



## Review Article

# Current advances of targeting epigenetic modifications in neuroendocrine prostate cancer

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Submission : 17-Aug-2020  
Revision : 05-Sep-2020  
Acceptance : 06-Oct-2020  
Web Publication : 24-Feb-2021

### ABSTRACT

Neuroendocrine prostate cancer (NEPC) is the most lethal malignancy of prostate cancer (PCa). Treatment with next-generation androgen receptor (AR) pathway inhibitors (ARPIs) has successfully extended patients' lifespan. However, with the emergence of drug resistance, PCa tumors increasingly adapt to potent ARPI therapies by transitioning to alternative cellular lineage. Such therapy-induced drug resistance is largely driven from the cellular plasticity of PCa cells to alter their phenotypes of AR independence for cell growth and survival. Some of the resistant PCa cells undergo cellular reprogramming to form neuroendocrine phenotypes. Recent evidences suggest that this cellular reprogramming or the lineage plasticity is driven by dysregulation of the epigenome and transcriptional networks. Aberrant DNA methylation and altered expression of epigenetic modifiers, such as enhancer of zeste-homolog 2, transcription factors, histone demethylases, are hallmarks of NEPC. In this review, we discuss the nature of the epigenetic and transcriptional landscapes of PCa cells which lose their AR independence and transition to the neuroendocrine lineage. We also discuss how oncogenic signaling and metabolic reprogramming fuel epigenetic and transcriptional alterations. In addition, the current state of epigenetic therapies for NEPC is addressed.

**KEYWORDS:** *Androgen receptor, Enhancer of zeste-homolog 2, Epigenetic, Neuroendocrine prostate cancer*

## INTRODUCTION

Prostate cancer (PCa) is the most common cancer in males and the second leading cause of cancer-related lethality worldwide [1]. PCa as diagnosed in the first place is often an androgen-driven disease which depends on the androgen receptor (AR)-mediated signaling for tumor growth [2]. Accordingly, treatments for the AR-sensitive PCa by androgen deprivation therapy (ADT) effectively reduce tumor growth [3]. Although nearly all PCa patients respond well to ADT, some PCa cells can eventually evade ADT and restore AR signaling even in the absence of androgen, reaching a state referred to as castration-resistant PCa (CRPC) and, consequently, about 20%–25% of patients will develop to the state of metastatic CRPC (mCRPC) [4]. These tumors are still relying on AR signaling for survival, therefore treatment with next-generation AR pathway inhibitors (ARPIs) such as abiraterone and enzalutamide has significantly improved patient's survival [5,6]. Mechanistically, abiraterone and enzalutamide can blunt AR signaling, respectively, by blocking *de novo* androgen biosynthesis and competitively binding to the AR. Accumulating evidences demonstrate that prolonged treatment of CRPC or mCRPC patients with ARPIs develops therapeutic

resistance. Typically, therapy resistance of the antiandrogens is mainly due to re-activation of AR by different mechanisms including genomic mutation, gene amplification, or rearrangement of the AR gene [7]. In a small proportion of patients, however, drug resistance is emerging and accompanied by a loss of AR function and its downstream signaling, leading to histological alterations that associate with the formation of epithelial–mesenchymal transition (EMT) or small-cell neuroendocrine carcinoma characteristics [8-10], which refers to neuroendocrine PCa (NEPC). NEPC has been characterized by the presence of a combination of neuroendocrine biomarkers and genomic and epigenetic features [Table 1].

In this review, we discuss the key concepts of how epigenetic and transcriptional dysregulation as a driver mechanism of cellular plasticity reprograms PCa cells to the neuroendocrine lineage (NEPC formation). We also highlight oncogenic signaling and metabolic changes which drive

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<b>Quick Response Code:</b> 	<b>Website:</b> <a href="http://www.tcmjmed.com">www.tcmjmed.com</a>
	<b>DOI:</b> 10.4103/tcmj.tcmj_220_20

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**How to cite this article:** Cheng WC, Wang HJ. Current advances of targeting epigenetic modifications in neuroendocrine prostate cancer. *Tzu Chi Med J* 2021;33:224-32.

**Table 1: List of proposed biomarkers associated with neuroendocrine prostate cancer**

Gene symbol	Gene name	Regulation*	Reference
<i>CHGA/CHGB</i>	Chromogranin A and B	+	[11]
<i>NSE/ENO2</i>	Neuron-specific enolase	+	
<i>SYP</i>	Synaptophysin synaptic vesicle protein p38	+	[11]
<i>AURKA</i>	Aurora kinase A	+	[11,12]
<i>N-MYC</i>	Neuroblastoma-derived Myc	+	[13]
<i>EZH2</i>	Enhancer of Zeste 2 (polycomb repressive complex 2 subunit)	+	[12,14]
<i>CALC1</i>	Calcitonin	+	[15]
<i>FOXA2</i>	Forkhead box A2	+	[16,17]
<i>SRRM4</i>	Serine/arginine repetitive matrix 4	+	[18]
<i>POU3F2/BRN2</i>	POU class 3 homeobox 2	+	[19]
<i>SOX2 and SOX11</i>	Sex-determining region Y-box 2 and 11	+	[19-21]
<i>TMPRSS2-ERG</i>	TMPRSS2-ERG gene rearrangement	Gene fusion	[22,23]
<i>PEG10</i>	Paternally expressed10	+	[24]
<i>SCG2/SCG3</i>	Secretogranin II and III	+	[25]
<i>ASH1/ASCL1</i>	Human achaete-scute homolog 1	+	[26]
<i>NCAM1/CD56</i>	Neural cell adhesion molecule	+	[8]
<i>DNMT</i>	DNA methyltransferase	+	[27]
<i>KDMs</i>	Histone demethylases	+	[28]
<i>ONECUT2</i>	Transcription factor, driver of NEPC	+	[29]
<i>AR</i>	Androgen receptor	-	[11]
<i>PSA/KLK3</i>	Prostate-specific antigen/kallikrein-3	-	[11]
<i>RB1</i>	Retinoblastoma tumor suppressor gene	Gene loss	[8]
<i>TP53</i>	Tumor protein p53	Gene loss	[30]
<i>REST</i>	RE1 silencing transcription factor	-	[31]
<i>PTEN</i>	Phosphatase and tensin homolog	-	[13,32]
<i>CCND1</i>	Cyclin D1	-	[33]
<i>FOXA1</i>	Forkhead box A1	-	[34]

\*Regulation: +, upregulated; -, downregulated. NE: Neuroendocrine, NEPC: Neuroendocrine prostate cancer

epigenetic changes related to NEPC. In addition, the current progresses of clinical trials of epigenetic drugs for advanced PCa are included.

### MECHANISMS OF THERAPY RESISTANCE AND NEUROENDOCRINE TRANS-DIFFERENTIATION

The underlying mechanisms of CRPC are largely caused by the genetic alterations of AR, including gene amplification of the AR locus and mutation of AR, resulting in the conversion of anti-androgens to agonists. In addition, the overexpression of an AR splice variant AR-V7, an alternative splicing variant lacking the C-terminal androgen-binding domain, results in an androgen-independent activation of AR signaling [9,35]. Apart from deregulating AR signaling in CRPC and mCRPC, additional resistant mechanisms giving rise to the use of next-generation ADI are emerging as a potential approach to target drug resistance. For instance, the overexpression of glucocorticoid receptor (GR) confers the enzalutamide-resistant PCa by bypassing AR signaling through the mechanism, of which GR substitutes for the AR-binding sites to activate similar transcriptional programs [36,37], supporting an adaptive mechanism of AR resistance in response to ARPI treatments [11,38,39].

Another mechanism of therapy resistance is associated with cellular lineage plasticity [40]. Increasing evidences have suggested that therapy resistance has been uncovered through which the PCa cells can evade AR pathway blockade by

lineage switching mechanism [41]. According to this mechanism, PCa cells acquire a specific cell lineage whose growth and survival are no longer controlled by the anti-AR drug target. An indication of the lineage switching (also referred to lineage plasticity) contributes to the anti-AR drug resistance followed by treatment of mCRPC with ARPIs and recurrence of the tumor cells exhibiting small-cell NEPC, a specific lineage that exhibits AR indifferent and resistant to current AR-targeted therapies.

### LINAGE PLASTICITY AND NEUROENDOCRINE TRANS-DIFFERENTIATION IN PROSTATE CANCER

Clinically, NEPC is an extremely aggressive cancer type of PCa, which appears resistant to any current therapies for this advanced PCa. NEPC also displays a high cell proliferation and tumor metastasis activity. Different from CRPC, which prefers to produce bone metastases, NEPC typically tends to metastasize to visceral organs such as liver and lung [42]. Histologically, NEPC displays small-cell phenotypes with high nuclear-to-cytoplasmic ratio and a high proliferative rate (indicated by Ki-67 staining). Immunohistochemical staining further indicates that NEPC specifically expresses neuroendocrine lineage markers, including synaptophysin, chromogranin A, CD56, and neuron-specific enolase, as well as the loss of the expression of AR and AR-responsive genes such as prostate-specific antigen [8,43,44]. Genetically, NEPC is also associated with genomic alterations in tumor suppressor genes

including *RBI* loss and mutation, *TP53* deletion, or *PTEN* inactivation [8,13,20,30,32]. Genome-wide sequencing studies have shown that the overall spectrum of somatic mutations between prostate adenocarcinoma and NEPC are surprisingly similar, [8,45], suggesting that additional mechanisms are required to coordinate with genomic alterations to reprogram cell fate to the neuroendocrine lineage. Not surprisingly, epigenomic abnormalities are involved in the neuroendocrine differentiation. Indeed, combining whole-genome bisulfite sequencing, a technology used for methylome analysis, and the transcriptomic analysis revealed that distinct cellular pathways are epigenetically dysregulated in NEPC, including cell adhesion, neuronal development, EMT, and the regulation and maintenance of stemness properties [46]. Taken together, numerous evidences suggest that neuroendocrine differentiation of PCa involves multiple cellular processes that are mediated by the transcription and epigenetic factors. In view of tumor microenvironment, different kinds of stroma cells in tumor itself or its surroundings, as well as other factors, also play vital roles in the induction of neuroendocrine trans-differentiation. In the following sections, we will briefly discuss the epigenetic abnormalities, regulation and maintenance of stemness [46], and EMT that have been shown to drive NEPC progression.

### EPITHELIAL-MESENCHYMAL TRANSITION AND NEUROENDOCRINE PROSTATE CANCER

It has been shown that neuroendocrine lineage plasticity and EMT [46] share a similar cellular mechanism, therefore it is not surprising that EMT-associated transcription factors have been implicated in NEPC. For instance, *ZEB1* [47], *Snail* [48], *Slug* [49], and *FOXC2* [50] promote neuroendocrine differentiation, highlighting the convergent transcriptional networks involved in NEPC and EMT. The implications of convergence between the pathways involved in EMT and NEPC suggest that therapies used for targeting EMT could also inhibit NEPC progression [51]. For example, monoclonal antibodies for targeting notch (e.g., rovalpituzumab and tarextumab) and stemness (disulfiram, an inhibitor of ALDH, which is overexpressed in cancer stem cells) are promising strategies for both EMT and NEPC.

### THE EPIGENETIC BASIS OF NEUROENDOCRINE PROSTATE CANCER

Currently available data supported that NEPC can emerge from CRPC or mCRPC following AR inhibitor treatments [8,52]. By closely examining the patient tumors during the course of disease progression using genome-wide sequencing studies, the overall spectrum of somatic mutations between prostate adenocarcinoma (PC or CRPC) and NEPC was found to be surprisingly similar [8,45]. Apart from the loss of *RBI* and *TP53* is known to contribute to progression of NEPC, the activation of pluripotency transcription factor *SOX2* and the epigenetic modifier (Enhancer of Zeste-Homolog 2 [*EZH2*]), a histone methyl transferase subunit of polycomb repressive complex 2 (PRC2), is involved in NEPC progression [45]. In addition, protein chromobox 2 (*CBX2*), a PRC1 component, acts as an epigenetic modulator involved in neuroendocrine

differentiation, the detailed mechanism of which is described below.

### ENHANCER OF ZESTE-HOMOLOG 2 AS A MASTER REGULATOR OF NEUROENDOCRINE PROSTATE CANCER REPROGRAMMING

*EZH2* is frequently overexpressed in PCa patients who have progressed to NEPC [8,11]. *EZH2* is the catalytic subunit of the PRC2, which mediates transcriptional silencing by depositing repressive histone marker, namely tri-methylation of histone H3 at lysine 27 (H3K27 me3) [53], to suppress specific gene expressions including lineage-switching factors and to promote stemness characteristics [54]. Recently, in PCa patients, elevated activity of *EZH2* (H3K27 me3 expression) has been reported in the majority of NEPC (87% NEPC vs. 46% adenocarcinoma) [8,55]. Functionally, *EZH2* cooperates with lineage-guiding transcription factors to epigenetically regulate gene expression and coordinate lineage trans-differentiation [13]. In the setting of NEPC, *EZH2* directly associates with N-Myc to transcriptionally repress genes that induce AR signaling-dependent CRPC [13]. Accordingly, conditional expression of N-Myc in prostate epithelial cells is sufficient to induce neuroendocrine differentiation [13,56], suggesting that N-Myc is an oncogenic driver of NEPC [13]. Consistently, N-Myc-overexpressed neuroblastomas are strongly dependent on *EZH2* for cell growth and survival [57], indicating that a regulatory interplay between N-Myc and *EZH2* drives the activation of neuronal differentiation. In the case of PCa, recent studies identified that N-Myc binds to the promoters of neuronal lineage-associated genes, where both repressive H3K27 me3 and active H3K4 me3 histone marks (bivalent chromatin state) were occupied. *EZH2* was required to maintain the bivalency chromatin state in the N-Myc-bound genes. Furthermore, knockdown of *EZH2* led to disassociation of N-Myc from the neuronal-associated genes in the NEPC organoid model [58].

*EZH2* has been reported to synergize with other epigenetic modifiers to promote chromatin remodeling, facilitating lineage plasticity. For instance, *EZH2* is linked to DNA methyltransferase (DNMT) activity via a scaffolding mechanism mediated by the long ncRNA *HOTAIR* [59]. Similarly, *EZH2* complexes with a H3K36 me2 methyltransferase, nuclear receptor-binding SET domain protein 2 (NSD2). NSD2 is overexpressed in NEPC [60] and functions to reprogram epigenome by reprogramming the binding distribution of *EZH2* [61]. NSD2 activity is also regulated by *EZH2*, which is required for the *EZH2*-mediated epigenetic reprogramming of PCa [62].

Recent studies are beginning to shed light on how *EZH2*-mediated epigenetic reprogramming facilitates neuroendocrine differentiation. In particular, *EZH2* can be activated by transcription factor 4 (TCF4), a key transcription factor in Wnt/ $\beta$ -catenin signaling, leading to the recruitment of H3K27 me3 histone marks on the miR-708 promoter [63]. Silencing of miR-708 induces the expression of neuronal [64], a key mediator for neuronal differentiation and stem cell-like factor CD44 [65]. Significantly, elevated

Wnt/ $\beta$ -catenin signaling is a feature of NEPC [66], and inhibiting TCF4 prevented NEPC trans-differentiation following androgen deprivation [63]. Moreover, EZH2 activity has been shown to be associated with cAMP-response element-binding protein (CREB) activation in PCa. Inhibition of EZH2 shows the efficacy of blocking CREB-induced H3K27 me3 and neuroendocrine differentiation [14]. EZH2 has also been reported to regulate the transcriptional activity of STAT3 [67], the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway [68], the MAPK/ERK pathway [69,70], and the SWI/SNF chromatin remodeling complexes [71]. Further studies are required to address the complexities between the canonical PRC2-bound and non-canonical functions of EZH2 during NEPC progression.

### **CHROMOBOX PROTEIN 2, A POLYCOMB REPRESSIVE COMPLEX 1 COMPONENT, ACTS AS A BRIDGE BETWEEN POLYCOMB REPRESSIVE COMPLEX 2 AND POLYCOMB REPRESSIVE COMPLEX 2 TO MEDIATE NEUROENDOCRINE PROSTATE CANCER PROGRESSION**

Another critical PRC1 component that has been implicated a coordinate regulatory role in NEPC progression is the chromodomain protein CBX2. Mechanistically, CBX2 directly associates with the histone suppressive marker H3K27 me3 through its chromodomain, and consequently represses the transcription of target genes. This indicates that CBX2 mediates chromatin condensation in an EZH2-independent manner [72]. Functionally, CBX2 can act as a linker between PRC2 and PRC1 [73], with a concomitant upregulation in NEPC. In the NEPC tumors and xenograft tumors of NEPC, CBX2 and EZH2 are consistently overexpressed [55], confirmed by a recent transcriptomic study by comparing clinical samples of NEPC and CRPC. Therefore, it appears that aberrant PRC1 and PRC2 activities are key features of NEPC, in which upregulation of CBX2 and EZH2 correlates with the downregulation of PRC target genes [55]. Interestingly, in the lung cancer model, overexpression of both CBX2 and EZH2 appears to be significantly associated with small-cell lung carcinomas (SCLCs) rather than that of non-SCLCs, suggesting that dysregulation of these epigenetic regulators plays a major driver for neuroendocrine differentiation [55].

### **REPRESSOR ELEMENT 1-SILENCING TRANSCRIPTION FACTOR**

In NEPC, repressor element (RE) 1-silencing transcription factor (REST) is a critical epigenetic regulator which is dysregulated in disease progression. REST functions as a transcription silencing factor and widely expressed in embryonic and pluripotent stem cells, and specifically in neuronal progenitors to modulate neuronal differentiation [74]. Mechanically, REST recruits several epigenetic co-repressors such as EZH2 (above mentioned) and lysine histone demethylase 1A (LSD1) to the RE site of neuronal genes to coordinately repress neuronal differentiation [31,75]. Clinical evidences have supported that REST downregulation is commonly observed in PCa tissues. In PCa cell lines, knockdown

of REST *in vitro* can attenuate AR signaling and elevate the expression of neuroendocrine markers, indicating a specific role of REST that suppresses neuroendocrine trans-differentiation [31]. Controversially, overexpression of REST in some NEPC patients has also been observed [31]. This paradox can be explained by the overexpression of SRRM4 (serine/arginine repetitive matrix protein 4), which promotes the formation of truncated REST lacking the transcriptional repressor domain [76]. Clinically, data from PCa tissues supported that SRRM4 is specifically overexpressed in neuroendocrine tumors, say, SRRM4 was overexpressed in 50% of NEPCs versus 3% of adenocarcinomas [11,13,75].

### **HISTONE DEMETHYLASES**

Histone methylation had been considered to be irreversible and a nonregulated event. Until the discovery of KDM1, the first histone demethylase, which removes the trimethyl mark from H3K27, was found to be a co-activator of AR [77]. Subsequently, Jumonji C (JmjC) domain-containing molecules were found to carry lysine demethylase activity [78]. Currently, there are 28 different JmjC domain-containing proteins that have been identified in human genome, of which 15 have been demonstrated to demethylate lysine residues in the H3 tail and one to demethylate the methylated arginine [79-81]. They are grouped into eight subfamilies (KDMs 1-8), with KDM8 being the newest and discovered in 2010 [82]. The most striking feature of lysine demethylases (KDM) is their exquisite specificity toward different lysine residues and different methylated forms [83,84]. Nearly all KDM family members (e.g., KDM1, KDM2A-C, KDM3A, C, KDM4A-D, KDM5A-C, KDM6B, C, and KDM8) have been found to be overexpressed in PCa, and several of these KDMs are correlated with a worse prognosis of the disease, suggesting an important regulatory role in PCa tumorigenesis by histone demethylation [83]. Currently, the well-characterized histone demethylases are KDM1 and KDM4 subfamilies. It has been shown that KDM1, KDM4A, B, and C can physically associate with AR and serve as AR co-activators [85,86]. Significantly, overexpression of KDM1s as well as KDM4A predicts the poor prognosis of PCa [80,87]. Thus, various evidences have suggested that histone demethylases have a close relationship with AR and are directly relevant to castration resistance of PCa. NEPC tumors exhibit elevated expression of the histone lysine demethylase KDM8, which functions to reprogram metabolism toward aerobic glycolysis [88]. The AR can directly recruit histone modifiers to remodel chromatin architecture and alter gene expression. LSD1 is an important regulator of AR transcriptional activity, facilitating the suppression of AR target genes via H3K4 demethylation [69]. Interestingly, LSD1-AR-mediated trans-activation is associated with loss of RB1 expression, which is an important consequence for NEPC [89]. In addition, LSD1 + 8a, an LSD1 alternative splicing variant, has been shown to promote neuronal gene expression. Mechanically, the aforementioned splicing factor SRRM4 promotes the expression of LSD1+8a splicing variant and involves NEPC progression [90].



## TARGETING EPIGENETIC REGULATORS IN NEUROENDOCRINE PROSTATE CANCER

Based on the progression of NEPC transcriptome and epigenome, targeting the epigenetic mechanism to reverse or delay neuroendocrine trans-differentiation is beginning to become promising. High EZH2 levels in NEPC and the association between the tumors and cellular plasticity are known to provide a rationale for developing epigenetic targeting strategies. Recent preclinical and clinical studies support this notion, as summarized in [Table 2].

EZH2 is the most well-documented dysregulated epigenetic factor in NEPC [8,55]. In preclinical NEPC models, EZH2 inhibitors have been shown to attenuate neuroendocrine

phenotypes and re-sensitize ARPI treatments [13,30,58]. For example, the EZH2 inhibitor PF-06821497 is currently being tested in a Phase I study in patients with advanced/mCRPC (NCT03460977). Similarly, the Phase Ib/II trial is assessing the utility of combining the EZH2 inhibitor, CPI-1205, with enzalutamide or abiraterone in patients with mCRPC (NCT03480646).

Aurora A inhibitors such as alisertib (MLN8237), which result in destabilization of N-Myc, have also shown some efficacy in clinical trials. A Phase II clinical trial of MLN 8237 (alisertib) in NEPC patients showed a modest clinical benefit (NCT01799278). A second Phase I/II clinical trial of MLN 8237 in combination with abiraterone in CRPC with neuroendocrine differentiation was terminated

**Table 2: Clinical trials for epigenetic therapies in prostate cancer**

Mechanism	Clinical trial ID	Agent	Phase	Indication	Clinical status
EZH2 inhibition	NCT03009344	Tazemetostat	I	Relapsed or refractory B-cell non-Hodgkin's lymphoma	Active, not recruiting
	NCT02875548	Tazemetostat	II	Diffuse large B-cell Lymphoma; advanced solid tumors	Completed
	NCT04179864	Tazemetostat + abiraterone/prednisone	I	mCRPC	Recruiting
	NCT03460977	PF-06821497	I	Relapsed/refractory SCLC, CRPC, and follicular lymphoma	Recruiting
	NCT02395601	CPI-1205	I	B-Cell lymphomas	Completed
	NCT03480646	CPI-1205 + enzalutamide	Ib/II	mCRPC	Active, not recruiting
	NCT01848067	CPI-1205 + abiraterone/prednisone	I/II	mCRPC	Completed
	NCT03525795	Alisertib (MLN8237) + abiraterone + prednisone	I/II	mCRPC	Completed
	NCT03525795	CPI-1205 + Ipilimumab	I/II	Advanced solid tumors	Active, not recruiting
	NCT01799278	MLN8237/alisertib	II	CRPC/NEPC	Completed; no appearance of new lesions for >1 month
BET inhibition	NCT02705469	ZEN003694	I	mCRPC	Completed; dose confirmation
	NCT04145375	I: ZEN003694 II: ZEN003694 + enzalutamide	I/II	mCRPC	Enrolling by invitation
	NCT02711956	I: ZEN003694 II: Enzalutamide	Ib/IIa	mCRPC	Active, not recruiting
	NCT04471974	Pembrolizumab (day 1) ZEN-3694 + enzalutamide (days 1- 21)	II	mCRPC	Not yet recruiting (2020/8 start)
	NCT02607228	GS-5829 + enzalutamide	Ib/II	mCRPC with ARPI	Completed
	NCT02698176	MK-8628	I	CRPC	Terminated
LSD1 inhibition	NCT02712905	INCB059872	I/II	Advanced malignancies; NEPC	Terminated
	NCT02217709	Phenelzine	II	Nonmetastatic recurrent prostate cancer	Active, not recruiting
	NCT01253642	Phenelzine + docetaxel	II	PCa with progressive disease	Terminated
DNMT inhibition	NCT00384839	Azacitidine for injectable suspension	II	PCa to hormonal therapy	Completed; PSA doubling time ≥3 months
	NCT03572387	5-AZA + ATRA	Pilot study	PCa with PSA-only recurrence	Recruiting
	NCT00503984	Azacitidine + docetaxel + prednisone	I/II	mPC	Terminated
	NCT03709550	Enzalutamide + decitabine	Ib/II	mCRPC	Not yet recruiting (2020/8 start)
	NCT02998567	Guadecitabine + pembrolizumab	I	CRPC solid tumors	Active, not recruiting

5-AZA: 5-azacitidine, ATRA: All-trans retinoic acid, CRPC: Castration-resistant prostate cancer, mCRPC: Metastatic CRPC, DNMT: DNA methyltransferase, PCa: Prostate cancer, ARPI: Androgen receptor pathway inhibitor, BET: Bromodomain and extra-terminal motif, NE: Neuroendocrine, NEPC: NE prostate cancer, SCLC: Small-cell lung carcinoma

early due to the severe cell toxicity and lack of clinical benefits (NCT01848067).

An epigenetic drug targeting BRD4, the BET family of chromatin readers and transcriptional regulators, is under clinical studies. Experimental data suggested that targeting BRD4 disrupts AR recruitment to its chromatin binding sites and reduces AR-dependent cell growth [91]. Treatment of BRD4 inhibitors in monotherapy or in combination with ARPIs in PCa exhibits anti-tumor activities [92-94]. A Phase I/II trial is ongoing to evaluate the efficacy of the BET inhibitor ZEN003694 in combination with enzalutamide in CRPC (NCT02711956). Immunotherapy with checkpoint inhibitors, such as anti-PD-1 and anti-PD-L1, has shown disappointing efficacy in PCa treatments [95]. In melanoma and ovarian cancer models, however, inhibition of EZH2 synergizes with immune checkpoint inhibitors to enhance the infiltration of T-cells (CD8+) to the tumor microenvironment and improve tumor killing [96]. This implicates, in PCa, that EZH2 inhibition may turn the immunologically cold prostate tumor hot. Clinical trials of EZH2 inhibitors in combination with Ipilimumab, a monoclonal antibody that activates the immune system by targeting CTLA-4, in patients with advanced solid tumors, are ongoing (NCT03525795).

Another epigenetic drug that targets LSD1 presents a promising efficacy in NEPC because LSD1 specifically overexpressed in androgen-independent PCa modulates FOXA1-dependent AR-associated reprogramming and activates stem cell-associated gene expressions [97,98]. Clinical trial of the LSD1 inhibitor INCB059872 is terminated due to the strategic business decision (NCT02712905). Another clinical trial of LSD1 inhibitor phenelzine is terminated because of low enrollment (NCT02217709 and NCT01253642).

In preclinical studies, inhibition of DNMTs, DNA methyltransferases, could re-sensitize ARPI-resistant neuroendocrine-like PCa cell lines [27,99], suggesting that the development of DNMT inhibitors may be an attractive therapeutic strategy for NEPC. Notably, the DNMT inhibitors such as decitabine and azacytidine are already approved by the FDA for the treatment of myelodysplastic syndromes and could therefore be re-purposed to NEPC. However, in Phase II clinical trials in CRPC, DNMT inhibitors did not show a strong efficacy of anti-tumor activity [100,101]. Currently, a clinical trial in combination of DNMT inhibitor decitabine with enzalutamide in mCRPC patients has been just launched this year (NCT03709550). In deeded, future clinical trials will be needed to assess the efficacy of DNMT-directed therapies in NEPC patients.

## CONCLUSION AND PERSPECTIVE

Numerous evidences have demonstrated that epigenetic and transcriptional dysregulation is central to the emergence and maintenance of lethal NEPC. Aberrant activities of master epigenetic regulators, such as DNMT1 and EZH2 and KDMs, as well as master transcription factors, such as N-Myc, facilitate chromatin remodeling to support the activation of lineage plasticity pathways under potent AR therapy. These epigenetic changes are increased, in part, through metabolic

reprogramming. The dependency of NEPC tumors on the epigenetic and transcriptional machinery provides an excellent opportunity to develop effective therapeutical intervention. Although epigenetic therapy seems to be promising, still, numerous challenges remain with respect to patient responses, timing, and combination with ARPIs and/or immunotherapy to refine clinical outcomes. Moreover, biomarker-driven treatment strategy for NEPC is urgent.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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