ERG and SPDEF. With PSA level, age, race and family history, the AUC for discriminating Gleason \( \geq 7 \) tumours from Gleason 6 tumours and benign disease was 0.73 in developmental and validation cohorts of 255 and 519 patients, respectively [123]. Last, the SelectMDx assesses urine for levels of HOXC6 and DLX1 overexpression, with KLK3 as the control. Shown in two prospective multicentre studies, it had an AUC for 0.90 for identifying Gleason \( \geq 7 \) tumours in developmental and validation cohorts of 492 and 371 patients, respectively [124]. These are promising diagnostic tools that with further validation may lead to better precision medicine and avoidance of unnecessary biopsies in the future.

**Summary**

Prostate cancer differs from RCC and MIBC in its often protracted clinical course, therefore, assigning prognostic value to specific genetic alterations is difficult. Although TCGA and others have produced molecular subtypes, the prognostic significance of the latter is unclear (Table 5). However, other studies have found alterations of note; for example, germline BRCA mutations may confer worse survival in locally advanced disease. Furthermore, high expression of ERK1/2, ETV1 and FOXA1 in samples has been found in different studies to predispose to recurrence post-radical prostatectomy. Given that comprehensive characterization of genetic alterations in prostate cancer is relatively new, it will be many years before studies can demonstrate long-term mortality outcomes corresponding to specific alterations. It will certainly be interesting to see how prognostic alterations are incorporated into current multivariate prediction models such as the PREDICT Prostate tool [125]. It will also be interesting to see how knowledge alterations are integrated into diagnostic biomarker assays, with some existing urinary assays already showing promising results.

Therapeutically, the main targeted therapy in prostate cancer for many years has been anti-androgen therapy, in modern times achieved with next-generation agents such as abiraterone and enzalutamide (Table 6). These agents have shown significant mortality benefit in high-profile trials, but efficacy has been hampered by the phenomenon of neuroendocrine transdifferentiation. Immunotherapy has been extensively studied but without the successes seen in RCC and MIBC, although there are many phase III trials in progress. In contrast, whilst PI3K targeting has been disappointing, trials for agents affecting DNA repair and MAPK pathways are promising but in preliminary stages.

Last, the role of SNPs should receive further attention, especially given good evidence that certain SNPs can predict toxicity post-radiotherapy.

**Testicular Germ Cell Tumours**

Testicular germ cell tumours are the most common cancer of young adult European men. TGCTs can be classified histologically as either seminoma, or a non-seminomatous germ cell tumour (NSGCT). NSGCTs consist of embryonal carcinoma (EC), choriocarcinoma, yolk sac tumour, and teratoma (mature or immature). NSGCTs can additionally be a mix of seminomatous and non-seminomatous components.

With current treatment, the prognosis for TGCT is excellent given its sensitivity to platinum-based chemotherapy. Seminomas are largely indolent tumours, whilst NSGCTs have a comparatively early onset with a higher mortality. Even with excellent treatment, however, it is important to develop tools by which we can stratify patients, identifying those who would not benefit from aggressive treatment and its adverse effects. Furthermore, treatment options for platinum-resistant tumours remain limited in the face of their poor prognosis, and these tumours would likely benefit from more targeted treatments.

**Commonly Altered Genes and Clinical Implications**

TCGA published its analysis of TGCTs in 2018, analysing samples from 137 primary tumours from 133 patients, with a median age of diagnosis of 31 [126]. Samples were compared with matched germline control DNA from the same patients. The 137 tumours comprised 72 seminomas (52.6%), and 65
NSGCTs (47.4%). Amongst NSGCTs, 18 were EC, nine were EC-dominant (>60% histology was EC-like), three were mature teratomas, 10 were mature teratoma-dominant, three were immature teratoma-dominant, five were yolk sac tumours, eight were yolk sac tumour-dominant, and nine were mixed with no dominant histology. Unlike the aforementioned TCGA analyses, in this paper, survival analyses were not performed [126]. Table 7 lists key alterations with their prognostic and therapeutic implications.

Testicular cancer overall had a low mutational frequency (0.5 mutations/Mb). Three significantly mutated driver genes were observed: KIT (18%), KRAS (14%) and NRAS (4%). These mutations all occurred exclusively in seminomas except for a NSGCT with 30% seminoma histology that contained a KRAS mutation. Of note, 13/17 men with cryptorchidism developed seminomas with amplifications of KIT, KRAS and MDM2 were significantly, recurrently focally amplified across both seminomas and NSGCTs [126]. Seminomas with amplifications of KRAS were also significantly more likely to possess wild-type KIT. NSGCTs were associated with significantly recurrently focally deleted genes GRID2/ATOH1, JARID2, NEGR1, PDE4D and PARK2.

DNA Methylation

Global methylation was low in seminomas, consistent with their primordial germ cell origin [126]. KIT-mutant seminomas were fully unmethylated, but wild-type KIT seminomas had some residual methylation.

High levels of methylation were demonstrated in ECs at non-canonical cytosine sites [126]. This corresponded with greater mRNA levels of DNMT3A/3B. Important tumour suppressors BRCA1, MGMT and RASSF1A were methylated and silenced exclusively in NSGCTs. A total of 24.6% of NSGCTs also had silencing of RAD51C, an important component of the homologous recombination DNA repair pathway, along with BRCA1. In addition, DNACJ15/MCMJ was found to be epigenetically silenced, an alteration associated with drug resistance in patients with breast or endometrial cancer, something which if investigated may have significance as a potential therapeutic biomarker [130].

### Table 7 Known genetic alterations in testicular germ cell tumours that affect prognosis, and/or have known or proposed therapeutic utility.

| Subtype | Altered gene, signature, or subgroup | Prognostic implication | Therapeutic implication |
|---------|-------------------------------------|------------------------|-------------------------|
| Overall | KIT                                 |                        | No significant clinical activity with imatinib [127] |
|         | PI3K pathway (KIT, KRAS, NRAS, PIK3CA, PIK3CD) | miR-371a-3p | Levels rise in TGCT with excellent sensitivity and specificity [136]. Adding miR-367-3p and miR-373-3p increases sensitivity and specificity further. |
|         | Hypermethylation (of BRCA1, MGMT, RAD51C and RASSF1A) | miR-367-3p | Levels correlate with tumour stage and recurrence [138] |
|         | CD274 (PD-L1) | miR-373-3p | Levels correlate with tumour stage [138] |
|         |                      | CD274 (PD-L1) | Mixed results in small preliminary studies [142-145] |

A common theory of testicular cancer tumourigenesis involves at least one whole-genome duplication event followed by chromosome arm deletion. In the TCGA analysis, all samples experienced at least one whole-genome duplication event, and 7.3% experienced two. Seminomas frequently experienced loss of chromosome 11q, whilst NSGCTs frequently experienced loss of chromosomes 19q, 15, 22, 19p, 10q, 8p, 2q and 8q [126]. At least one isochromosome 12p was also observed in 87% of tumours, with the remaining tumours all being seminomas.

KIT, KRAS and MDM2 were significantly, recurrently focally amplified across both seminomas and NSGCTs [126].
Epigenetic therapy could have a future role in TGCT management, particularly NSGCTs, through inducing hypomethylation and subsequent immunogenicity. The DNA methyltransferase inhibitor guadecitabine has shown significant anti-tumour activity in animal models of cisplatin-resistant TGCTs [131]. 5-azacitidine has also shown pro-apoptotic effects and partial restoration of cisplatin-sensitivity in NSGCT cell lines, suggesting that combination therapy with chemotherapy and DNA demethylating agents could be promising [132]; however, clinical studies are lacking. In a single-arm phase II study of hydralazine with magnesium valproate before chemotherapy in solid tumours, the one patient with TGCT studied had reduced chemoresistance and stable clinical response [133]. There are currently no ongoing trials investigating epigenetic therapy in TGCT.

The common silencing of BRCA1 and RAD51C in NSGCTs could make these tumours candidates for PARP inhibition. PARP inhibitor monotherapy has also been shown in vitro to strongly enhance cisplatin sensitivity of cisplatin-resistant, homologous recombination-defective EC cell lines [134]. The only trial ongoing is a phase II study investigating the combination of gemcitabine, carboplatin and the PARP inhibitor veliparib in refractory TGCT (Table 8).

**microRNAs**

A high expression of multiple miRNAs was observed in ECs, including the miR-519 cluster, associated with high expression in embryonic stem cells. All other TGCT subtypes had low expression [126].

miR-371a-3p was dramatically overexpressed in TGCTs compared with other tumour types, particularly in seminomas, ECs, and mixed NSGCTs [126]. In contrast, it had low expression in yolk sac tumours and minimal expression in teratomas. However, miR-375 was overexpressed in teratomas, as well as yolk sac tumours and mixed tumours with teratoma or yolk sac elements. miR-375 was minimally expressed in seminomas and ECs.

MicroRNA could have importance as biomarkers alongside the existing use of alpha-fetoprotein (AFP), hCG and lactate dehydrogenase (LDH). These are markers with good sensitivity and specificity and are currently used in diagnosis, classification, staging and prognosis; however, only 60% of TGCTs have raised tumour markers at diagnosis [135]. These tumour markers also poorly correlate with residual lesion histology post-chemotherapy. In a recent, large, prospective, multicentre study of 616 patients and 258 controls, miR-371a-3p detection had a sensitivity of 90.1% and a specificity of 94.0% for diagnosing TGCTs [136]. In contrast, AFP, hCG and LDH all had sensitivities under 50% in seminomas, and only slightly higher in non-seminomas. This miRNA may therefore be particularly useful for patients with seminoma,

![Table 8: Ongoing targeted therapy trials in testicular germ cell tumours on the basis of known genetic alterations.](image)
who are often marker-negative. There is also evidence that addition of miR-367-3p and miR-373-3p to the testing panel increases sensitivity and specificity further [137].

Levels of miR-371a-3p, as well as miR-367-3p and miR-373-3p also correlate with tumour staging, suggesting potential utility [138]. In addition, miR-371a-3p levels decline following surgery for localized malignant disease and for chemotherapy, and therefore could be utilised to monitor tumour burden during chemotherapy or as surveillance. miR-371a-3p levels have also been shown to rise in relapse [139].

Importantly, raised levels of miR-371a-3p (sensitivity 100%, specificity 54%), and the combined miR-371a-3p/miR-373-3p signature, are highly predictive of viable tumour post-chemotherapy compared to traditional markers [138]. It is common to treat metastatic TGCT with chemotherapy followed by retroperitoneal lymph node dissection (RPLND) if the residual mass is >1 cm$^2$. However, RPLND only benefits patients with viable residual TGCT (55–60% cases), whilst the remaining 40–45% with residual fibrosis or necrosis are exposed to significant morbidity from this major operation [140]. In one study, no patients with residual lesions ≤3 cm and negative miR-371a-3p were found to have viable tumour [138]. With regular monitoring, it may therefore be possible to avoid RPLND in these patients. The major limitation, however, is the use of miR-371a-3p in managing teratomas owing to its minimal expression, and in discriminating between necrosis or fibrosis, and teratomas using miR-371a-3p levels. However, miR-375, which is overexpressed in teratomas but has low expression in healthy young men, could find use as a marker [141].

Aside from further validation in large prospective studies, a significant remaining challenge for miRNA use, however, is understanding their behaviour in and importance to individual histological subtypes.

**Immune Infiltration**

The highest immune infiltration was observed in KIT-mutant seminomas, with gene signatures corresponding to infiltration by several T-cell types [126]. With this, seminomas also had higher levels of T-cell receptors, as well as higher diversity of B-cell receptors and T-cell receptors. In addition, there were higher levels of known cancer-testis-antigen genes in seminomas. Although the role of lymphocytes in TGCT is unclear, these observations could suggest a polyclonal antigen-driven immune response around the tumour. Comparatively, wild-type KIT tumours had slightly lower immune infiltration, with NSGCTs having the lowest levels of immune infiltration.

TGCTs show expression of CD274 (PD-L1) in 73% of seminomas and 64% of NSGCTs, suggesting that immune checkpoint inhibition could be important [142]. In the first PD-L1-targeting clinical trial, 12 patients with platinum-refractory NSGCT were given pembrolizumab monotherapy, which did not lead to any clinically meaningful activity [143]. However, this study was limited by its small size; furthermore, only two patients had positive PD-L1 staining. In a separate retrospective study of seven patients with platinum-refractory NSGCT, two (28%) achieved long-term tumour responses with nivolumab or pembrolizumab [144]. Preliminary results from a phase II trial investigating the anti-CD30 drug brentuximab vedotin in nine relapsed patients with TGCT showed an overall response rate of 22.2% and a 6-month overall survival of 85.7% [145]. This and other promising phase II immunotherapy trials are ongoing (Table 8).

**Summary**

Comparatively little research has been performed in TGCTs, perhaps given its excellent survival rates with current treatment. However, there are certainly some high priority areas needing further work, particularly platinum-resistant metastatic disease, and in reducing morbidity. Although prognostic markers in TGCTs are not well established, diagnostic markers in the form of miRNAs have great potential. miR-371a-3p, miR-367-3p and miR-373-3p may find significance as a diagnostic panel, certainly one that could outperform the currently used AFP, hCG and LDH markers. miR-371a-3p and miR-373-3p may also identify viable tumour post-chemotherapy, thereby helping establish if further treatment or RPLND is needed or not. In regard to treatment itself, immunotherapy may represent an option for treating platinum-resistant metastatic disease due to frequently observed increased expression of CD274 (PD-L1).

The results of phase II trials are awaited.

**Conclusions**

Consortiums like TCGA are performing key research that undoubtedly will help personalize and improve cancer treatment. In RCC, MIBC, prostate cancer, and TGCTs, numerous alterations with prognostic significance have been identified in various histological and molecular subtypes, which are now being targeted in trials. Although we are far from it, it is hoped in the future that we can provide accurate prognostic information and highly targeted therapy on the basis of a patient’s tumour genetics. Until then, numerous trials are underway investigating targeted agents. These should focus on stratifying patients by particular alteration or molecular subtypes to determine how individual groups of patients respond to these targeted therapies. This will be vital for achieving optimized, personalized patient care in urological oncology. We also await the publication of work from other important
consortiums such as the 100 000 Genomes Project and PanProstate Cancer Group, whose work will also further shape our understanding.

**Conflicts of Interest**
None declared.

**References**

1. Turajlic S, Xu H, Litchfield K et al. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx renal. Cell 2018; 173:595–610.e11

2. Ricketts CJ, De Cubas AA, Fan H et al. The cancer genome atlas comprehensive molecular characterization of renal cell carcinoma. Cell Rep 2018; 23: 313–26.e5

3. Choueiri TK, Vaziri SA, Jaeger E et al. von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. J Urol 2008; 180: 860–5

4. Sun M, Marconi L, Eisen T et al. Adjuvant vascular endothelial growth factor-targeted therapy in renal cell carcinoma: a systematic review and pooled analysis. Eur Urol 2018; 74: 611–20

5. Chen W, Hill H, Christie A et al. Targeting renal cell carcinoma with a HIF-2 antagonist. Nature 2016; 539: 112–7

6. Courtney KD, Infante JR, Lam ET et al. Phase I dose-escalation trial of PT2385, a first-in-class hypoxia-inducible factor-2alpha antagonist in patients with previously treated advanced clear cell renal cell carcinoma. J Clin Oncol 2018; 36: 867–74

7. Hakimi AA, Ostrovnya I, Reva B et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA research network. Clin Cancer Res 2013; 19: 3259–67

8. Marques-Magalhaes A, Graca I,Henrique R, Jeronimo C. Targeting DNA methyltransferases in urological tumors. Front Pharmacol 2018; 9: 366

9. Joseph RW, Kapur P, Serie DJ et al. Loss of BAP1 protein expression is an independent marker of poor prognosis in patients with low-risk clear cell renal cell carcinoma. Cancer 2014; 120: 1059–67

10. da Costa WH, Fares AF, Bezerra SM et al. Loss of BAP1 expression in metastatic tumor tissue is an event of poor prognosis in patients with metastatic clear cell renal cell carcinoma. Urol Oncol 2019; 37: 78–85

11. Kapur P, Pena-Llopis S, Christie A et al. Effects on survival of BAP1 and PBRM1 mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation. Lancet Oncol 2013; 14: 159–67

12. Varela I, Tarpey P, Raine K et al. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. Nature 2011; 469: 539–42

13. Hsieh JJ, Chen D, Wang PI et al. Genomic biomarkers of a randomized trial comparing first-line everolimus and sunitinib in patients with metastatic renal cell carcinoma. Eur Urol 2017; 71: 405–14

14. Beuselinck B, Job S, Becht E et al. Molecular subtypes of clear cell renal cell carcinoma are associated with sunitinib response in the metastatic setting. Clin Cancer Res 2015; 21: 1329–39

15. Fay AP, de Velasco G, Ho TH et al. Whole-exome sequencing in two extreme phenotypes of response to VEGF-targeted therapies in patients with metastatic clear cell renal cell carcinoma. J Natl Compr Canc Netw 2016; 14: 820–4

16. Miao D, Margolis CA, Gao W et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. Science 2018; 359: 801–6

17. Chen F, Zhang Y, Senbabaoglu Y et al. Multilevel genomics-based taxonomy of renal cell carcinoma. Cell Rep 2016; 14: 2476–89

18. Motzer RJ, Penkov K, Haanen J et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019; 380: 1103–15

19. Rini BI, Plimack ER, Stus V et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med 2019; 380: 1116–27

20. Mollica V, Di Nunno V, Gatto I et al. Novel therapeutic approaches and targets currently under evaluation for renal cell carcinoma: waiting for the revolution. Clin Drug Investig 2019; 39: 503–19

21. Grimm MO, Bex A, De Santis M et al. Safe use of immune checkpoint inhibitors in the multidisciplinary management of urological cancer: the European Association of Urology Position in 2019. Eur Urol 2019; 76: 368–80

22. Shuch B, Bratslavsky G, Shih J et al. Impact of pathological tumour characteristics in patients with sarcomatoid renal cell carcinoma. BJU Int 2012; 109: 1600–6

23. Cheville JC, Lohse CM, Zincke H et al. Sarcomatoid renal cell carcinoma: an examination of underlying histologic subtype and an analysis of associations with patient outcome. Am J Surg Pathol 2004; 28: 435–41

24. Bi M, Zhao S, Said JW et al. Genomic characterization of sarcomatoid transformation in clear cell renal cell carcinoma. Proc Natl Acad Sci USA 2016; 113: 2170–5

25. Golshayan AR, George S, Heng DY et al. Metastatic sarcomatoid renal cell carcinoma treated with vascular endothelial growth factor-targeted therapy. J Clin Oncol 2009; 27: 235–41

26. Michaelson MD, McKay RR, Werner L et al. Phase 2 trial of sunitinib and gemcitabine in patients with sarcomatoid and/or poor-risk metastatic renal cell carcinoma. Cancer 2015; 121: 3435–43

27. Schoffski P, Wozniak A, Escudier B et al. Crizotinib achieves long-lasting disease control in advanced papillary renal-cell carcinoma type 1 patients with MET mutations or amplification. EORTC 90101 CREATE trial. Eur J Cancer 2017; 87: 147–63

28. Choueiri TK, Vaishampayan U, Rosenberg JE et al. Phase II and biomarker study of the dual MET/VEGFR2 inhibitor foretinib in patients with papillary renal cell carcinoma. J Clin Oncol 2013; 31: 181–6

29. Choueiri TK, Plimack E, Arkenau HT et al. Biomarker-based phase II trial of savolitinib in patients with advanced papillary renal cell cancer. J Clin Oncol 2017; 35: 2993–3001

30. Cancer Genome Atlas Research Network, Linehan WM, Spellman PT et al. Comprehensive molecular characterization of papillary renal-cell carcinoma. N Engl J Med 2016; 374: 135–45

31. Farber NJ, Kim CJ, Modi PK, Hon JD, Sadimim ET, Singer EA. Renal cell carcinoma: the search for a reliable biomarker. Transl Cancer Res 2017; 6: 620

32. Song JB, Morrissey JJ, Mobley JM et al. Urinary aquaporin 1 and periplin 2: can these novel markers accurately characterize small renal masses and help guide patient management? Int J Urol 2019; 26: 260–5

33. Oto J, Plana E, García-Tormo F et al. Urinary microRNAs: looking for a new tool in diagnosis, prognosis, and monitoring of renal cancer. Curr Urol Rep 2020; 21: 1–8

34. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature 2014; 507: 315–22

35. Robertson AG, Kim J, Al-Ahmadie H et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell 2017; 171:540–56.e25

36. Hussain MH, MacVicar GR, Petrylak DP et al. Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/neu-positive urothelial carcinoma: results of a multicenter phase II National Cancer Institute trial. J Clin Oncol 2007; 25: 2218–24

37. Powles T, Huddart RA, Elliott T et al. Phase III, Double-blind, randomized trial that compared maintenance lapatinib versus placebo
after first-line chemotherapy in patients with human epidermal growth factor receptor 1/2-positive metastatic bladder cancer. J Clin Oncol 2017; 35: 48–55.

38. Hussain M, Daignault S, Agarwal N et al. A randomized phase 2 trial of gemcitabine/cisplatin with or without cetuximab in patients with advanced urothelial carcinoma. Cancer 2014; 120: 2684–93.

39. Choudhry NJ, Campanile A, Antic T et al. Afinitor activity in platinum-refractory metastatic urothelial carcinoma in patients with ERBB alterations. J Clin Oncol 2016; 34: 2165–71.

40. Milowsky MI, Iyer G, Regazzi AM et al. Phase II study of everolimus in metastatic urothelial carcinoma. BJU Int 2013; 112: 462–70.

41. Iyer G, Hanrahant AJ, Milowsky MI et al. Genome sequencing identifies a basis for everolimus sensitivity. Science 2012; 338: 221.

42. Grivas P, Mortazavi A, Picus J et al. Mocetinostat for patients with previously treated, locally advanced/metastatic urothelial carcinoma and inactivating alterations of acetyltransferase genes. Cancer 2019; 125: 533–40.

43. Seiler R, Ashab HAD, Erho N et al. Impact of molecular subtypes in muscle-invasive bladder cancer on predicting response and survival after neoadjuvant chemotherapy. Eur Urol 2017; 72: 544–54.

44. Rosenberg JE, Hoffman-Censits J, Powles T et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016; 387: 1909–20.

45. Sieffker-Radtke A, Necchi A, Park SH et al. First results from the primary analysis population of the phase 2 study of erdafitinib (ERDA; [N-42756493] in patients (pts) with metastatic or unresectable urothelial carcinoma (mUC) and FGFR alterations (FGFRalt). J Clin Oncol 2018; 36: 450.

46. O’Donnell P, Goldman JW, Gordon MS et al. 621 a phase I dose-escalation study of MFG1877S, a human monoclonal anti-fibroblast growth factor receptor 3 (FGFR3) antibody, in patients (pts) with advanced solid tumors. Eur J Cancer 2012; 48: 191–2.

47. Bellmunt J, Pal SK, Picus J et al. Safety and efficacy of docetaxel + b-701, a selective inhibitor of FGFR3, in subjects with advanced or metastatic urothelial carcinoma. J Clin Oncol 2017; 35(15_suppl): 4540.

48. Kilgour E, Ferry D, Saggese M et al. Exploratory biomarker analysis of a phase I study of AZD4547, an inhibitor of fibroblast growth factor receptor (FGFR), in patients with advanced solid tumors. J Clin Oncol 2014; 32(15_suppl): 11010.

49. Voss MH, Hierro C, Heist RS et al. Debio 1347, an oral FGFR inhibitor: Results from a first-in-human, phase I dose-escalation study in patients with FGFR genomically activated advanced solid tumors. J Clin Oncol 2017; 35(15_suppl): 2500.

50. Joerger M, Soo R, Cho BC et al. Phase I study of the pan-fibroblast growth factor receptor FGFR inhibitor BAY 1163877 with expansion cohorts for subjects based on tumor FGFR mRNA expression levels. Ann Oncol 2016; 27: 1–36.

51. Pal SK, Rosenberg JE, Hoffman-Censits JH et al. Efficacy of BGJ398, a fibroblast growth factor receptor 1–3 inhibitor, in patients with previously treated advanced urothelial carcinoma with FGFR3 alterations. Cancer Discov 2018; 8: 812–21.

52. Rosenberg JE, Hoffman-Censits J, Powles T et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 2016; 387: 1909–20.

53. Balar AV, Galsky MD, Rosenberg JE et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet 2017; 389: 67–76.

54. Bellmunt J, de Wit R, Vaughn DJ et al. Pembrolizumab as second-line therapy for advanced urothelial carcinoma. N Engl J Med 2017; 376: 1015–26.

55. Tabayoyong W, Kamat AM. Current use and promise of urinary markers for urothelial cancer. Curr Urol Rep 2018; 19: 96.

56. Ou Z, Li K, Yang T et al. Detection of bladder cancer using urinary cell-free DNA and cellular DNA. Clin Transl Med 2020; 9: 47.

57. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. Cell 2015; 163: 1011–25.

58. Robinson D, Van Allen EM, Wu YM et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015; 161: 1215–28.

59. Rodrigues LU, Rider L, Nieto C et al. Coordinate loss of MAP3K7 and CHD1 promotes aggressive prostate cancer. Cancer Res 2015; 75: 1021–34.

60. Mateo J, Carreira S, Sandhu S et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015; 373: 1697–708.

61. Abida W, Bryce AH, Vogelzang J et al. Preliminary results from TRITON2: a phase 2 study of rucaparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) associated with homologous recombination repair (HRR) gene alterations. Ann Oncol 2018; 29(suppl_8): viii271–viii302.

62. Sanchez A, Schoenfeld JD, Nguyen PL et al. Common variation in BRCA1 may have a role in progression to lethal prostate cancer after radiation treatment. Prostate Cancer Prostatic Dis 2016; 19: 197–201.

63. Castro E, Goh C, Leongamornlert D et al. Effect of BRCA mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. Eur Urol 2015; 68: 186–93.

64. Liu W, Xie CC, Thomas CY et al. Genetic markers associated with early cancer-specific mortality following prostatectomy. Cancer 2013; 119: 2405–12.

65. Schwartz S, Wongsuvapat J, Trigwell CB et al. Feedback suppression of PI3Kalpha signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kbeta. Cancer Cell 2015; 27: 109–22.

66. Armstrong AJ, Halabi S, Healy P et al. Phase II trial of the PI3 kinase inhibitor buparlisib (BKM-120) with or without enzalutamide in men with metastatic castration resistant prostate cancer. Eur J Cancer 2017; 81: 228–36.

67. Graham I, Banda K, Torres A et al. A phase II study of the dual mTOR inhibitor MLN0128 in patients with metastatic castration resistant prostate cancer. Invest New Drugs 2018; 36: 458–67.

68. Statz CM, Patterson SE, Mockus SM. mTOR inhibitors in castration-resistant prostate cancer: a systematic review. Target Oncol 2017; 12: 47–59.

69. Nichols NG, Nazarian R, Zhao SG et al. MEK-ERK signaling is a therapeutic target in metastatic castration resistant prostate cancer. Prostate Cancer Prostatic Dis 2019; 22: 531–8.

70. Beardsley EK, Hotte SJ, North S et al. A phase II study of sorafenib in combination with bicalutamide in patients with chemotherapy-naïve castration resistant prostate cancer. Invest New Drugs 2012; 30: 1652–9.

71. Meyer A, Cygan P, Tolzien K et al. Role of sorafenib in overcoming resistance of chemotherapy-failure castration-resistant prostate cancer. Clin Genitourin Cancer 2014; 12: 100–5.

72. Butler DE, Marlein C, Walker HF et al. Inhibition of the PI3K/AKT/mTOR pathway activates autophagy and compensatory Ras/Raf/MEK/ERK signalling in prostate cancer. Oncotarget 2017; 8: 56698–713.

73. Pettersson A, Graff RE, Bauer SR et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2012; 21: 1497–509.

74. Segales I, Juanpere N, Lorenzo M et al. Strong cytoplasmic ETV1 expression has a negative impact on prostate cancer outcome. Virchows Arch 2019; 475: 457–66.

75. Qi M, Liu Z, Shen C et al. Overexpression of ETV4 is associated with poor prognosis in prostate cancer: involvement of uPA/uPAR and MMPa. Tumour Biol 2015; 36: 3565–72.

76. Liu D, Takhar M, Alshalahf M et al. Impact of the SPOP mutant subtype on the interpretation of clinical parameters in prostate cancer. J
92 Hussain M, Asim M. 89 Lee JK, 83 Fraser M, Houlahan KE. 82 Marques-Magalhaes A. 86 Beer TM. Small EJ, Davies AH, Boysen G, Tsourlakis MC, Rydzewska LHM.

prostate cancer: results from NCI 9012. Lancet Oncol 2018; 29: 536–378.

2016; 25: 1615–25.

85 correlates of the epigenetic landscape of prostate cancer. Carcinogenesis 2017; 38: 1180–7.

81 Marques-Magalhaes A, Graca I, Henrique R, Jeronimo C. Targeting DNA methyltransferases in urological tumors. Front Pharmacol 2018; 9: 366.

82 Houlahan KE, Shah YJ, Gusev A et al. Genome-wide germline correlates of the epigenetic landscape of prostate cancer. Nat Med 2019; 25: 1615–26.

83 Lee JK, Phillips JW, Smith BA et al. N-Myc drives neuroendocrine prostate cancer initiated from human prostate epithelial cells. Cancer Cell 2016; 29: 536–47.

84 Fraser M, Sabelnykova VY, Yamaguchi TN et al. Genomic hallmarks of localized, non-indolent prostate cancer. Nature 2017; 541: 359–64.

85 Rydzewska LHM, Burdett S, Vale CL et al. Adding abiraterone to androgen deprivation therapy in men with metastatic hormone-sensitive prostate cancer: a systematic review and meta-analysis. Eur J Cancer 2017; 84: 88–101.

86 Beer TM, Armstrong AJ, Rathkopf DE et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014; 371: 424–33.

87 Hussain M, Fizazi K, Saad F et al. Enzalutamide in men with nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2018; 378: 2465–74.

88 Smith MR, Saad F, Chowdhury S et al. Apalutamide treatment and metastasis-free survival in prostate cancer. N Engl J Med 2018; 378: 1408–18.

89 Fizazi K, Shore N, Tammela TL et al. Darolutamide in nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2019; 380: 1233–46.

90 Asiim M, Tarish F, Zecchini HI et al. Synthetic lethality between androgen receptor signalling and the PARP pathway in prostate cancer. Nat Commun 2017; 8: 374.

91 Clarke N,Wiechno P, Alekseev B et al. Olaparib combined with abiraterone in patients with metastatic castration-resistant prostate cancer: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2018; 19: 975–86.

92 Hussain M, Daignault-Newton S, Twardowski PW et al. Targeting androgen receptor and DNA repair in metastatic castration-resistant prostate cancer: results from NCI 9012. J Clin Oncol 2018; 36: 991–9.

93 Davies AH, Beltran H, Zoubi A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. Nat Rev Urol 2018; 15: 271–86.

94 Small EJ, Aggarwal RR, Huang J et al. Clinical and genomic characterization of metastatic small cell/neuroendocrine prostate cancer (SCNC) and intermediate atypical prostate cancer (IAC): Results from the SU2C/PCF/AACRWest Coast Prostate Cancer Dream Team (WCDT). J Clin Oncol 2016; 34: 5019.

95 Beltran H, Rickman DS, Park K et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011; 1: 487–95.

96 Beltran H, Prandi D, Mosquera JM et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016; 22: 298–302.

97 Tan HL, Sood A, Rahimi HA et al. Rb loss is characteristic of prostatic small cell neuroendocrine carcinoma. Clin Cancer Res 2014; 20: 890–903.

98 Dardenne E, Beltran H, Benelli M et al. N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. Cancer Cell 2016; 30: 563–77.

99 Ku SY, Rosario S, Wang Y et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. Science 2017; 355: 78–83.

100 Krens SL, Osterr H, Stock R et al. Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys 2010; 78: 1292–300.

101 Krens SL, Stock R, Stone N et al. A 2-stage genome-wide association study to identify single nucleotide polymorphisms associated with development of erectile dysfunction following radiation therapy for prostate cancer. Int J Radiat Oncol Biol Phys 2013; 85: e21–28.

102 Krens SL, Stone NN, Stock RG, Rath L, Osterr H, Rosenstein BS. A 2-stage genome-wide association study to identify single nucleotide polymorphisms associated with development of urinary symptoms after radiotherapy for prostate cancer. J Urol 2013; 190: 102–8.

103 Ahmed M, Dorling L, Krens S et al. Common genetic variation associated with increased susceptibility to prostate cancer does not increase risk of radiotherapy toxicity. Br J Cancer 2016; 114: 1165–74.

104 Krens SL, Dorling L, Fachal L et al. Meta-analysis of genome wide association studies identifies genetic markers of late toxicity following radiotherapy for prostate cancer. EBioMedicine 2016; 10: 150–63.

105 Krens SL, Stock RG, Stone NN et al. Genome-wide association study identifies a region on chromosome 11q14. 3 associated with late rectal bleeding following radiation therapy for prostate cancer. Radiother Oncol 2013; 107: 372–6.

106 Fachal L, Gómez-Caamaño A, Barnett GC et al. A three-stage genome-wide association study identifies a susceptibility locus for late radiotherapy toxicity at 2q24.1. Nat Genet 2014; 46: 891–4.

107 Krens SL, Kundu S, Ob JH et al. The prediction of radiotherapy toxicity using single nucleotide polymorphism — based models: a step toward prevention. Semin Radiat Oncol 2015; 25: 281–91.

108 Kearns JT, Lapin B, Wang E et al. Associations between iCOGS single nucleotide polymorphisms and upgrading in both surgical and active surveillance cohorts of men with prostate cancer. Eur Urol 2016; 69: 223–8.

109 Eedes RA, Kote-Jarai Z, Giles GG et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 2008; 40: 316–21.

110 Benafí S, Kote-Jarai Z, Eedes RA. A review of prostate Cancer genome-wide association studies (GWAS). Cancer Epidemiol and Biomarkers Prev 2018; 27: 845–57.

111 Elhage O, Galustian C, Dasgupta P. Immune checkpoint blockade—a treatment for urological cancers? BJU Int 2016; 4: 498–500.

112 Reimers MA, Slane KE, Pachynski RK. Immunotherapy in metastatic castration-resistant prostate cancer: past and future strategies for optimization. Curr Urol Rep 2019; 20: 64.

113 Kwon ED, Drake CG, Scher HI et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol 2014; 15: 700–12.

114 Beer TM, Kwon ED, Drake CG et al. Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naïve castration-resistant prostate cancer. J Clin Oncol 2017; 35: 40–7.
Antonarakis ES, Shaukat F, Velho PI et al. Clinical features and therapeutic outcomes in men with advanced prostate cancer and DNA mismatch repair gene mutations. *Eur Urol* 2019; 75: 378–82

Abida W, Cheng ML, Armenia J et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. *JAMA Oncol* 2019; 5: 471–8

Karzai F, Van der Weele D, Madan RA et al. Activity of durvalumab plus olaparib in metastatic castration-resistant prostate cancer in men with and without DNA damage repair mutations. *J Immunother Cancer* 2018; 6: 141

Boudadi K, Sufman DL, Anagnostou V et al. Ipilimumab plus nivolumab and DNA-repair defects in AR-V7-expressing metastatic prostate cancer. *OncoTarget* 2018; 9: 28561

Sakellariou C, Elhage O, Papaevangelou E et al. Prostate cancer cells enhance interleukin-15-mediated expansion of NK cells. *BJU Int* 2020; 125: 89–102

Gorchkakov AA, Kulezmim SV, Kochneva GV, Taranin AV. Challenges and prospects of chimeric antigen receptor T-cell therapy for metastatic prostate cancer. *Eur Urol* 2020; 77: 299–308

Koo KM, Mainwaring PN, Tomlins SA, Trau M. Merging new-age biomarkers and nanodiagnostics for precision prostate cancer management. *Nat Rev Urol* 2019; 16: 302–17

Sanda MG, Feng Z, Howard DH et al. Association between combined TMPRSS2: ERG and PCA3 RNA urinary testing and detection of aggressive prostate cancer. *JAMA Oncol* 2017; 3: 1085–93

McKiernan J, Donovan MJ, O’Neill V et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA Oncol* 2016; 2: 882–9

Van Neste L, Hendriks RJ, Dijskstra S et al. Detection of high-grade prostate cancer using a urinary molecular biomarker–based risk score. *Eur Urol* 2016; 70: 740–8

Thurtle DR, Greenberg DC, Lee LS, Huang HH, Pharoah PD. Gnanapragasam VJ. Individual prognosis at diagnosis in nonmetastatic prostate cancer: development and external validation of the PREDICT Prostate multivariable model. *PLoS Med* 2019; 16: e1002758

Shen H, Shih J, Hollern DP et al. Integrated molecular characterization of testicular germ cell tumors. *Cell Rep* 2018; 23: 3392–406

Einhorn LH, Brames MJ, Heinrich MC, Corless CL, Madani A. Phase II study of imatinib mesylate in chemotherapy refractory germ cell tumors expressing KIT. *Ann J Clin Oncol* 2006; 29: 12–3

Mego M, Svetlovskova D, Miskovska V et al. Phase II study of everolimus in refractory testicular germ cell tumors. *Urol Oncol* 2016; 34: 122.e17–e22

Fenner M, Oing C, Dieing A et al. Everolimus in patients with multiply relapsed or cisplatin refractory germ cell tumors: results of a phase II, single-arm, open-label multicenter trial (RADITI) of the German Testicular Cancer Study Group. *J Cancer Res Clin Oncol* 2019; 145: 717–23

Fernandez-Cabezudo MI, Faour I, Jones K et al. Deficiency of mitochondrial modulator MCJ promotes chemoresistance in breast cancer. *JCI. Insight* 2016; 1: e86873. [https://doi.org/10.1172/jci.insight.86873](https://doi.org/10.1172/jci.insight.86873)

Albany C, Hever-Jardine MP, von Herrmann KM et al. Refractory testicular germ cell tumors are highly sensitive to the second generation DNA methylation inhibitor guadecitabine. *OncoTarget* 2017; 8: 2949–59

Oing C, Verem I, Mansour W, Bokemeyer C, Dushlovoy S, Honecker F. S-Azacitidine exerts prolonged pro-apoptotic effects and overcomes cisplatin-resistance in non-seminomatous germ cell tumour cells. *Int J Mol Sci* 2018; 20: 21

Candelaria M, Gallardo-Rincón D, Arce C et al. A phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors. *Ann Oncol* 2007; 18: 1529–38

Cavallo E, Graziani G, Antinozzi C et al. Reduced proficiency in homologous recombination underlies the high sensitivity of embryonal carcinoma testicular germ cell tumors to Cisplatin and poly (adp-ribose) polymerase inhibition. *PLoS ONE* 2012; 7: e51563

Barlow LJ, Badalato GM, McKiernan JM. Serum tumor markers in the evaluation of male germ cell tumors. *Nat Rev Urol* 2010; 7: 610–7

Dieckmann KP, Radtke A, Gecz i et al. Levels of MicroRNA-371a-3p (M371 Test) as a new biomarker of testicular germ cell tumors: results of a prospective multicentric study. *J Clin Oncol* 2019; 37: 1412–23

van Aghoven T, Looijenga LHI. Accurate primary germ cell cancer diagnosis using serum based microRNA detection (ampTSmiR test). *OncoTarget* 2016; 8: 58037–49

Leao R, van Aghoven T, Figueiredo A et al. Serum miRNA predicts viable disease after chemotherapy in patients with testicular nonseminoma germ cell tumor. *J Urol* 2018; 200: 126–35

Terbuch A, Adiprasito JB, Stiegelbauer V et al. MRI-371a-3p serum levels are increased in recurrence of testicular germ cell tumor patients. *Int J Mol Sci* 2018; 19: 3130

Krege S, Beyer J, Souchon R et al. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European Germ Cell Cancer Consensus Group (EGCCCG): part II. *Eur Urol* 2008; 53: 497–513

Zhang H, Yang H, Zhang C et al. Investigation of microRNA expression in human serum during the aging process. *J Gerontol A Biol Sci Med Sci* 2015; 70: 102–9

Fankhauser CD, Curioni-Fontecedro A, Allmann V et al. Frequent PD-L1 expression in testicular germ cell tumors. *Br J Cancer* 2015; 113: 411–3

Adra N, Einhorn LH, Althouse SK et al. Phase II trial of pembrolizumab in patients with platinum refractory germ-cell tumors: a Hoosier Cancer Research Network Study. *Gu14-206. Ann Oncol* 2018; 29: 209–14

Zschabitz S, Lasitschka F, Hadaschik B et al. Response to anti-programmed cell death protein-1 antibodies in men treated for platinum refractory germ cell cancer relapsed after high-dose chemotherapy and stem cell transplantation. *Eur J Cancer* 2017; 76: 1–7

Necchi A, Magazza D, Anichini A et al. An open-label, single-group, phase 2 study of brentuximab vedotin as salvage therapy for males with relapsed germ-cell tumors (GCT); Results at the end of first stage (FM12GCT01). *J Clin Oncol* 2016; 34(2_suppl): 480

Correspondence: Prokar Dasgupta, Department of Urology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK. E-mail: prokar.dasgupta@kcl.ac.uk

**Abbreviations:** AFP, alpha-fetoprotein; AR, androgen receptor; AUC, area under the curve; ccRCC, clear-cell RCC; chRCC, chromophobe RCC; CIMP, CpG island methylator phenotype; CIS, carcinoma in situ; EC, embryonal carcinoma; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FH, fumarate hydratase; HIF, hypoxia-inducible factor; MIBC, carcinoma invading bladder muscle; miRNA, microRNA; NSGCT, non-seminomatous germ cell tumour; PDC, pyruvate dehydrogenase complex; pRCC, papillary RCC; RCT, randomized controlled trial; RPLND, retroperitoneal lymph node dissection; SNP, single-nucleotide polymorphism; TCGA, The Cancer Genome Atlas; TGCT, testicular germ cell tumour; TKI, tyrosine kinase inhibitor; UC, urothelial carcinoma; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.