



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/88617/>

Version: Accepted Version

Article:

Santoni, M., Scarpelli, M., Mazzucchelli, R. et al. (2015) Current Histopathologic and Molecular Characterisations of Prostate Cancer: Towards Individualised Prognosis and Therapies. *European Urology*. ISSN: 0302-2838

<https://doi.org/10.1016/j.eururo.2015.05.041>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

Take-home message

Data on *TMPRSS2-ERG* and *AR-V7* may pave the way for personalised therapy for prostate cancer (PCa) patients. Comprehensive molecular profiling can help identify multiple PCa subtypes and driving alterations. Translating these findings into clinical practice is still challenging.

Current Histopathologic and Molecular Characterisations of Prostate Cancer: Towards Individualised Prognosis and Therapies

Matteo Santoni ^a, Marina Scarpelli ^b, Roberta Mazzucchelli ^b, Antonio Lopez-Beltran ^c, Liang Cheng ^d, Jonathan I. Epstein ^e, Stefano Cascinu ^a, Alberto Briganti ^f, James W. Catto ^g, Francesco Montorsi ^{f,h}, Rodolfo Montironi ^{b,*}

^a Medical Oncology, Università Politecnica delle Marche, Azienda Ospedaliero-Universitaria Ospedali Riuniti Umberto I, GM Lancisi, G Salesi, Ancona, Italy

^b Section of Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, United Hospitals, Ancona, Italy

^c Department of Pathology and Surgery, Faculty of Medicine, Cordoba, Spain

^d Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

^e Departments of Pathology, Urology, and Oncology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

^f Department of Urology, IRCCS San Raffaele Hospital, Milan, Italy

^g Academic Urology Unit, University of Sheffield, Sheffield, UK

^h Vita-Salute San Raffaele University, Milan, Italy

* Corresponding author. Pathological Anatomy, Polytechnic University of the Marche Region, School of Medicine, United Hospitals, Via Conca 71, I-60126 Torrette, Ancona, Italy. Tel.: +39 0715964830; Fax: +39 071889985.

E-mail: r.montironi@univpm.it

Keywords: Prostate cancer; Radical prostatectomy; Prognosis; Prognostic grade grouping; Epstein; Personalised therapy

Radical prostatectomy (RP) is a common treatment for prostate cancer (PCa) [1]. The surgical specimen plays an important role in disease annotation and the prediction of future events. Its evaluation should include a definition of morphologic characteristics with prognostic and therapeutic value and a personalised pathology report based on the latest international guidelines. The morphologic evaluation should correlate with and explain pre- and intraoperative findings (Fig. 1a and 1b). Advances in molecular biology mean there will soon be a need to match histopathologic findings with molecular features that could improve prognostication and individualise treatment [2].

This editorial updates the contemporary role of the uropathologist in the era of personalised medicine in the evaluation of the morphologic and molecular characteristics of PCa and their clinical significance.

The uropathologist in the personalised medicine era

The macroscopic evaluation of RP specimens should include quality indicators of the surgical procedure, such as specimen integrity, including missing parts, and should take into account the type of surgical procedures, such as nerve sparing and the approach used (open vs minimally invasive). The uropathologist should also consider the effects of previous treatment and/or surgical procedures, such as transurethral resection of the prostate or radiation therapy/focal therapy, and the presence of tissue other than prostate (ie, rectal wall).

Microscopic evaluation, based on the latest international guidelines, should include (1) tumour multifocality, the index tumour, and tumour extent; (2) histopathologic type; (3) Gleason score

and grade grouping; and (4) TNM stage including surgical margin status and lymphovascular invasion (LVI).

Tumour multifocality, index tumour, and tumour volume

Although PCa is usually multifocal (Fig. 1b), the index lesion (mostly defined as the largest tumour) is considered crucial in driving outcomes. Gleason grade, tumour volume, and stage are mostly determined by the index lesion because secondary foci are usually small well-differentiated lesions. Recording tumour volume using a quantitative estimate is recommended, although most studies demonstrating this measure do not provide independent prognostic information beyond standard pathologic parameters [3–5]. Recent data suggest multiparametric magnetic resonance imaging performs well at predicting pathologic features of the index lesion (Fig. 1a), regardless of tumour multifocality [6]. Ahmed et al recently reported a single-centre prospective study in which 56 patients with multifocal PCa were treated only for the largest and highest grade tumour (index tumour). Index lesion ablation was associated with little toxicity, and >80% of patients were without clinically significant cancer at 12 mo [7].

The index lesion can be hard to define, and so studies are under way to examine their genetics and thus better define each lesion. Further advances in molecular studies may define or redefine the index tumour as the most aggressive biologically, rather than the largest or most poorly differentiated [8]. Although recording tumour volume using some quantitative estimate is recommended, tumour volume does not provide independent prognostic information once other standard pathologic parameters are known.

Lindberg et al [9], by searching for metastatic-specific DNA alterations in several regions of the prostate, identified the area that gave rise to metastases. The metastasising component probably originated from prostatic ducts via an invasive component with Gleason score $4 + 4 = 8$ highly

related to the intraductal carcinoma component although located at some distance. Such a finding supports the fact that intraductal carcinoma is a morphologic marker of aggressive disease and a major step forward on the origin of PCa and on the mechanisms of metastatic spread [10].

Histopathologic type

More than 95% of all prostate carcinomas are referred to as acinar, microacinar, usual, or conventional type. Several variants of PCa have been described including neuroendocrine differentiation, ductal, mucinous, signet ring cell-like, sarcomatoid carcinoma, adenosquamous, and other cancers (some deceptively benign looking). Although relatively uncommon, these variants have prognostic and therapeutic importance. A novel morphologic classification of PCa with neuroendocrine differentiation (NE) was recently published [11]. NE differentiation is implicated in hormonal escape, androgen receptor (AR) independence, and resistance to AR antagonist enzalutamide [12].

Transdifferentiation from a prostate epithelial luminal cell type to a NE-like phenotype is a complex multifactorial process that is considered the result of suppressing androgen levels [13]. However, the prognostic significance of NE differentiation in typical PCa is controversial. As suggested by Sargos et al, “The exploration of the different pathways implicated in the neuroendocrine differentiation of prostate cancers is essential for the comprehension of castration-resistance mechanisms” [14].

Gleason score and prognostic grade grouping

Gleason score can predict findings in RP, biochemical failure, local or distant recurrence in untreated patients as well as in patients who undergo RP, radiation therapy, or other therapies. In 2005 the International Society of Urological Pathology (ISUP) organised a consensus conference where the Gleason grading system was updated to reflect contemporary practice. In 2010 it was

recognised that PCa grading needs further modifications [15]. In 2013 a new grade grouping, which accurately reflects prognosis and is clearly understood by physicians and patients alike, was proposed by Epstein [16]: Gleason score ≤ 6 (prognostic grade group I), Gleason score $3 + 4 = 7$ (prognostic grade group II), Gleason score $4 + 3 = 7$ (prognostic grade group III), Gleason score $4 + 4 = 8$ (prognostic grade group IV), and Gleason score 9–10 (prognostic grade group V). The ISUP had a meeting on PCa grading in November 2014 in Chicago, IL, USA. The advantages of this prognostic grade grouping were presented by Jonathan I. Epstein: A statistically different time of recurrence-free progression, suggesting that this grouping system should be considered the new standard for PCa patients. There was overwhelming support from the pathologists and clinicians present for utilisation of a five-tier grading prognostic system based on grouping of the 2005 modified Gleason grading system and the 2010 amendments to the diagnostic criteria for Gleason pattern 3. The five-tier grade group system was recently also accepted by the World Health Organisation *Classification of Tumours* scheduled for publication in 2016.

TNM stage including surgical margin status and lymphovascular invasion

The pT2 substaging, surgical margin status, and LVI represent three important topics for uropathologists and clinicians. In contrast to the clinical importance of cT2 cancer substaging [17], the lack of clinical relevance of T2 pathologic substaging suggests that a future TNM revision should just include pT2. Patients could be stratified based on clinical, molecular, or other qualitative and quantitative morphologic features including prognostic grade grouping or tumour volume. A revision of the TNM is expected later in 2015.

The definition of a positive surgical margin (PSM; ie, R1) is ink on tumour cells and can be extraprostatic (failure to widely excise tumour with extraprostatic extension) or intraprostatic

(through surgical breach of the tumour). The presence of a PSM at RP has been linked to increased risk of biochemical recurrence and need for adjuvant therapy. The association of Gleason grade at the site of PSM with subsequent clinical progression among patients with Gleason score 7 PCa has been investigated [18] and shown to add prognostic information along with the extent of cancer at the margin. A close association between PSM and mortality outcomes [19,20], as well as the prognostic significance of the presence of benign prostatic tissue at the resection margin [21,22], is still controversial.

It is of utmost importance to precisely identify the presence of tumour cells at the level of the surgical margin because this is a decisive factor in determining the future therapeutic approach [2]. A technology to reduce such surgery-related morbidities, especially in localised PCa, should be developed. An imaging technique associated with a tumour biomarker to allow clean-up of residual neoplastic cells would be useful [23]. This could be obtained by intraoperative prostate-specific membrane antigen (PSMA)-based imaging for the visualisation of PCa. This technique consists of the use of a humanised anti-PSMA monoclonal antibody conjugated to an indocyanine green derivative to assess PCa extracapsular extension [23].

LVI is defined as the unequivocal presence of tumour cells within a vascular or lymphatic endothelial-lined space or as the presence of tumour emboli in small intraprostatic vessels. LVI has been associated with decreased time to biochemical progression, distant metastases, and overall survival after RP. In addition, LVI has been shown to act as an independent predictor of disease recurrence when controlling for other pathologic variables known to influence clinical outcome [24]. Figure 1c is the map distribution of the tumour whose pathology report is summarised in Table 1.

Update on molecular characteristics and their clinical significance

PCa is a heterogeneous disease that may be defined by molecular subtypes [25]. Research is currently focussing on the pathogenetic and prognostic role of the transmembrane protease, serine 2-v-ets avian erythroblastosis virus E26 oncogene homolog (*TMPRSS2-ERG*) fusion and AR splice variant 7 (AR-V7).

ERG (ETS-related gene) and *TMPRSS2* are members of the ETS family of genes, implicated in embryonic tissue development and cancer progression. Approximately 50% of usual PCa and small cell carcinoma harbour *TMPRSS2-ERG* gene fusion and/or alternative *ERG* gene rearrangements [26]. Danila et al evaluated *TMPRSS2-ERG* status in circulating tumour cells (CTCs) from patients treated with abiraterone acetate, showing no correlation with prostate-specific antigen decline and clinical outcome [27]. Interestingly, *TMPRSS2-ERG* gene fusion has been proposed as a diagnostic and prognostic urinary biomarker for PCa [28]. Urinary *TMPRSS2-ERG* gene fusion has been shown to correlate with biopsy Gleason score and clinical tumour stage in patients with PCa [28].

It has been speculated that the presence of the *TMPRSS2-ERG* fusion protein could correlate with a better response to androgen deprivation therapy (ADT) compared with *TMPRSS2-ERG*-negative tumours. *ERG* is implicated in in vitro and in vivo resistance to taxanes [29]. At present, two phase 2 studies are assessing the predictive value of *TMPRSS2-ETS* fusion status in PCa patients treated with enzalutamide (PREMIERE-SOGUG, NCT02288936) or abiraterone acetate (ABIGENE, NCT01858441). The results of these prospective studies will allow a better characterisation of the role of *TMPRSS2-ETS* in guiding treatment decisions.

AR splice variants are common in castration-resistant PCa tissue/cells and have been implicated as a potential mechanism of resistance to ADT. In 2014 Antonarakis et al reported the detection of AR-V7 from CTCs in patients treated with AR antagonists [30]. They showed that AR-V7 in

CTCs from patients with castration-resistant PCa was associated with resistance to enzalutamide and abiraterone. At the American Society of Clinical Oncology Genitourinary Cancers Symposium 2015, the same group presented the results on the role of AR-V7 detection in patients treated with taxanes, showing the lack of a significant association with sensitivity to taxanes [31]. Based on these findings, AR-V7 may represent a predictive biomarker to select the optimal treatment sequencing for patients with metastatic castration-resistant PCa.

Conclusions

A close collaboration among urologists, uropathologists, genitourinary radiologists, and uro-oncologists in the evaluation of prostate specimens is improving our knowledge on the morphologic and molecular backgrounds for individualised prognosis and therapies in patients with PCa. Data on *TMPRSS2-ERG* and AR-V7 may pave the way to personalised therapy for PCa patients. Comprehensive molecular profiling from RP samples as well as from prostate biopsies can help identify multiple PCa subtypes and driving alterations. Translating these findings into clinical practice is still challenging.

Conflicts of interest: The authors have nothing to disclose.

References

[1] Montironi R, Cheng L, Lopez-Beltran A, et al. Joint appraisal of the radical prostatectomy specimen by the urologist and the uropathologist: together, we can do it better. *Eur Urol* 2009;56:951–5.

- [2] Montironi R, Santoni M, Lopez-Beltran A, Cheng L, Moch H, Scarpelli M. Morphologic and molecular backgrounds for personalized management of genito-urinary cancers: an overview. *Curr Drug Targets* 2015;16:96–102.
- [3] Epstein JI, Carmichael M, Partin AW, et al. Is tumor volume an independent predictor of progression following radical prostatectomy? A multivariate analysis of 185 clinical stage B adenocarcinoma of the prostate with 5 years of follow-up. *J Urol* 1993;149:1478–85.
- [4] Salomon L, Levrel O, Anastasiadis AG, et al. Prognostic significance of tumor volume after radical prostatectomy: a multivariate analysis of pathological prognostic factors. *Eur Urol* 2003;43:39–44.
- [5] Van Oort IM, Witjes JA, Kok DE, et al. Maximum tumor diameter is not an independent prognostic factor in high-risk localized prostate cancer. *World J Urol* 2008;26:237–41.
- [6] Le JD, Tan N, Shkolyar E, et al. Multifocality and prostate cancer detection by multiparametric magnetic resonance imaging: correlation with whole-mount histopathology. *Eur Urol* 2015;67:569–76.
- [7] Ahmed HU, Dickinson L, Charman S, et al. Focal ablation targeted to the index lesion in multifocal localised prostate cancer: a prospective development study. *Eur Urol*. In press. DOI:10.1016/j.eururo.2015.01.030
- [8] Barbieri CE, Demichelis F, Rubin MA. The lethal clone in prostate cancer: redefining the index. *Eur Urol* 2014;66:395–7.
- [9] Lindberg J, Kristiansen A, Wiklund P, Grönberg H, Egevad L. Tracking the origin of metastatic prostate cancer. *Eur Urol* 2015;67:819–22.
- [10] Gundem G, Van Loo P, Kremeyer B, et al. The evolutionary history of lethal metastatic prostate cancer. *Nature* 2015;520:353–7.

- [11] Epstein JI, Amin MB, Beltran H, et al. Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. *Am J Surg Pathol* 2014;38:756–67.
- [12] Ferrari AC, Liu X-M, Alshalalfa M, et al. Effect of enzalutamide (EZ) exposure time on androgen receptor (AR) splice variant (SV) expression and gene transcription correlates in androgen-dependent (AD) prostate cancer (PC) cells [abstract e22066]. *J Clin Oncol* 2014;32(Suppl).
- [13] Beltran H, Tomlins S, Aparicio A, et al. Aggressive variants of castration-resistant prostate cancer. *Clin Cancer Res* 2014;20:2846–50.
- [14] Sargos P, Ferretti L, Gross-Goupil M, et al. Characterization of prostate neuroendocrine cancers and therapeutic management: a literature review. *Prostate Cancer Prostatic Dis* 2014;17:220–6.
- [15] Epstein JI. An update of the Gleason grading system. *J Urol* 2010;183:433–40.
- [16] Pierorazio PM, Walsh PC, Partin AW, Epstein JI. Prognostic Gleason grade grouping: data based on the modified Gleason scoring system. *BJU Int* 2013;111:753–60.
- [17] Ramos CG, Carvalhal GF, Smith DS, et al. Clinical and pathological characteristics, and recurrence rates of stage T1c versus T2a or T2b prostate cancer. *J Urol* 1999;16:1525–9.
- [18] Viers BR, Sukov WR, Gettman MT, et al. Primary Gleason grade 4 at the positive margin is associated with metastasis and death among patients with Gleason 7 prostate cancer undergoing radical prostatectomy. *Eur Urol* 2014;66:1116–24.
- [19] Emerson RE, Koch MO, Daggy JK, Cheng L. Closest distance between tumor and resection margin in radical prostatectomy specimens: lack of prognostic significance. *Am J Surg Pathol* 2005;29:225–9.

- [20] Viers BR, Sukov WR, Gettman MT, et al. Primary Gleason grade 4 at the positive margin is associated with metastasis and death among patients with Gleason 7 prostate cancer undergoing radical prostatectomy. *Eur Urol* 2014;66:1116–24.
- [21] Kernek KM, Koch MO, Daggy JK, Juliar BE, Cheng L. The presence of benign prostatic glandular tissue at surgical margins does not predict PSA recurrence. *J Clin Pathol* 2005;58:725–8.
- [22] Kohl SK, Balaji KC, Smith LM, et al. Clinical significance of benign glands at surgical margins in robotic radical prostatectomy specimens. *Urology* 2007;69:1112–6.
- [23] Nakajima T, Mitsunaga M, Bander NH, Heston WD, Choyke PL, Kobayashi H. Targeted, activatable, in vivo fluorescence imaging of prostate-specific membrane antigen (PSMA) positive tumors using the quenched humanized J591 antibody-indocyanine green (ICG) conjugate. *Bioconjug Chem* 2011;22:1700–5.
- [24] Baydar DE, Baseskioglu B, Ozen H, Geyik PO. Prognostic significance of lymphovascular invasion in clinically localized prostate cancer after radical prostatectomy. *ScientificWorldJournal* 2008;8:303–12.
- [25] Fisher KW, Montironi R, Lopez-Beltran A, et al. Molecular foundations for personalized therapy in prostate cancer. *Curr Drug Targets* 2015;16:103–14.
- [26] Wang L, Williamson SR, Zhang S, et al. Increased androgen receptor gene copy number is associated with TMPRSS2-ERG rearrangement in prostatic small cell carcinoma. *Mol Carcinog*. In press. DOI:10.1002/mc.22162
- [27] Danila DC, Anand A, Sung CC, et al. TMPRSS2-ERG status in circulating tumor cells as a predictive biomarker of sensitivity in castration-resistant prostate cancer patients treated with abiraterone acetate. *Eur Urol* 2011;60:897–904.

- [28] Leyten GH, Hessels D, Jannink SA, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *Eur Urol* 2014;65:534–42.
- [29] Galletti G, Matov A, Beltran H, et al. ERG induces taxane resistance in castration-resistant prostate cancer. *Nat Commun* 2014;5:5548.
- [30] Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med* 2014;371:1028–38.
- [31] Antonarakis ES, Lu C, Chen Y, et al. AR splice variant 7 (AR-V7) and response to taxanes in men with metastatic castration-resistant prostate cancer (mCRPC) [abstract 138]. *J Clin Oncol* 2015;33(Suppl 7).

Figure legend

Fig. 1 – (a) The vertical bars represent the biopsy cores and their locations in relation to the prostate drawing in the background. The bars in black represent the positive cores (ie, biopsies with cancer) including the extension of cancer. The prostate in the background is subdivided into zones according to the guidelines of multiparametric magnetic resonance imaging (mpMRI) evaluation. The red area with the number 5 inside represents an area identified as “Clinically significant cancer is highly likely to be present” (Prostate Imaging Reporting and Data System [PI-RAD] score 5). A combination of bars with an indication of cancer-positive cores, including the extent, and a drawing of the prostate with the results of an mpMRI evaluation, when available, is what should be sent to clinicians. (b) Radical prostatectomy specimen processed with the whole-mount technique. The dotted areas represent the location of the cancer foci. There are two cancer foci. The index nodule (dominant nodule) is present in the body of the prostate, right side, in two consecutive whole-mount sections. It shows the features of a significant cancer (Gleason score $3 + 4 = 7$; volume: 0.9 ml). It corresponds to the mpMRI area identified as PI-RAD score 5 in (a). The additional nodule is in the opposite side of the prostate; it is present in one whole-mount section only and shows the features of an insignificant cancer (Gleason score $3 + 3 = 6$; volume: 0.4 ml). (c) Prostate map graphically representing the whole mount sections and the location of the two tumour foci, one already identified by mpMRI and both detected with the prostate biopsies and characterised histologically. The tumour focus in red corresponds to the significant cancer (index tumour) and the green to the insignificant cancer. Such a prostate map is sent to clinicians together with the pathology report that

includes the macroscopic and microscopic evaluations as well as a summary of the analysis, as seen in Table 1.

L = left side of the prostate gland; R = right side of prostate gland.

Table 1 – Radical prostatectomy specimen: personalised pathology report

Specimen	Report
pT2c (m, 2) R0 LV10 NX MX	
Total tumour volume	1.3 ml
Volume of the index nodule (dominant nodule)	0.9 ml (greatest diameter: 1.1 cm)
Volume of the additional tumour nodule	0.4 ml
Summary of the GS and grades	
GS of the index nodule (dominant nodule)	3 + 4 = 7 (% of tumour with Gleason grade 4: 10%)
GS of the additional tumour nodule	3 + 3 = 6

GS = Gleason score.

Figure 1

