Men with prostate cancer have variable treatment outcomes, ranging from cure to death, that are not well explained by current clinical prognostic factors. While prostate cancer has long been recognised as a heterogenous disease, recent technological advances in genomics have enabled key insights into intra-patient, intra-tumour and inter-patient heterogeneity of prostate cancer. For example, using whole genome sequencing (WGS) of the prostate and metastatic sites in patients with metastatic castration-resistant prostate cancer (mCRPC), Gundem et al reported that, at least in some patients, there may be a complex pattern of metastatic spread, including more than one prostate cancer subclone moving to a metastatic site as well as metastasis-to-metastasis spread. In some cases, with up to 10 separate cancer sites sampled in the same patient, no two sites had the same clonal or subclonal composition, suggesting significant intra-patient heterogeneity within this disease. 

The surge in genomic information and an understanding of tumour heterogeneity in prostate cancer are helping to inform prognosis, treatment decisions and identification of new targeted therapies.

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Using a validated bioinformatics approach to estimate the intra-tumoural heterogeneity of prostatectomy samples, Morris et al were able to demonstrate, in a multivariate analysis, that higher intra-tumoural heterogeneity was associated with an increased risk of death (hazard ratio [HR] 5.6, p=0.016) and was a stronger prognostic factor than PSA level, tumour stage or resection margin status. However, the most therapeutically relevant recent developments are related to inter-patient heterogeneity of prostate cancer. In particular, the Stand Up To Cancer-Prostate Cancer Foundation (SU2C-PCF) International Prostate Cancer Dream Team has shown, through in-depth sequencing of 150 patients with mCRPC, that 89% of individuals harboured clinically actionable mutations. These mutations could be grouped into five broad categories: androgen receptor (AR) pathway (62.7%); phosphoinositol-3-kinase (PI3K) pathway (49%); cell cycle regulators (25%); Wnt/beta-catenin signalling pathway (18%); and DNA repair aberrations (19.3%). As inherited mutations in DNA-repair genes are associated with increased risks of lethal prostate cancer, Pritchard et al used a 20-gene targeted sequencing panel to determine the prevalence of germline DNA-repair mutations. The frequency of 11.8% (82/692) was surprisingly high, demonstrating that a sizeable proportion of DNA aberrations found in mCRPC are indeed heritable. The remainder of this article focuses on how this knowledge of inter-patient heterogeneity is being exploited to improve therapeutic options, response assessment and clinical trial design.

**ANDROGEN RECEPTOR SPLICE VARIANTS**

Inhibition of the AR signalling pathway remains the cornerstone of therapy for mCRPC, but patients eventually become resistant to both first- and second-line hormonal treatments. Antonarakis et al demonstrated that patients with the AR-V7 splice variant, a constitutively active version of the AR that lacks the ligand binding domain, are unlikely to benefit from second-generation hormonal treatments (abiraterone [Zytiga] or enzalutamide). In a pilot study, it was shown that AR-V7 positivity did not seem to predict for taxane chemotherapy resistance. In a further retrospective analysis, Scher et al have demonstrated that patients positive for AR-V7, as detected by anti-AR-V7 on circulating tumour cells, had a better overall survival if treated with a taxane compared to hormonal therapy (HR 0.24; 95% CI 0.10–0.57; p=0.035), and that the presence of AR-V7 increased with more exposure to systemic therapy.

Separately, Welti et al demonstrated in intra-patient paired tissue biopsies that the expression of AR-V7 increased between the hormone-sensitive and castration-resistant prostate cancer samples, and that high AR-V7 predicts for worse overall survival. They also demonstrated that the AR-V7 antibody used (epitope aa630-645, the AR-V7 cryptic region) for both the Scher and Welti studies also detected as yet unidentified off-target proteins that could limit the potential utility of this antibody in future studies. Development of more specific antibodies to AR-V7 and other AR splice variants, corroborative tests and prospective confirmation of these trial results are all being pursued (Figure 2).

**PI3K/PTEN LOSS AND ACTIVATED AKT SIGNALLING**

The PI3K (phosphoinositide 3-kinase) signalling pathway is critical for the growth of prostate cancer and targeting the intermediaries of this cascade such as PI3K, Akt and mTOR (mechanistic target of rapamycin) has shown some activity in early phase studies. Furthermore, loss of PTEN, the phosphatase that negatively regulates the PI3K pathway, was associated with decreased overall survival in patients treated with abiraterone (overall survival PTEN status negative versus positive; 13.9 versus 20.7 months; HR 1.75, p=0.004). There is significant cross-regulation between the PI3K pathway and the AR signalling pathway in prostate cancer, such that inhibition of each individual pathway causes a compensatory upregulation of the other. However, when both pathways are blocked, this leads to marked tumour regression in a xenograft mouse model. This has led to the development of several combination trials in patients progressing on abiraterone or enzalutamide who decline chemotherapy.

At the American Society of Clinical Oncology (ASCO) annual meeting in 2016, Professor de

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**Figure 2. Domain structure of the androgen receptor cancer-associated missense mutations (a) and splice variants (b). Androgen receptor splice variants with the in-frame variant specific amino acids derived from the alternative splicing events which can be detected with antibodies. Adapted from Watson PA et al, 2015**

(a) [Domain structure of the androgen receptor cancer-associated missense mutations](#).

(b) [Domain structure of the androgen receptor splice variants](#).
Bono presented the results of a phase 2 trial with three-way randomisation of abiraterone in combination with either the Akt inhibitor ipatasertib (Ipat; 200mg or 400mg) or placebo. The trial recruited 253 patients; the drug combination was generally well tolerated, with high dose Ipat plus abiraterone demonstrating a trend to improved radiological progression-free survival (rPFS) compared with placebo and abiraterone (median 8.2 versus 6.4 months; HR 0.75, p=0.17).11

Shortly following the BUG meeting, data pertaining to the co-primary endpoint of rPFS in patients with PTEN-deficient prostate cancer was presented at the European Society for Medical Oncology (ESMO) 2016 congress. There was no difference in rPFS for patients receiving abiraterone plus either dose of Ipat, although it should be noted that the phase 2 trial was intentionally not powered to demonstrate a statistically significant difference between the treatment groups. Despite this, there was a clear improvement in rPFS when Ipat was added to abiraterone (Ipat 400mg/abiraterone [n=25] versus placebo/abiraterone [n=21]; 11.5 versus 4.6 months; HR 0.39, \( p=0.0064 \)) in patients with PTEN-deficient prostate cancer. This is the first clinical evidence to support PTEN loss as a predictive biomarker for therapy with ipatasertib plus abiraterone in mCRPC, and will need to be validated in a phase 3 clinical study.11

DNA DAMAGE REPAIR DEFICIENCY (DDRD)

There are distinct single-strand and double-strand DNA repair mechanisms that are redundant, meaning that they are able to compensate for each other if one of them is defective. Farmer et al reported a synthetic lethal interaction between high-fidelity double-strand DNA repair defects (defective homologous recombination due to BRCA1 or BRCA2 mutations) and inhibition of polyadenosine diphosphate ribose polymerase [PARP] by olaparib.12

Building on this observation, Mateo et al assessed the response to olaparib in mCRPC as part of a two-stage multicentre phase 2 clinical study (TOPARP).13 In the first stage, 50 mCRPC patients pretreated with docetaxel chemotherapy went on to receive 400mg olaparib twice daily until progression, with the primary endpoint being response rate. Treatment was well tolerated, and of the 49 patients that were assessable for response, there were 16 responders. 14/16 responding patients were shown to have aberrations in DNA repair genes, most commonly BRCA2, ATM and FANCA. As part of the planned analysis, a DNA repair mutational signature was developed, and biomarker positivity also correlated with rPFS (positive versus negative; 9.8 versus 2.7 months; log rank \( p<0.001 \)). This interesting biomarker is being used as a selection marker for the second stage of the TOPARP study, which is currently recruiting. Multiple PARP inhibitors are now being evaluated, based on the TOPARP data, in registration trials in men suffering from mCRPC.

MISMATCH REPAIR DEFICIENCY (MMRD)

From the SU2C-PCF Dream Team patient cohort of 150 mCRPC, four patients were shown to have hypermutation, three of whom had aberrations in mismatch repair genes.1 This corroborates the work of Pritchard et al, who sequenced samples of mCRPC from a warm autopsy program and demonstrated that 7/60 patients had hypermutation and mismatch MMRD.14 Evidence from endometrial and colon cancer suggests that a proportion of MMRD tumours have high mutation burden, increased immune cell infiltration and improved response rates to immune checkpoint inhibitors. For example, in a phase 2 study, Le et al treated 32 mCRPC patients (11 MMR-proficient and 21 MMR-deficient) with pembrolizumab, a PD-1 immune checkpoint inhibitor, and demonstrated that the MMR-deficient patients had a better objective response rate (4/10 [40%] versus 0/18 [0%]) and 20-week rPFS rate (78% versus 11%; HR 0.1, \( p<0.001 \)) than MMR-proficient patients.15

In keeping with the hypothesis discussed at the BUG meeting 2016, that MMRD-prostate cancer may be sensitive to checkpoint inhibition, two separate groups have subsequently presented early data on patients with mCRPC responding to pembrolizumab. Hansen et al demonstrated that the 3/23 mCRPC patients who responded to pembrolizumab monotherapy had a T-cell inflamed signature.16 Graff et al showed that in 20 mCRPC patients who had previously progressed on enzalutamide, there were four responders when pembrolizumab was added to the enzalutamide, with at least one patient, who was shown to be MMR-deficient, having a rapid and deep biochemical response.17,18 Several commercial and investigator-initiated studies are currently being set up to test which patients may benefit from immune checkpoint blockade including MMR as a biomarker (eg the PERSEUS and NEPTUNE trials).

CONCLUSIONS

Heterogeneity of prostate cancer has marked implications for the diagnosis, treatment stratification and development of resistance. However, the surge in genomic information and an understanding of heterogeneity, coupled with carefully designed clinical trials (Box 1) will help inform prognosis, treatment decisions and identification of new therapies.

Declaration of interests

Johann de Bono is an employee of The Institute of Cancer Research that has a commercial interest in abiraterone, PARP inhibitors for the treatment of homologous recombination DNA repair defective cancers and PI3K/AKT inhibitors including AZD5363. He has served as an advisory board member for many Pharma and Biotech companies including Astellas, AstraZeneca, Genentech/Roche, GSK, Merck Sharp & Dohme, Merck Serono and Sanofi-Aventis. Mark Linch receives research funding from BMS, Astellas and Sanofi and has served as an advisory board member for Janssen Pharmaceuticals.
In recognition of the significant phenotypic heterogeneity of prostate cancer, it has been recommended that dedicated trials should be designed for different groups of prostate cancer patients, defined by their pattern of spread.

As individual lesions within the same patient may be biologically distinct, it has been recommended that the annotation of disease progression includes a breakdown of new sites of disease or progression of existing sites, and to increase the number of lesions reported to five per organ rather than the standard RECIST 1.1 (Response Evaluation Criteria in Solid Tumours) reporting of a total of five lesions.

In recognition of the heterogeneity-driven mixed treatment response, whereby some lesions are progressing while the majority are stable or responding, treatment should be discontinued when the patient is no longer clinically benefiting (NLCB) rather than strictly at the first evidence of progression.

PCWG3 emphasises the importance of serial biological profiling of the disease using minimally invasive blood–based assays of tumour material, imaging or biopsy of a metastatic tumour site to identify and target mechanisms of primary or adaptive resistance, and to better enable treatment selection to be based on disease biology.

PCWG3 strongly endorses incorporating detailed molecular assessments of tumours into clinical trial strategies to better understand the disease biology, and to identify predictors of sensitivity to a specific therapy.

REFERENCES