



# Androgen-targeted therapy-induced epithelial mesenchymal plasticity and neuroendocrine transdifferentiation in prostate cancer: an opportunity for intervention

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Androgens regulate biological pathways to promote proliferation, differentiation, and survival of benign and malignant prostate tissue. Androgen receptor (AR) targeted therapies exploit this dependence and are used in advanced prostate cancer to control disease progression. Contemporary treatment regimens involve sequential use of inhibitors of androgen synthesis or AR function. Although targeting the androgen axis has clear therapeutic benefit, its effectiveness is temporary, as prostate tumor cells adapt to survive and grow. The removal of androgens (androgen deprivation) has been shown to activate both epithelial-to-mesenchymal transition (EMT) and neuroendocrine transdifferentiation (NEtD) programs. EMT has established roles in promoting biological phenotypes associated with tumor progression (migration/invasion, tumor cell survival, cancer stem cell-like properties, resistance to radiation and chemotherapy) in multiple human cancer types. NEtD in prostate cancer is associated with resistance to therapy, visceral metastasis, and aggressive disease. Thus, activation of these programs via inhibition of the androgen axis provides a mechanism by which tumor cells can adapt to promote disease recurrence and progression. Brachyury, Axl, MEK, and Aurora kinase A are molecular drivers of these programs, and inhibitors are currently in clinical trials to determine therapeutic applications. Understanding tumor cell plasticity will be important in further defining the rational use of androgen-targeted therapies clinically and provides an opportunity for intervention to prolong survival of men with metastatic prostate cancer.

**Keywords: prostate cancer, epithelial-to-mesenchymal transition, neuroendocrine, androgen deprivation therapy, castrate resistant, tumor cell plasticity, brachyury, Axl**

## INTRODUCTION

Prostate cancer is the most prevalent malignancy in men, and ranks second as the cause of cancer-related deaths in the developed world (1, 2). Advanced prostate cancer is initially treated with androgen deprivation therapy (ADT) and subsequently with newer generation androgen-targeted therapies (ATT), approaches which rely on the central role of androgens in tumor development and growth. In the majority of patients, castrate resistant prostate cancer (CRPC) develops and tumor progression occurs despite treatment. The development of agents that more effectively block androgen receptor (AR) activity, such as enzalutamide and abiraterone, has greatly enhanced the clinical armamentarium and extended survival (3–6). Nonetheless, advanced prostate cancer remains incurable. Tumor cell plasticity induced by androgen

deprivation may play a critical role in disease progression, and potentially provides an additional opportunity to further improve cancer control.

## PROGRESSION TO CASTRATE RESISTANCE

While the exact mechanisms underlying the development of CRPC are not yet known, it arises when cancer cells can either maintain AR signaling in the absence of normal levels of ligand or no longer require activation of this pathway for survival and proliferation. There are a number of mechanisms that can produce this outcome, including altered functionality of the AR due to genomic events, resulting in either promiscuous (7, 8), constitutively activated (9, 10), or hypersensitive (11, 12) states; intraprostatic production of androgens by tumor cells themselves (13); and

altered growth factor and/or microenvironment signaling (14–18). Despite the development of multiple strategies that effectively target the androgen axis, disease progression is inevitable. This is underpinned by the accumulation of further genomic abnormalities, outgrowth of different clonal populations of tumor cells, and the adaptive response of cancer cells to therapy. In this review, we focus on adaptive changes induced by therapy, specifically epithelial-to-mesenchymal plasticity (EMP) and neuroendocrine transdifferentiation (NEtD), which may contribute to the development of advanced disease (Figure 1). A better understanding of these processes will contribute to the development of new therapeutic strategies that may potentially enhance the efficacy of androgen-targeted agents and delay disease progression.

### EPITHELIAL-TO-MESENCHYMAL PLASTICITY

Epithelial-to-mesenchymal transition (EMT) is a process by which adherent, polar cells with an otherwise epithelial phenotype develop more migratory and invasive properties through altered gene expression (19–23). Both EMT and the related process mesenchymal-to-epithelial transition are physiological mechanisms important in development and tissue repair. However, when differentiated epithelium begins to display mesenchymal characteristics it is often a sign of disease progression in cancers (19, 24–27). EMT is commonly characterized by the loss of epithelial markers (typically E-cadherin, epithelial cytokeratins, and desmosomes), and gain of mesenchymal markers (such as N-cadherin, vimentin, and fibronectin) and transcriptional repressors of E-cadherin (Twist1, Snai1, Snai2, Zeb1, Zeb2) (20, 21). EMT has been associated with advanced prostate cancer, and correlated with aggressive behavior and therapy resistance in primary tumors (17, 28–30).

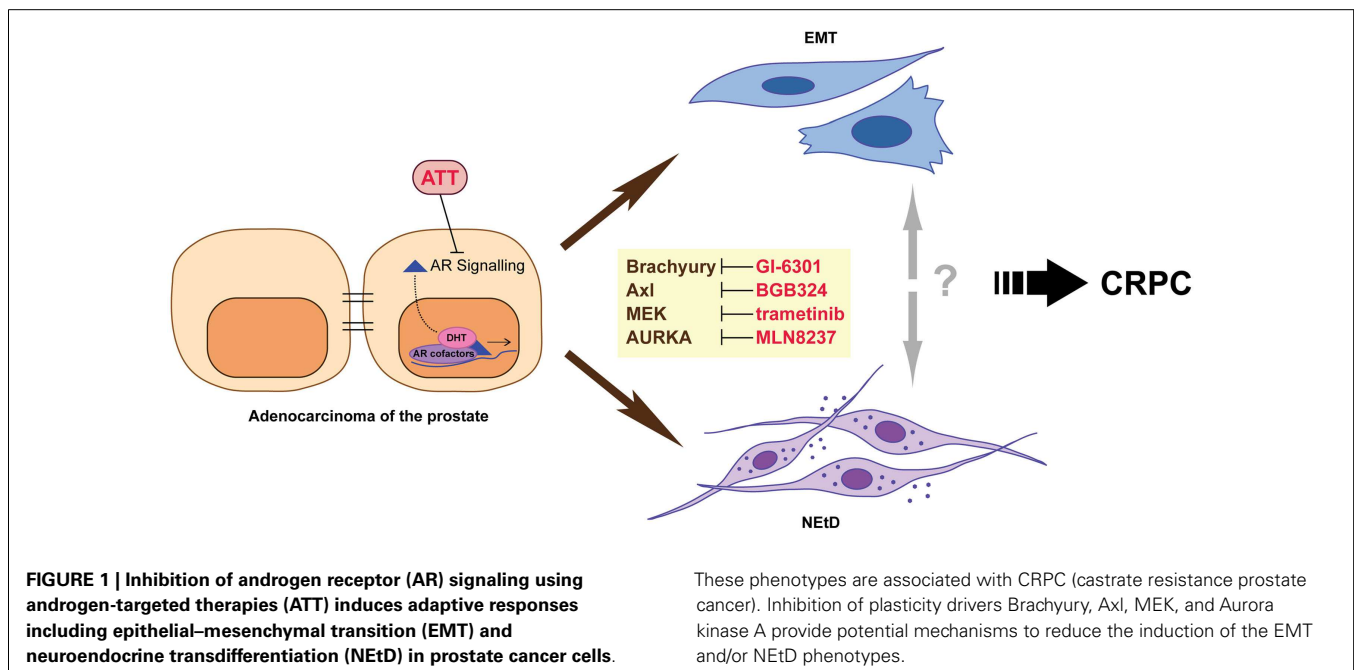
### NEUROENDOCRINE TRANSDIFFERENTIATION

While men may present with prostate cancer demonstrating various neuroendocrine features (31), the prevalence of

neuroendocrine differentiation increases following ADT and in CRPC (32–37). These cells not only express neuropeptides, reminiscent of the normal NE cells of the prostate, but also proteins that are characteristic of prostate epithelial cells [such as prostatic acid phosphatase cytokeratin 8/18 and/or epithelial adhesion molecules and proliferation markers (38, 39)], while AR expression is typically absent or low (40). Importantly, the number of NE-like prostate cancer cells is positively associated with the duration of hormone deprivation therapy (32–34). There are several hypotheses for the origin of NE-like prostate cancer cells. It has been postulated that NE-like cancer cells can arise during disease progression from NE cells of the prostate (41). However, the observation that genetic aberrations are common to both the adenocarcinoma and NE-like cells (42–45) suggests that this is not likely to be a common mechanism. An alternative explanation is that a common progenitor prostate cancer stem cell gives rise to both the NE-like and adenocarcinoma components and both these components continue to evolve and respond to selective pressures in parallel (42, 44, 46, 47). In contrast, NEtD is a process that can enable prostatic adenocarcinoma cells to gain NE characteristics without relying on genetic divergence. NEtD can occur after prolonged androgen deprivation, and has recently been reported in a patient derived xenograft (48). This mechanism would potentially enable tumor cells to reduce ATT-induced apoptosis and thus provide an adaptive pathway that would contribute to the development of CRPC (41).

### ANDROGENS SUPPRESS NEUROENDOCRINE TRANSDIFFERENTIATION

Evidence of NEtD has been observed in both *in vitro* and *in vivo* studies. LNCaP cells, an androgen dependent prostate cancer cell line derived from a lymph node metastasis, undergo NEtD when exposed to media lacking androgens (39, 49–51). In low-androgen conditions, LNCaP cells take on an altered elongated neuron-like



phenotype, gain cytoplasmic secretory granules, and undergo growth arrest. This is accompanied by an increase in expression of NE markers and a decrease in AR and PSA levels. This transdifferentiation is reversible with the addition of androgens (DHT) to the media, an observation consistent with the identical allelic profiles of NETd LNCaP and parental LNCaP cells. Silencing of the AR using siRNA also induces NETd in LNCaP cells, suggesting that AR signaling suppresses NETd (52). *In vivo* studies also provide support for the NETd model. Castration of nude mice bearing prostate cancer xenografts LNCaP, PC-295, CWR22, and PC-310 increased the number of tumor cells expressing NE markers, consistent with induction of NETd (53–56). Furthermore, implantation of primary patient tumor tissues from a population of adenocarcinoma cells implanted under the renal capsule of castrated mice appear to undergo an NETd en masse as an adaptive response (48).

### Therapy-Induced EMT

There is accumulating evidence supporting that ADT may induce an EMT, and that this is particularly prominent with the newer generation ATT. ADT has been associated with an increase in the expression of mesenchymal markers N-cadherin, vimentin, Zeb1, Twist1, and Snai2, with a concomitant loss of E-cadherin in patient derived xenografts and clinical prostate tumors (17, 57–59). Traditionally, investigations have primarily focused on the effects of targeting AR signaling in prostate cancer cells; however, ADT/ATT is not specific to tumor cells alone. Recent reports demonstrate significant effects of ADT/ATT on the tumor microenvironment, including stromal and immune cells (15, 18, 60). For instance, elevated numbers of tumor associated macrophages have been reported in men undergoing ADT (60), and these cells have been shown to promote local invasion and metastatic dissemination of tumor cells in response to ADT (18, 57–60). Hence, the implications of targeting the androgen axis and its effect on the multiple cell types comprising the tumor microenvironment needs to be assessed when considering therapeutic interventions.

### Therapy-Induced EMT and Neuroendocrine Transdifferentiation as Clinical Targets

Therapeutically targeting regulators of EMP/NETd is an attractive concept that has recently matured to clinical trials (Figure 1). Brachyury is a transcription factor required for the developmental EMT that generates mesoderm by converting epithelial cells into migratory mesenchymal cells (61). In tumor cells, including prostate cancer, Brachyury also induces EMT and an invasive phenotype (62–65). Furthermore, Brachyury is overexpressed at both the transcript and protein level in clinical prostate cancer specimens, and nuclear expression is associated with metastasis (66). While the regulation of Brachyury by androgen-targeted therapies has not been addressed, Brachyury motifs were highly enriched in AR bound promoters when LAPC-4 cells were grown in the presence of AR antagonist flutamide (67). Furthermore, *in silico* bioinformatic analysis using transcriptional profiles from clinical prostate cancer specimens and clustering Brachyury co-expressed genes by functional role/signaling pathways demonstrated an enrichment for regulation of neuron differentiation and nervous system development (68). An inverse relationship

between Brachyury and E-cadherin expression, with a concomitant positive correlation of Brachyury with EMT promoting genes FN1, Snai1, IL8, and TGF- $\beta$  was also observed. Thus, we hypothesize that targeting Brachyury in the context of ATT may modulate the emergence of both a neuroendocrine phenotype and EMP by preventing, for example, the induction of Brachyury mediated release of migration/invasion promoting soluble factors into the tumor microenvironment (62, 68, 69). GI-6301 (Tarmogen) is a Brachyury vaccine (70) currently in Phase I clinical trial in patients with metastatic or unresectable locally recurrent cancers who have failed previous therapy or have no further therapeutic options (NCT01519817). Recent assessment of data from patients with advanced chordoma in this trial demonstrated safety and a confirmed partial response (71), and data from the larger cohort are eagerly awaited.

The receptor tyrosine kinase Axl is implicated in the Snai1-, Snai2-, IL6-, and STAT3-mediated activation of EMT (72, 73) as well as the metastasis promoting AKT/NF- $\kappa$ B and AKT/Snai2 pathways (73, 74) in multiple cancer types. Targeting Axl has shown promise in preclinical models of cancer progression (75–77), and clinical trials are currently underway. BGB324 is a small molecule inhibitor of the Axl receptor tyrosine kinase developed to block EMT with the goal of inhibiting drug-resistance and metastasis. Recent Phase Ia data have demonstrated BGB324 to be safe and well tolerated, and Phase Ib studies commenced in non-small cell lung cancer and acute myeloid leukemia in 2014. Cabozantinib is another tyrosine kinase inhibitor targeting Axl, as well as EMT promoting kinases VEGFR2, RET, KIT, FLT-1/3/4, c-MET, and Tie-2 (78–80). Clinically significant regression of metastatic tumors in CRPC patients was achieved with cabozantinib treatment in a Phase II trial (81). Of course the precise molecular mechanism underpinning this efficacy is not clear and likely involves inhibition of multiple tyrosine kinases in several cell types. Trials investigating whether cabozantinib is a useful addition to ADT in the control of prostate cancer are currently underway (NCT01630590).

MEK inhibitors may also be useful in managing therapy-induced EMP/NETd. *In vitro*, MEK inhibitor PD98059 blocked the acquisition of NE-like morphology and prevented the increase in NSE levels usually observed in LNCaP-C33 cells induced to undergo NETd by androgen-depletion (82). Ectopic expression of constitutively active AR in LNCaP cells inhibited RAF/MEK/ERK-induced NSE expression (83), demonstrating the central regulatory role of AR in constraining the emergence of this phenotype. Furthermore, the RAF/MEK/ERK pathway has been shown to be necessary for the induction of Twist1, Snai1, and N-cadherin in multiple cancer models (84, 85). A neoadjuvant trial examining the effect of short-term MEK inhibition (trametinib) prior to radical prostatectomy in the context of ADT on markers of EMT (N-cadherin, vimentin) has recently commenced (NCT01990196).

Finally, Aurora kinase A (AURKA) inhibitors may also be effective in inhibiting ATT-induced EMP/NETd as they suppress both EMT and NETd *in vitro* and *in vivo* (86, 87). In cancer cells, AURKA has been demonstrated to play an important role in the genesis of a more mesenchymal phenotype via down-regulation of E-cadherin and up-regulation of vimentin (88). Clinical trials

examining the role of the inhibitors in prostate cancer are currently ongoing (NCT01799278, NCT01094288).

Despite independent lines of evidence implicating key factors in both EMT and NETD, the functional and molecular relationship between these states in prostate cancer has not been extensively explored. McKeithen et al. (89) have demonstrated that the well-established EMT-inducing transcription factor Snai1 induced both EMT and NETD in LNCaP cells as defined by morphology and marker expression. However, as the data are mostly presented as analyses of bulk populations of cells, it is not possible to determine whether EMT and NETD phenotypes are co-expressed within individual cells, and are thus intimately linked, or whether these transdifferentiation processes are independent of each other and become activated by influences such as neighboring cells, local microenvironmental cues, or cell intrinsic factors.

### CONCLUDING REMARKS

Multiple factors are clearly involved in the progression to CRPC during treatment with ATT. Studies over the past two decades have associated blockade of the androgen axis with the increased prevalence of neuroendocrine prostate cancer. These observations, in combination with recent reports of androgen deprivation modulating EMP, suggest novel strategies for therapeutic intervention. Further studies will be required to determine whether these adaptive response pathways have a functional role in the progression to CRPC or are simply a consequence of removing the differentiation pressure imposed by active androgen signaling on prostate cells. Moreover, revealing if and how these plasticity pathways intersect in the androgen-targeted environment will be an intriguing area for future research. Improved understanding of the molecular pathways underlying the adaptive responses to ATT provides opportunities to investigate whether targeted inhibition of these pathways will delay tumor progression and thus improve outcomes for men with prostate cancer.

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