Aggressive Variants of Castration-Resistant Prostate Cancer

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Abstract

A subset of patients with advanced castration-resistant prostate cancer may eventually evolve into an androgen receptor (AR)–independent phenotype, with a clinical picture associated with the development of rapidly progressive disease involving visceral sites and hormone refractoriness, often in the setting of a low or modestly rising serum prostate-specific antigen level. Biopsies performed in such patients may vary, ranging from poorly differentiated carcinomas to mixed adenocarcinoma-small cell carcinomas to pure small cell carcinomas. These aggressive tumors often demonstrate low or absent AR protein expression and, in some cases, express markers of neuroendocrine differentiation. Because tumor morphology is not always predicted by clinical behavior, the terms “anaplastic prostate cancer” or “neuroendocrine prostate cancer” have been used descriptively to describe these rapidly growing clinical features. Patients meeting clinical criteria of anaplastic prostate cancer have been shown to predict for poor prognosis, and these patients may be considered for platinum-based chemotherapy treatment regimens. Therefore, understanding variants within the spectrum of advanced prostate cancer has important diagnostic and treatment implications. Clin Cancer Res; 20(11); 2846–50. ©2014 AACR.

On July 31, 2013, the Prostate Cancer Foundation assembled a working committee on the molecular biology and pathologic classification of neuroendocrine prostate cancer (NEPC). The committee consisted of genitourinary oncologists, urologists, urological surgical pathologists, basic scientists, and translational researchers, with expertise in this field. One area that was extensively discussed was whether transformation to anaplastic/NEPC is related to therapy. Aggressive variants of prostate cancer are increasingly recognized in the clinic. This could be due to greater awareness, patients living longer due to more effective therapies and followed for their natural history of disease, or the development of NEPC as a mechanism of resistance to current therapies. Recent clinical experience and preclinical data suggest that transformation to androgen receptor (AR)–independent prostate cancer likely occurs as one potential mechanism of adaptive resistance to AR-targeted therapies in a minority of patients. While this may be the case, anecdotal experiences were discussed in which patients with anaplastic or small cell NEPC responded to androgen deprivation or newer AR-targeted therapies; thus, clinical heterogeneity can be observed even between patients with this disease. In practice and depending on the clinical scenario, we often recommend hormonal therapy first or concurrently with chemotherapy. The group, due to absence of data, discouraged the term "therapy-related" neuroendocrine or small cell prostate cancer due to concern that clinicians or patients may withhold potentially effective hormonal therapies concerned that it could make things worse. It was generally thought that hormonal therapy should be considered in the appropriate clinical context.

There are deficiencies associated with the currently used terminologies. "Anaplastic prostate cancer" is not generally accepted by surgical pathologists because the term anaplastic is a well-recognized histopathologic term used to denote pleomorphic cytology. Although "anaplastic" in this setting is used to describe clinical features and does not require pathology, using the term anaplastic implies a histologic correlate when it is not typically present within this disease spectrum. "NEPC" is also debated as an appropriate term to describe this clinical phenotype, as the term is currently not well defined and implies that small cell carcinoma or a predominantly neuroendocrine histology must be seen on biopsy, when it is clear that many cases with this clinical phenotype do not demonstrate classical morphology or immunohistochemical profiles (i.e., chromogranin, synaptophysin, neuron-specific enolase; NSE).
In addition, neuroendocrine features or immunohistochemical expression can be seen in prostate cancer that does not show such aggressive behavior (i.e., prostatic adenocarcinoma with paneth cell–like differentiation). Because clinical features of anaplastic/NEPC most often correlate with AR independence, "AR-negative prostate cancer" has also been proposed. However, there are frequently mixed tumors with both AR-positive and AR-negative cells present, and less frequently hybrid tumors with dual expression of both neuroendocrine markers and AR in the same tumor cells. Therefore, based on this as well as lack of access to metastatic biopsies in many cases and inter- and intra-patient clinical and pathologic heterogeneity, the term AR negative was too limiting. In practice, all of the above terms may describe the same or overlapping clinical scenario, and as there is greater understanding, acceptance, and refinement of the pathologic and molecular classification, it is anticipated that tumors within this clinical category may be reclassified.

Although not all patients with the anaplastic/neuroendocrine clinical phenotype have neuroendocrine features present on biopsy, patients that do in fact demonstrate small cell or predominantly neuroendocrine carcinoma on biopsy in this clinical setting have a universally poor prognosis, typically lack AR expression, and may share common molecular features even with those that do not show classical morphology. Therefore, understanding the biologic underpinnings driving progression from castrate-resistant prostate cancer (CRPC) to NEPC is considered a priority research focus for this group. This knowledge can aid the identification of biomarkers for earlier detection of NEPC cells undergoing trans-differentiation and in understanding the molecular mechanisms underlying trans-differentiation. Biopsy should be considered for diagnostic purposes in patients with CRPC that develop rapid progression (especially to visceral organs such as brain or liver) in the setting of a low or modestly elevated serum prostate-specific antigen (PSA) level. Serial biopsies are also useful in helping understand clonal evolution and in identifying multiple clones or subclones that may emerge under selective pressures of treatment. We discussed the utility of neoadjuvant trials, the two Stand Up To Cancer-PCF Dream Team samples to evaluate metastatic tumors before and after potent AR-targeted therapies (abiraterone and enzalutamide), incorporation of metastatic biopsies into ongoing clinical trials, and the rapid autopsy programs. The University of Washington (Seattle, WA) and other rapid autopsy programs have served as an invaluable resource to study resistance phenotypes, investigate disease heterogeneity, and develop patient-derived xenografts. Data were presented at the workshop from the University of Washington program showing that the clinical features of patient-derived NEPC xenografts were highly concordant with the emerging phenotype we are seeing in the clinic (rapid doubling time, visceral metastases, low PSA). These xenografts have been molecularly characterized and can be

**Translational Relevance**

Often under-recognized, transformation to an androgen receptor–independent neuroendocrine or anaplastic prostate cancer can be seen in later stages of the disease and has diagnostic and treatment implications. Therefore, understanding the pathologic and molecular features underlying this clinical phenotype is an unmet need in oncology. To this end, we gathered a team of experts in the field to summarize current findings and implications in the context of novel therapies and emerging clinical and molecular data. We discuss emerging data and describe challenges and future directions in identifying and treating neuroendocrine or anaplastic prostate cancer and in correlating clinical phenotypes with pathologic and molecular features.
An improved pathologic classification of NEPC is a clinical unmet need. At this time, there exists variability in reporting between expert pathologists, which is likely even more pronounced in community centers. One reason for this is the lack of extensive literature due to emerging recognition and previous lack of access to treated or metastatic tissues. Furthermore, the clinical spectrum encompassing focal neuroendocrine differentiation of primary prostate cancer, paneth cell change, treatment-induced changes, pure small cell or large cell carcinoma of the prostate, and hybrid phenotypes are not fully addressed by the current classification system. To this end, a Pathology Consensus Statement with recommendations for nomenclature and immunohistochemical studies was published (Table 1) and is to be published as a companion manuscript as a result of this workshop (Epstein JI, Amin M, et al. American Journal of Surgical Pathology. In press.).

There is likely a biologic continuum with conventional prostate adenocarcinoma evolving from a hormone naïve state to a CRPC/mixed tumor to NEPC. Therefore, biopsies may only provide snapshots in time and may commonly demonstrate cancers in transition with mixed histopathologic and molecular profiles. With this caveat, characteristic molecular alterations of NEPC include loss of AR and androgen-regulated protein expression, induction of neuroendocrine and neural programs, loss of tumor suppressors (TP53, RB1, PTEN, which can also be seen in AR-driven CRPC), activation of mitotic programs, tumor suppressors (TP53, RB1, PTEN, which can also be seen in a subset of CRPC, most often through genomic instability. The combination of RB1 and TP53 loss has been implicated as key driving events in the pathogenesis of NEPC. RB1 is a tumor suppressor lost in the majority of NEPC but also seen in a subset of CRPC, most often through genomic deletion. TP53 is another tumor suppressor frequently mutated in NEPC as well as CRPC, leading to accumulation of the p53 protein in cancer cells. The combination of Rb and TP53 deficiency in promoting transformation to NEPC is supported by a conditional mouse model of Rb and TP53 deficiency in mice, which rapidly develop invasive metastatic cancer of mixed phenotype (both AR and synaptophysin positive); after cas- tration, the tumors transform to NEPC. The SV40 large T- cell antigen (TRAMP) prostate cancer mouse model also develops neuroendocrine small cell type prostate cancer as

Table 1. Pathologic classification of neuroendocrine differentiation in prostate carcinoma

<table>
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<th>Classification</th>
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<tr>
<td>Usual prostate adenocarcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma with paneth cell neuroendocrine</td>
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<tr>
<td>differentiation</td>
</tr>
<tr>
<td>Carcinoid tumor</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
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<tr>
<td>Large cell neuroendocrine carcinoma</td>
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<tr>
<td>Mixed (small and large cell) neuroendocrine carcinoma—aicinar adenocarcinoma</td>
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utilized for the development of coclinical trials and validation of therapeutic targets (True and colleagues; unpublished data).

Immunohistochemical studies for neuroendocrine markers are often clinically performed by pathologists to support the diagnosis in patients with clinical or histologic suspicion of NEPC. Markers include chromogranin, synaptophysin, CD56, NSE, and bombesin, though none are diagnostic or particularly reliable, and are contingent on the specificity of the antibodies and the thresholds for visual interpretation of the staining. Expert pathologists surveyed at the workshop generally agreed that of these commonly used immunohistochemical markers, synaptophysin is most sensitive and chromogranin A is most specific for NEPC. CD44 is a cell surface adhesion molecule highly expressed in cancer stem cells and in NEPC (14) and associated with tumor metastasis (15, 16), suggesting that there may be stem cell–like functions of neuroendocrine cells in prostate cancer and potentially reversion to a primitive stem-like state in pure NEPC. Induction of neuronal differentiation regulators and genes associated with a neural phenotype are also commonly seen in NEPC. These include ASCL1 (17), MYCN (6), and others associated with axon guidance and synapse signaling (17, 18). Furthermore, reduced expression of the transcription factor REST, a repressor of neuronal differentiation, is commonly observed in NEPC, associated with upregulation of neuronal genes, and is implicated as a key regulator in driving NEPC disease progression (18). New data were also presented at the workshop evaluating gene expression profiles of NEPC cell lines and tumors from published datasets, revealing that NEPC tumors show induction of a specific brain signature profile (Ayala and colleagues; unpublished data). Incorporating these genes into molecular classifiers and/or targeting nerves through denervation models or other therapeutic approaches are areas of active investigation.

The combination of RB1 and TP53 loss has been implicated as key driving events in the pathogenesis of NEPC. RB1 is a tumor suppressor lost in the majority of NEPC but also seen in a subset of CRPC, most often through genomic deletion. TP53 is another tumor suppressor frequently mutated in NEPC as well as CRPC, leading to accumulation of the p53 protein in cancer cells. The combination of Rb and TP53 deficiency in promoting transformation to NEPC is supported by a conditional mouse model of prostate cancer which also mimics NEPC (19). Inactivation of either gene alone leads to PIN, but double KO mice rapidly develop invasive metastatic cancer of mixed phenotype (both AR and synaptophysin positive); after castration, the tumors transform to NEPC. The SV40 large T-cell antigen (TRAMP) prostate cancer mouse model also develops neuroendocrine small cell type prostate cancer as.
a result of RB1 and TP53 inactivation (20). Rb is involved in transcriptional regulation of mitotic checkpoint genes and also contributes to prostate cancer progression through modulation of androgen signaling (21). TP53 is important in mediating the IL8-CXCR2 signaling pathway, and loss of TP53 stimulates interleukin (IL)-8–induced cellular proliferation of AR-negative prostate cancer (22). Biologic mechanisms underlying how RB1 and TP53 may cooperate and also potentially interact with other oncogenic pathways to drive NEPC and potential therapeutic implications are yet to be fully elucidated and are areas of active research. IHC to evaluate for both loss of RB1 protein and overexpression of TP53 is clinically feasible and warrants further investigation as biomarkers for distinguishing NEPC from other subtypes within CRPC (23).

NEPC tends to be highly proliferative with Ki-67 rates of >50%. Although not routinely assessed, Ki-67 is sometimes utilized by expert pathologists to support the diagnosis. Recent work has identified several cell-cycle genes as frequently amplified and/or overexpressed in NEPC, including UBE2C, cyclin D1, AURKA, AURKB, and PLK1 (6, 24). Altered mitotic programs likely play a role in driving uncontrolled growth and disease progression. Importantly the cell-cycle aurora kinases, polo-like kinase (PLK1), as well as RB1 are potentially targetable and may represent novel therapeutic targets for NEPC. Furthermore, elucidating how these molecular markers or others may predict platinum sensitivity and how they may associate with response or resistance to other treatments for CRPC such as taxanes warrants further study. The histone deacetylase EZH2 is also highly expressed in NEPC (6, 25) and hypermethylation of key genes within the NEPC genome may be associated with the cellular plasticity seen during trans-differentiation; the role of epigenetic modification in promoting NEPC progression is an area of research (Beltran and colleagues; unpublished data). The role of the tumor microenvironment and data supporting MET as a potential targetable driver of NEPC was also presented as an area of active investigation (Tomlins and colleagues; unpublished data).

In the last few years, there have been significant advances in our understanding and treatment of patients with advanced prostate cancer. With the widespread clinical introduction of new effective systemic therapies, patients are living longer. Recognizing that we now have choices, it becomes essential to understand variants within the spectrum of CRPC to select patients for appropriate therapies. Neuroendocrine or anaplastic prostate cancer is one important variant and these patients may be considered for platinum chemotherapy or clinical trial enrollment. With a better understanding of the biologic mechanisms driving progression toward this phenotype, recent development of novel preclinical models to study trans-differentiation, increased clinical awareness, and access to metastatic tumors, novel molecular biomarkers and targeted therapies are rapidly being developed for this distinct subclass.

**Disclosure of Potential Conflicts of Interest**

H. Beltran reports receiving commercial research grants from Astellas and Millennium Pharmaceuticals. S. Tomlins reports receiving speakers bureau honoraria from VentanaMedicalSystems/Roche; has ownership interest (including patents) in Gen-Probe, Inc/Hologic and Ventana Medical Systems/Roche; and is a consultant/advisory board member for Ventana Medical Systems/Roche. C. Logothetis is a consultant/advisory board member for, and reports receiving other commercial research support and speakers bureau honoraria from Astellas, Bristol-Myers Squibb, Exelixis, Johnson & Johnson, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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