Aggressive Variants of Castration Resistant Prostate Cancer

Himisha Beltran\textsuperscript{1,2}, Scott Tomlins\textsuperscript{3}, Ana Aparicio\textsuperscript{4}, Vivek Arora\textsuperscript{5}, David Rickman\textsuperscript{6,2}, Gustavo Ayala\textsuperscript{7}, Jiaoti Huang\textsuperscript{8}, Lawrence True\textsuperscript{9}, Martin E. Gleave\textsuperscript{10}, Howard Soule\textsuperscript{11}, Christopher Logothetis\textsuperscript{4}, Mark A. Rubin\textsuperscript{6,2}

\textsuperscript{1}Division of Hematology and Medical Oncology, Weill Cornell Medical College, New York, NY.
\textsuperscript{2}Institute for Precision Medicine, New York Presbyterian-Weill Cornell Medical College, New York, NY.
\textsuperscript{3}Department of Pathology, University of Michigan, Ann Arbor, MI.
\textsuperscript{4}Department of Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX.
\textsuperscript{5}Department of Oncology, Memorial Sloan Kettering, New York, NY.
\textsuperscript{6}Department of Pathology and Laboratory Medicine, Weill Cornell Medical College
\textsuperscript{7}Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center, Houston, TX.
\textsuperscript{8}Department of Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA.
\textsuperscript{9}Department of Pathology, University of Washington, Seattle, WA.
\textsuperscript{10}Prostate Cancer Prostate Centre, Vancouver, BC. Canada.
\textsuperscript{11}Prostate Cancer Foundation, Santa Monica, CA.

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Corresponding Authors:

Mark A. Rubin
Weill Medical College of Cornell University
1300 York Avenue, C-440
New York, NY 10065 U.S.A.
E-mail: rubinma@med.cornell.edu
Telephone: +1 (212) 746-6313

Himisha Beltran
Weill Medical College of Cornell University
525 East 68th Street, Box 403
New York, NY 10065 U.S.A.
E-mail: hip9004@med.cornell.edu
Telephone: +1 (646)-962-2072
**Translational Relevance:** Often under-recognized, transformation to an androgen receptor (AR) 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.
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**Abstract:** A subset of patients with advanced castration resistant prostate cancer (CRPC) 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 (PSA) level (1, 2). 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. Since tumor morphology is not always predicted by clinical behavior, the terms “anaplastic prostate cancer” or “neuroendocrine prostate cancer” have been employed 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(3). Therefore, understanding variants within the spectrum of advanced prostate cancer has important diagnostic and treatment implications.

On July 31, 2013, the Prostate Cancer Foundation assembled a working committee on the molecular biology and pathological 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/ neuroendocrine prostate cancer 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 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 neuroendocrine prostate cancer 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. “Neuroendocrine prostate cancer” 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 (ie., chromogranin, synaptophysin, neuron specific enolase). Additionally, 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). Since clinical features of anaplastic/neuroendocrine prostate cancer 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 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 and in the development of novel therapeutic strategies.
There is extensive preclinical data demonstrating the role of neuroendocrine differentiation in promoting the growth of prostate adenocarcinoma through paracrine and endocrine secreted factors, and potentially also through transmission of signals through of nerve-like conduction. In addition to promoting surrounding adenocarcinoma growth, transformation to a pure NEPC can also occur clinically. There are two hypotheses of how NEPC arises: 1) NEPC cells share the same cell of origin with normal neuroendocrine cells of the prostate, or 2) adenocarcinoma cells undergo trans-differentiation into NEPC cells. The majority of evidence to date favors a trans-differentiation model. In this model, adenocarcinomas treated extensively with androgen deprivation therapies develop into a NEPC as a mechanism of adaptive response/tumor resistance. There is extensive *in vitro* and *in vivo* xenograft data demonstrating trans-differentiation of LNCaP cells to a neuroendocrine phenotype when exposed to various stimuli (e.g., androgen depletion, cAMP, cytokines, growth factors)(4). More recently, trans-differentiation has been observed in a patient-derived prostate adenocarcinoma xenograft model that develops small cell NEPC after medical castration; genomic profiling at various time-points during trans-differentiation suggests clonal evolution(5). Furthermore, several *in situ* studies of patient NEPC tumors showing mixed features have demonstrated molecular concordance between adenocarcinoma and NEPC foci with respect to ERG gene rearrangement and TP53 status(6, 7), which also supports a same cell of origin or trans-differentiation model. Recently, xenograft models have also identified epithelial to mesenchymal transition (EMT) with loss of AR expression as a mechanism of resistance to both castration therapy and enzalutamide, offering another conceptualization of prostate cancer transdifferentiation to an AR independent state(8, 9).
One of the major limitations in the past for studying advanced prostate cancer has been the lack of post-treatment biopsies. The importance of collection of tumor samples for molecular profiling from patients at various time points during therapy and progression is a significant area of need in clinically identifying patients undergoing transformation 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 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, enzalutamide), incorporation of metastatic biopsies into ongoing clinical trials, and the rapid autopsy programs. The UW and other rapid autopsy programs have served as an invaluable resource to study resistance phenotypes, investigate disease heterogeneity, and develop patient-derived xenografts. Data was presented at the Workshop from the UW 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 utilized for the development of co-clinical trials and validation of therapeutic targets (True and colleagues, unpublished data).

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 proposed (Table 1) and is to be published as a companion manuscript as a result of this Workshop (Epstein, Amin et al, 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, and genomic instability.

Genomic rearrangement of the ERG gene is present in approximately 50% of NEPC(6, 10-12), similar to the frequency in prostate adenocarcinoma(13), and distinguishes NEPC from small cell carcinomas of other primary sites. Therefore, the ERG break-apart fluorescence in situ hybridization (FISH) assay to evaluate for ERG fusion is clinically useful in confirming prostate origin in cases of unknown primary. Immunohistochemistry (IHC) for ERG should not be replaced for FISH in cases of suspected NEPC, as protein expression of ERG is androgen driven, and thus ERG is low or absent in NEPC due to low AR expression. Similarly, other androgen
regulated proteins (such as PSA, PSMA) are variably expressed but usually low in NEPC and tend to disappear in cases of pure small cell prostate carcinoma. Whether assessment of AR status using a panel of protein markers by IHC is clinically useful in the management of CRPC and selecting therapies (AR targeted therapy versus chemotherapy) is compelling but yet to be clinically validated.

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, neuron specific enolase (NSE), 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 IHC 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 up-regulation of neuronal genes, and is implicated as a key regulator in driving NEPC disease progression(18). New data was 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). 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 IL8 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 are clinically feasible and warrant further investigation as biomarkers for distinguishing NEPC from other subtypes within CRPC(23).
NEPC tends to be highly proliferative with Ki67 rates of >50%. Although not routinely assessed, Ki67 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, Rubin, 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 in order 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 towards 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.

REFERENCES

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