

## Review

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Review

**Ricardo Leão<sup>a–d</sup>**  
**Célia Domingos<sup>e, f</sup>**  
**Arnaldo Figueiredo<sup>c, d</sup>**  
**Robert Hamilton<sup>b</sup>**  
**Uri Tabori<sup>a</sup>**  
**Pedro Castelo-Branco<sup>a, e–g</sup>**

<sup>a</sup>Arthur and Sonia Labatt Brain Tumor Research Center, The Hospital for Sick Children, and <sup>b</sup>Division of Urology, Department of Surgery, Princess Margaret Cancer Center, University of Toronto, Toronto, ON, Canada; <sup>c</sup>Serviço de Urologia e Transplantação Renal, Centro Hospitalar Universitário Coimbra EPE, and <sup>d</sup>Faculty of Medicine, University of Coimbra, Coimbra, <sup>e</sup>Regenerative Medicine Program, Department of Biomedical Sciences and Medicine, and <sup>f</sup>Centre for Biomedical Research (CBMR), University of Algarve, and <sup>g</sup>Algarve Biomedical Center, Campus Gambelas, Faro, Portugal

# Cancer Stem Cells in Prostate Cancer: Implications for Targeted Therapy

## Keywords

Cancer stem cells · Prostate cancer · Targeted therapy

## Abstract

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and the second most common cause of cancer-related mortality among men in the developed world. Conventional anti-PCa therapies include surgery, radiation, hormonal ablation, and chemotherapy. Despite increasing efforts, these therapies are not effective for patients with advanced and/or metastatic disease. In most cases, cancer therapies fail due to an incomplete depletion of tumor cells, resulting in tumor relapse. The cancer stem cell (CSC) hypothesis is an emerging model that explains many of the molecular characteristics of oncological disease as well as the tendency of cancers to relapse, metastasize, and develop resistance to conventional therapies. CSCs are a reservoir of cancer cells that exhibit properties of self-renewal and the ability to reestablish the heterogeneous tumor cell population. The existence of PCa stem cells offers a theoretical explanation for many uncertainties

regarding PCa and also for treatment resistance and disease progression once clinical cure is achieved. Therapies targeting CSCs might therefore lead to more effective cancer treatments, divergent from a traditional anti-proliferative approach, based on tumor bulk reduction accompanied by CSC-specific inhibition. Here, we focus on reviewing the historical perspective as well as concepts regarding stem cells and CSCs in PCa. In addition, we will report possible strategies and new clinical approaches that address the CSC-based concept of tumorigenesis in PCa.

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## Introduction

Prostate cancers (PCa) are highly heterogeneous tumors containing multiple independent and genetically distinct clones [1–4]. The cellular heterogeneity might

R.L. and C.D. contributed equally to this work.

**KARGER**

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E-Mail [karger@karger.com](mailto:karger@karger.com)  
[www.karger.com/uin](http://www.karger.com/uin)Pedro Castelo Branco  
Department of Biomedical Sciences and Medicine  
University of Algarve, Campus Gambelas, Ed 7  
8005-139 Faro (Portugal)  
E-Mail [pjbranco@ualg.pt](mailto:pjbranco@ualg.pt)

pose clinical challenges due to therapy resistance and tumor relapse.

Current treatments for clinically localized PCa are based on surgical excision of the prostate (radical prostatectomy) and radiation therapy [2–5]. By contrast, advanced-stage PCa is usually treated with androgen-deprivation therapy, which reduces tumor burden and/or circulating prostate-specific antigen (PSA) to low or undetectable levels [2–6]. Patients with advanced PCa initially respond to hormone therapy; however, due to androgen mechanisms, a significant number of patients will progress to recurrent castration-resistant PCa and eventually die from metastatic disease [2–7].

Resistance to androgen deprivation and subsequently to chemotherapy remains the main cause for treatment failure and mortality in patients with PCa [7–10]. Treatment resistance in cancer may result from the survival of a subpopulation of tumor cells. These cells may subsequently lead to relapse, disease progression, and eventually systemic disease [3, 7]. This is particularly true for PCa since these tumors present a heterogeneous population of cells [3, 7–9].

One premise that explains this cellular heterogeneity and treatment resistance is the “cancer stem cell” (CSC) hypothesis [4, 7], which postulates that human tumor cancers cells are organized hierarchically, and only a subset of cancer cells are endowed with tumor-initiating and long-term tumor-propagating capability [3, 8]. These tumor-initiating cells are termed CSCs and possess many phenotypic and functional properties associated with normal stem cells [3, 7].

Therefore, it is not surprising that many PCa stem cell (PCSC) populations have now been identified and that emerging evidence suggests a critical role for them in disease relapse and progression [1, 2, 4, 11].

### Stem Cells

The discovery of hematopoietic stem cells emerged from the study of hematopoiesis [12]. Hematopoietic cells have the capacity for unlimited self-renewal as well as differentiation into all lineages of mature cells and thereby sustain a permanent source of blood cells [13]. The physiological function of “normal” stem cells is to maintain tissue homeostasis, tissue regeneration and repair, as well as controlling proliferation and differentiation necessary for the proper functioning of organs. In addition to hematopoietic stem cells, adult stem cells have

been identified in bone marrow, muscle, intestine, brain, skin/hair follicles, heart, lung, mammary glands, and prostate [14–16].

Adult stem cells reside in a quiescent state, regulated by cell cycle regulatory genes (p21, p18, p63) [13, 17]. When they leave the quiescent state, these cells self-renew and differentiate into other cells (usually restricted to the respective tissue type) controlled by intrinsic and extrinsic regulatory mechanisms [12, 17, 18].

### Prostate and Prostate Stem Cells

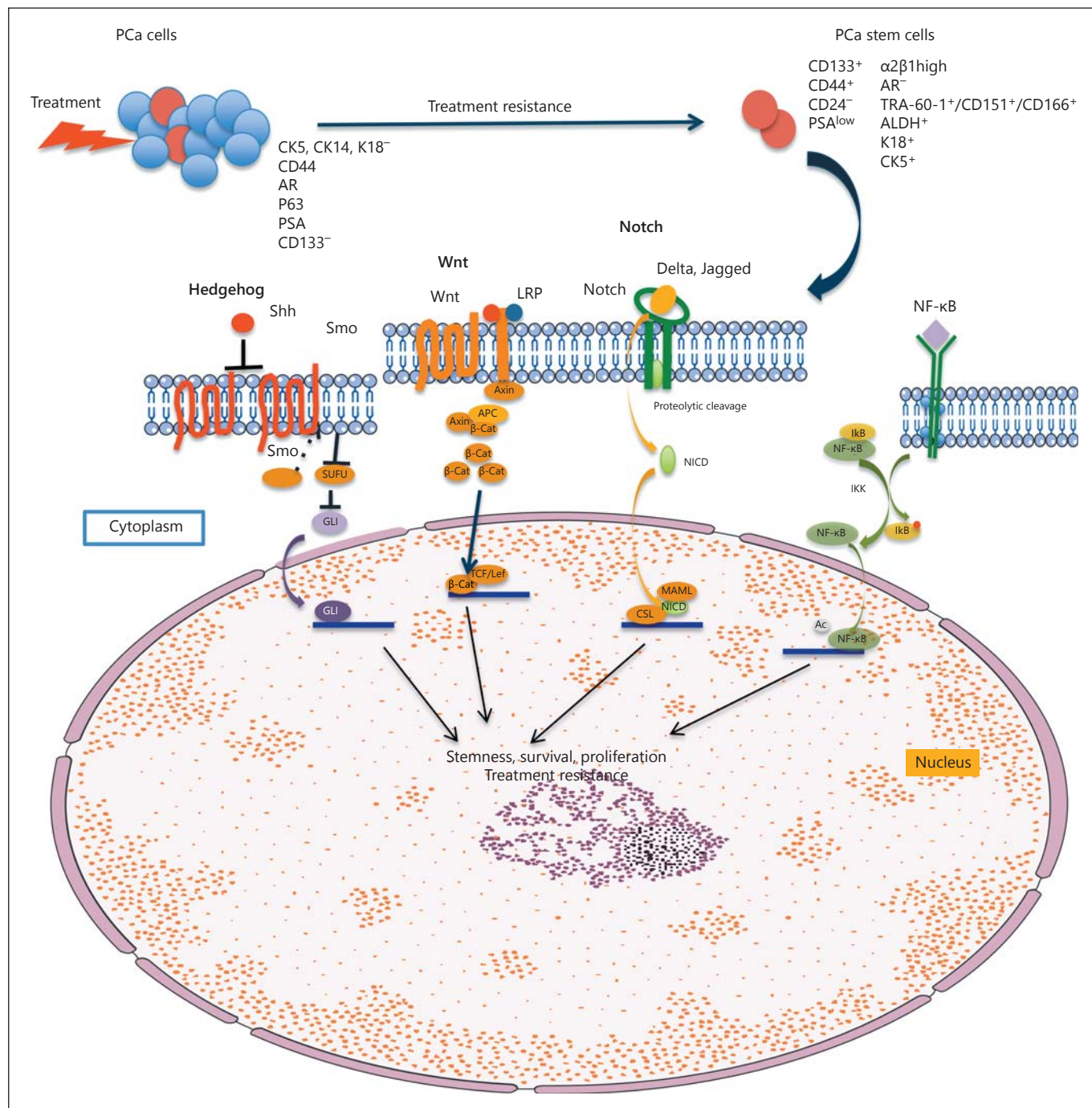
The prostate gland is an endodermal tissue that develops during late embryogenesis from the anterior urogenital sinus epithelium in a dihydrotestosterone-dependent process [2, 19–21]. In early embryonic development, the prostate is comprised of a multilayered epithelium surrounded by mesenchyma [2, 19, 22].

There are 3 main cell types that constitute normal mature prostatic epithelium: luminal secretory cells, proliferative basal cells, and neuroendocrine cells [4, 19, 23, 24]. These cells are morphologically distinguishable by the expression of specific markers [25, 26].

The embryonic origin of the prostate (from the urogenital sinus) may explain the large presence of prostate stem cells within this organ [27]. The existence of a stem cell subpopulation in the prostate was first identified in the 1980s [22]. The most widely accepted model proposes the presence of stem cells in the basal cell compartment [18, 19, 25, 26, 28]. Additionally, prostate epithelial cells (with an intermediate phenotype) can be observed in adult prostates, suggesting that the developmental relationship between basal and secretory cells is maintained into adult life and that the capacity for renewal and differentiation is still present [18, 19, 25, 26].

Basal cells typically show higher proliferation rates than secretory cells. In studies of androgenic suppression, the prostatic epithelium (consisting mainly of secretory cells) undergoes apoptosis [22, 23, 29]. In contrast, basal cells are able to survive in low androgen environments, demonstrating that the high renewal capacity of the prostatic epithelium is due to the basal cells, which reconstitute the secretory cell compartment in response to androgen readministration [9, 22, 23, 29]. The preferential survival of basal cells led to the hypothesis that the stem cells reside within the basal cell layer of the prostate gland [9, 22].

Although the model in which renewal and differentiation of prostatic epithelium occurs in the basal cells is cur-



**Fig. 1.** Prostate cancer (PCa) cells–associated and PCa stem cells–associated pathways.

rently widely accepted, there are some authors who propose a different model in which the secretory cells are capable of sustaining self-renewal and differentiation, without any contribution from basal cells [30, 31].

The tumorigenic properties of prostate stem cells, isolated from patients undergoing radical prostatec-

tomy, have been studied in vitro and in vivo (by implanting them into immunocompromised animals) assessing their capacity for extensive proliferation, self-renewal, differentiation, and invasion (Fig. 1) [32, 33].



## PCa Stem Cells

Cancer is recognized as a heterogeneous disease with a variety of phenotypes. This heterogeneity is an intrinsic characteristic that contributes to therapy failure. One theory that explains tumor heterogeneity is the CSC hypothesis [4, 7, 34–36]. This hypothesis postulates that only a small subpopulation of cancer cells within a tumor possesses the capacity to regenerate the tumor. This subset of tumor-propagating cells, defined as CSCs, share a number of characteristics with normal stem cells such as self-renewal, high proliferative potential, and the capacity to generate the heterogeneous lineages of cancer cells that make up the tumor [4–8, 36, 37]. Thus, the CSC hypothesis suggests that tumor cell lineages are hierarchical with a unique self-renewing population of cells at the top of that hierarchy (Fig. 1) [4].

Research into CSCs has progressed rapidly and concomitantly with advances in stem cell biology, novel methods for their identification, purification, and characterization have been developed as well as the creation of animal models [4, 6, 8, 34, 37]. The first demonstration of CSCs in any cancer was in 1977 when Bonnet and Dick [12] demonstrated the initiation of human acute leukemia myeloid from a population of cells expressing cell surface markers CD44<sup>+</sup> and CD38<sup>−</sup> in NOD/SCID mice. This suggested that normal primitive cells are targets for leukemic transformation [38]. CSCs have since been reported in a wide spectrum of solid tumors including breast, pancreas, colon, lung, brain, and prostate [4, 8, 35].

CSCs express various specific surface markers, including cell-adhesion molecules (e.g., CD24, CD44, CD133, and hyaluronic acid), HER2, aldehyde dehydrogenase (ALDH) activity and transcription factors including OCT-4 and SOX-2 at different levels from the bulk tumor population [35, 37, 39, 40]. Human prostatic cells (within the basal layer) express cell surface marker CD133 (hematopoietic stem cell marker) and stem cell antigen-1, which reveals their stem cell properties [9, 28, 41, 42].

PCa cells are highly organized and only a subset of these cells retains the capacity for tumor initiation and long-term tumor-propagating activity, supporting the CSC hypothesis [3, 5]. These tumor-initiating cells display phenotypic and functional features characteristic of normal prostate stem cells and are involved in tumor initiation, metastasis, and drug resistance [5]. Thus, PCa may originate from stem cells or dividing progenitor cells that become CSCs as a consequence of genetic mutations or changes in the tumor microenvironment [3, 5]. PCSCs could account for resistance to androgen-deprivation

therapy-resistant cells that give rise to castrate resistant PCa (CRPC), since they possess self-renewal and tumor-propagating capabilities and also lack or have very low androgen receptors expression [4, 28].

The cellular origins of PCa are still under debate (Fig. 1). The most common prostate tumors express high levels of K8, K18, androgen receptors, and PSA but low levels of basal cell markers such as p63, suggesting that the disease arises from luminal cells [32, 42, 43]. Other studies suggest that the disease is derived from intermediate cells that have acquired the ability to self-renew [32].

Isolation of PCSCs has been reported by several different groups. Collins et al. [44] first isolated a CSC population from patients undergoing radical prostatectomy. The PCSCs displayed a significant capacity for self-renewal as well as the ability to regenerate the phenotypically mixed populations of non-clonogenic cells [10]. The PCSCs had a CD44/ $\alpha$ 2 $\beta$ 1<sup>high</sup>/CD133<sup>+</sup> phenotype and showed high clonogenic and invasive capacity, while phenotypically basal and genetically unstable [44, 45].

Rajasekhar et al. developed a study for better understanding PCSCs. They found that CSCs expressing human pluripotent stem cell marker TRA-60-1<sup>+</sup>/CD151<sup>+</sup>/CD166<sup>+</sup> had high capacity for self-renewal and differentiation, and were able to recapitulate the original tumor heterogeneity in serial xenotransplantations, indicating a tumor cell hierarchy in PCa development [46].

Recently, it has been shown that 55% of prostate tumors harbor a gene fusion between the prostate-specific TMPRSS2 gene and the ERG oncogene (TMPRSS2:ERG) [47, 48]. Polson et al. [49] demonstrated the presence and expression of TMPRSS2:ERG in PCSCs, which would provide ERG-driven survival advantages. Moreover, ALDH<sup>+</sup> PCa cells have been shown to exhibit several CSC characteristics such as clonogenicity, migration, tumorigenicity, and propensity to form metastases in vivo [3, 10]. Furthermore, PCSCs have also been shown to express Oct4 and Sox2 along with Nanog in several PCa tissue samples [3, 28].

Recent reports indicate that miRNAs play a significant role in CSC regulation, including PCSCs, and may explain some of the molecular regulatory mechanisms of CSCs [50]. The aberrant expression of miRNAs in cancer suggests that they function as either oncogenes or tumor-suppressor genes [51]. miRNAs such as miR-470, miR-296, and miR-134 are involved in regulating target genes essential for pluripotency and stem-cell function including Oct4, NANOG, and SOX2 [50, 52, 53].

Moreover, a miRNA expression signature specific for CSC populations has been confirmed in several cancers

including PCa. The majority of miRNAs discovered in PCa act as, or target, tumor-suppressor genes. These miRNAs are underexpressed in the PCSCs leading to the disinhibition of various stem-cell properties, such as clonogenic expansion, tumor regeneration, and metastasis [50, 52].

One important capability of CSCs is the epithelial-mesenchymal transition (EMT). This event plays an important role in promoting cell migration and the development of metastasis. Several miRNAs, such as the miRNA-200 family, miRNA132/212, miRNA-205, and miRNA-203 promote the epithelial state and have been shown to inhibit the EMT [50, 51, 54, 55].

Thus, the identification of PCSCs and their cell-specific markers is a crucial step in understanding PCa ontogenesis and in developing new therapeutic approaches [15, 37, 56, 57].

### CSCs as Therapeutic Targets in PCa

The CSC hypothesis provides an explanation for tumor initiation, progression, conventional therapy-resistance, recurrence, and metastasis [4, 6–8, 34, 35, 37, 58]. CSCs display self-renewal capacity, resistance to cytotoxic agents (e.g., chemotherapy or androgen ablation in prostate), competency to proliferate, and the ability to be both clonogenic and tumorigenic [19, 37, 56, 58].

The CSC hypothesis suggests that standard therapies have incomplete and temporary effects on the bulk tumor, and that tumors tend to relapse due to the multiple resistant mechanisms existing in CSCs [15, 37, 56]. CSCs therefore constitute a cellular reservoir that persists after anti-proliferative therapies [12, 37, 48, 59]. The inherent plasticity of CSCs makes them more adept to survive in a foreign environment. Additionally, genetic instability in CSCs likely provides a selective advantage in adapting to foreign sites [15].

Current therapeutic approaches for PCa remain insufficient for some patients with progressive disease. Although tumors initially respond well to androgen reduction, the duration of response is short, approximately 12–33 months. At this point, a population of cells resistant to androgen-deprivation therapy emerges [43]. Even after chemotherapy (and after a period of good clinical response) there is further clinical progression [43].

Thus, the development of PCSC-specific anticancer drugs constitutes an attempt to innovate in the treatment in PCa [60, 61].

### Specific Targeting of Pathways Involved in PCSCs

Signaling pathways that are upregulated in stem cells and that are specific to their functionality represent a hypothetical target for drugs addressing CSCs. The fact that many forms of cancer share the same expression pattern in certain pathways suggests that targeting of CSC signaling pathways may be an extraordinary therapeutic strategy. Currently, the pathways that demonstrate therapeutic potential in PCSCs are the Hedgehog (Hh), Wnt, Notch, and NF- $\kappa$ B pathways. Also, ABC transporters and tumor microenvironment seem to be potential targets for CSC depletion (Fig. 1) [35, 37, 59, 62].

#### Hh Pathway

The Hh pathway is critical in embryogenesis but is significantly less active in adults. However, it plays a crucial role in regulating CSCs in various human cancers including PCa by regulating target genes involved in proliferation, survival, metastasis, and auto-regulation [37, 63–65]. Furthermore, the Hh pathway promotes multidrug resistance by increasing the transcription of the ABC transporter proteins ABCB1 and ABCG2 in PCa [63, 64]. Thus, the inhibition of the Hh signaling pathway may result in the depletion of CSCs [37, 63].

Hh inhibitors have shown promising results in vitro. Sonidegib (LDE-225) inhibits spheroid formation and self-renewal of CSCs by suppressing the pluripotency-maintaining factors Nanog, Oct4, Sox2, and c-Myc and also inhibits tumor growth in PCa cells [63]. GANT-61 is a cell-permeable hexahydropyrimidine compound that is an inhibitor of GLI-mediated gene transactivation. GLI-mediated transcription is the final step in the Hh signaling pathway; therefore, GANT-61 halts Hh pathway effects. GANT-61 has shown selective inhibition of GLI1 and GLI2 in many cancer cell lines. In a xenograft mouse model of PCa, GANT-61 reduced tumor growth and proliferation and strongly reduced the expression of PTCH1 mRNA [66, 67]. These inhibitors are currently in preclinical development in numerous tumor types including PCa [68, 69].

At the clinical level, the Food and Drug Administration has already approved the first Hh inhibitor for clinical use, vismodegib (GDC-0449) [62, 64, 68–70]. Five clinical trials are currently testing Hh inhibitors in PCa treatment (Table 1). In the NTC01163084 study, vismodegib is being tested in stages IIA and IIB PCa as a neoadjuvant treatment in conjunction with the anti-androgen leuprolide acetate. Itraconazole is being used in hormone-sensitive

**Table 1.** Clinical trials targeting PCa cells-associated pathways

Clinical trial	Drug	Target population	Objectives	Approval stage	Associated pathway
NCT01163084	LHRHa vs. LHRHa plus vismodegib followed by surgery	Clinical stage T1c or T2 with high-grade disease (Gleason's 8–10) on initial biopsy and PSA >10 ng/mL, or clinical stage T2b–T2c with Gleason's grade ≥7	Compare tumor involvement in both arms	Phase 1, phase 2	Hedgehog
NCT01787331	Itraconazole	Patients with non-castrate, non-metastatic, biochemically relapsed PCa after prior definitive local therapy	Determine the proportion of patients who achieve ≥50% decline in PSA	Phase 2	Hedgehog
NCT02111187	LDE225 vs. no treatment (before surgery)	High risk PCa patients (Gleason ≥8; PSA >20 ng/mL; clinical stage ≥T3)	Change from baseline in tissue GLI1 expression; pathological effect of pre-surgical treatment with LDE225	Phase 1	Hedgehog
NCT02054793	Itraconazole and orteronel	Castrate resistant PCa	To determine the safety and tolerability of the combination regimen: orteronel + itraconazole	Phase 1, phase 2	Hedgehog
NCT02115828	Vismodegib	mCRPC with accessible metastatic lesions for tumor biopsy	Proportion of mCRPC patients treated with vismodegib who achieve a pharmacodynamics response in tumor biopsies	Phase 0	Hedgehog
NCT02655952	Foxy-5	Metastatic PCa (for which no curative therapy exists)	Establish the recommended dose for a clinical phase II study and enable further development of foxy-5 as a first in class antimetastatic cancer drug	Phase 1	Wnt
NCT02020291	Foxy-5	Metastatic PCa (for which no curative therapy exists)	Determine the safety, tolerability and pharmacology safety of foxy-5	Phase 1	Wnt
NCT01608867	OMP-54F28	Solid tumors including PCa (confirmed malignancy that is metastatic or unresectable)	Determine the safety, pharmacokinetics and immunogenicity of OMP-54F28 in subjects with previously treated solid tumors	Phase 1	Wnt
NCT01200810	Notch signalling pathway inhibitor RO4929097 + bicalutamide	Recurrent PCa stage IV PCa	Compare time for PSA progression	Phase 1	Notch
NCT02757365	Aspirin Levofloxacin	PSA >10 ng/mL; prostate biopsy showing BPH with infiltrated lymphocytes	Evaluate the role of aspirin in PCa prevention	Phase 2	NF-κB
NCT00118092	Tanespimycin	Hormone refractory PCa patients	Evaluate drug response in terms of: PSA response; overall survival; disease specific survival	Phase 2	NF-κB
NCT01695473	BKM120	High-risk (confirmed by prostate biopsy), localized PCa	Determine the proportion of men with downstream target inhibition of PI3K in prostate tumor tissue	Phase 2	NF-κB

PCa, prostate cancer; PSA, prostate specific antigen; mCRPC, metastatic castration-resistant Pca; BPH, benign prostatic hyperplasia.

PCa (NCT018787331) and in combination with Orteronel (NCT02054793) for CRPC in patients previously treated with curative intent. LDE225 is being evaluated in high-risk patients as a neoadjuvant treatment (NCT02111187) and finally Vismodegib (NCT02115828) is being tested in patients with metastatic lesions.

### Wnt Signaling Pathway

The Wnt signaling pathway is involved in multiple biological processes including embryogenesis, development, cell proliferation, cell survival, and cell differentiation. The canonical Wnt signaling pathway plays a critical role in self-renewal and maintenance of stem cells [37]. In

PCa, the Wnt signaling pathway has been linked to the progression of androgen-independent disease and bone metastasis [45, 48, 71].

The inhibition of the Wnt pathway results in the down-regulation of CSCs and constitutes an interesting target for treatment [37]. The Wnt pathway can be inhibited by Wnt inhibitory factors, Wnt antagonists, or via conditional knockout of  $\beta$ -catenin [37].

A number of therapeutic agents targeting the Wnt pathway are under investigation and monoclonal antibodies (mAb) against the Wnt cascade have been tested with promising anti-tumor activity [35, 72]. A small molecule inhibitor, Wnt inhibitor 3289–8625, has also been shown to inhibit the growth of PC3 PCa cells in vitro [73].

Vantictumab (OMP-18R5), a monoclonal antibody that blocks canonical Wnt/ $\beta$ -catenin signaling by binding to 5 of the FZD receptors, and LGK974 (a small molecule inhibitor of porcupine) are being tested in solid tumors in combination with chemotherapeutic drugs such as docetaxel and paclitaxel [72–75]. LGK974 is a potent inhibitor of porcupine – a membrane-bound O-acyltransferase that is required for the palmitoylation of Wnt ligands and which is crucial in the process of Wnt ligand secretion [76].

Foxy-5 is another Wnt-inhibiting agent that is being tested in 2 clinical trials. It is a hexapeptide that activates Wnt-5a-mediated signaling. Increased Wnt-5a signaling may inhibit endothelial tumor cell migration and invasion and may decrease metastasis. Foxy-5 has been tested in metastatic PCa patients for whom standard therapies were not effective (NCT02020291 and NCT02655952); however, the results of these studies are not yet known.

Finally, OMP-54F28, a fusion protein of the FZD8 ligand-binding domain that binds to all Wnt ligands, is now being tested in a phase I trial for treatment of solid tumors including PCa (NCT01608867) [72].

### *Notch Signaling Pathway*

The Notch signaling pathway regulates stem cell maintenance and differentiation. It contributes to angiogenesis, proliferation, differentiation, and apoptosis [37, 77]. In hypoxic microenvironments, the hypoxia-inducible factor activates the Notch pathway and the expression of transcription factors such as Oct4. This pathway controls stem cell self-renewal and pluripotency, enabling CSCs to survive and proliferate [77].

The Notch signaling pathway is often over-activated in cancers, including PCa [37]. The silencing of Notch ex-

pression in mouse prostate inhibits morphogenesis, growth, and differentiation during development. In addition, it inhibits prostate regrowth triggered by hormone replacement in castrated mice [78].

Several agents targeting Notch signaling are being trialed including the  $\gamma$ -secretase inhibitor (GSI), siRNAs, and mAb against Notch receptors and Notch ligands [79].

GSI was initially tested in T-Acute Lymphocytic Leukemia cell lines and later in prostate, breast, and lung cell lines and was found to suppress cancer growth [80]. Notch inhibitors have been tested in combination with conventional cytostatic agents or targeted drugs in phase I or phase II evaluations [79].

There are currently 2 clinical trials studying the effects of GSI in cancer. One of these is specific for PCa patients (NCT01200810; Table 1). This study combines the anti-androgen bicalutamide with GSI (RO4929097) in patients whose PCa recurs after surgery or radiation and compares the time to PSA progression in both arms [80].

### *NF- $\kappa$ B Pathway*

The NF- $\kappa$ B pathway is upregulated in CSCs and is important for apoptosis resistance in tumor cells [35, 48, 77, 81]. It has been shown that the activation of NF- $\kappa$ B signaling in PCa cells correlates with PCa progression, chemoresistance, recurrence, and metastasis [82, 83]. Thus, inhibiting NF- $\kappa$ B can suppress chemoresistance, and mediate antitumor responses while enhancing the sensitivity of tumor cells to other anticancer drugs [82]. Several studies have focused on targeting the NF- $\kappa$ B pathway on its own or in combination with conventional agents [84].

Bortezomib is a proteasome-inhibitor that was first approved for use in multiple myeloma. While its effects are complex, it has an overall inhibitory effect on the NF- $\kappa$ B pathway. Preclinical studies have shown that bortezomib induces cell growth inhibition and apoptosis in a broad range of cancer cell lines in vitro and in various animal xenograft models, including PCa [84].

Phase I, II, and III clinical studies, demonstrate that bortezomib causes chemo/radio-sensitization and can overcome drug resistance when combined with conventional therapeutic agents or radiation. In patients with advanced androgen-independent PCa, bortezomib alone shows anti-tumor activity (phase I) [85]. However, various phase II trials investigating bortezomib alone or in combination with prednisone or docetaxel in patients with CRPC have shown disappointing results. Patients with biochemical recurrence after definitive local therapy



for PCa, treated with bortezomib alone before androgen deprivation, however have shown stabilization of PSA levels [84, 85].

IKK inhibitors are another class of NF- $\kappa$ B inhibiting agents. The IKK-inhibitors PS1145 and BMS345541 have shown promising results by inducing cellular apoptosis, inhibiting invasion of PCa cells in vitro, and reducing proliferation in androgen receptor-expressing PCa cell lines [84].

There are currently 3 clinical trials targeting the NF- $\kappa$ B pathway in PCa (Table 1). NCT02757365 is evaluating the efficiency of aspirin in preventing the occurrence of PCa. The rationale for this trial is that inflammation may play an important role in the development of PCa, by unknown mechanisms. Aspirin has already been shown to prevent several inflammation-related tumors. In this study, investigators are exploring the effects of anti-inflammatory therapy on progression from inflammation to PCa and from androgen-dependent PCa to CRPC.

NCT00118092 is a phase II trial that is evaluating the effect of 17-(allylamino)-17-demethoxygeldanamycin, a heat shock protein 90 (HSP90) inhibitor used in the treatment of patients with metastatic PCa who did not respond to previous hormone therapy. The enrollment for this trial was prematurely stopped because of insufficient PSA response [86].

Finally, clinical trial NCT01695473 is testing a potent and highly selective pan-class I PI3K inhibitor (BKM120) that acts downstream of NF- $\kappa$ B. It is being tested in patients with high-risk, localized PCa [87].

#### *ABC Transporters*

CSCs express high levels of ABC transporters that protect CSCs from chemotherapeutic agents, thereby contributing to multidrug resistance [9, 35, 37]. ABCG2 inhibitors make CSCs more susceptible to chemotherapeutic agents, thereby allowing the use of drugs that were previously used unsuccessfully [14, 35, 88].

The first P-gp efflux pump inhibitor was verapamil, a widely used calcium channel blocker [89]. Simultaneous treatment with verapamil and antitumor drugs, such as vincristine, vinblastine, docetaxel, and paclitaxel has shown promising therapeutic effects [35, 89]. Verapamil, shows anti-proliferative action in LNCaP cells through the inhibition of K<sup>+</sup> channels, suggesting that P-gp could be a target for new pharmacological agents directed against PCa cell proliferation [90].

Cyclosporin A (CsA) is also a Pgp inhibitor that has antitumor effects in various cancers including PCa. Lee et

al. [91] demonstrated that CsA might have a survival benefit in metastatic PCa that relapses and progresses to CRPC. It has also been shown that a combination of CsA with EGFR or AKT inhibitors may have clinical application due to its efficiency in inhibiting cancer growth. Another study demonstrated the potential of CsA in inactivating the NFATc1 (nuclear factor of activated T-cells) in hormone-naïve PCa and also in CRPC [92].

The estrogen-receptor modulator tamoxifen has been evaluated in a preliminary phase II study to access its therapeutic efficacy in metastatic PCa patients who have progressed in spite of classical endocrine therapy using LHRH-analogs and/or anti-androgens. Results showed a decrease of greater than 50% in PSA serum levels within the first 2 months of therapy in 20% of the patients [93].

PSC 833 is an immunosuppressive agent that interferes with the function of the multi-drug resistance pump, and consequently plays a key role in chemoresistance. This drug, combined with estramustine, etoposide, ketoconazole, suramin, or vinorelbine, has demonstrated synergistic effects in PCa cell lines [94].

In spite of these successes, many early-phase I/II studies attempting Pgp inhibition using first-generation, non-specific Pgp inhibitors, such as verapamil, CsA, and tamoxifen failed to demonstrate an improvement in overall drug efficacy, primarily due to poor potency. With the development of second-generation agents like PSC833 (valspodar) and VX-710 (biricodar), there has been an increase in potency. However, results remain unpromising [95].

Recent studies using chemotherapeutic drugs in combination with modulators of ABC drug transporters have shown some benefit [89, 96]. As yet, however, there are no clinical trials targeting PCSCs using ABC transporter inhibitors.

#### *Microenvironment*

Normal stem cells reside in a specialized cellular location known as the niche, which provides a microenvironment that maintains a stem-like state. Studies suggest that CSCs also rely on a niche, the CSC niche, which controls self-renewal and differentiation [22, 39, 97, 98]. The CSC niche provides a microenvironment that maintains the balance between quiescence and self-renewal and responds dynamically to physiological requirements essential to homeostasis [22]. This interaction is supported by the fact that loss of the niche environment leads to the loss of CSCs [97].

The tumor microenvironment also protects CSCs from drug-induced apoptosis, and is responsible for drug



resistance [35, 59, 98–100]. The CSC microenvironment is responsible for abnormal signaling pathway activation, including the Wnt, Hh, and TGF- $\beta$  pathways [101]. In addition, the microenvironment is hypothesized to be involved in metastasis by the induction of the EMT, leading to the dissemination and invasion of other tissues [97, 101].

Currently, several novel therapies that disrupt signaling pathways within tumor microenvironments are under investigation. These agents disrupt the “crosstalk” between epithelial cells, stromal cells, and the ECM necessary for PCa progression and metastasis [35, 102].

## Discussion and Conclusion

PCa is a highly prevalent disease and is of biological, clinical, social, and also economic interest, motivating a growing body of translational research. Tumor recurrence, disease progression, and metastasis remain obstacles to improving survival rates in patients with PCa.

Advanced cancer is a major problem in clinical oncology. Progressive, recurrent, and metastatic cancers are clinically challenging. Driven by the need for novel and improved treatment modalities for advanced PCa, the development of therapeutic strategies targeting PCSCs is becoming a major focus of research. The CSC concept of tumorigenesis suggests that therapies specifically targeting CSCs may lead to more effective cancer treatments and this has provided a stimulus for new therapeutic strategies beyond the traditional anti-proliferative agents [33, 103, 104]. This new approach is based on the concept of tumor bulk reduction accompanied by CSC-specific inhibition.

Recent advances in CSC biology have accelerated translational research aimed at depleting CSCs. Targeted therapies are being developed for cell surface markers, signaling pathways, and the CSC cellular microenvironment (niche). Angiogenesis is a cornerstone of the tumor microenvironment and is important in CSC survival and drug resistance.

Preclinical data have demonstrated that targeting pathways involved in CSC homeostasis has meaningful results. Hh, Wnt, Notch, and NF- $\kappa$ B pathway inhibitors inhibit CSC renewal, suppress pluripotency of stem cell factors (Oct4, Sox2, c-Myc, and Nanog), and inhibit tumor growth both in vivo and ex vivo.

These exciting preclinical results have led to the development of clinical trials targeting these specific pathways. A majority of ongoing clinical trials are

phase II studies. Together, these studies address all stages of PCa disease (localized, high-risk, hormone-refractory, and metastatic) as well as disease prevention (aspirin).

Currently, there are active clinical trials addressing all the pathways mentioned in this manuscript. Wnt pathway inhibitors (Foxy-5 and OMP-54F28) are being tested in patients with metastatic disease for whom all other therapies have been ineffective (phase I studies). This makes intuitive sense given that the Wnt pathway is involved in androgen-independent mechanisms and bone metastasis. Hh pathway inhibitors have received more attention from clinicians and industry with a significant number of ongoing clinical trials. This may be justified, given the promising results obtained with vismodegib in basal cell carcinoma and other cancers. In PCa, vismodegib is being studied as a neoadjuvant for localized disease as well as in metastatic disease.

Studies using aspirin and itraconazole, as inhibitors of the Hh and NF- $\kappa$ B pathways, respectively, represent an attempt to harness known drugs with few side effects. In these studies, the Hh pathway has been targeted using itraconazole alone or in combination with orteronol. The use of orteronol to block male sex hormone production was hypothesized to increase reliance on the Hh pathway, thus creating a synergistic effect. Unfortunately, this study was closed prior to enrollment. The single clinical trial targeting Notch signaling was also terminated early.

These pathways have been shown to be important in PCa, promoting tumor growth through CSC proliferation, creating a favorable microenvironment for tumor growth and metastasis, and contributing to therapy resistance.

The targeting of PCSCs via the Wnt, Hh, Notch, NF- $\kappa$ B pathways as well as the targeting of the CSC niche is an exciting area of research and may become an important addition to traditional treatment regimens. Although the clinical results have not yet lived up to pre-clinical hopes, there are still many avenues to be explored. Targeting PCSCs in multidrug combined regimens will shift the paradigm of PCa treatment and might be a breakthrough in the treatment of both early and progressive disease.

## Disclosure Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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