NEOPLASIA
www.neoplasia.com

Volume 15 Number 1 January 2013 pp. 1–10 1

Concurrent *AURKA* and *MYCN* Gene Amplifications Are Harbingers of Lethal Treatment-Related Neuroendocrine Prostate Cancer[1,2]

Juan Miguel Mosquera[*,†,3], Himisha Beltran[*,‡,3], Kyung Park[†], Theresa Y. MacDonald[†], Brian D. Robinson[†], Scott T. Tagawa[*,‡], Sven Perner[§], Tarek A. Bismar[¶], Andreas Erbersdobler[#], Rajiv Dhir[**], Joel B. Nelson[††], David M. Nanus[*,‡] and Mark A. Rubin[*,†]

*Weill Cornell Cancer Center, Weill Medical College of Cornell University, New York, NY; [†]Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY; [‡]Department of Medicine, Weill Medical College of Cornell University, New York, NY; [§]Institute of Pathology, University of Bonn, Bonn, Germany; [¶]Departments of Pathology and Laboratory Medicine and Oncology, University of Calgary, Calgary, Canada; [#]Institute of Pathology, University of Rostock, Rostock, Germany; **Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [††]Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Abstract

Neuroendocrine prostate cancer (NEPC), also referred to as anaplastic prostate cancer, is a lethal tumor that most commonly arises in late stages of prostate adenocarcinoma (PCA) with predilection to metastasize to visceral organs. In the current study, we explore for evidence that Aurora kinase A (*AURKA*) and N-myc (*MYCN*) gene abnormalities are harbingers of treatment-related NEPC (t-NEPC). We studied primary prostate tissue from 15 hormone naïve PCAs, 51 castration-resistant prostate cancers, and 15 metastatic tumors from 72 patients at different stages of disease progression to t-NEPC, some with multiple specimens. Histologic evaluation, immunohistochemistry, and fluorescence *in situ* hybridization were performed and correlated with clinical variables. *AURKA* amplification was identified in overall 65% of PCAs (hormone naïve and treated) from patients that developed t-NEPC and in 86% of metastases. Concurrent amplification of *MYCN* was present in 70% of primary PCAs, 69% of treated PCAs, and 83% of metastases. In contrast, in an unselected PCA cohort, *AURKA* and *MYCN* amplifications were identified in only 5% of 169 cases. When metastatic t-NEPC was compared to primary PCA from the same patients, there was 100% concordance of *ERG* rearrangement, 100% concordance of *AURKA* amplification, and 60% concordance of *MYCN* amplification. In tumors with mixed features, there was also 100% concordance of *ERG* rearrangement and

Address all correspondence to: Dr Juan Miguel Mosquera or Dr Mark A. Rubin, Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10065. E-mail: jmm9018@med.cornell.edu, rubinma@med.cornell.edu

[1]This work was supported by NCI R01 CA152057 (M.A.R.), Early Detection Research Network NCI U01 CA111275 (J.M.M. and M.A.R.), and the Prostate Cancer Foundation (H.B. and M.A.R.). M.A.R. is a co-inventor of the patent on the detection of gene fusions in prostate cancer, filed by the University of Michigan and the Brigham and Women's Hospital. The diagnostic field of use for ETS gene fusions has been licensed to Hologic Gen-Probe. The authors declare that there are no relationships that could be construed as resulting in an actual, potential, or perceived conflict of interest with regard to the current manuscript submitted for review.
[2]This article refers to supplementary materials, which are designated by Table W1 and Figures W1 and W2 and are available online at www.neoplasia.com.
[3]These authors contributed equally to this work.
Received 17 September 2012; Revised 19 November 2012; Accepted 20 November 2012

DOI 10.1593/neo.121550

94% concordance of *AURKA* and *MYCN* co-amplification between areas of NEPC and adenocarcinoma. *AURKA* and *MYCN* amplifications may be prognostic and predictive biomarkers, as they are harbingers of tumors at risk of progressing to t-NEPC after hormonal therapy.

Neoplasia (2013) 15, 1–10

Introduction

The development of neuroendocrine prostate cancer (NEPC, also referred to as anaplastic prostate cancer) is thought to drive approximately 25% of the nearly 34,000 cases per year of lethal prostate cancer in the United States [1]. However, data from autopsy studies suggest that the incidence of NEPC may be significantly underestimated [2]. It is known that the amount of neuroendocrine differentiation increases with disease progression and correlates with patient exposure to long-term androgen deprivation therapy. Preclinical studies also support transformation of prostate adenocarcinoma (PCA) cells into neuroendocrine cells when depleted of androgen *in vitro* and in xenograft models [3–7]. Therefore, with the introduction of new highly potent androgen receptor (AR)–targeted therapies into the clinic, the incidence of treatment-related NEPC (t-NEPC) might escalate. Patients who develop t-NEPC have an aggressive clinical course and often develop visceral metastases, and most survive less than 1 year [8]. Because neuroendocrine cells do not express AR or secrete prostate-specific antigen (PSA), the PSA level tends to be low or does not elevate in proportion to clinical progression. Elevated serum markers of neuroendocrine differentiation, such as chromogranin A and neuron-specific enolase (NSE), may support the diagnosis.

The prostate cancer–specific *ERG* gene rearrangement occurs in approximately 50% of t-NEPC [9–11] and there is concordance of *ERG* status and other molecular abnormalities between PCA and t-NEPC foci of mixed tumors [11]; these data strongly suggest that there is a similar cell of origin of PCA and t-NEPC. This also distinguishes t-NEPC from neuroendocrine tumors arising from other anatomic sites and suggests that molecular studies of PCA may provide insight into events that occur early before the development of t-NEPC.

We recently demonstrated that the cell cycle kinase, Aurora kinase A, and the transcription factor, N-myc, cooperate to induce neuroendocrine differentiation in prostate cancer [12]. Furthermore, treatment of NEPC models with an Aurora kinase inhibitor resulted in significant tumor shrinkage and reversal of the neuroendocrine phenotype, thereby providing rationale for clinical evaluation of an Aurora kinase inhibitor for patients with t-NEPC. In that study, we also showed that concurrent overexpression and amplification of the Aurora kinase A gene (*AURKA*) and N-myc gene (*MYCN*) in metastatic t-NEPC was significantly higher (40%) when compared to an unselected cohort of localized PCA (5%). Interestingly, one of the patients who progressed from PCA to t-NEPC had amplification of *AURKA* and *MYCN* in his primary hormone naïve PCA, suggesting that these molecular events occur early in the disease [12].

In the current study, we examined the histologic spectrum of t-NEPC and evaluated *AURKA* and *MYCN* amplifications in primary prostate tumors and metastases from 72 patients who developed lethal t-NEPC.

Materials and Methods

Case Selection

Pathology material from 72 patients who clinically developed NEPC was evaluated. Clinical parameters for the diagnosis of NEPC included rapid progression of the disease with visceral metastases in the setting of low (≤10 ng/ml) or modestly rising PSA and/or elevated neuroendocrine serum markers (chromogranin A > 5× upper limit of normal, NSE > 2× upper limit of normal). All patients received androgen deprivation therapy before disease progression toward NEPC. Cases were identified at different collaborating institutions under Institutional Review Board (IRB)-approved protocols for the purpose of this study. The clinical information collected for each patient included the age at diagnosis of PCA, clinical stage, type of primary and systemic therapy, interval of time between initial diagnosis and castration-resistant state, interval of time between castration-resistant prostate cancer (CRPC) and metastatic disease including sites of metastases, and death.

For comparison purposes, an unselected cohort of 169 patients with localized PCA who underwent radical prostatectomy (RP) at Weill Cornell Medical Center was used. In addition, two pathology specimens from patients with primary (*de novo*) mixed small cell carcinoma with prostatic adenocarcinoma were assessed, and six prostatectomy cases of hormone naïve, localized PCA with Paneth cell–like neuroendocrine change were included in the study as separate controls of low-grade neuroendocrine differentiation [13].

A summary of clinical characteristics of patients included in the study is presented in Table 1.

Pathologic Evaluation

Formalin-fixed paraffin-embedded tissue of the aforementioned cases was available. Regarding archival material from the 72 patients who clinically progressed to t-NEPC, different specimens were available corresponding to different stages of disease (see below). Hematoxylin and eosin (H&E)–stained slides from surgical resections and biopsies were reviewed by study pathologists (J.M.M., K.P., B.D.R., and M.A.R.). Pathologic evaluation included Gleason score of untreated tumors (prostate biopsy, transurethral resection, and/or prostatectomy specimens), histologic examination of metastases and treated prostate tumors, and pathologic tumor stage. Classification of the spectrum of neuroendocrine tumors, both primary and metastatic PCAs, was applied using the definitions used in lung classification [14,15]. Briefly, tumors with neuroendocrine morphology were small cell carcinoma (pure or combined) and large cell neuroendocrine carcinoma, and non–small cell carcinomas were poorly differentiated adenocarcinomas of the prostate with or without neuroendocrine differentiation. Poorly differentiated adenocarcinomas were considered to have neuroendocrine differentiation when more than 30% of tumor cells were positive for synaptophysin or chromogranin A.

Table 1. Clinicopathologic Characteristics of Patients Who Developed t-NEPC and Controls.

Archival material studied from patients who developed t-NEPC (*n* = 72), 100%
Primary hormone naïve PCA only (*n* = 11), 15.3%
Primary hormone naïve PCA, treated PCA, and subsequent metastases (*n* = 1), 1.4%
Primary hormone naïve PCA and subsequent metastases (*n* = 3), 4.2%
Gleason score of localized PCA: 3+3=6 to 5+5=10
Pathologic stage of localized PCA: pT2c N0 to pT3a N1
Treated PCA only (*n* = 49), 68.0%
Treated PCA and subsequent metastases (*n* = 1), 1.4%
Metastases only* (*n* = 7), 9.7%
Age at diagnosis of PCA: 42 to 84 years (median = 65 years)
Time interval to progression to CRPC: 2 to 10 years (median = 4 years)
Overall survival after clinical diagnosis of NEPC: 8 to 14 months (median = 12 months)
Treatments received as monotherapy or in combination: RP, radiation therapy, androgen deprivation therapy including MDV3100 or abiraterone, chemotherapy (carboplatin + taxol, carboplatin + etoposide, docetaxel, irinotecan, docetaxel + radium-223 chloride) (Table W1)
Unselected cohort of patients with localized PCA who underwent RP (*n* = 169), 100%
Age at diagnosis of PCA: 42 to 75 years (median = 62 years)
Gleason score of localized PCA: 3+3=6 to 4+5=9
Pathologic stage of localized PCA: pT2a N0 to pT3b N0
Primary (*de novo*) NEPC (mixed small cell carcinoma and PCA) (*n* = 2), 100%
Age at diagnosis of *de novo* NEPC: 65 and 67 years
Pathologic stage (RP performed): pT3a N0 both cases
Localized PCA with Paneth cell–like neuroendocrine differentiation (*n* = 6), 100%
Age at diagnosis of PCA: 54 to 74 years (median = 65 years)
Gleason score of localized PCA: 3+3=6 to 4+5=9
Pathologic stage of localized PCA: pT2a N0 to pT3b N1

*Fifteen metastatic sites included retroperitoneum (one), colon (one), bladder (three), brain (two), pleura (one), pelvic soft tissue (two), liver (one), and bone (four).

Archival tissue from the 72 patients who developed t-NEPC included 11 primary hormone naïve PCA cases only (matched treated PCA or metastasis unavailable), 1 case of hormone naïve PCA with available tissue of treated PCA and metastases, 3 cases of hormone naïve PCA and subsequent metastases after treatment (matched treated PCA unavailable), 49 treated PCA only (matched hormone naïve PCA or metastases unavailable), 1 treated PCA and subsequent metastases (matched hormone naïve PCA unavailable), and 7 cases of metastases only (matched hormone naïve or treated PCA unavailable) (Table 1 and Figure W1*A*). A total of 15 metastatic tumors from 12 patients were interrogated. Sites of metastases included retroperitoneum (*n* = 1), colon (*n* = 1), bladder (*n* = 3), brain (*n* = 2), pleura (*n* = 1), pelvic soft tissue (*n* = 2), liver (*n* = 1), and bone (*n* = 4). Some patients presented with synchronous metastases at other anatomic locations including peritoneum, lungs, and stomach.

Overall, the assessed pathology material of these patients, some with multiple specimens from different stages in progression toward t-NEPC, included 15 hormone naïve clinically localized PCAs and 66 treated tumors: 51 treated PCAs and 15 metastases, the latter being from 12 patients (see Figure W1*A*).

A subset of 19 neuroendocrine tumors from non-prostate origin was also interrogated for *AURKA* and *MYCN* amplifications and included primary small cell carcinoma of lung (*n* = 12) and bladder (*n* = 2), metastatic small cell carcinoma of lung to cerebellum (*n* = 1), well-differentiated neuroendocrine tumor ("typical carcinoid") of bowel (*n* = 1), metastatic well-differentiated neuroendocrine tumor ("typical carcinoid") of bowel to liver (*n* = 1) and lung (*n* = 1), and mammary ductal carcinoma *in situ* with neuroendocrine differentiation (*n* = 1).

Fluorescence In Situ *Hybridization*

To assess *AURKA* and *MYCN* amplifications and *PTEN* status, we used a locus-specific probe plus reference probe fluorescence *in situ* hybridization (FISH) assays as previously described [12,16]. The reference probe was located at 10q25 (BAC RP11431P18), spanning a stable region of the chromosome. Amplification was defined as the presence of four or more copies on average for gene-specific (*AURKA* or *MYCN*) signals per nucleus compared to two reference signals. *ERG* rearrangement was assessed using dual-color break-apart interphase FISH assay as described previously [17,18]. At least 50 nuclei were evaluated per tissue section using a fluorescence microscope (Olympus BX51; Olympus Optical, Tokyo, Japan).

Immunohistochemistry

Immunohistochemistry (IHC) stain was performed in a subset of 44 cases using antibodies for synaptophysin (Clone SP11 from Lab Vision/Thermo Fisher Scientific, Kalamazoo, MI) and chromogranin A (Clone LK2H10 from Biogenex, Fremont, CA), following vendors' specified optimal dilutions for IHC. In a subset of 15 cases (primary PCA of patients who developed t-NEPC), IHC for Aurora kinase A was performed (ab13824 from Abcam Inc, Cambridge, MA; 1:800 dilution).

Results

Clinical Characteristics of Patients Who Progressed to t-NEPC

Full clinical information was available in 43 of 72 patients and partial clinical information in the remaining 29 patients. The age at diagnosis of PCA ranged from 42 to 84 years (median = 65 years). Time interval to progression to CRPC ranged from 2 to 10 years (median = 4 years), time on androgen deprivation therapy ranged from 1 to 11 years (median = 4 years), and overall survival after clinical diagnosis of NEPC ranged from 8 to 14 months (median = 12 months).

Treatment modalities received since diagnosis of PCA encompassed one or more of the following: RP, radiation therapy (external beam radiation and brachytherapy), androgen deprivation therapy such as luteinizing hormone-releasing hormone analogs, luteinizing hormone-releasing hormone antagonists, and anti-androgens including MDV3100 and abiraterone, and chemotherapy protocols with carboplatin + paclitaxel, carboplatin + etoposide, docetaxel, irinotecan, docetaxel + radium-223 chloride.

Detailed treatment received including hormonal and chemotherapy in a subset of patients along with survival, pathology, and FISH data is presented in Table W1.

The age at diagnosis of PCA in the unselected cohort of 169 patients used for comparison (RP only) ranged from 42 to 75 years (median = 62 years).

Histopathology

Microscopic evaluation of 51 treated PCA cases and 15 metastases demonstrated three major histologic groups: 1) pure neuroendocrine prostate carcinoma, which included small cell carcinoma (*n* = 18) and large cell neuroendocrine carcinoma (*n* = 1); 2) poorly differentiated adenocarcinoma with (*n* = 21) or without (*n* = 8) neuroendocrine differentiation; 3) mixed neuroendocrine carcinoma and adenocarcinoma (*n* = 18). Among the latter group of 18 cases with mixed morphology, the neuroendocrine carcinoma component included areas of small cell carcinoma (*n* = 15) and large cell neuroendocrine carcinoma (*n* = 3) (Table 2). The spectrum of NEPC, primary and metastatic, is illustrated in Figure 1.

The prostate specimens from two patients who presented with primary (*de novo*) NEPC corresponded to one case of mixed small

Table 2. Three Histologic Groups of Treated PCA and Metastases from Patients Who Developed t-NEPC.

Treated PCA cases (*n* = 51) and metastatic sites (*n* = 15) examined (*n* = 66), 100%
1. Pure neuroendocrine carcinoma (*n* = 19), 28.8%
Small cell carcinoma (*n* = 18)
Large cell neuroendocrine carcinoma (*n* = 1)
2. Mixed neuroendocrine carcinoma and adenocarcinoma (*n* = 18), 27.2%
With areas of small cell carcinoma (*n* = 15)
With areas of large cell neuroendocrine carcinoma (*n* = 3)
3. Poorly differentiated adenocarcinoma (*n* = 29), 44.0%
With neuroendocrine differentiation (*n* = 21)
Without neuroendocrine differentiation (*n* = 8)

cell carcinoma with areas of PCA Gleason score 5 + 4 = 9 (prostate needle biopsies) and one case of mixed large cell neuroendocrine carcinoma with areas of PCA with ductal features (transurethral resection of prostate).

The Gleason scores of 15 hormone naïve PCAs (i.e., initial diagnosis of patients who later developed t-NEPC) ranged from 3 + 3 = 6 to 5 + 5 = 10, and their pathologic tumor stage ranged from pT2c N0 to pT3a N1. Areas of benign prostate tissue were also identified in these 15 specimens.

The Gleason scores of the unselected cohort of 169 cases of localized PCA ranged from 3 + 3 = 6 to 4 + 5 = 9, and their pathologic

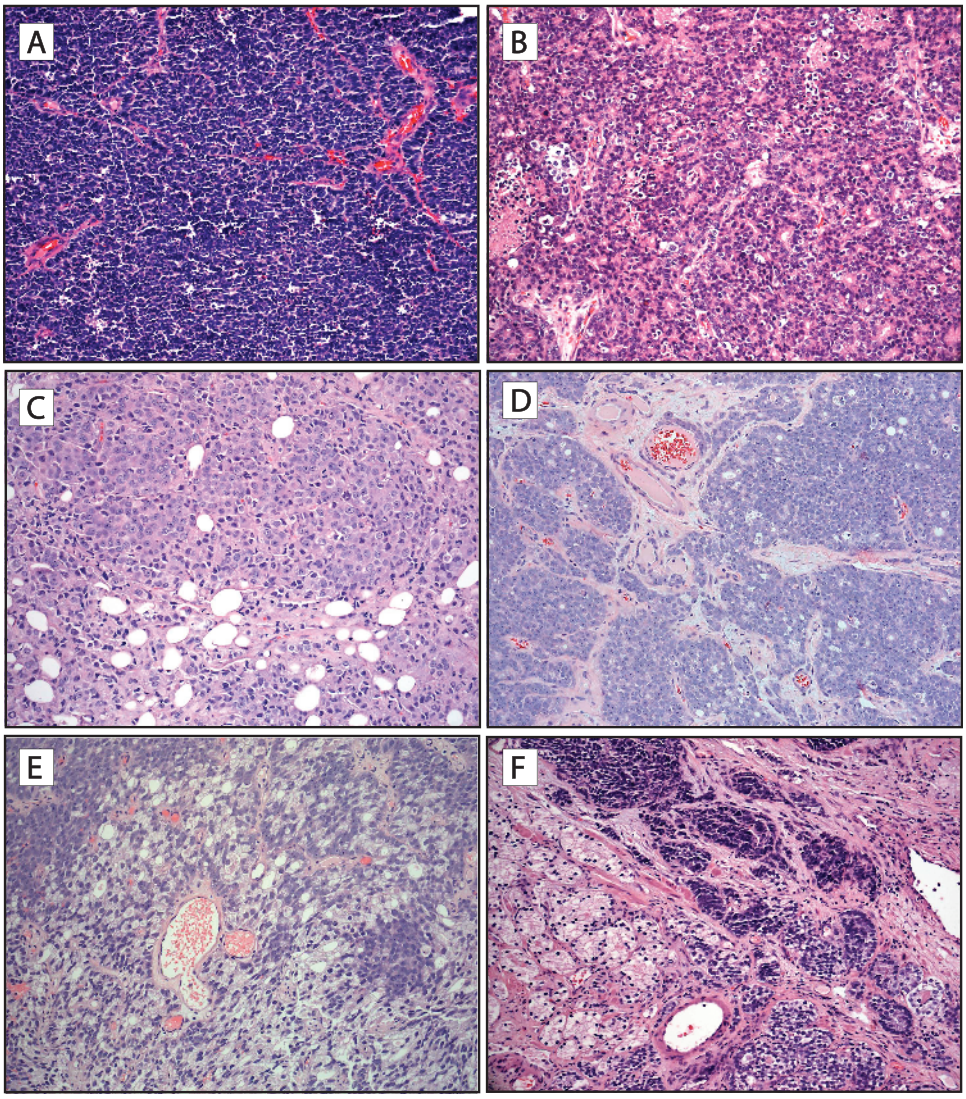

Figure 1. Morphologic spectrum of t-NEPC. (A) Small cell carcinoma of the prostate. The tumor is composed of sheets of uniform cells with scant cytoplasm, hyperchromatic nuclei, coarse chromatin, and unapparent nucleoli. (B) Large cell neuroendocrine carcinoma of the prostate. Tumor is composed of sheets and ribbons of cells with abundant cytoplasm, large nuclei with coarse chromatin, brisk mitotic activity, and foci of necrosis; pseudorosettes are also apparent. (C) Metastatic poorly differentiated adenocarcinoma of the prostate *without* neuroendocrine differentiation, treated (metastatic CRPC). Sheets of tumor cells with pale eosinophilic cytoplasm and abundant mitotic figures are seen within fibroadipose tissue. (D) Poorly differentiated adenocarcinoma of the prostate *with* neuroendocrine differentiation, treated (CRPC). Note the vaguely organoid pattern of tumor cells, which have amphophilic cytoplasm and prominent nucleoli. (E) Poorly differentiated adenocarcinoma of the prostate with focal areas of neuroendocrine differentiation, treated (CRPC). Areas of tumor cells with neuroendocrine differentiation are interspersed and demonstrate basophilic appearance. (F) Mixed t-NEPC and adenocarcinoma of prostate, treated (CRPC). Areas of small cell carcinoma and poorly differentiated adenocarcinoma are seen (H&E stain, original magnification, ×200).

tumor stage ranged from pT2a N0 to pT3b N0. In addition, archival material from this control cohort also included 50 benign prostate tissue samples.

Gleason scores of the six PCA cases with Paneth cell–like neuroendocrine differentiation ranged from 3 + 3 = 6 to 4 + 5 = 9 (Table 1).

FISH and IHC Results

In the group of primary hormone naïve PCA cases from patients who clinically progressed to t-NEPC, *AURKA* amplification was identified in 10 of 15 (67%) cases, seven of which (70%) also had concurrent *MYCN* amplification. Protein overexpression of Aurora kinase A was confirmed by IHC in five of such seven cases. Aurora kinase A overexpression was seen as multifocal and scattered positive nuclei, as illustrated in Figure W2. The presence of *AURKA* or *MYCN* amplification was not associated with neuroendocrine marker (synaptophysin and chromogranin A) expression in these primary PCA cases.

Among t-NEPC cases, *AURKA* amplification was identified in 29 of 46 (63%) treated PCAs and in 12 of 14 (86%) metastases that were assessable. Concurrent *MYCN* amplification was present in 20 of 29 treated tumors (69%) and in 10 of 12 metastases (83%) that were evaluable (see Figure W1*B*).

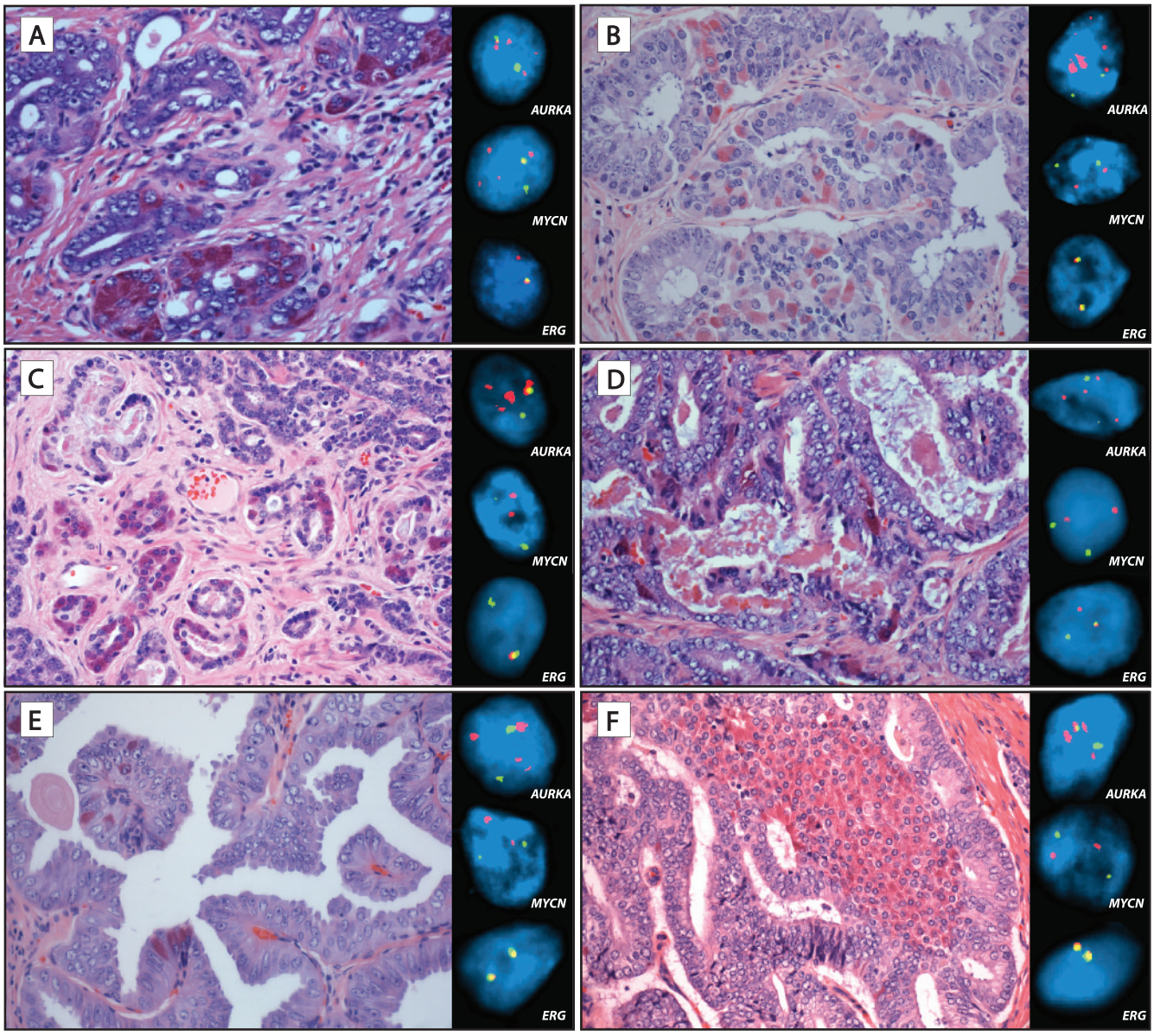

Figure 2. Prostate cancer with Paneth cell–like neuroendocrine differentiation harbors *AURKA* amplification. (A–F) Six cases of localized prostate cancer with Paneth cell–like change were identified and used as separate controls of low-grade neuroendocrine differentiation. On H&E stain, tumor cells with Paneth cell–like neuroendocrine differentiation are easily identified and contain distinct large eosinophilic granules in the cytoplasm. One case (A) demonstrated *AURKA* and *MYCN* amplifications. The other five cases (B–F) harbored *AURKA* amplification only (insets). *ERG* rearrangement, one through insertion (D) and one through deletion (A), is identified in two of these cases (insets). Clusters of tumor cells with Paneth cell–like neuroendocrine differentiation are located around asterisks, and more focal areas are marked with arrowheads (H&E stain, original magnification, ×400; FISH images, original magnification, ×600).

Table 3. Results of *AURKA* and *MYCN* Amplifications by FISH.

Group	Assessable Cases/Total Cases	*AURKA* Amplification	Concurrent *MYCN* Amplification
Hormone naïve PCA of patients who developed t-NEPC	15/15	67% (10/15)	70% (7/10)
Treated PCA (CRPC and t-NEPC)	46/51	63% (29/46)	69% (20/29)
Metastatic t-NEPC	14/15	86% (12/14)	83% (10/12)
Control cohort of patients with localized PCA	169/169	5%	100% (8/8)
Primary (*de novo*) NEPC	2/2	50% (1/2)	100% (1/1)
PCA with Paneth cell–like neuroendocrine differentiation	6/6	100% (6/6)	17% (1/6)

In only two of all 75 specimens assessable by FISH did *MYCN* gain occurred without concurrent *AURKA* amplification; both cases previously treated with hormonal therapy, one was obtained from prostate and the other from a bladder mass.

In contrast, *AURKA* amplifications were identified only in 5% (8 of 169 cases) of the unselected PCA cohort, with concurrent *MYCN* amplification identified in 7 of 169 of cases (not seen in absence of *AURKA* amplification). Particularly noteworthy is the fact that *AURKA* amplification was detected in all six cases of PCA with Paneth cell–like neuroendocrine differentiation, one of them with concurrent *MYCN* amplification (Figure 2).

Of the two *de novo* NEPC cases, one showed *AURKA* amplification with concurrent *MYCN* polysomy, and the other one was negative for either amplification. The results of *AURKA* and *MYCN* amplifications by FISH are summarized in Table 3.

AURKA and *MYCN* amplifications were detected in more than 95% of nuclei evaluated on each positive case. No *AURKA* or *MYCN* amplification was detected in benign prostate tissue (n = 50). In all cases of primary PCA, the presence of *AURKA* and *MYCN* amplifications was independent of other clinical prognostic features (Gleason grade, serum PSA, and stage) including neuroendocrine marker expression (chromogranin A and synaptophysin) by IHC.

In the five cases where metastatic t-NEPC was compared to primary PCA from the same patient, either hormone naïve or treated PCA, there was 100% concordance (five of five matching cases) of *AURKA* amplification. *MYCN* amplification was present in three of five cases (60% concordance), with metastatic t-NEPC demonstrating *MYCN* amplification at all times. Histologic and molecular findings of three of these cases are illustrated in Figure 3. In prostate tumors with mixed features, there was 94% concordance in *AURKA*/*MYCN* amplification between areas of neuroendocrine carcinoma and adenocarcinoma. An example of such combined histomorphology is highlighted in Figure 4, with both areas showing *AURKA* and *MYCN* amplifications.

Overall, *ERG* rearrangement was observed in 29 of 69 (42%) assessable tumors (PCA and metastases) in the t-NEPC cohort, 10 through insertion and 19 through deletion. *PTEN* deletion was observed in 14 of 49 (29%) assessable cases, eight of which were also *ERG* rearranged. There was no association between *AURKA*/*MYCN* amplification and *ERG* rearrangement or *PTEN* deletion status. Among the five cases of metastatic t-NEPC with matching PCA (hormone naïve or treated), four were positive for *ERG* rearrangement in the PCA and corresponding metastases, and one was negative for such gene rearrangement in both sites. This 100% concordance of *ERG* rearrangement is compatible with a clonal origin of t-NEPC, identical to those findings observed in tumors with mixed features (Figure 4).

In neuroendocrine tumors from non-prostate origin, *AURKA* amplification was detected in 10 of 11 (91%) assessable primary small cell carcinomas of lung including the metastasis to cerebellum and in one assessable primary small cell carcinoma of the bladder. *MYCN* amplification was detected in seven of these cases (64%), always in the presence of *AURKA* amplification. In contrast, *AURKA*/*MYCN* amplifications were not seen in well-differentiated neuroendocrine tumor ("typical carcinoid") of bowel and metastases or in ductal carcinoma *in situ* with neuroendocrine differentiation.

Discussion

The findings of the current study suggest that a broader definition of NEPC is desirable to capture the wide spectrum of this disease. Although the occurrence of *de novo* NEPC is rare, the incidence of NEPC that secondarily arises with disease progression and after therapy appears common and is associated with poor clinical outcome [19–25]. Given emerging preclinical and clinical evidence supporting promotion of neuroendocrine transformation by androgen deprivation therapies [3–7,26,27], we propose that these secondary NEPC tumors should be termed t-NEPC. Widely under-recognized, especially as patients with advanced stage disease are rarely biopsied to make the diagnosis, progression toward an AR-negative neuroendocrine phenotype is one proposed mechanism by which tumors acquire resistance to hormonal therapies. Thus, the landscape of advanced prostate cancer is evolving as novel potent AR-targeted therapies enter widespread clinical use (e.g., abiraterone acetate, MDV3100, TAK700) and patients develop resistance, and the incidence of t-NEPC will presumably escalate. Alternative mechanisms of resistance also include up-regulation or continued activation of the AR [28]. Recognition of resistance associated with the development of an AR-negative t-NEPC *versus* an AR-activated CRPC is essential, as this affects how patients may respond to subsequent therapies; for instance, patients with t-NEPC would be less likely to respond to hormonal agents and may better respond to chemotherapy or enrollment in a clinical trial.

Although there are some limitations regarding clinical data presented in the current study, this retrospective cohort represents the largest tissue collection of t-NEPC reported to date. The main purpose of this study was to generate hypotheses regarding novel biomarkers and insight into the pathogenesis of t-NEPC. This work will be the basis for future clinical studies, including the prospective evaluation of *AURKA* and *MYCN* in primary tumors and metastases from patients with t-NEPC. If validated in prospective trials, these biomarkers would be useful for clinical decision making. For this purpose, defining the histomorphology and molecular characterization of t-NEPC become critical steps toward understanding the spectrum of disease, especially as efforts are made to incorporate the latest discoveries into the clinic, either as potential targets or in the form of significant biomarkers, both for diagnostic and prognostic uses. Planned clinical trials incorporating metastatic tumor biopsies for

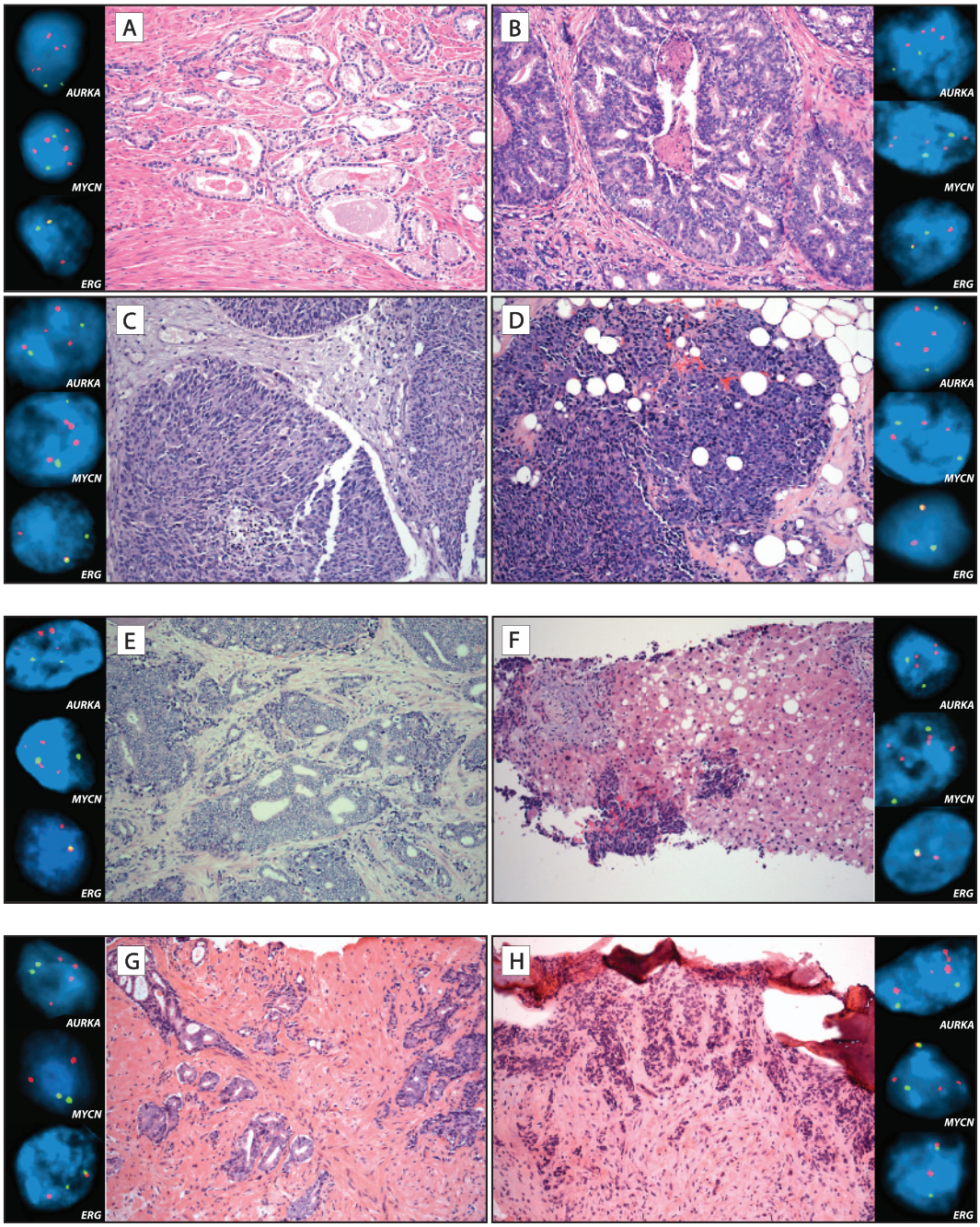

Figure 3. *AURKA* and *MYCN* amplifications in primary prostatic adenocarcinoma predict the development of t-NEPC. (A–D) Top panel illustrates several specimens from a patient at different stages of disease progression to t-NEPC. (A and B) Images of hormone naïve prostate cancer with areas of Gleason score 3 + 3 = 6 (A) and 4 + 5 = 9 (B) at initial diagnosis. Concurrent *AURKA* (upper inset) and *MYCN* (middle inset) amplifications are present in both areas. (C) Subsequent metastasis/local recurrence in the bladder demonstrates poorly differentiated adenocarcinoma without neuroendocrine differentiation, exhibiting both *AURKA* and *MYCN* amplifications (upper and middle insets, respectively). (D) Five years after treatment, the patient presents with metastatic large cell neuroendocrine carcinoma in pelvic soft tissue. The tumor has organoid appearance focally forming pseudorosettes, and cells have abundant cytoplasm and prominent nucleoli. The tumor has both *AURKA* and *MYCN* amplifications (upper and middle insets, respectively). Clonal origin is confirmed by *ERG* rearrangement through translocation in all tumors (lower inset). (E and F) Center panel illustrates prostatectomy specimen from a patient with initial diagnosis of PCA Gleason score 4 + 5 = 9 (E), which has concurrent *AURKA* and *MYCN* amplifications (upper and middle insets, respectively). A liver biopsy 7 years after (F) shows metastatic small cell carcinoma, which harbors *AURKA* and *MYCN* co-amplification as well. Clonal origin is confirmed by *ERG* rearrangement through deletion in both tumors (lower inset). (G and H) Lower panel illustrates needle biopsies from a patient with initial diagnosis of (G) PCA Gleason score 3 + 4 = 7 with intraductal spread (IDC-P) with amplification of *AURKA* (upper inset) but not *MYCN* (middle inset). Eight years after initial diagnosis and intermittent treatment, the patient developed pancytopenia and bone lytic lesions, which biopsy demonstrates (H) metastatic small cell carcinoma (frozen tissue artifact present), consistent with spread from known prostatic primary. In addition to *AURKA* amplification (upper inset), clonal origin is confirmed by *ERG* rearrangement through translocation in both tumors (lower inset). The metastatic tumor demonstrates *MYCN* amplification (middle inset) (H&E stain, original magnification, ×200; FISH images, original magnification, ×600).

patients with CRPC including those patients with NEPC treated with an Aurora kinase inhibitor will be valuable for prospective correlation of pathologic findings and genomic sequencing results with clinical features and will help further define t-NEPC.

Former studies have described the phenotypic features of metastatic hormone-refractory PCA to bone [29] and visceral sites [30,31], and of pure small cell carcinoma of prostate, which occurrence is rare (less than 1% of PCA cases) [32]. Here, we highlight that most treated prostate tumors and metastases from patients who clinically developed NEPC, including *de novo* NEPC cases, are part of a morphologic spectrum that encompasses pure neuroendocrine histology and mixed tumors with areas of poorly differentiated adenocarcinoma. Although the term anaplastic small cell carcinoma has also been used clinically to describe tumor progression in patients with CRPC [33,34], the histologic features of treated prostate tumors from patients who progress to NEPC, both primary and metastatic, are heterogeneous. The term t-NEPC may be a more useful descriptor for the clinical scenario of patients with CRPC with rapid disease progression (visceral and/or lytic bone metastases) and low serum PSA, especially in the setting of potent androgen deprivation therapy.

Some molecular characteristics of CRPC (e.g., *ERG*, *PTEN* status) have been described earlier [30,35–38]. In our series, *ERG* rearrangement was observed in 42% of cases, which is at similar frequency as reported in other cohorts. However, *ERG* rearrangement occurred more often through deletion (as opposed to translocation), a finding that has been previously associated with an aggressive behavior [37,39]. These findings also support clonal origin of t-NEPC and acinar prostate cancer, previously demonstrated by concordance of *ERG* rearrangement in tumors with mixed features [9–11] and in primary PCA and metastatic NEPC [8] (Figure 4). This has important clinical implications, as it suggests that adenocarcinomas may harbor molecular lesions before the development of t-NEPC, which is relevant toward the development of novel biomarkers and identifying patients at high risk for progression.

We previously identified higher expression and amplification of *AURKA* and *MYCN* in NEPC in contrast to hormone naïve PCA [12]. In the current study, *AURKA* amplification was also detected in overall 65% primary PCA from patients that later developed t-NEPC, with concurrent *MYCN* amplification in a substantial proportion of cases. This is highly significant, especially when compared to 5% frequency of *AURKA* and *MYCN* amplifications observed in an unselected primary PCA population. Furthermore, these alterations were independent of Gleason score, PSA level, or pathologic tumor stage at initial diagnosis, suggesting that they add prognostic value. In the current study, we also did not observe any correlation with Gleason score. Importantly, *AURKA* and *MYCN* amplifications were also seen in low-grade Gleason 3 + 3 = 6 tumors, the type of tumors that might be considered for active surveillance. Because *AURKA* and *MYCN* alterations arise early, other genetic changes are clearly also important for disease progression. There was also high concordance of *AURKA* and *MYCN* amplifications (100% and 60%, respectively) when ensuing t-NEPC or later metastases were also interrogated (Figure 3); this suggests that *AURKA* and *MYCN* alterations are acquired early and persist during disease progression. The importance is two-fold: *AURKA* is targetable (i.e., Aurora kinase A inhibitors), and *AURKA* and *MYCN* amplifications may represent new prognostic and predictive biomarkers because, as demonstrated in our current study, their presence identifies patients with PCA who are at risk of progressing to t-NEPC after androgen deprivation therapy. These patients may therefore benefit from early intervention with an Aurora kinase A inhibitor.

Paneth cell–like change in PCA has been suggested to represent a low-grade neuroendocrine differentiation with generally favorable prognosis [13]. Noteworthy, all six cases of PCA with these features harbored *AURKA* amplification. This particular histomorphology of PCA may be enriched for *AURKA* amplification, with resultant significant clinical implication (Figure 2). This might therefore suggest that these tumors may not be low grade. In the prior study by Tamas and Epstein [13], patients with PCA with Paneth cell–like neuroendocrine differentiation including tumors with Gleason pattern 5 had no evidence of progression with mean and median follow-ups ranging from 42.5 to 60.5 months. These criteria were used to assign PCA with Paneth cell–like neuroendocrine differentiation to a category of favorable prognosis. Given the high association with *AURKA* amplification and the development of t-NEPC illustrated in our current study, future studies with longer follow-up and detailed

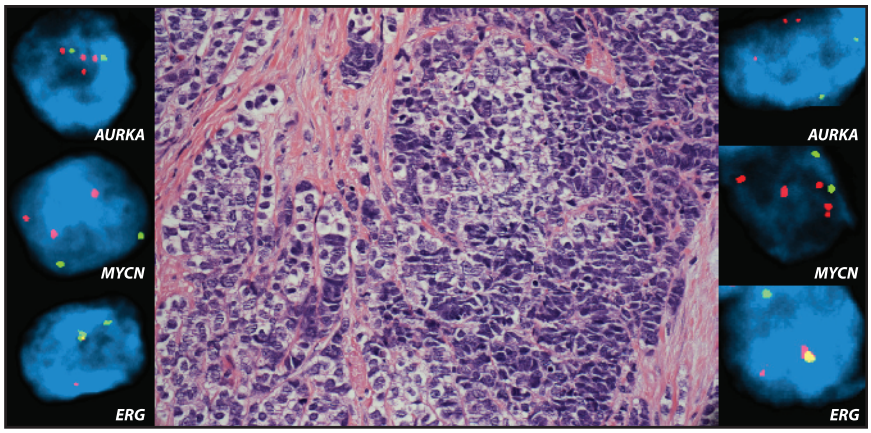

Figure 4. Concordance of *AURKA* and *MYCN* amplifications in tumors with mixed areas of neuroendocrine carcinoma and poorly differentiated adenocarcinoma. Representative image of local recurrence of castration-resistant prostatic carcinoma with areas of mixed small cell carcinoma (right) and adenocarcinoma (left). Both areas demonstrate concordance of *AURKA* and *MYCN* amplifications (upper and middle insets, respectively). Clonal origin is supported by *ERG* rearrangement through translocation in both areas (lower inset) (H&E stain, original magnification, ×200; FISH images, original magnification, ×600).

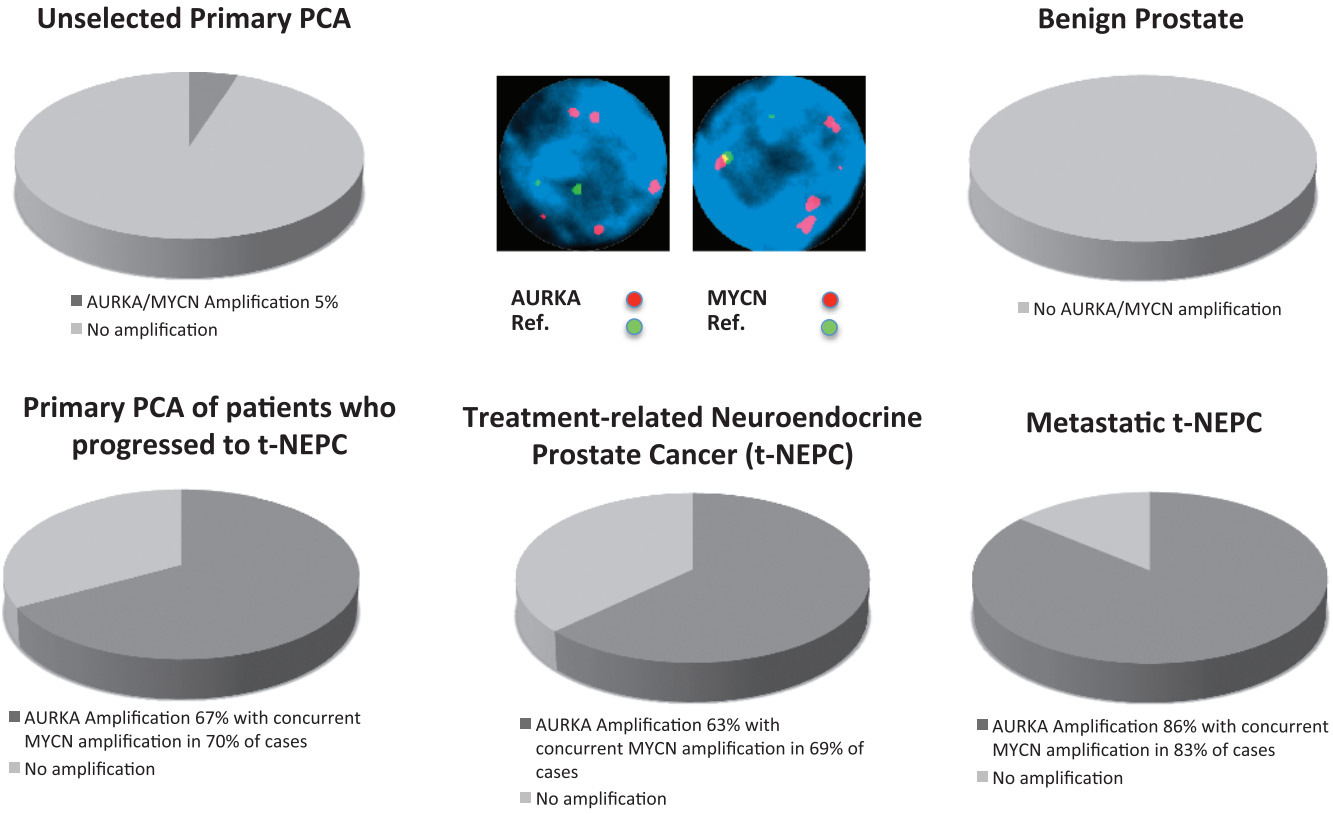

Figure 5. Concurrent *AURKA* and *MYCN* gene amplifications are harbingers of lethal t-NEPC. *AURKA* and *MYCN* gene amplifications evaluated by FISH are not present in benign prostate tissue and identified only in 5% of unselected primary prostate cancers. In contrast, 67% of primary tumors from patients who clinically develop t-NEPC harbor *AURKA* amplification, 70% of which also demonstrate concurrent *MYCN* amplification. Similar frequency of *AURKA*/*MYCN* amplification is present in t-NEPC. Metastatic t-NEPC harbors *AURKA* amplification in 86% of cases, with 83% *MYCN* co-amplification.

hormonal therapy are needed. PCA with Paneth cell–like neuroendocrine differentiation should best be viewed for now as uncertain clinical behavior.

AURKA and *MYCN* amplifications were also identified in small cell carcinomas of non-prostate origin but not in well-differentiated neuroendocrine tumors. This warrants further exploration for the role of *AURKA* and *MYCN* in small cell carcinomas of other primary sites. In addition, it demonstrates that both *AURKA* and *MYCN* amplifications may be good markers of neuroendocrine differentiation, but unlike the recurrent *ETS* rearrangements or *SPOP* mutations [40] they are not PCA specific.

Finally, this study also highlights the divide between the clinical presentation of NEPC and the pathologist's view of NEPC. Surgical pathologists only rarely encounter NEPC in their routine clinical practice due in part to the rare nature of *de novo* small cell prostate cancer, selection bias toward cases that are amenable to surgery and radiation, and the near universal absence of systematic biopsies for men with CRPC. More recently, clinical protocols are incorporating biopsies during the treatment course of CRPC. As exposed in this study and other recent studies that have evaluated metastatic samples, there is a wider range of morphology than normally encountered in hormone naïve prostate cancer. As a result, there is a significant gap in knowledge as to how treatment alters the course of many solid tumors but particularly prostate cancer, when hormonal or taxane-based therapy can be administered on the basis of clinical symptoms of bone pain, radiology images, or elevated PSA results without first obtaining a tissue diagnosis. We anticipate that given the more aggressive approach to obtaining biopsies on clinical trials for CRPC, we will need to more formally address the pathologic and molecular changes that occur as a result of targeted therapies. Therefore, we would advocate keeping a more encompassing broad view for the definition of NEPC until we better understand the biology and response to treatment through emerging clinical trials with mandatory tissue biopsies.

In summary, t-NEPC is a clinical entity that has an array of histologic features including pure neuroendocrine morphology (small cell carcinoma in most cases) and mixed tumors with poorly differentiated adenocarcinoma component. *AURKA* and *MYCN* amplifications occur early and are present in hormone naïve tumors from patients who ultimately progress to t-NEPC after androgen deprivation therapy (Figure 5). Therefore, *AURKA* and *MYCN* amplifications may be prognostic and predictive biomarkers, as they are harbingers of tumors at risk of progressing to t-NEPC after hormonal therapy and may identify patients that could potentially benefit from Aurora kinase inhibitor therapy.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, and Forman D (2011). Global cancer statistics. *CA Cancer J Clin* **61**(2), 69–90.

[2] Brawn PN and Speights VO (1989). The dedifferentiation of metastatic prostate carcinoma. *Br J Cancer* **59**(1), 85–88.

[3] Ismail AH, Landry F, Aprikian AG, and Chevalier S (2002). Androgen ablation promotes neuroendocrine cell differentiation in dog and human prostate. *Prostate* **51**(2), 117–125.

[4] Ito T, Yamamoto S, Ohno Y, Namiki K, Aizawa T, Akiyama A, and Tachibana M (2001). Up-regulation of neuroendocrine differentiation in prostate cancer after androgen deprivation therapy, degree and androgen independence. *Oncol Rep* **8**(6), 1221–1224.

[5] Shen R, Dorai T, Szaboles M, Katz AE, Olsson CA, and Buttyan R (1997). Transdifferentiation of cultured human prostate cancer cells to a neuroendocrine cell phenotype in a hormone-depleted medium. *Urol Oncol* **3**(2), 67–75.

[6] Wright ME, Tsai MJ, and Aebersold R (2003). Androgen receptor represses the neuroendocrine transdifferentiation process in prostate cancer cells. *Mol Endocrinol* **17**(9), 1726–1737.

[7] Yuan TC, Veeramani S, Lin FF, Kondrikou D, Zelivianski S, Igawa T, Karan D, Batra SK, and Lin MF (2006). Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. *Endocr Relat Cancer* **13**(1), 151–167.

[8] Beltran H, Tagawa ST, Park K, MacDonald TY, Milowsky MI, Mosquera JM, Rubin MA, and Nanus DM (2012). Challenges in recognizing treatment-related neuroendocrine prostate cancer. *J Clin Oncol* **30**(36), e386–e389.

[9] Guo CC, Dancer JY, Wang Y, Aparicio A, Navone NM, Troncoso P, and Czerniak BA (2011). TMPRSS2-ERG gene fusion in small cell carcinoma of the prostate. *Hum Pathol* **42**(1), 11–17.

[10] Lotan TL, Gupta NS, Wang W, Toubaji A, Haffner MC, Chaux A, Hicks JL, Meeker AK, Bieberich CJ, De Marzo AM, et al. (2011). ERG gene re-arrangements are common in prostatic small cell carcinomas. *Mod Pathol* **24**(6), 820–828.

[11] Williamson SR, Zhang S, Yao JL, Huang J, Lopez-Beltran A, Shen S, Osunkoya AO, MacLennan GT, Montironi R, and Cheng L (2011). ERG-TMPRSS2 rearrangement is shared by concurrent prostatic adenocarcinoma and prostatic small cell carcinoma and absent in small cell carcinoma of the urinary bladder: evidence supporting monoclonal origin. *Mod Pathol* **24**(8), 1120–1127.

[12] Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, Wang YW, Sheikh KL, Terry S, Tagawa ST, et al. (2011). Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discov* **1**(6), 487–495.

[13] Tamas EF and Epstein JI (2006). Prognostic significance of Paneth cell-like neuroendocrine differentiation in adenocarcinoma of the prostate. *Am J Surg Pathol* **30**(8), 980–985.

[14] Travis WD (2010). Advances in neuroendocrine lung tumors. *Ann Oncol* **21**(suppl 7), vii65–vii71.

[15] Travis WD, Linnoila RI, Tsokos MG, Hitchcock CL, Cutler GB Jr, Nieman L, Chrousos G, Pass H, and Doppman J (1991). Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma. An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am J Surg Pathol* **15**(6), 529–553.

[16] Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, Sboner A, Esgueva R, Pflueger D, Sougnez C, et al. (2011). The genomic complexity of primary human prostate cancer. *Nature* **470**(7333), 214–220.

[17] Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, Tchinda J, Tomlins SA, Hofer MD, Pienta KG, et al. (2006). TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res* **66**(17), 8337–8341.

[18] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, et al. (2005). Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310**(5748), 644–648.

[19] Aprikian AG, Cordon-Cardo C, Fair WR, Zhang ZF, Bazinet M, Hamdy SM, and Reuter VE (1994). Neuroendocrine differentiation in metastatic prostatic adenocarcinoma. *J Urol* **151**(4), 914–919.

[20] Aprikian AG, Cordon-Cardo C, Fair WR, and Reuter VE (1993). Characterization of neuroendocrine differentiation in human benign prostate and prostatic adenocarcinoma. *Cancer* **71**(12), 3952–3965.

[21] Abrahamsson PA (1999). Neuroendocrine cells in tumour growth of the prostate. *Endocr Relat Cancer* **6**(4), 503–519.

[22] Abrahamsson PA (1999). Neuroendocrine differentiation in prostatic carcinoma. *Prostate* **39**(2), 135–148.

[23] Abrahamsson PA, Falkmer S, Falt K, and Grimelius L (1989). The course of neuroendocrine differentiation in prostatic carcinomas. An immunohistochemical study testing chromogranin A as an "endocrine marker". *Pathol Res Pract* **185**(3), 373–380.

[24] di Sant'Agnese PA (1998). Neuroendocrine differentiation in prostatic carcinoma: an update. *Prostate Suppl* **8**, 74–79.

[25] di Sant'Agnese PA and Cockett AT (1996). Neuroendocrine differentiation in prostatic malignancy. *Cancer* **78**(2), 357–361.

[26] Berruti A, Mosca A, Porpiglia F, Bollito E, Tucci M, Vana F, Cracco C, Torta M, Russo L, Cappia S, et al. (2007). Chromogranin A expression in patients with hormone naïve prostate cancer predicts the development of hormone refractory disease. *J Urol* **178**(3 pt 1), 838–843; quiz 1129.

[27] Hirano D, Okada Y, Minei S, Takimoto Y, and Nemoto N (2004). Neuroendocrine differentiation in hormone refractory prostate cancer following androgen deprivation therapy. *Eur Urol* **45**(5), 586–592; discussion 592.

[28] Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, and Sawyers CL (2004). Molecular determinants of resistance to antiandrogen therapy. *Nat Med* **10**(1), 33–39.

[29] Roudier MP, True LD, Higano CS, Vesselle H, Ellis W, Lange P, and Vessella RL (2003). Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum Pathol* **34**(7), 646–653.

[30] Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, Macvicar GR, Varambally S, Harwood J, Bismar TA, et al. (2004). Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. *Cancer Res* **64**(24), 9209–9216.

[31] Rubin MA, Putzi M, Mucci N, Smith DC, Wojno K, Korenchuk S, and Pienta KJ (2000). Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. *Clin Cancer Res* **6**(3), 1038–1045.

[32] Wang W and Epstein JI (2008). Small cell carcinoma of the prostate. A morphologic and immunohistochemical study of 95 cases. *Am J Surg Pathol* **32**(1), 65–71.

[33] Schwartz LH, LaTrenta LR, Bonaccio E, Kelly WK, Scher HI, and Panicek DM (1998). Small cell and anaplastic prostate cancer: correlation between CT findings and prostate-specific antigen level. *Radiology* **208**(3), 735–738.

[34] Rubenstein JH, Katin MJ, Mangano MM, Dauphin J, Salenius SA, Dosoretz DE, and Blitzer PH (1997). Small cell anaplastic carcinoma of the prostate: seven new cases, review of the literature, and discussion of a therapeutic strategy. *Am J Clin Oncol* **20**(4), 376–380.

[35] Bismar TA, Yoshimoto M, Duan Q, Liu S, Sircar K, and Squire JA (2012). Interactions and relationships of PTEN, ERG, SPINK1 and AR in castration-resistant prostate cancer. *Histopathology* **60**(4), 645–652.

[36] Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, et al. (2012). The mutational landscape of lethal castration-resistant prostate cancer. *Nature* **487**(7406), 239–243.

[37] Han B, Mehra R, Suleman K, Tomlins SA, Wang L, Singhal N, Linetzky KA, Palanisamy N, Zhou M, Chinnaiyan AM, et al. (2009). Characterization of ETS gene aberrations in select histologic variants of prostate carcinoma. *Mod Pathol* **22**(9), 1176–1185.

[38] Mehra R, Tomlins SA, Yu J, Cao X, Wang L, Menon A, Rubin MA, Pienta KJ, Shah RB, and Chinnaiyan AM (2008). Characterization of TMPRSS2-ETS gene aberrations in androgen-independent metastatic prostate cancer. *Cancer Res* **68**(10), 3584–3590.

[39] Attard G, Clark J, Ambroisine L, Fisher G, Kovacs G, Flohr P, Berney D, Foster CS, Fletcher A, Gerald WL, et al. (2008). Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. *Oncogene* **27**(3), 253–263.

[40] Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, White TA, Stojanov P, Van Allen E, Stransky N, et al. (2012). Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet* **44**(6), 685–689.

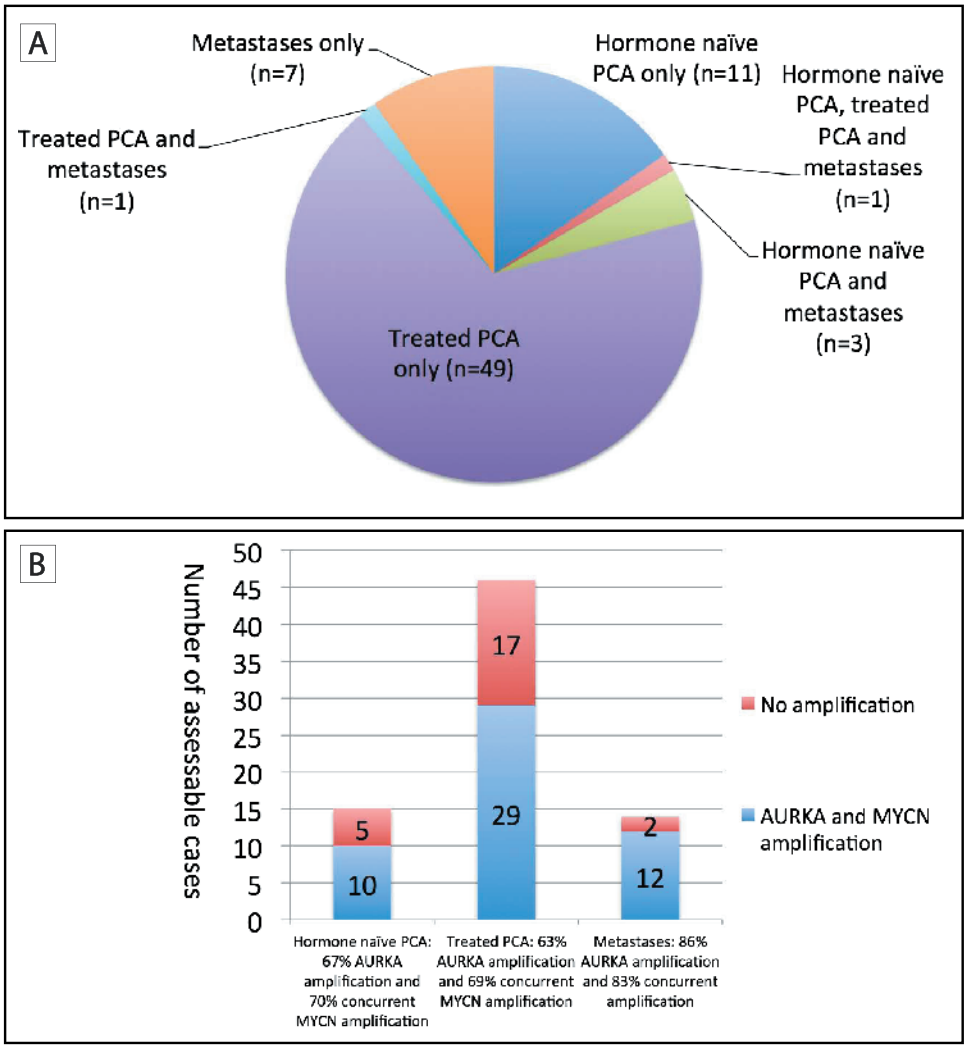

Figure W1. Summary of prostate cancer specimens interrogated for *AURKA* and *MYCN* gene amplifications in the current study. (A) Tumors from 72 patients at different stages of disease progression to t-NEPC were studied: 15 hormone naïve prostate cancers, 51 treated prostate cancer cases, and 15 metastases from 12 patients. Some patients had multiple specimens. (B) Results of *AURKA* and *MYCN* gene amplifications evaluated by FISH in assessable cases of hormone naïve PCA, treated PCA, and metastases.

Table W1. Clinicopathologic Characteristics, Treatment and Follow-up of Patients Who Developed t-NEPC.

Case	Site	Clinical Course	Time on Hormonal Therapy (years)	Age (years)	Survival (years)	Diagnosis	Pathology	FISH			
								ERG	PTEN	AURKA	NMYC
2	Retroperineal mass and bladder	RP 1992, XRT 1995, ADT 2000, anaplastic recurrence pelvic mass + RP lymph nodes (LN), low PSA (4 ng/ml); 2001—J591, estramustane, cytoxan, taxol, carboplatin-taxol, nizoral-adriamycn	2	76	10 (14 months since dx anaplastic)	Poorly diff AdenoCa NED	PCA in retroperineal mass and bladder from cysto/transrectal prostate biopsies; s/p RP and hormonal therapy and radiation	T	DEL	AMP	Polysomy
7	Prostate (autopsy case)	ADT only with suppressed PSA but rapidly developed widely met CRPC to LN, stomach, iliac fossa, bladder, bone with PSA (9 ng/ml), autopsy case	2	88 at dx, 90 at death	2	Poorly diff AdenoCa	PCA with treatment effect in prostate and multiple metastases from autopsy	T	DEL	AMP	AMP
8	Prostate (autopsy case)	RP 1997 (age 43), XRT, ADT, docetaxel, irinotecan, widely metastatic autopsy to liver, peritoneum, lung, LN, autopsy case	9	42 at dx, 56 at death	12	Poorly diff AdenoCa	PCA in prostate area and multiple metastases from autopsy	T + D	–	–	AMP
10	Prostate	RP, XRT, ADT, three to four lines chemotherapy	2	dx 64	13, three from mets, one from NEPC dx	AdenoCa	Primary PCA Gleason 7 (3 + 4) from biopsies	–	–	–	–
14	Prostate (metastatic disease)	Presented with metastatic disease 2009, PSA 33, prostate bx Gleason 4 + 4; developed CRPC 2010, ADT, MDV3100, docetaxel + radium-223	3	dx age 61	Alive	AdenoCa	Primary PCA Gleason 7 (4 + 3) from biopsies	T	–	–	–
16	Bladder Met/Prostate	RP 1990 for PSA 50, Gleason 4 + 3, 1/10 LN, ADT 1991→bladder recurrence 2010, PSA 0.03, elevated chromogranin 1617 and NSE 15	11	66 at dx, now 78	Alive	AdenoCa	PCA with treatment effect from TURB; positive PSMA and negative PSA, CGA, and SYP	–	DEL	AMP	AMP
17	Spinal Metastasis	RP 1999 for Gleason 4 + 4, XRT 2000, bone mets 2004, ADT, CRPC 2007, J591, docetaxel, abiraterone × 9 months, carboplatin-taxol, cord compression and brain mets 2011	7	dx age 56, mets age 61, death 68	12	AdenoCa met	Metastatic PCA to brain and spinal cord; positive PSMA and negative PSA	–	–	AMP	–
18	Spinal Metastasis	XRT + ADT 2004 for Gleason 5 + 5, PSA 26, ADT, CRPC 2006, docetaxel, J591	4	dx age 65, died age 69	4	Poorly diff AdenoCa	Metastatic PCA from spinal tumor; weakly positive for PSA	Polysomy	Homo Del	AMP	AMP
19	Prostate (metastatic disease)	ADT, cisplatin-etoposide, carboplatin-taxol, palliative XRT bone	1	56	2	Mixed small cell and 4 + 5	PCA Gleason 9 (4 + 5) with ductal features and focal small cell diff. from TURP; negative PSA, and positive focal CGA and diffuse NSE	T + D	DEL	Polysomy	–
20	Prostate (metastatic disease)	XRT for Gleason 4 + 3, multiple TURPS, ADT, bone mets, liver mets	4	dx age 67, NEPC age 77	10	Mixed (small cell + AdenoCa)	PCA Gleason 10 (5 + 5) and majority of tumor consisting of small cell undifferentiated variant (90%) from TURP; primary	del both 5′ signals	Homo Del	Polysomy	–

Table W1. (*continued*)

Case	Site	Clinical Course	Time on Hormonal Therapy (years)	Age (years)	Survival (years)	Diagnosis	Pathology	FISH			
								ERG	PTEN	AURKA	NMYC
21	Prostate (metastatic disease)	RP for Gleason 7 LN+ in 1996, ADT, LN/liver mets 1999 with TURP at that time t-NEPC, cisplatin-etoposide, carboplatin-taxol, pelvic RT	3	dx age 58, NEPC age 61	3	Small cell Ca	Small cell anaplastic carcinoma from TURP; negative PSA, CGA, SYP, and PAP	T	–	AMP	AMP
23	Prostate (metastatic disease)	RP 2006 for Gleason 5 + 4, 3/19 LN, mets 2006 with PSA 0.06, ADT, docetaxel, cisplatin-etoposide, J591, carboplatin-taxol	5	dx age 54	Alive	AdenoCa (3 + 3)	PCA Gleason 9 (5 + 4) from RP	T	–	AMP	AMP
24	Prostate (metastatic disease)	RP 2008 Gleason 4 + 3 pT3a, bone mets 2009, cord compression 2009 elevated chromogranin 191, NSE 23	1	59	1 diagnosis→death	AdenoCa (5)	PCA Gleason 9 (4 + 5) from RP	–	–	–	–
25	Prostate (metastatic disease)	XRT 2002 Gleason 8, ADT 2006, local recurrence 2009 NEPC, chromogranin >7000 NSE 65, cisplatin-etoposide, salvage RP 2010	4	dx 2002 age 62, death age 71	9 (14 months NEPC to death)	Small cell Ca	Undifferentiated NE carcinoma from prostate biopsies; strong CD56, moderate TTF-1; negative PSAP and PSMA	T + D	–	AMP	AMP
27	Prostate (metastatic disease)	RP, salvage XRT, intermittent ADT, R-CHOP (for lymphoma), pt later developed liver mets NEPC with elevated CEA	5	59	6	AdenoCa (4 + 3)	Primary PCA Gleason 7 (4 + 3); liver metastasis with NEPC	T + D	–	AMP	AMP
30	Pleural metastasis	XRT 1989, treated ADT 1990s, docetaxel, abiraterone—liver, lung, and pleura mets with low PSA (8 ng/ml), carboplatin-paclitaxel	10	dx age 71, NEPC age 81	22 from dx to death, 8 months from NEPC to death	Small cell Ca met	Small cell carcinoma	T + D, ploidy	–	AMP	AMP
74	Prostate (metastatic disease)	Intermittent ADT 1992, XRT, DES, docetaxel + CNTO, docetaxel + revlimid, ipilumimab *versus* placebo, cabazitaxel, then abiraterone. cytopenias despite dropping PSA, BM c/w NEPC, rapidly progressed and died in hospice 1 month later	10	dx 51, died 71	20	AdenoCa	Well-differentiated adenocarcinoma; TURP specimen, confirmed primary PCA Gleason 6 (3 + 3) and 8 (4 + 4) in biopsies from 1993, bone metastasis in 2011 with NEPC	T	Homo Del	AMP	AMP
75	Prostate and pelvic mass	XRT + ADT 2008 for Gleason 4 + 4; developed local recurrence 2011, NEPC 6.6 × 5.2 cm pelvic mass with local ext to bladder/colon, carboplatin/etoposide	4	dx 75	Alive	AdenoCa (4 + 4)	Primary PCA Gleason 8 (4 + 4) and 6 (3 + 3), biopsies	–	–	AMP	AMP

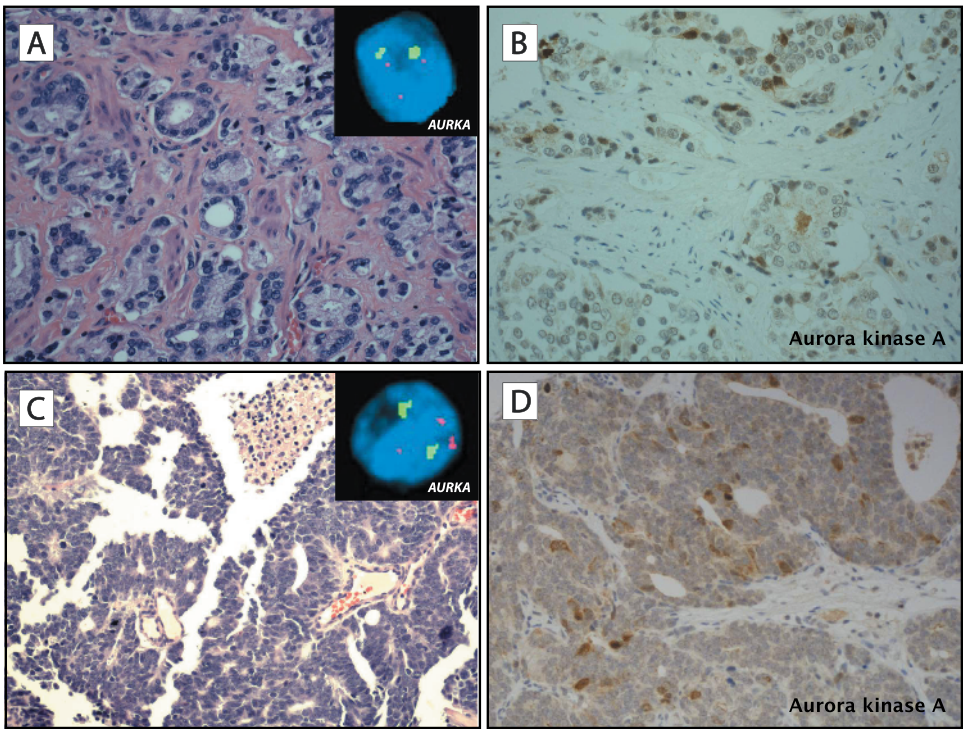

Figure W2. Aurora kinase A overexpression is present in primary PCA with *AURKA* amplification from patients who later developed t-NEPC. Aurora kinase A overexpression by IHC was detected in five of seven primary PCAs with *AURKA* gene amplification from patients who clinically develop t-NEPC. Illustrated here are two of such cases. (A) Primary prostatic adenocarcinoma, Gleason score 4 + 3 = 7 with *AURKA* amplification (inset) from a 59-year-old patient who, 6 years after initial diagnosis, developed t-NEPC. (B) Overexpression of Aurora kinase A is present. (C) Primary prostatic adenocarcinoma, Gleason score 5 + 4 = 9 with *AURKA* amplification (inset) from a 65-year-old patient who, 9 years after initial diagnosis, developed t-NEPC. (D) Overexpression of Aurora kinase A is present (H&E and IHC stains of A and B, original magnification, ×400; H&E and IHC stains of C and D, original magnification, ×200; FISH images, original magnification, ×600).