Neuroendocrine Prostate Cancer: Subtypes, Biology, and Clinical Outcomes

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Abstract
Neuroendocrine prostate cancer (NEPC) encompasses various clinical contexts, ranging from the de novo presentation of small cell prostatic carcinoma to a treatment-emergent transformed phenotype that arises from typical adenocarcinoma of the prostate. The development of resistance to potent androgen receptor signaling inhibition may be associated with the emergence of aggressive phenotype, advanced castration-resistant NEPC. Clinically, small cell prostate cancer and NEPC are often manifested by the presence of visceral or large soft tissue metastatic disease, a disproportionately low serum prostate-specific antigen level relative to the overall burden of disease, and a limited response to targeting of the androgen signaling axis. These tumors are often characterized by loss of androgen receptor expression, loss of retinoblastoma tumor suppressor copy number or expression, amplification of Aurora kinase A and N-Myc, and activation of the PI3K pathway. However, a consensus phenotype-genotype definition of NEPC has yet to emerge, and molecularly based biomarkers are needed to expand on traditional morphologic and immunohistochemical markers of NEPC to fully define the spectrum of this aggressive, androgen receptor-independent disease. Emerging studies implicate a shared clonal origin with prostatic adenocarcinoma in many cases, with the adaptive emergence of unique cellular programming and gene expression profiles. Ongoing clinical studies are focused on developing novel targeted therapeutic approaches for this high-risk, lethal subset of disease, to improve on the limited durations of response often observed with traditional platinum-based chemotherapy. U Natl Compr Canc Netw 2014;12:719–726

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Learning Objectives
Upon completion of this activity, participants will be able to:
• Describe the diagnostic and histopathologic criteria of NEPC
• Discuss the clinical features and outcomes of NEPC
• Outline treatment options for patients with NEPC

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Neuroendocrine prostate cancer (NEPC) is a lethal form of prostate cancer, with most patients dying within 1 to 2 years of diagnosis.1,2 Pure localized small cell carcinoma of the prostate gland is rare (<1% of cases); focal neuroendocrine differentiation admixed with adenocarcinoma is more commonly observed in approximately 5% to 10% of cases, depending on the criteria and biomarkers used.3 In the localized disease setting, an increasing proportion of neuroendocrine differentiation confers an adverse prognosis independent of Gleason grade and tumor stage.4,5 In the metastatic, castration-resistant disease state, treatment-emergent NEPC (t-NEPC) can be observed at the development of resistance to androgen deprivation therapy (ADT) and after progression while taking potent androgen receptor signaling inhibitors, including abiraterone and enzalutamide. Prior studies indicate that t-NEPC detected by immunohistochemical staining is found in approximately 20% to 30% of metastatic castration-resistant tumors.6 The true incidence of NEPC may be higher because of the recent introduction of more potent androgen receptor signaling inhibitors, underrecognition as a result of tumor heterogeneity, the limited number of metastatic tumor biopsies performed, the lack of a uniform consensus definition based on histology or biomarker expression, and frequent misclassification as high-grade prostate adenocarcinoma, especially in tumors with mixed histologies. More accurate detection and an increased understanding of the true prevalence of NEPC is needed, along with improved molecular and clinicopathologic classifications and novel therapeutic targeted approaches. This article reviews the diagnostic and histopathologic criteria, clinical features and outcomes, molecular characteristics, and therapeutic approaches toward NEPC.

Diagnosis, Nomenclature, and Histopathologic Classification

The terminology used to describe NEPC has varied in previous literature, and a more standardized set of definitions are needed. Pure small cell carcinoma represents a subset of NEPC, which is currently defined according to histopathologic features discussed herein. NEPC in turn represents a subset of a broader clinically defined prostate cancer phenotype that displays relative resistance to androgen receptor signaling inhibition (Figure 1). This clinically defined phenotype has been previously described as anaplastic prostate cancer; however, aggressive variant may be a more appropriate term, because many, if not all, of the tumors do not display anaplastic pathologic features, and some may represent high-grade adenocarcinomas without neuroendocrine differentiation.

The histopathologic features of NEPC resemble those of neuroendocrine tumors of other sites of origin. An updated classification schema based on morphologic characteristics divides NEPC into the following categories: small cell carcinoma, large cell NEPC,8 adenocarcinoma with Paneth cells (rare),9 carcinoid tumors (rare),10 and adenocarcinoma admixed with neuroendocrine differentiation, which can exist either in primary untreated disease or, more commonly, as a treatment-emergent phenomenon on the development of resistance to ADT and secondary androgen receptor signaling inhibitors11 (Figure 2 and Table 1). ETS transcription factor family gene rearrangements are present in 60% to 70% of NEPC tumors, which are not observed in small cell cancers and neuroendocrine tumors originating from other anatomic sites. If present, this translocation can help confirm a prostate origin in cases of neuroendocrine tumors of unknown primary.12 The morphologic characteristics of small cell prostate cancer are similar to those of other small cell tumors, consisting of sheets of uniform cells with a high nuclear/cytoplasmic ratio, frequent mitotic figures, and diffuse infiltration with poorly circumscribed margins. Large cell neuroendocrine cancer is a less common variant of primary NEPC, consisting of solid sheets of cells with large nuclei and abundant, eosinophilic cytoplasm. The Paneth cell subtype is identified on hematoxylin-eosin stained pathology sections based on its intense eosinophilic staining pattern with large cytoplasmic granules. Carcinoid tumors represent low-grade, well-differentiated tumors with a uniform cellular pattern. The clinical significance of these histologic subtypes is based on the differential grade and proliferative index seen with each subtype (Table 1). Higher-grade subtypes, including small and large cell NEPC, with or without admixed adenocarcinoma, display a high proliferation index and more aggressive phenotype that often necessitates initiation of cytotoxic chemotherapy early in the treatment course.

Immunohistochemical biomarkers augment the sensitivity of detecting small cell and NEPC based on morphologic criteria alone (Figure 1). Induction
of chromogranin A and synaptophysin expression, loss of androgen receptor and prostate-specific antigen (PSA) expression, and upregulation of CD56 can help distinguish NEPC from prostatic adenocarcinoma. However, significant overlap and heterogeneity may occur, especially in cases of mixed histologies or with poorly differentiated high-grade adenocarcinomas. For example, PSA and androgen receptor expression are observed in approximately one-quarter of tissue samples consisting predominantly of NEPC. Serum-based biomarkers, including chromogranin and neuron-specific enolase, may further aid in the detection of neuroendocrine differentiation, especially when metastatic disease sites are not accessible for tissue acquisition, but correlation with immunohistochemical positivity or molecular biomarkers is not very robust and has not been rigorously ascertained. Higher serum chromogranin levels may be associated with adverse disease outcomes, but do not seem to correlate with treatment response to standard cytotoxic chemotherapy in the metastatic setting.

The cellular origin of NEPC remains under debate. The expression of chromogranin A, synaptophysin, and CD56 is observed in neuroendocrine cells and neural tissue found normally scattered throughout the benign prostate gland. In addition, poorly differentiated prostate cancers have a proclivity for perineural invasion, suggesting mimicry of a neural phenotype and, potentially, that NEPC arises from malignant transformation of preexisting neuroendocrine or neural tissue within the prostate gland. Alternatively, a transdifferentiation model has been proposed, whereby NEPC arises from prostate adenocarcinoma, and is supported by shared ETS transcription family gene rearrangements in tissues with mixed neuroendocrine/adenocarcinoma histologies and preclinical studies of prostate cancer cell lines (discussed herein) that support the plasticity between neuroendocrine and adenocarcinoma differentiation.

Clinical Features

Pure primary NEPC is a rare entity (<1%) with an incidence of 35 per 10,000 people each year, and frequently presents with symptoms related to locally invasive or metastatic disease at the time of diagnosis, including bowel or bladder invasion; hydronephrosis; visceral metastatic disease to the liver, lung, and central nervous system; and predominantly lytic bone metastases. Given the propensity for brain metastases, routine MR brain imaging is reasonable to consider in patients with NEPC. FDG-PET/CT scans are frequently positive in NEPC, and may be useful to obtain in select patients to evaluate for the presence of occult distant metastatic disease before initiating locally directed therapy. Paraneoplastic syndromes are infrequent but more common than in prostatic adenocarcinoma, and include parathyroid hormone (PTH)–related protein–induced hypercalcemia, Cushing disease, and limbic encephalopathy.

The development of resistance to potent androgen signaling inhibitors in the metastatic setting can be associated with the development of t-NEPC. Preliminary studies indicate the presence of t-NEPC in approximately 30% of metastatic castration-resistant tumors. It has been suggested that the incidence of t-NEPC may be increasing with the widespread introduction of potent androgen signaling inhibitors, such as abiraterone and enzalutamide, into clinical practice. Before considering sequential androgen
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neuroendocrine transdifferentiation

AURKA

AURKA protein in all NEPC tumors and amplification of Aurora kinase A (AURKA) and N-myc (MYCN); loss of Rb; upregulation of EZH2; and downregulation of REST (regulator of neuronal gene expression). The TMPRSS2-ERG translocation is the most common gene rearrangement in prostate adenocarcinoma, occurring in 40% to 60% of cases. In 2011, Lotan et al showed that ERG gene rearrangements were present in a similar frequency of small cell prostate cancer tissues. Furthermore, concordance with respect to ERG gene rearrangement was seen between the acinar and small cell components of the available prostate samples, supporting a model of transdifferentiation toward NEPC from a common cellular origin. Downregulation of androgen receptor–driven TMPRSS2-ERG protein expression may represent a potential mechanism of neuroendocrine transdifferentiation. In a preclinical model, knockdown of ERG expression led to decreased PSA and increased chromogranin A levels. Preliminary studies suggest that enzalutamide-mediated blockade of androgen receptor–driven expression of the TMPRSS2-ERG fusion protein may promote the emergence of secondary NEPC. Induction of neuroendocrine markers has long been observed with antiandrogens in tissue culture; however, the precise mechanisms for this phenotypic change are unclear.

Several recently reported studies of human NEPC tissue samples have identified potential novel molecular markers of neuroendocrine differentiation. One of the hallmarks of NEPC is loss of androgen receptor, androgen receptor knockdown in an androgen receptor–sensitive cell line has been shown to correspond with neuroendocrine differentiation. Moreover, Lapuk et al identified decreased expression of REST, a transcription factor that represses neuronal differentiation, in NEPC. They further found REST binding sites on 28 of 50 transcriptionally active genes in NEPC and validated their findings in a cohort of 218 prostate tumors, in which they found REST downregulation in 50% of NEPC tumors.

A separate study published by Beltran et al showed overexpression of the AURKA protein in all NEPC tumors and amplification of the AURKA gene in 4 of 7 NEPC tumors. Interestingly, inhibition of AURKA

Molecular Characterization and Modeling of NEPC With Small Cell Features

Recent studies have revealed several molecular signatures of NEPC, including ERG gene rearrangement; amplification of Aurora kinase A (AURKA) and N-myc (MYCN); loss of Rb; activation of PI3K, Akt, and β-catenin; and weak prostate-specific antigen staining (C) on immunohistochemistry. From Bitting RL, Schaeffer D, Somarelli JA, et al. The role of epithelial prostate cancer metastasis Rev, in press; with permission.

Figure 2 Prostate cancer histology transforming from adenocarcinoma to neuroendocrine carcinoma over the course of treatment. Initial prostate biopsy showed high-grade prostate adenocarcinoma (A). Over the course of treatment, disease transformed into neuroendocrine differentiation, with strong synaptophysin staining (B) and weak prostate-specific antigen staining (C) on immunohistochemistry. From Bitting RL, Schaeffer D, Somarelli JA, et al. The role of epithelial plasticity in prostate cancer dissemination and treatment resistance. Cancer Metastasis Rev, in press; with permission.

receptor–targeted therapy in metastatic castration-resistant prostate cancer (eg, abiraterone after disease progression on enzalutamide, or vice versa), it is useful to determine whether any evidence of neuroendocrine transdifferentiation is present, which would predict for a lower likelihood of benefit with continued androgen receptor–targeted therapies. Clues from the patient’s presentation that suggest the presence of neuroendocrine differentiation include a limited response duration to primary ADT (<6 months); high PSA nadir on ADT (>4 ng/mL); visceral metastatic disease, including to the lung, liver, and central nervous system; predominantly lytic bone metastases; and elevated serum markers of neuroendocrine differentiation, including chromogranin and neuron-specific enolase; and high levels of serum prostate-specific antigen (CSE) serum levels. Serum markers of NEPC should be checked in patients with castration-resistant prostate cancer with the clinical features of neuroendocrine differentiation outlined earlier. Although these clinical features may be suggestive of NEPC, the diagnosis currently remains histopathologic. Therefore, biopsy should be strongly considered whenever clinical features and/or serum markers are suggestive of neuroendocrine differentiation. Standard pathologic evaluation should quantify the level of expression of chromogranin, synaptophysin, and neuron-specific enolase, and determine whether small cell features are present. A diagnosis of neuroendocrine differentiation has both prognostic and therapeutic implications, as discussed herein. In the research setting, biopsies of metastatic tumors are used to evaluate potential novel molecular biomarkers of neuroendocrine differentiation.
in this study led to a loss of neuroendocrine differentiation, providing for the possibility of a mechanistic link between AURKA signaling and reversible NEPC. AURKA is known to stabilize N-myc, and amplification of MYCN was found in 40% of NEPC cases compared with only 4% of prostate adenocarcinoma cases.24

In addition, the PI3K/Akt-mTOR pathway has been shown to be necessary for NEPC formation. Inhibition of PI3K led to decreased neuron-specific enolase expression, and transfection of activated Akt led to increased neuron-specific enolase expression, indicating a reversible NEPC activation.25 In a transgenic mouse model of prostate cancer using the TRAMP-C2 cell line, activation of Akt was shown to be necessary for NEPC differentiation, with a concurrent increase in β-catenin expression.26 When PI3K/Akt inhibitors were incorporated, β-catenin was not expressed and neuroendocrine differentiation was not seen.26

The plasticity of neuroendocrine transdifferentiation is supported by several independent preclinical studies and clinical studies of patients with non–small cell lung cancer (NSCLC). Recent studies have described the transformation of NSCLC into small cell histologic variants at the time of developing epidermal growth factor receptor (EGFR) inhibitor resistance.27 This histologic transformation was found to be reversible in several cases on EGFR inhibitor withdrawal and treatment with platinum-based chemotherapy, indicating the plasticity of this histologic transformation. Knockdown of androgen receptor expression in the androgen-sensitive LNCaP prostate cancer cell line induced increased neuroendocrine differentiation in a temporally related manner.28 Whether this neuroendocrine differentiation is reversible in patients with NEPC through stopping androgen receptor–targeted therapy is not known. Conversely, reversion back to the adenocarcinoma phenotype with loss of neuroendocrine markers was observed during therapy with an AURKA or mTOR inhibitor.26 These data suggest that potent inhibitors of key oncogenic pathways that regulate cellular differentiation, such as the EGFR and androgen receptor pathways, may lead to reversible histologic variants with altered biology and treatment response. The plasticity of neuroendocrine transdifferentiation with the application of treatment selection pressure has implications for future treatment strategies, which are discussed in the following section.

**Modeling of NEPC/Small Cell Prostate Cancer**

Several models of NEPC have been created that have yielded additional insights into the molecular drivers of neuroendocrine differentiation. Patient-derived xenografts were developed from a single patient with pelvic soft tissue metastatic castration-resistant prostate cancer, and implanted into CB17 SCID mice.29 Four of these xenograft tumors developed into large cell NEPC, and the other 4 developed into small cell NEPC. This finding suggests that large cell NEPC exists as an intermediate state in the spectrum of differentiation between prostate adenocarcinoma and pure small cell NEPC.29 The xenografts showed an absence of androgen receptor expression and loss of Rb and cyclin D1 expression.30 The xenograft model was validated in 24 NEPC and 44 tissue samples from patients with prostate adenocarcinoma; 96% of human NEPC samples showed loss of Rb and cyclin D1, compared with 23% of prostate adenocarcinoma samples.31 These data suggest that Rb loss may be a suitable molecular biomarker for NEPC, albeit with some overlap with adenocarcinoma.

The transgenic adenocarcinoma of the murine prostate (TRAMP) model in the Friend virus B (FVB)
Treatment of Small Cell Carcinoma and Prostate Cancer With Neuroendocrine Differentiation

The prognosis of small cell carcinoma of the prostate is poor. More than half of patients present with metastatic disease at diagnosis; the remainder almost always present with locally advanced disease, for which radiation therapy is usually recommended. Given the propensity for the development of distant metastatic disease, concurrent platinum-based chemotherapy is usually recommended, consistent with treatment guidelines for limited-stage small cell carcinoma of the lung. However, distant relapse is common, often presenting as visceral metastases in the liver, lung, and central nervous system, along with the development of predominantly lytic bone metastases.

Advanced small cell prostate cancer and prostate cancer with extensive neuroendocrine differentiation are both highly proliferative subsets of prostate cancer and, as such, frequently respond rapidly to cytotoxic chemotherapy with minimal (if any) response to androgen deprivation. Pure small cell carcinoma with distant metastases should largely be treated with up-front chemotherapy rather than ADT. In prostate adenocarcinoma admixed with extensive neuroendocrine differentiation, it is reasonable to consider a trial of ADT, potentially in combination with cytotoxic chemotherapy. Pure small cell carcinoma is frequently treated with platinum plus etoposide combinations; admixed adenocarcinoma with neuroendocrine differentiation is frequently treated with docetaxel, which treats both the neuroendocrine and the adenocarcinoma components, or a combination of carboplatin plus docetaxel. Platinum-based treatment should be strongly considered in cases involving more extensive neuroendocrine differentiation and/or small cell features within the tumor biopsy. These regimens are based on retrospective series and single-arm phase II studies, including a prior series of 21 patients with metastatic small cell prostate cancer treated with a combination of platinum plus etoposide, in which 62% of patients experienced an objective tumor response. However, the duration of response was transient, with a median survival of less than 12 months in this series.

Several prospective clinical trials have investigated combination chemotherapeutic approaches. A single-arm phase II study investigated the use of carboplatin plus doxorubicin in combination with prednisone in 113 patients with 1 or more of 7 prespecified criteria designed to capture anaplastic or aggressive phenotype prostate cancer: (1) histologic evidence of small cell prostate cancer (pure or mixed), (2) exclusively visceral metastases, (3) predominantly lytic bone metastases, (4) bulky pelvic soft tissue masses greater than 5 cm, (5) low serum PSA level (<10 ng/mL) in combination with high volume (>20) bone metastases, (6) positive immunohistochemistry staining of chromogranin or synaptophysin A or elevated serum markers of neuroendocrine differentiation plus elevations in lactate dehydrogenase (LDH), CEA, or malignant hypercalcemia, or (7) short-interval (<6 months) response to primary ADT. After disease progression on carboplatin plus doxorubicin, patients were treated with cisplatin plus etoposide. Median overall survival for the entire study cohort was 16 months (95% CI, 13.6–19.0), and the proportion of patients who were progression-free after 4 cycles of carboplatin plus docetaxel and subsequent treatment with cisplatin plus etoposide (N=71) was 65.4% and 33.8%, respectively. The trial results suggest that continued platinum-based chemotherapy with the addition of etoposide may be a reasonable second-line therapy in patients with NEPC who experience disease progression on platinum plus taxane combinations, but further study of this combination is required. The heterogeneous patient population is reflected in the variable prognostic weight of each of the 7 criteria. Somewhat surprisingly, of the baseline
7 criteria, only the presence of bulky pelvic soft tissue disease greater than 5 cm was associated with both inferior progression-free and overall survivals. Although the presence of exclusive visceral metastatic disease was not associated with overall and progression-free survivals, this finding may reflect an underpowered trial as opposed to a negative association. Serum levels of LDH and CEA seemed to have more prognostic value than levels of chromogranin A or immunohistochemical positivity of chromogranin A and synaptophysin, illustrating that biomarkers other than traditional NEPC biomarkers may be prognostic. Immunohistochemistry staining for one or both of these markers was positive in 56.9% of the 51 samples available for analysis. The heterogeneity of the patient population and variability of biomarker expression highlight the need for novel molecular-based biomarkers to identify biologically distinct subsets of NEPC and thereby develop more-effective targeted therapies.

The recently discovered molecular alterations in t-NEPC, including the coamplification of AURKA and MYCN, may provide a therapeutic window. Preclinical studies indicate significant activity of AURKA inhibitors in NEPC models, and an ongoing phase II study is evaluating the efficacy of the AURKA inhibitor MLN8237 in patients with metastatic NEPC (ClinicalTrials.gov identifier: NCT01799278). Prospective studies will be needed to validate AURKA gene amplification as predictive and/or prognostic biomarkers to guide treatment selection and as an enrichment criterion or stratification factor in future clinical studies. Given the molecular complexity of these tumors, strategies combining the inhibition of multiple pathways will likely be needed.

The plasticity toward neuroendocrine transdifferentiation observed with the application of androgen signaling inhibition in preclinical models suggests that transdifferentiation toward a neuroendocrine state may occur before overt clinical resistance to androgen signaling inhibition is seen, and that dual therapy targeting the 2 populations of cancer cells may provide therapeutic benefit. This hypothesis warrants testing in future clinical studies.

Summary
Prostate cancer with small cell features and neuroendocrine differentiation is an often lethal variant of the disease, occasionally presenting de novo, but more frequently developing in the setting of androgen receptor–targeting resistance. Emerging translational and preclinical studies are beginning to molecularly define this lethal subset of disease. These data should help improve on current histopathologic criteria and guide the development of predictive and/or prognostic biomarkers. Current treatment generally entails platinum-based chemotherapy and consideration of clinical trial enrollment; however, novel therapies are needed to lengthen response durations and improve long-term outcomes for this high-risk subset of patients with prostate cancer.

References
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Posttest Questions

1. Based on morphologic characteristics, which of the following categories can NEPC divide into?
   a. Adenocarcinoma with Paneth cells
   b. Small cell carcinoma
   c. Carcinoid tumors
   d. Large cell NEPC
   e. All of the above

2. True or False: Biopsy should be strongly considered whenever feasible to evaluate for the presence of neuroendocrine components.

3. NEPC is commonly treated with:
   a. Antiandrogens
   b. Platinum-based chemotherapy
   c. Immunotherapy
   d. Bisphosphonates