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SHORT COMMUNICATION

Metastatic castration resistant prostate cancer with squamous cell, small cell, and sarcomatoid elements—a clinicopathologic and genomic sequencing-based discussion

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Abstract

Histologic variants are uncommon but well reported amongst cases of prostatic adenocarcinoma, including those in the setting of hormonal and/or chemoradiation therapy and castration resistance. However, the spectrum of morphologic phenotypes and molecular alterations present in such histologic variants are still incompletely understood. Herein, we describe a case of metastatic prostatic adenocarcinoma with hormonal and chemoradiation therapy-associated differentiation, displaying a combination of squamous cell, small cell, and sarcomatoid elements. The morphologic, immunohistochemical, and molecular observations are discussed with attention given to the gene alterations present, including in *TP53, NF1, AR, PTEN*, and *RB1*. Finally, we will compare our findings with those observed in uncommonly reported similar cases so as to detail the molecular underpinnings of such processes which may carry therapeutic implications.

Keywords Prostate cancer · Small cell · Squamous · Sarcomatoid · Castration resistant · Sequencing

Introduction

Variant morphologic patterns in prostatic carcinomas are relatively uncommon, and may appear as mixed components of recurrent conventional adenocarcinoma, de novo or after treatment. A host of infrequent patterns have been described, including prostatic ductal adenocarcinoma, small cell carcinoma, mucinous (colloid) carcinoma, squamous cell carcinoma, and sarcomatoid carcinoma.

Squamous cell carcinoma of the prostate is a rare entity, representing less than 1% of all prostate carcinomas, and typically presents after hormonal and/or radiation therapy for prostatic adenocarcinoma; components of squamous cell carcinoma are often associated with advanced disease at presentation and may predict refractoriness to hormonal therapy [1, 2]. Small cell carcinoma of the prostate is also associated with advanced stage disease, visceral metastases, and poor survival. Sarcomatoid carcinoma of the prostate often presents as bulky recurrence following radiation therapy, and the presence of sarcomatoid carcinoma with or without heterologous epithelioid elements is considered to be associated with poor survival [1]. When identified in isolation, these variant patterns often necessitate the exclusion of secondary malignancy from metastasis or adjacent spread. These variant features have been rarely identified simultaneously in combination as components of conventional prostatic adenocarcinoma [3–5]. However, the biologic underpinnings of these variant morphologies and their association with post-treatment recurrence are incompletely understood, and further characterization may help to develop

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more efficient targeted therapies for such malignancies with a poor prognosis.

In this report, we present the case of a 47-year-old man with metastatic prostatic adenocarcinoma treated with hormone therapy, chemotherapy, and radiation therapy. Post-treatment biopsy of a right iliac wing mass revealed metastatic prostatic adenocarcinoma with squamous cell, small cell, and sarcomatoid features. We also present and discuss the results of integrative clinical sequencing as it pertains to targeted therapies for this unique case.

Materials and methods

Patient consent and approval was attained via our MION-COSEQ enrollment process; the study was reviewed and approved by the University of Michigan Institutional Review Board. Hemotoxylin and eosin (H&E) staining was performed at the University of Michigan Health System using routine laboratory methods. Immunohistochemistry by the Department of Pathology at the University of Michigan Health System was performed using BenchMark ULTRA automated stainer and the ultraView Universal DAB Detection Kit (Ventana Medical Systems, Oro Valley, AZ). The following primary antibodies were used: PSA (polyclonal; predilute, Ventana Medical Systems); and NKX3.1 (CP422B, polyclonal; 1:50 predilute, Biocare Medical, Concord, CA) with appropriate positive and negative controls. H&E slides were reviewed and protein expression of ERG, PSA, and NKX3.1 was evaluated by two study pathologists (A.L. and R.M.). Strong diffuse cytoplasmic staining for PSA, and nuclear staining for NKX3.1 was considered positive.

Needle biopsies were snap frozen in OCT. High quality DNA and RNA was isolated from the core needle biopsies and germline DNA was isolated from patient matched normal tissue. Samples were processed to perform comprehensive next generation sequencing (whole-exome capture sequencing of tumor and normal DNA, and capture transcriptome sequencing of tumor RNA). Briefly, exome libraries of tumor and matched normal genomic DNAs were generated using the Illumina TruSeq DNA Sample Prep Kit, following the manufacturer's instructions. RNA-Seq transcriptome libraries were prepared following Illumina's TruSeq RNA protocol, using 2 μg of total RNA. RNA integrity was measured using an Agilent 2100 Bioanalyzer. The quality and quantity of the resulting exome and transcriptome libraries were analyzed using an Agilent 2100 Bioanalyzer and DNA 1000 reagents. Paired-end libraries were sequenced with the Illumina HiSeq 2500. Reads that passed the chastity filter of Illumina BaseCall software were used for subsequent analysis. MI-ONCOSEQ bioinformatic pipelines were used to detect the following classes of mutations: somatic and germline variant calls including single-nucleotide variants and insertion/deletions (indels), copy number alterations, gene fusions and outlier gene expression [6]. Potentially actionable mutations are defined as any genomic findings discovered during sequencing that could lead to a (1) change in patient management by providing a targetable molecular aberration; (2) change in diagnosis or risk stratification and/or; (3) cancer-related germline findings that inform about a potential cancer risk to patient family members.

Results

Clinical history and sequence of events

A 47-year-old male without significant past medical history or family medical history presented with intermittent occipital headaches and progressive difficulty swallowing, left tongue deviation, and changes in the quality of his voice. While initial work up including brain MRI and neck CT was unrevealing, repeat neck CT after 3 months showed a soft tissue mass at the anterior skull base involving adjacent osseous structures as well as multiple lytic spinal vertebral lesions. Subsequent laboratory evaluation demonstrated anemia, thrombocytopenia, and a markedly elevated prostate-specific antigen (PSA) of 2231.4 ng/mL. Bone marrow biopsy revealed complete marrow replacement by metastatic prostatic adenocarcinoma as described below.

The patient was initiated on the gonadotropin-releasing hormone (GnRH) receptor antagonist Degarelix, the androgen receptor inhibitor Bicalutamide, and the synthetic gonadotropin releasing hormone Lupron. In addition, the patient also received palliative radiation therapy to the base of skull lesion. Serial PSA measurements decreased, suggesting some degree of castrate sensitivity. The patient also underwent six cycles of the cytotoxic chemotherapy with docetaxel with continued reduction of PSA levels. However, after approximately 6 months of treatment, PSA levels increased, and the patient was deemed to be castrate resistant. Bicalutamide was removed from his treatment regimen and he began palliative radiation therapy to his spinal metastases. The antiandrogenic medication Abiraterone was thence instituted along with prednisone.

He was readmitted nearly a year after his initial biopsy diagnosis with a rapidly expanding lumbar mass and began palliative radiation therapy to this new lesion. Repeat MRI revealed a new area of disease within the right iliac wing eroding through cortex with a large extraosseous component. This soft tissue component was biopsied for sequencing purposes, and the biopsy revealed metastatic prostatic adenocarcinoma as described below.

Pathologic findings

The initial bone marrow biopsy demonstrated complete replacement by metastatic prostatic adenocarcinoma. Morphologic evaluation exhibited conventional acinar differentiation (primarily cribriform prostatic adenocarcinoma) without features of any histologic variants, as well as associated desmoplastic stromal reaction and remodeling of trabecular bone (Fig. 1a, b). Immunohistochemical stains for NKX3.1 and prostate-specific antigen (PSA) demonstrated diffuse and positive expression in the malignant cells (Fig. 1c, d). The corresponding peripheral blood smear not surprisingly identified leukoerythroblastic features, including granulocytic left shift, teardrop poikilocytosis of the red blood cells with frequent nucleated forms, consistent with myelophthisis.

The most recent biopsy of the right iliac wing soft tissue mass demonstrated metastatic prostatic adenocarcinoma with squamous cell, small cell, and sarcomatoid features along with a minor component of conventional acinar type adenocarcinoma with focal comedonecrosis (Fig. 2a, b). The squamous cell component exhibited infiltrative nests of moderately differentiated pink polygonal squamous cells with squamous whorls and keratin formation (Fig. 2d, e). The small cell component displayed sheets of cells with small hyperchromatic nuclei (similar size to conventional component), high nuclear to cytoplasmic ratio, relatively fine chromatin without prominent nucleoli and scant cytoplasm. In some areas, the small cell component was seen to merge with areas of abrupt keratinization and/or surround squamous nests (Fig. 2d, e). The sarcomatoid component exhibited relatively dispersed high-grade, fusiform cells with spindled to epithelioid hyperchromatic nuclei, set in a fibrotic stroma (Fig. 2c). Focally the cells demonstrated frank anaplasia with multiple nuclei in horseshoe and wreath-like arrangements (Fig. 2f). Immunohistochemical assessment of this biopsy demonstrated positive NKX3.1 expression in the conventional adenocarcinoma component

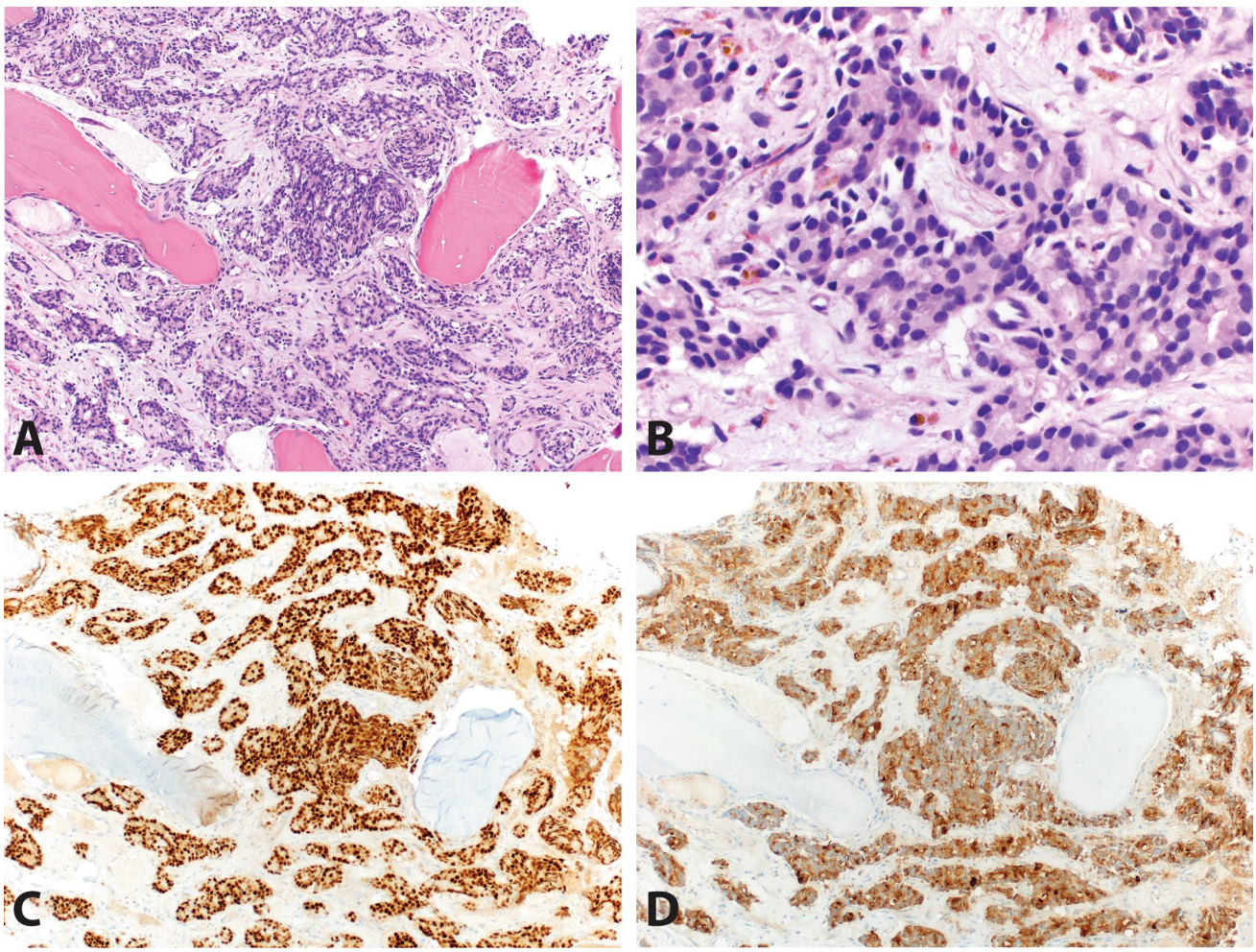
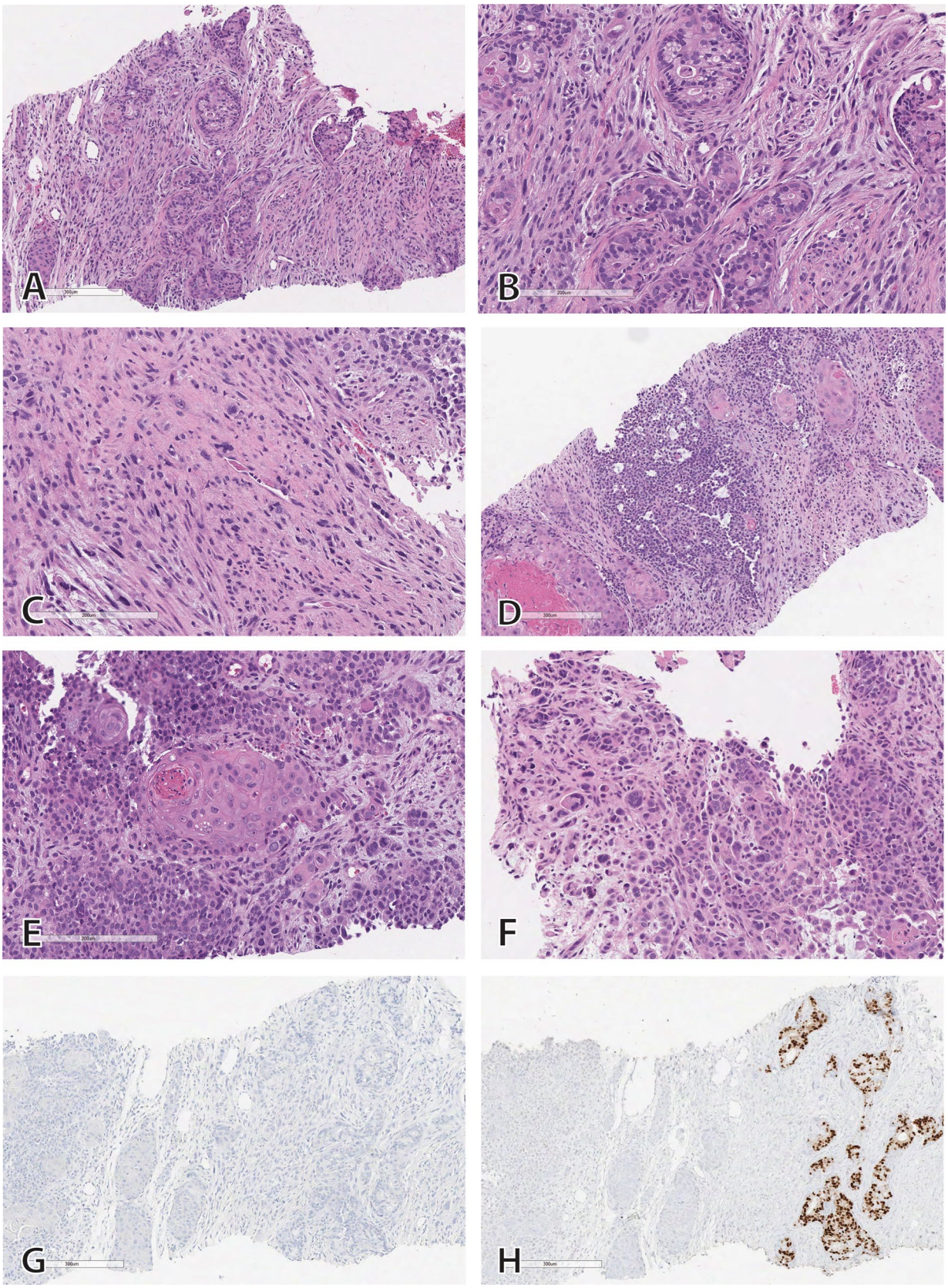


Fig. 1 Initial bone marrow biopsy demonstrating complete replacement by metastatic prostatic adenocarcinoma. **a** H&E stain 10×; **b** H&E stain, 40×; **c** NKX3.1 stain, 10×; **d** PSA stain, 10×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◂**Fig. 2** Iliac wing soft tissue mass biopsy demonstrating metastatic prostatic adenocarcinoma. **a, b** Conventional component (H&E, 10× and 20×). **c** Sarcomatoid component (H&E, 20×). **d, e** Squamous and small cell components (H&E, 10× and 20×). **f** Anaplastic component (H&E, 20×). **g** PSA stain (10×). **h** NKX3.1 stain highlighting positivity in conventional component only (10×)

only, while PSA expression was negative in all the components, including the conventional areas (Fig. 2g, h).

Sequencing and ancillary studies

Genomic sequencing of the tissue from the right iliac wing mass biopsy demonstrated a number of somatic alterations. Point mutations were identified in genes encoding tumor protein p53 (*TP53*) (p.E286K with copy loss, allelic fraction 62%) and neurofibromin 1 (*NF1*) (splice acceptor of exon 47 with copy loss [c.7063-2A>G], allelic fraction 52%), as well as bromodomain and WD repeat domain containing 3 (*BRWD3*) (p.H1141L, allelic fraction 68%), chromodomain helicase DNA-binding protein 9 (*CHD9*) (p.R1227* stop-gain, allelic fraction 53%), DNA methyltransferase 1 (*DNMT1*) (p.P1320H, allelic fraction 40%), protein tyrosine phosphatase receptor type M (*PTPRM*) (p.S352I, allelic fraction 39%), sulfotransferase family 1A member 1 (*SULT1A1*) (p.M60I, allelic fraction 18%), and transmembrane channel like 9 (*TMC8*) (p.G103R, allelic fraction 42%). A frameshift insertion was also identified in the cyclin-dependent kinase 20 gene (*CDK20*) (p.H298fs, allelic fraction 61%). Copy number alterations identified include homozygous deletions of phosphatase and tensin homolog (*PTEN*, chromosome 10), RB transcriptional corepressor 1 (*RB1*, chromosome 13), and mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*, chromosome 5). No pathogenic germline variants were detected. No ETS (E26 transformation-specific) fusion transcript was detected.

The tumor also showed outlier expression of the squamous cell marker tumor protein p63 (TP63) and demonstrated extremely low expression of androgen receptor (AR) via RNA sequencing.

Discussion

Variant histologic patterns in prostatic adenocarcinoma are rare but disproportionately associated with prior hormonal and/or radiation treatment for prostatic adenocarcinoma. In our case, a 47-year-old male developed prostatic adenocarcinoma with squamous cell, small cell, sarcomatoid, as well as conventional morphologies. Prior work suggests an association between chemoradiation and/or hormonal therapy and the subsequent development of squamous cell, small cell, and sarcomatoid elements [1–5]; however, the molecular foundations and genetic triggers for the development of these variants are still relatively unknown. Among the histologic variants identified in our current case, there is some literature suggesting multipotential cell origination. While investigators initially believed squamous cell carcinoma of the prostate to represent carcinomatous evolution from benign squamous metaplasia [7], the epidemiological rarity and strong association with antecedent radiation and/or hormonal therapy has made authors suggest that squamous cell carcinoma may arise from multipotential conventional prostatic adenocarcinoma cells [1]. Additionally, previous studies have speculated that small cell carcinoma of the prostate may evolve from a subset of multipotent non-neuroendocrine prostatic tumor cells based on co-expression of prostate-specific and neuroendocrine markers [1]. More recent theories speculating the origin of small cell carcinoma include a transdifferentiation phenomenon in which the pathogenesis behind the development of resistance to androgen deprivation therapy may involve development of a neuroendocrine phenotype. The transition from hormone-sensitive to castrate-resistant cancer is thought to be related to a process referred to as neuroendocrine transdifferentiation which refers to a transition from an epithelial-like phenotype in the cancer to a neuroendocrine-like phenotype, and is believed to be a resistance mechanism that may occur as a result of androgen deprivation therapy [8, 9]. Finally, sarcomatoid carcinoma of the prostate may also likely arise from cells with epithelial and mesenchymal potential, as spindled areas of these tumors have demonstrated desmosomes upon ultrastructural examination [10].

Such concepts of tumor evolution may be applied to the morphological, immunohistochemical, and molecular findings in the current case. Prostate cancer is fundamentally an androgen-driven disease with the upstream stimulation of the androgen receptor (AR) leading to tumor proliferation and downstream PSA expression (measured as rising serum PSA). Castration resistance is associated with a rising serum PSA and/or progression of metastases despite castration levels of testosterone. Disease progression in castration-resistant prostate cancer is often driven by reactivation of AR signaling, which can be treated with potent AR-targeted therapies: Abiraterone acetate (which our patient received) and Enzalutamide, among others. For many patients, disease progression after potent AR-therapies may occur in different ways such as: (1) secondary alterations involving the AR gene (e.g., amplification or activating point mutations, AR splice variants) lead to restored AR signaling; (2) bypass or crosstalk mechanisms (for example, glucocorticoid receptor, PI3K/AKT pathway activation) lead to restored AR signaling despite nonfunctional and/or downregulated AR; and (3) the tumor becomes less dependent on AR signaling and grows despite suppressed AR signaling without a concordant rise in PSA.

In our case, the extremely low expression of *AR*, and the rising PSA despite castration levels of testosterone points to continued AR signaling via a bypass mechanism, which we attributed to the mutation in *NF1* (Neurofibromin 1). While *NF1* amplifications have been demonstrated in a subset of neuroendocrine prostate cancer, *NF1* mutations are seen in fewer than 5% of prostate cancer cases [11, 12]. Complete loss of NF1 can lead to activation of Ras signal transduction pathway, which regulates a cascade of downstream pathways, including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB), and mammalian target of rapamycin (mTOR) kinase; this may explain how our patient's tumor was able to resume downstream AR signaling independent of the androgen receptor. In addition, the relative rarity of *NF1* mutation within conventional prostatic adenocarcinoma in the literature coupled with its identification in this patient with squamous/small cell/sarcomatoid components, raises the possibility of a causal association in such cases. The additional mutations in *TP53, RB1*, and *PTEN* are known to be fairly frequent in metastatic and poorly differentiated disease; based on the recent literature, alterations in these genes also likely played a contributory role in the development of a range of histologic appearances in this case, mostly likely reflecting variably differentiating phenotypes [13–15].

Sequencing results from this case also generated clinically actionable information, including potential eligibility for ongoing clinical trials. The *NF1* loss-of-function mutation status opens potential eligibility to NCT02465060, a clinical trial of the MEK inhibitor trametinib for refractory *NF1*-mutant tumors. In addition, the *PTEN* deletion opens up potential eligibility to NCT02465060 and NCT02215096 (PI3K-beta inhibitor GSK2636771 without and with concurrent enzalutamide), NCT01884285 (PI3K inhibitor AZD8186 as monotherapy and incombination with mTOR inhibitor AZD2014), NCT02576444 (mTOR inhibitor AZD2014 plus olaparib), NCT02761694 (pan-AKT inhibitor ARQ 751), and NCT02961283 (B-RAF and PI3K inhibitor ASN003).

Future studies will continue to elucidate the molecular underpinnings of divergent differentiation, treatment-related changes, and castration-resistance in advanced stage prostate cancer; moreover, this work may benefit future prognostic stratification and patient selection for sequencing to identify targeted therapies. Whether *NF1* mutation is consistently and specifically associated with the above-reported divergent histologic features in prostatic adenocarcinoma, de novo and/or post-therapy, remains to be investigated in bigger cohorts and future studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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