

# The Role of Lineage Plasticity in Prostate Cancer Therapy Resistance

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## Abstract

Lineage plasticity has emerged as an important mechanism of treatment resistance in prostate cancer. Treatment-refractory prostate cancers are increasingly associated with loss of luminal prostate markers, and in many cases induction of developmental programs, stem cell-like phenotypes, and neuroendocrine/neuronal features. Clinically, lineage plasticity may manifest as low PSA progression, resistance to androgen receptor (AR) pathway inhibitors, and sometimes small cell/neuroendocrine pathologic features observed on metastatic biopsy. This mechanism is not restricted to prostate cancer as other malignancies also demonstrate lineage plasticity during resistance to targeted therapies. At present, there is no established therapeutic approach for patients with advanced prostate cancer developing lineage plasticity or small cell neuroendocrine prostate cancer (NEPC) due to knowledge gaps in the underlying biology. Few clinical trials

address questions in this space, and the outlook for patients remains poor. To move forward, urgently needed are: (i) a fundamental understanding of how lineage plasticity occurs and how it can best be defined; (ii) the temporal contribution and cooperation of emerging drivers; (iii) preclinical models that recapitulate biology of the disease and the recognized phenotypes; (iv) identification of therapeutic targets; and (v) novel trial designs dedicated to the entity as it is defined. This Perspective represents a consensus arising from the NCI Workshop on Lineage Plasticity and Androgen Receptor-Independent Prostate Cancer. We focus on the critical questions underlying lineage plasticity and AR-independent prostate cancer, outline knowledge and resource gaps, and identify strategies to facilitate future collaborative clinical translational and basic studies in this space.

## Introduction

Lineage plasticity is a biological process that occurs during normal development and later as a mechanism that promotes cell survival when adapting to their environment, evading stress, or repairing tissues. Plasticity may manifest as reversible or irreversible changes in cellular "identity," whereby cells take on an alternative morphologic, phenotypic, or epigenetic state (1). In cancer, lineage plasticity facilitates carcinogenesis, metastasis, and treatment resistance (2). During therapy-related lineage plasticity, differentiated tumor cells acquire new phenotypes, in some cases reverting back to a more "stem-like" state followed by redifferentiating toward an alternative "cell fate" in order to bypass

therapeutic pressure. This versatility of cellular state is particularly prominent in cancer types with effective therapies that target major growth programs and lineage-directing factors (e.g., BRAF-mutant melanoma, EGFR-mutant lung cancer, AR-driven prostate cancer). In these cases, early "targetable" genomic alterations are often retained, but expression of the pressured target is suppressed. Despite preserving a molecular memory of their differentiated cancer cell precursor, alternative lineage programs facilitate subsequent tumor progression.

Prostate cancer is a malignancy driven by androgen receptor (AR) signaling, and AR-targeted therapies are commonly used to treat patients at all stages of the disease. Prostate tumors most frequently

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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Clin Cancer Res 2019;25:6916-24

doi: 10.1158/1078-0432.CCR-19-1423

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### Translational Relevance

Lineage plasticity associated with loss of androgen receptor signaling dependence and the acquisition of alternative lineage programs occurs in up to 20% of advanced prostate cancer patients as a mechanism of treatment resistance, with important clinical and therapeutic implications. We discuss our current understanding of mechanisms underlying this process and outline a path toward the development of novel biomarkers and trials for this molecularly distinct subset of patients.

display an adenocarcinoma morphology reminiscent of normal luminal prostate architecture, with higher levels of disorganization associated with advanced tumor grade. Downstream markers of canonical AR activity, such as PSA, are used as clinical biomarkers to confirm a diagnosis of prostate cancer and for disease monitoring. Although several prostate cancer drugs are effective at lowering androgen levels and/or blocking the AR directly, metastatic prostate cancers universally develop treatment resistance (3). Acquired resistance is typically due to reactivation of AR signaling mediated, in part, by genomic mutation, amplification, or structural rearrangement of the AR gene itself. However, loss of AR expression and/or downstream signaling occurs in an estimated 15% to 20% of castration-resistant tumors (4–6). In extreme cases, tumors may reprogram toward alternative pathways adopting features of neuroendocrine, neuronal, or other lineages. Clinically, these cancers are notable for attenuated AR signaling and a range of histologies of which the most common exhibit small cell neuroendocrine carcinoma characteristics. At present, the definition and mechanistic underpinnings of this subset remain ill-defined, and there are no established therapeutic approaches for small cell/NEPC or other phenotypes. Few clinical trials address lineage plasticity, and the outlook for patients remains poor.

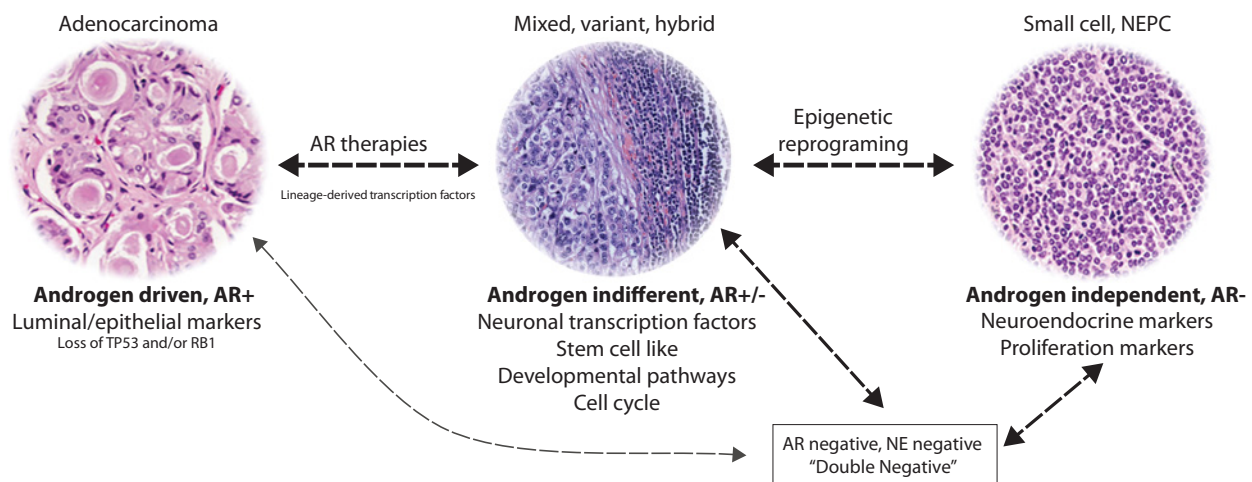
To address the challenges resulting from lineage plasticity, the NCI organized a Workshop focused on Lineage Plasticity and Androgen Receptor-Independent Prostate Cancer. Five working

groups were assembled prior to the workshop to formulate questions underlying basic, translational, and clinical knowledge gaps and to develop approaches to address them (Supplementary Information). This Perspective generated as a result of the workshop summarizes concepts, data, deficiencies, and opportunities that drive critical questions underlying lineage plasticity and AR-independent prostate cancer.

### Gap 1: A Fundamental Understanding of How Lineage Plasticity Occurs

Lineage plasticity is a term that implies that cells are capable of reprogramming their identity by acquiring an alternative lineage state and that this process is at some point plastic, or reversible. Whether AR-independent treatment resistance is mediated through an intermediate stem-like state, an "epithelial-mesenchymal transition" (EMT), or through direct transdifferentiation to acquire new characteristics and the extent of the reversibility of these processes are not well understood. Tumors with mixed or overlapping features expressing both AR and AR pathway genes as well as neuroendocrine markers ("hybrid" or "amphicrine" tumors), or those lacking both AR and neuroendocrine markers ("double negative" tumors) may further represent distinct disease states or a continuum (Fig. 1). Lineage programs such as gastrointestinal, squamous, and others have also been described (7). Identifying subsets within AR-independent disease provides expanding insights into the unique biology of AR-independent therapy resistance as well as potentially distinct therapeutics, such as the preferential activation of fibroblast growth factor signaling in "double negative" castration-resistant prostate cancer (CRPC; ref. 4).

The transition of prostate cancer away from a luminal epithelial phenotype is facilitated by genomic loss of the tumor suppressors *RB1* and *TP53*, leading to changes in stem cell, developmental, and EMT programs, mediated in part by the lineage pluripotency transcription factor *SOX2* (8–10). Downregulation of *REST* (a transcription factor that normally silences the expression of neuron-specific genes in nonneuronal cells) through splicing



**Figure 1.**

Schematic of the proposed molecular events and transition states underlying lineage plasticity that occurs during CRPC progression from an AR-positive, AR-driven prostate adenocarcinoma (luminal phenotype) toward an AR-negative, AR-independent cellular state (e.g., small cell/neuroendocrine).

regulated by SRRM4 (11, 12), as well as activation of lineage-associated transcription factors such as N-myc (13, 14), One-cut2 (15, 16), and BRN2 (17), and derepression of developmental genes such as PEG10 (18) also occur. These changes in cellular programs associate with an increase in cellular proliferation and amplification or overexpression of cell-cycle regulators (e.g., aurora kinase, polo-like kinase; refs. 13, 19), leading to aggressive tumor growth and often visceral metastatic spread. Lineage tracing studies (20) have supported a luminal cell of origin of neuroendocrine prostate cancer cells that arise upon potent androgen blockade, supporting a "transdifferentiation" process. Important questions concerning the timing, cooperation, and feedback of the drivers of transdifferentiation processes and their relationships to oncogenic programs and AR silencing remain to be addressed. Further, identification of noncanonical AR programs associated with AR-positive, PSA low tumors as well as genes derepressed by AR inhibition could provide biological insights into other potential early mediators of lineage plasticity. Epigenetic modifications including changes in DNA methylation and upregulation of the polycomb complex gene enhancer of zeste 2 (EZH2) also play a role in silencing the luminal program and in reprogramming lineage (8, 10). Targeting epigenetic programs may result in some degree of reversibility or reversion back to a more luminal state. Metabolic shifts mediated in part by loss of PKC $\lambda$ /i (21), which support proliferation and epigenetic changes, as well as changes in tumor hypoxic state (15) are also observed during NEPC progression. A better understanding of the fundamental cellular and molecular interactions that drive lineage plasticity and the role of the tumor microenvironment, metabolic alterations, and changes in epigenetic state requires further focus and study. This will help to further refine the working definition of this cellular state in prostate cancer.

#### Working definition of lineage plasticity in prostate cancer

A progressive state of CRPC associated with the loss of AR-regulated lineage characteristics and in some situations the acquisition of new phenotypes (e.g., neuroendocrine features). Plasticity is driven by intrinsic and/or acquired alterations in the biological activities of tumor cells and the tumor microenvironment that involve metabolic, genetic, and epigenetic changes. The consequences of these changes comprise a gene expression profile/phenotype consistent with AR/androgen independence and sustained proliferation.

#### Open questions

- Is loss of canonical AR signaling equivalent to AR independence? For instance, could the presence of AR in cases with low AR signaling still drive a noncanonical program to sustain tumor growth?
- How many lineages are there and how do we best recognize and define them?
- What degree of reversibility underlies lineage plasticity? Could targeting epigenetic alterations completely revert back toward a luminal state, and/or do genetic factors "fix" tumors in an alternate state?
- What are the mechanisms and mediators of tumor microenvironment regulation that underlie lineage plasticity—aging, senescence, inflammation, metabolism, oncogenic, and nononcogenic biological processes; and the cellular dynamics of immune cells, fibroblasts, adipocytes, endothelial, and neuronal cells that effect this process?

- How do lineage determining transcription factors interact or cooperate during prostate cancer progression?
- What extent do factors related to the metastatic environment (e.g., liver, bone) and/or oncogene-associated or therapy-induced senescence contribute to lineage plasticity?

## Gap 2: Determining the Temporal Contribution and Cooperation of Emerging Drivers

How AR-directed therapies affect clonal evolution, clonal selection, and the evolutionary bottlenecks that shape lineage plasticity is not well established. Mapping these processes have important clinical implications for biomarkers and therapeutics. For instance, hormone-naïve high-grade localized prostate cancers with low PSA levels may express neuroendocrine genes and harbor lower AR signaling (22), which may represent a high-risk patient population for developing lineage plasticity when later treated with AR pathway inhibitors. *RB1* loss, with or without *TP53* mutation or deletion, also occurs in a subset of prostate adenocarcinomas along the disease spectrum (23, 24), and may identify patients for aggressive surveillance and/or early intervention therapeutic strategies. The timing and cooperation of lineage-derived transcription factors further shape downstream programs as well as the epigenetic landscape. Whether intermittent AR inhibition, potentially alternating with therapies that target these alterations to induce differentiation, could delay the onset of lineage plasticity or prevent its emergence due to reconstitution of the tumor ecosystem with luminal lineage cells is intriguing and requires further study.

Understanding how mixed CRPC tumors that express both AR and neuroendocrine (or other) markers respond to AR therapies, and whether double-negative and small cell NEPC share treatment vulnerabilities such as sensitivity to platinum chemotherapy have important clinical implications for therapy selection. Assessment of the impact of emerging therapies (and combination approaches) on the prevention or reversal of phenotype or for their impact on tumor kill will help refine treatment goals; the readouts from targeting epigenetic alterations or alternative splicing, for instance, may differ from the goals of targeting proliferation or downstream effects of fixed genomic events. Serial metastatic biopsies or liquid biopsies will be useful for understanding the sequence of aberrations in patients and, when combined with clinical features such as PSA trends and sites of radiologic progression, have the potential to improve the diagnosis of patients progressing through the various biological disease states and to help refine clinical endpoints. One proposed translational research strategy for identifying the context and impact of *RB1* alterations is highlighted in Box 1.

#### Open questions

- What are the relationships between lineage drivers and oncogenic programs?
- Does the presence of *RB1* and/or *TP53* aberrations in prostate adenocarcinoma predispose patients toward developing lineage plasticity and small cell/neuroendocrine features?
- Is there an intermediate clinical state where intervention may be effective in preventing or reversing lineage plasticity?
- Do PSA dynamics adequately reflect AR signaling in patients?

**Box 1. One of the proposed translational research strategies developed at the workshop to address the role and cooperation of *RB1* and *TP53* in driving lineage plasticity in prostate cancer and other Rb1-deficient tumors (e.g., small cell lung cancer, SCLC) and an approach toward the development of novel biomarker-driven therapeutics.**

What are the vulnerabilities in NEPC versus SCLC and other RB-deficient cancers?

**Aim 1:** Develop a series of collaborating institutions with large clinical volumes of CRPC patients and clinical databases to determine clinical parameters associated with RB loss  $\pm$  TP53 loss and type of alteration (mutation; copy loss). Compare with other dual RB/p53 loss cancer types (SCLC).

**Aim 2:** Develop tissue and blood-based markers (IHC/ISH/transcriptional/proteomic/metabolic signatures) that detect/identify and associate with specific RB1  $\pm$  TP53 aberrations.

**Aim 3:** Develop isogenic models of RB  $\pm$ TP53 deficiency in prostate cancer backgrounds [cell lines, patient-derived xenograft (PDX), organoids] to assess: (i) molecular consequence for E2F and TP53 signaling, (ii) biological impact, and (iii) relevance for reflecting clinical observations as per Aim 1.

**Aim 4:** Utilize models developed in Aim 3 to screen for vulnerabilities/synthetic lethality using high-throughput strategies (e.g., CRISPR screening, FDA-approved compound screening) with the goal of establishing the foundation for the next phase of clinical testing

**Aim 5:** Refine strategies for accurately assessing RB and TP53 status in liquid biopsy, and develop longitudinal studies to determine the impact on progression from prostate cancer to CRPC to NEPC.

- How and when is the AR lost in the context of disease progression and how do specific therapeutics influence resistance pathways?
- Are there strategies to maintain cell differentiation and AR dependency while still restraining tumor growth?
- Do increased DNA damage response gene activities, or other specific transcriptional signatures contribute to the lineage plasticity program, and can these activities be exploited therapeutically?
- Are there synthetic lethal therapeutic approaches that may be exploited to target lineage plasticity and AR-independent prostate cancer?
- What degree of intratumoral heterogeneity is seen within and across metastases in individual patients?

**Gap 3: Preclinical Models That Recapitulate Biology of the Disease and the Recognized Phenotypes**

Preclinical models that represent the prostate cancer disease spectrum are essential for understanding biology and for the

**Box 2: Glossary**

*AR signaling (canonical):* androgen receptor signaling that occurs through ligand (androgen)-mediated or ligand-independent means, resulting in activation of downstream programs critical for prostate growth, development, and function

*AR signaling independent or AR indifferent:* sustained growth of prostate tumor cells that is not driven by or reliant on downstream canonical AR signaling

*Cellular identity or differentiation:* a means of classifying cells based on their phenotype or physiologic function

*Cellular determination:* the process by which a cell becomes specialized to perform a specific function

*Clonality:* cells that share a common ancestry

*Differentiation:* the process by which a stem cell or progenitor cell matures into a cell with a specific identity. Dedifferentiation is when a differentiated cell loses its matured cellular identity to become less mature and more stem-like

*Double-negative prostate cancer:* a subset of castration-resistant prostate cancer that does not express the AR and also does not express neuroendocrine markers

*Epigenome/epigenetics:* changes in gene expression and cellular phenotype caused by mechanisms other than changes in DNA

*Epithelial–Mesenchymal Transition:* a process by which epithelial cells lose their cell–cell adhesion and gain migratory and invasive properties to become more like mesenchymal cells, which may facilitate cancer growth and metastatic spread

*Embryonic stem cells:* pluripotent stem cells that have the potential to differentiate into multiple cell types and are capable of self-renewal

*Lineage determining transcription factors:* proteins that bind to DNA, either alone or in cooperation with other partners, to control transcriptional output of a cell type to regulate its phenotype and functional characteristics

*Lineage reprogramming:* the conversion of a mature, terminally differentiated cell type into another mature cell type with or without undergoing dedifferentiation

*Lineage tracing:* A method that delineates all progeny produced by a single cell or a group of cells

*Multipotent:* Potential of a cell to form different lineages

*Plasticity:* Ability of a cell to convert from one cell type to another. This may refer to the potential of a differentiated cell to dedifferentiate and then redifferentiate into a new state

*Progenitor:* ancestor cell that gives rise to a specific type of differentiated cell

*Neuroendocrine:* cells that are neural and endocrine in structure or function. In the pathologic classification of neoplasms, this designation is typically based on tumor morphology rather than function. Well-differentiated neuroendocrine tumors (e.g., carcinoids) and poorly differentiated neoplasms (e.g., small cell carcinoma) have distinct biology and may arise in various anatomic sites

*Pluripotent:* A cell that is capable of developing into any cell type

*Small cell carcinoma:* morphologic definition based on pathologic review of tumor hematoxylin and eosin–stained slides demonstrating small blue cells, high-grade features, scant cytoplasm, and distinct nuclear features (i.e., fine chromatin,

lacking prominent nucleoli). Protein expression of classical neuroendocrine markers such as chromogranin or NSE may be present but is not required

*Stem-like*: cells that possess characteristics similar to normal stem cells, such as the ability to give rise to other cell types within a tumor

*Transdifferentiation*: reprogramming of a differentiated cell of one lineage into a differentiated cell of another lineage

development of novel therapeutics. Currently, the number and types of cell lines that recapitulate lineage plasticity are limited, though there are PDX and organoid models of AR-negative and neuroendocrine prostate cancer that have been described and characterized (refs. 25–29; Table 1). Dynamic *in vivo* models, such as a PDX model that changes phenotype from an AR-positive adenocarcinoma to an AR-negative NEPC (30), and xenografts that lose AR signaling dependence during enzalutamide resistance (17) have been used to capture the lineage plasticity process. Genetically engineered mouse models such as the TRAMP model and others designed to alter key molecular events, including loss

**Table 1.** Preclinical models

Model	Source	Pathologic and molecular features
NCI-H660 cell line	Lymph node metastasis	Small cell carcinoma; AR-negative; PSA-negative; TMPRSS2-ERG gene fusion-positive; synaptophysin, CD56, NSE-positive
LuCAP 49, 93, 145.1, 145.2 patient derived xenograft (PDX) and PDX-organoid models	Omental metastasis (LuCAP 49), TURP (LuCAP 93), liver (LuCAP 145.1), lymph node (LuCAP 145.2)	Neuroendocrine histology; AR-negative; synaptophysin-positive
MDA PCa 144-4, 144-13, 155-2, MDA PCa 177-0, MDA PCa 189-1 PDX models	Salvage pelvic exenteration (MDA 144, MDA 155-2), prostate (MDA 177, MDA 189-1)	Small cell and large cell carcinoma; Aggressive variant clinical features; TMPRSS2-ERG gene fusion positive (MDA 144)
LTL352 and LTL370 PDX models	Urethral (LTL352) and penile (LTL370) metastasis	Small cell carcinoma; AR-negative; PSA-negative; synaptophysin, NSE-positive
LTL331 transdifferentiation PDX model	Primary prostate cancer (LTL331) PDX model that develops castration-resistant NEPC <i>in vivo</i> (LTL331R)	Primary high-grade adenocarcinoma (LTL331) and neuroendocrine prostate cancer histology (LTL331R); TMPRSS2-ERG fusion-positive; LTL331: AR-positive; LTL331R: AR-negative, PSA-negative, Synaptophysin-positive, chromogranin-positive, CD56-positive
Enzalutamide-resistant LNCaP (42D, 42F)	Cell lines developed from enzalutamide-resistant LNCaP xenograft tumors	AR-positive; PSA low; chromogranin, synaptophysin-positive
MSK-PCA4 Patient-Derived Organoids	Pleural effusion	Neuroendocrine features; AR low; Synaptophysin-positive
WCM Patient-Derived Organoids	Metastatic lesions liver (155), bone (154), lymph node (1,078), soft tissue (1,262)	Small cell carcinoma; AR-negative; PSA-negative; synaptophysin, NSE, chromogranin-positive
TRAMP mouse	C57BL/6 mice expressing the rat probasin driving expression of SV40 large and small T antigens in prostatic epithelial cells	Adenocarcinoma to small cell carcinoma; Rb1 and Tp53 loss; Visceral metastases
p53 <sup>PE-/-</sup> /Rb <sup>PE-/-</sup> mouse	Conditional knockout of p53 and Rb (p53 <sup>PE-/-</sup> ; Rb <sup>PE-/-</sup> ) from the epithelium of all lobes of the mouse prostate	Small cell carcinoma; Rb1 and Tp53 loss
N-Myc-myrAKT1 mouse	Human prostate basal cells overexpressing NMYC and AKT1 implanted subcutaneously in NOD-SCID-IL2R $\gamma$ null (NSG) mice	Neuroendocrine features; AR low; chromogranin, synaptophysin-positive
Pten <sup>f/f</sup> ;LSL-MYCN <sup>+/+</sup> mouse	GEMM mice carrying MYCN gene integrated into the ROSA26 (LSL-MYCN) locus, a Tmprss2-driven tamoxifen-activated Cre recombinase and a Pten conditional knockout allele	Divergent differentiation Neuroendocrine features; AR low/AR-negative; chromogranin, synaptophysin-positive; visceral metastases with castration
PBCre4:Pten <sup>f/f</sup> :Rb1 <sup>f/f</sup> (DKO), PBCre4:Ptenf/f:Rb1f/f:Trp53f/f (TKO)	GEMM mice, PBCre4 transgene is used to delete floxed alleles specifically in prostate epithelium	Neuroendocrine features with metastases; AR-positive/AR low; Synaptophysin-positive
Npp53 mice	GEMM mice, inducible Nkx3.1 <sup>CreERT2</sup> driver to delete PTEN and TP53 genes in adult prostate epithelium	Abiraterone-resistant; neuroendocrine features- transdifferentiation (lineage tracing); AR low/AR-negative; Synaptophysin-positive
Ptenf/f-Prkcif/f-PbCre4+ mice (DKO)	GEMM mice, Ptenf/f-PbCre4+ mouse line (PTEN KO) with PTEN specifically deleted in the prostate epithelium, crossed with Prkcif/f mice	Neuroendocrine features; AR low/AR-negative; chromogranin, synaptophysin-positive

NOTE: Table of preclinical models that display lineage plasticity, small cell /NEPC histologic or molecular features, and/or AR-signaling indifference.

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of *PTEN*, *TP53*, and *RB1*, and/or gain of *MYCN*, are also useful tools to study the cooperation and timing of emerging tumor suppressors and oncogenes (9, 10, 14, 20, 21, 31). Optimizing and sharing protocols for model development and optimization was discussed as an unmet need, especially as fresh tumor biopsies are more commonly performed clinically and may be used for patient-derived model generation.

#### Open questions

- What are the preclinical models that can be manipulated in such a way to reflect transition from androgen-dependent to -independent states and further transitions that encompass new differentiation programs such as neuroendocrine phenotypes?
- Are the genomics, transcriptomics, epigenetics, and metabolomics of these model systems representative of advanced human prostate cancer? Do they change over time or with conditions?
- Can we develop a series of paired PDX-organoids with morphologic and molecular information of organoids, PDXs, and human tumor of origin representing the complex molecular landscapes of prostate cancer?
- How do we use model systems to assess reversibility and thereby understand the degree of plasticity?
- What are "the human models" that can be utilized to compare and contrast with the animal models (e.g., rapid autopsies, CTCs, cfDNA, etc.)?

### Gaps 4 and 5: Identification of Therapeutic Targets and Novel Trial Designs Dedicated to the Entity as It Is Defined

Given the relatively high reported frequency of small cell NEPC post AR-directed therapy, the National Comprehensive Cancer Network (NCCN) guidelines now recommend consideration of metastatic biopsy for any CRPC patient to look for small cell transformation. If found, patients could be considered for platinum-based chemotherapy based on extrapolation of clinical data for small cell lung cancer and supported by recent platinum-chemotherapy studies in aggressive variant prostate cancer (32). Although these practice guidelines have recently changed, the diagnosis of lineage plasticity remains a clinical challenge due to a lack of standardized or widely accepted clinical or pathologic criteria. Although pure small cell carcinoma defined by morphology is most often congruent between pathologists, those with mixed/hybrid or with varied degrees of neuroendocrine differentiation are often subject to interobserver variability (33).

The diagnosis of other phenotypes may require more detailed studies that involve IHC or other measures of gene expression. There are no standard criteria for when to perform ancillary studies such as IHC for classical neuroendocrine markers (e.g., chromogranin, synaptophysin), AR protein, PSA, or other markers (34, 35). There are also times when AR is expressed in NEPC, but the canonical AR transcriptome (including downstream targets like PSA) is low; identification of downstream genes that may be activated by AR in this setting would be informative and potentially help refine definitions. The incidence of AR-negative or AR-low CRPC without NEPC features or alternative lineage CRPC and the degree of heterogeneity within this spec-

**Table 2.** Pathology morphology and available ancillary testing

Morphology
%tumor
%glandular
%small cell
Protein studies ( <b>bold</b> indicates existing CLIA-validated biomarkers)
Molecular/Cell cycle
• <b>Rb</b> (Rb function: cyclin D1/p16)
• <b>P53</b> (p53 function: p21)
• <b>Ki-67</b> (discovery for threshold)
Lineage markers
• <b>Chg</b> (Canonical)
• <b>Syp</b> (Canonical)
• <b>CD56</b>
• <b>FOXA2</b>
• <b>INSM1</b>
• ASCL
• <b>Cytokeratin—Cam5.2</b>
• <b>Neurofilament</b>
Androgen signaling
• <b>AR</b> (canonical)
• AR-v7
• <b>PSA</b>

NOTE: Prioritization of pathology assessment when lineage plasticity is suspected. Tissue should be evaluated for morphologic characteristics that may support small cell carcinoma, neuroendocrine differentiation, or other histologies. Possible ancillary protein studies to consider performing by IHC are listed, with markers in **bold** indicating existing Clinical Laboratory Improvement Amendments (CLIA) grade tests.

trum are also not known. The workshop discussed a path toward the standardization of tumor morphology nomenclature and systematic prioritization of ancillary testing in metastatic CRPC (Table 2).

Given the current challenges of performing tumor biopsies and the variability in morphologic features that occur after therapy, platinum-based chemotherapy trials have been conducted for patient with aggressive clinical features even in the absence of tumor biopsy (32). Notably, these patients defined by clinical features suggestive of AR independence often harbor combined somatic tumor alterations involving *PTEN*, *TP53*, and/or *RB1*, similar to what has been observed in small cell NEPC (29).

With a loss of AR expression, tumors may also lose other related proteins including prostate-specific membrane antigen (PSMA). This manifests clinically as PSMA-negative lesions on PSMA PET imaging. Low or heterogeneous PSMA PET-CT combined with FDG positivity identifies patients with poor prognosis (36), therefore representing a potential noninvasive means to identify AR-independent resistance. However, there are still many unknowns regarding PSMA regulation and PSMA imaging characteristics along prostate cancer progression and therapy resistance. Multi-institutional collaborative studies combining PSMA with FDG PET imaging, in combination with tumor biopsies of discordant lesions and liquid/tissue molecular assessment, were designed at the workshop.

There is no known effective next-line therapy for patients with small cell NEPC especially after platinum chemotherapy. Although second-line SCLC regimens may be considered, their data in prostate cancer are scarce. Notably, although loss of AR activity eliminates the AR pathway as a therapeutic target, the acquisition of new characteristics that associate with NEPC and other phenotypes exposes new targets and vulnerabilities. Available drugs targeting the AURKA/MYCN or AURK/RB1 axis, LSD1,

EZH2, DLL3, and approaches to target the immune landscape (e.g., vaccine, TGF $\beta$ i, IL8i, and immune checkpoint inhibitors) are in development for NEPC, as well as drugs targeting FGF/MAPK for double-negative CRPC. The design of rational combination or cotargeting strategies, such as EZH2 inhibition in combination with AR pathway inhibitors or immunotherapy, may also have value. Ultimately, defining the appropriate inclusion criteria and endpoints for trials focused on lineage plasticity will be critical. Trials will require multicenter collaboration and molecularly based biomarker inclusion and careful patient selection. Incorporation of emerging molecular biomarkers in combination with clinical features may help distinguish subcategories of AR-independent CRPC and inform the development of liquid and imaging biomarker approaches for clinical trial design.

### Open questions

- What are the clinical and pathologic differences between small cell carcinoma and CRPC with neuroendocrine differentiation? Are IHC markers required and do they capture AR activity?
- Is the increased detection of small cell/NEPC due to increased awareness or is this due to more potent AR-directed therapies? Will the incidence of NEPC increase with recent approvals of potent AR-targeted drugs earlier in the disease (i.e., metastatic castrate-sensitive prostate cancer and nonmetastatic castration-resistant prostate cancer)?
- How well does loss of PSMA or PSMA heterogeneity on imaging in combination with FDG PET-CT noninvasively identify patients developing lineage plasticity? What other radiologic or radiomic (i.e., subvisual tools) may be applied for the early detection of lineage plasticity?
- Platinum sensitivity is also mediated by germline or somatic alterations involving DNA repair genes, which occurs in approximately 20% of CRPC independent of histology. What is the degree of overlap between DNA repair deficiency and lineage plasticity?
- Is reversal of phenotype or tumor kill the primary goal of therapy? What clinical endpoints may be used to assess these outcomes?
- What would a multiarm clinical trial design look like for patients developing lineage plasticity and AR-independent prostate cancer? Which clinical or molecular features are ready to use as inclusion criteria?

### Lessons from Other Cancer Types (the Scope of the Problem)

Similar to prostate cancer, malignancies arising from other anatomic sites, tissues, and cell types also develop lineage plasticity as a mechanism of therapy resistance. For instance, 5% to 15% of *EGFR*-mutant lung adenocarcinomas transform to SCLC histology during acquired resistance to *EGFR*-targeted therapies with retention of the original *EGFR* mutation (37). Cases of small cell transformation in lung cancer have also been reported after immunotherapy (38). *RB1* and *TP53* genomic alterations are universally present in both *de novo* and transformed SCLC and rarely present in unselected lung adenocarcinomas other than those that later develop histologic transdifferentiation (39–41). Whether preexisting *RB1* and *TP53* loss may be used as biomar-

kers to identify high-risk lung adenocarcinoma patients for alternative treatments is yet to be determined. Understanding whether this predisposition for lineage plasticity is also the case for the subset of castration-resistant prostate adenocarcinomas and localized prostate cancers that harbor *TP53/RB1* loss has important implications for the early detection and management. In melanoma, phenotypic switching with distinct cellular populations (e.g., invasive vs. proliferative) and developmental cell states (i.e., MITF low or high) frequently coexisting and dynamically regulated plays a central role in metastasis and therapy resistance (42–44). This switching is hypothesized to be largely regulated by epigenetic modifiers, hypoxia, and the tumor microenvironment, but specific factors remain to be fully elucidated. Similar to prostate cancer, much is still to be learned in melanoma plasticity regarding the number and types of phenotypic states that coexist and their interface with the genetics of the tumor (i.e., do some genomic lesions make the cells more or less responsive to plasticity signals?). In pancreatic ductal adenocarcinoma, lineage heterogeneity associates with poor outcomes; lineage tracing studies have pointed to an acinar cell of origin of neuroendocrine cells, and this is regulated by *MYC* and epigenomic changes in response to environmental signals (45). A subset of breast cancers also develop lineage plasticity, and in some cases, the luminal phenotype and/or estrogen receptor expression are lost with disease progression; this process may be mediated in part by subpopulations of cells with stem-like properties (46). Given these disease parallels, emerging mechanisms underlying plasticity, stemness, and cellular reprogramming during therapy resistance are pointing to shared mechanisms and potential targets across malignancies.

### Summary

It is increasingly recognized that a subset of prostate cancers evades AR-targeted therapies through the development of lineage plasticity. This is associated with loss of AR or AR signaling, frequent *RB1/TP53* loss of function, and activation of alternative lineage programs including neuronal, neuroendocrine, stem-like, and developmental pathways. In some instances, lineage plasticity occurs directly through transdifferentiation processes, whereas in other situations, tumor cells dedifferentiate to a stem-like state followed by reprogramming to a new phenotype. Continued tumor evolution under treatment pressures may occur through clonal selection. Although there are many open questions, recent studies have identified newly relevant biological pathways and actionable targets. Addressing the outlined gaps in knowledge will ultimately accelerate the translation of new biologic discoveries into the clinic.

### Disclosure of Potential Conflicts of Interest

H. Beltran is a consultant/advisory board member for Janssen, Sanofi Genzyme, and Astellas. H.I. Scher is a consultant/advisory board member for Amgen and Janssen. E.Y. Yu is a consultant/advisory board member for Amgen, Dendreon, Bayer, Merck, AstraZeneca, QED, Incyte, Tolmar, EMD Serono, Pharmaclics, Seattle Genetics, and Janssen. A. Dicker holds ownership interest (including patents) in Oncohost, is a consultant/advisory board member for Roche, Oncohost, Google, Dreamit Ventures, EMD Serono, Janssen, Cybrexa, and Self Care Catalysts, and reports receiving other remuneration from Wilson Soncini. R. White is a consultant/advisory board member for N-of-One, Inc. E.J. Small holds ownership interest (including patents) in Fortis Therapeutics and Harpoon Therapeutics, and is a consultant/advisory board member for Janssen, Fortis Therapeutics, Tolero Pharmaceuticals, and Beigene Therapeutics. T. Lotan

reports receiving other commercial research support from GenomeDx and Roche, and is a consultant/advisory board member for Janssen. C.L. Sawyers holds ownership interest (including patents) in Novartis, Enzalutamide, and Apalutamide, and is a consultant/advisory board member for Novartis, Agios, Beigene, Blueprint, Column Group, Nextech, Housey Pharma, Foghorn, KSQ, PMV, ORIC, and Petra. C.M. Rudin reports receiving commercial research grants from Daiichi Sankyo, and is a consultant/advisory board member for AbbVie, Amgen, Ascentage, Astra Zeneca, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Genentech/Roche, Ipsen, Loxo, Pharmamar, Vavotek, Bridge Medicines, and Harpoon Therapeutics. M.A. Rubin reports receiving commercial research grants from Janssen and Eli Lilly, speakers bureau honoraria from Roche and Novartis, and holds ownership interest (including patents) in the field of NEPC. P.S. Nelson is a consultant/advisory board member for Janssen, Astellas, and Roche. No potential conflicts of interest were disclosed by the other authors.

## Supplementary Information

Pre-meeting document with Working Group summaries and questions

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## Acknowledgments

This article represents a consensus perspective arising from the NCI Workshop on Lineage Plasticity and Androgen Receptor-Independent Prostate Cancer (held December 2018), a joint effort by the NCI's Division of Cancer Treatment and Diagnosis, Division of Cancer Biology, Center for Cancer Research, and Coordinating Center for Clinical Trials together with investigators of the NCI SPOEs in Prostate Cancer and the Prostate Cancer Task Force of the NCI Genitourinary Steering Committee. It was cochaired by H. Beltran, W. Dahut, and P.S. Nelson. The goal was to assemble leaders in the field to share scientific insights and identify emerging opportunities in basic, translational, and clinical research. We thank Tamara Walton with her help in the planning and coordination of the workshop. This perspective is dedicated to Dr. Andrew Hruszkewycz, recently deceased, whose scientific expertise, dedication, and genuine passion were instrumental in the success of this workshop.

Received May 7, 2019; revised June 7, 2019; accepted July 25, 2019; published first July 30, 2019.

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