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Extracting the Benefit of Nexrutine® for Cancer Prevention

Suleman S. Hussain[1,2], **Darpan Patel**[4,5], **Rita Ghosh**[1,2,3,4], and **Addanki P. Kumar**[1,2,3,4,6,*]

[1]Department of Urology, The University of Texas Health Science Center, San Antonio, TX

[2]Department of Pharmacology, The University of Texas Health Science Center, San Antonio, TX

[3]Department of Molecular Medicine, The University of Texas Health Science Center, San Antonio, TX

[4]Cancer Therapy and Research Center, The University of Texas Health Science Center, San Antonio, TX

[5]School of Medicine, Health Rest & Care System Management, School of Nursing, The University of Texas Health Science Center, San Antonio, TX

[6]South Texas Veterans Health Care System, San Antonio, TX

Abstract

The current standard of care for prostate cancer includes hormone therapy, radiation therapy and radical prostatectomy, each with its own set of undesirable side effects. In this regard there is an unmet need to develop strategies that can prevent or delay the development of clinical prostate cancer. One potential area involves the use of natural compounds involving botanicals. Along these lines we have found that Nexrutine®, a dietary supplement derived from *Phellodendron amurense* bark extract, has prostate cancer prevention activity. The “extract” nature of this botanical, which constitutes a blend of several active protoberberine alkaloids, allows it to target several pathways deregulated in prostate cancer simultaneously. In this review, we will emphasize the prospective translational benefit of Nexrutine® as a chemopreventive agent for prostate cancer management. The potential of Nexrutine® was first identified and has subsequently been most exhaustively studied with reference to prostate cancer. Therefore the focus of this review is on the use of Nexrutine® in prostate cancer. In addition we have summarized the emerging evidence regarding the use of Nexrutine® in other tumor models to demonstrate the potential benefits of Nexrutine®.

Keywords

Botanicals; Chemoprevention; Herbal extracts; Natural products; Nexrutine® Prostate cancer

Address for correspondence Addanki P Kumar, PhD, University of Texas Health, Science Center, 8403 Floyd Curl Drive, San Antonio, TX 78229, Tel: 210-562-4116, Fax: 210-562-4133, kumara3@uthscsa.edu.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

Suleman S. Hussain, Darpan Patel, Rita Ghosh and Addanki P. Kumar declare that they have no conflict of interest.

Introduction

Prostate cancer (PCA) is the second leading cause of cancer-related deaths in American men (1). Early localized disease has a 5-year survival rate of almost 100 percent, with a myriad of treatment approaches such as active surveillance, radiation therapy, hormone therapy and radical prostatectomy (1, 2). Unfortunately these strategies are associated with several undesirable side effects and are limited by progression to metastatic castration-resistant prostate cancer (CRPC) (3, 4). Notably, the FDA recently approved 6 new drugs, which improve overall survival or bone metastasis-free survival for CRPC patients (Table 1) (5). However, the survival benefit is limited with a suggested reactivation of the androgen receptor (AR) axis (6–8). Poor quality of life for patients undergoing these treatments underscores the need to find alternative strategies that can improve quality of life and or prevent the development of PCA in the first place.

A long latency is involved in the development of PCA including proliferative inflammatory atrophy (PIA), low and high-grade prostatic intraepithelial neoplasia (PIN) that finally culminates into clinically significant PCA (9). Recent advances in technologies that detect alterations (next gen sequencing, transcriptome sequencing) in various cancer causing pathways, have improved our understanding of the molecular mechanisms involved in PCA (10). This sets the stage to use the ‘long latent development period’ to test preventive agents using mechanism-based markers.

Prostate Cancer Chemoprevention

The emergence of the field of cancer chemoprevention reiterates the old proverbial saying, “Prevention is better than cure”. Sporn *et al.* originally defined chemoprevention as the application of natural, synthetic or biological modalities to prevent, contain or reverse the initiation of carcinogenesis or progression of localized cancer to metastatic disease (11). The recent addition of a ‘delay’ in initiation or progression has added an extra dimension to this definition (12). Chemoprevention can be generally classified into three categories depending on the stage of cancer when the intervention begins. Primary chemoprevention refers to the use of chemopreventive agent to healthy and high risk population; secondary chemoprevention is used to prevent or delay progression of premalignant lesions to invasive cancer, while tertiary chemoprevention targets tumor recurrence and metastasis for patients undergoing successful treatment of local disease (12, 13). The FDA approval of 10 drugs for cancer risk reduction including tamoxifen, raloxifene for breast cancer and HPV vaccines for cervical cancer signifies the rising surge of cancer prevention (14).

The untapped potential for PCA prevention led to large-scale clinical trials using 5α-reductase inhibitors. Prostate Cancer Prevention Trial (PCPT) and Reduction by Dutasteride of Prostate Cancer Events (REDUCE) were randomized placebo-controlled trials using the 5α-reductase inhibitor finasteride and dutasteride respectively (15, 16). The PCPT was a large-scale trial with 18,882 men, but the final analysis included only 9060 men due to early study termination and men declining the end of study biopsies (16). It is also important to note the higher rates of non-adherence (14.7 % vs. 10.8 %) and increased sexual functions in the finasteride group (16). A drawback of the study design was the lack of baseline

determination of 5α-reductase levels, which may have affected the treatment outcome. Recently, an 18-years follow-up of the PCPT trial showed that use of finasteride for a period of about 7 years had no significant difference in overall survival compared to placebo, further questioning potential use of finasteride in the clinic (17). While the finasteride trial showed that it could prevent lower grade cancer, it also identified high-grade tumors (Gleason 8–10) in the treatment group (16). Subsequently it has been suggested that finasteride helps in the detection of these high-grade tumors (18, 19). However, it does not fulfill the premise on which cancer chemoprevention as discussed above is based.

Dietary supplements as prospective chemopreventives

Diet is a modifiable risk factor, which can impact the progression of indolent disease to clinically significant PCA (20, 21). Epidemiological studies have suggested that the incidence of PCA is much lower in Asian populations consuming phytonutrients-rich diet compared to their western counterparts (22). Further, increased cancer prevalence in Asian populations that have migrated to the west, underscores the importance of diet, lifestyle and environmental factors in increased risk of PCA (22–24). Interestingly, cancer incidence data from Surveillance, Epidemiology, End Results (SEER) registries showed that PCA was the most common malignancy in a majority of Asian American men in the United States (25). In this regard, dietary supplements such as lycopene, selenium, vitamins, soy isoflavones, green tea polyphenols, and silibinin are some of the phytoconstituents tested in various preclinical and clinical settings for their chemopreventive capabilities in PCA (20, 26–28). The Selenium and Vitamin E Cancer Prevention Trial (SELECT) which randomly assigned 35,533 men to selenium, Vitamin E, selenium and Vitamin E or placebo groups was concluded after the 7-year interim analysis because of lack of benefit in PCA risk reduction (29). Selenium was administered in the form of selenomethionine in the SELECT trial, although the smaller Nutritional Prevention of Cancer (NPC) trial which showed chemopreventive potential of selenium used selenized yeast containing methyl selenocysteine suggesting the value of choice of selenium that maybe beneficial (30). Further, the variability in the endogenous levels of selenium in the study population could have affected the outcome. An increase in PCA incidence was noted in the Vitamin E arm, which may be due to the higher dose of Vitamin E used in SELECT compared to earlier trials (29). Interestingly, two recently published follow-up studies of the SELECT and PCPT trials have suggested that circulating Vitamin D can prevent clinically relevant PCA (31, 32). However, this effect was limited only to the African-American population in the SELECT trial with other men showing increased PCA risk. As we learn these lessons there is a general need to design more effective chemoprevention trials with careful consideration given to study design, formulation, dosage and patient selection criteria.

Phytoceuticals (plant derived chemicals) are a rich source of number of FDA approved drugs. Strikingly, approximately 50% of FDA approved drugs for cancer are natural products or their derivatives, which includes the taxanes and vinca alkaloids widely used in current cancer therapy (33). Herbal extracts used in Traditional Chinese medicine (TCM) and Ayurvedic medicine are anecdotal for treatment of various pathological conditions including cancer (34, 35). Although these extracts are under explored they provide the starting advantage of having minimal systemic toxicity. In addition to potential use as

chemopreventives they may also be beneficial in reducing the dose of current toxic treatments and delay therapeutic resistance.

Nexrutine®

Nexrutine® is an inexpensive over the counter dietary supplement used to relieve joint pain. It is derived from *Phellodendron amurense*, more commonly known as the cork tree, which is native to Asia and belongs to family Rutaceae (36, 37). Isoquinoline alkaloids like berberine, palmatine, phellodendrine, jatrrorhizine and magnoflorine and the liminoid, limonin (Figure 1A) are considered as active components of this extract that exhibit biological activity (36, 38, 39). In traditional Chinese medicine, the bark extract is referred to as 'Huang Bai' and has been used for centuries to treat inflammatory conditions including psoriasis, gastroenteritis, abdominal pain, and diarrhea and also used as an anti-bacterial (36, 40). It has also been shown to be useful for symptoms of osteoarthritis and shows potential as a neuroprotective agent in Alzheimer's disease (40, 41). Relora® (Next Pharmaceuticals, Salinas CA) is a proprietary formulation of *Phellodendron amurense* bark extract standardized to berberine and is believed to reduce stress and anxiety (42). Using activity-guided fractionation of Nexrutine®, we found that butanol fraction (F3) recapitulates the anti-proliferative and NF-κB inhibitory activity of the extract. Further, ultra-performance liquid-chromatography tandem mass spectrometry (UPLC–MS/MS) with multi reaction monitoring (MRM) analysis identified berberine and palmatine as the active constituents present in F3 (43).

In vitro evaluation in PCA: One extract, many properties

Compared with other natural compounds, the use of Nexrutine® is relatively unexplored in the arena of chemoprevention. However, in the past decade the number of reports demonstrating anti-cancer properties of Nexrutine® has been on the rise with reports of its testing in melanoma, multiple myeloma, prostate, pancreatic, breast and nonmelanoma skin cancer (37, 43–50). Studies from our laboratory first discovered Nexrutine® has anti-proliferative properties against PCA cell lines irrespective of their androgen dependence status (37). Subsequent studies showed that Nexrutine® also inhibited invasion and anchorage independent growth of androgen independent PCA cell lines (37, 47). Mechanistic investigations revealed that Nexrutine® exerts its biological effects by modulating key cell-survival pathways such as PI3K/AKT and STAT3/NF-κB signaling leading to apoptosis (37, 43,44, 48, 50). Introduction of a constitutively-active form of AKT blocked the anti-proliferative effect of Nexrutine® in PCA cells, implying PI3K/AKT pathway as a target of Nexrutine® (37). Similarly, Nexrutine® decreased phosphorylation and DNA binding activity of CREB, a transcription factor downstream of PI3K/AKT signaling and elevated in high-Gleason grade human prostate tumors (37, 48). CREB transcriptionally regulates expression of a plethora of genes involved in various cellular processes including cell proliferation, survival, apoptosis, inflammation, invasion and metastasis (51). Nexrutine® was shown to inhibit NF-κB reporter and DNA binding activity in androgen independent PC-3 cells (43). Treatment with Nexrutine® reduced levels of Cyclin D1 (cell cycle), and COX-2 (inflammatory mediator) (44, 48). It is noteworthy to mention that, an integrated oncogenomic analysis of prostate tumors revealed that the

PI3K/AKT pathway was deregulated in 42% of primary tumors and almost 100% of metastatic tumors (52). Alterations in *PTEN*, *PIK3CA*, *PHLPP*, and *INPP4B* genes are associated with poor prognosis and progression to metastatic. CRPC. Further, there is reciprocal cross-talk between PI3K and AR signaling; whereby inhibiting AR with anti-androgens causes increased PI3K/AKT signaling (53). Similarly, combined inhibition of AKT and AR delays progression to castrate resistant disease, underscoring the advantage of targeting the PI3K/AKT signaling in PCA (54). PI3K/AKT signaling mediated activation of NF-κB was shown to mediate PCA cell proliferation (55). Strikingly, nuclear expression of NF-κB is found to correlate with biochemical recurrence in PCA patients (56). Therefore, ability of Nexrutine® to modulate multiple critical deregulated signaling pathways will have enormous therapeutic benefit.

Preclinical Evaluation

Chemopreventive potential of Nexrutine® was evaluated in preclinical animal models (43, 44,46, 47, 50). Intervention with Nexrutine® not only prevented development of early-stage prostate tumors but also metastatic lesions in transgenic adenocarcinoma of mouse prostate (TRAMP) mice (47). Remarkably, intervention with Nexrutine® resulted in statistically significant increase in bone mineral density (BMD) in these animals (47). Bone is the most frequent site of metastasis in human PCA patients and is associated with increased pain and skeletal complications (57). Validation of these observations in large-scale studies will have significant impact to improve the quality of life for these patients. In addition, Nexrutine®-mediated *in vivo* effects were shown to be associated with decreased levels of Cyclin D1, AKT/CREB and NF-κB activation (43, 44).

From bench to bedside

Radiation therapy (RT) and radical prostatectomy are the common modes of treatment for localized PCA but are associated with several side effects with biochemical recurrence in the high and intermediate risk groups (58). Interestingly, Nexrutine® supplementation prior to RT inhibited progression of prostate tumors to poorly differentiated stage in TRAMP mice with no prominent toxicity (Hussain *et al*., unpublished data). Low dose radiation combined with Nexrutine® showed similar inhibition of surviving fraction as high dose radiation in androgen independent PC-3 cells (Hussain *et al*., unpublished data). Encouraged by the pre-clinical efficacy of Nexrutine®, Swanson *et al.* tested whether the supplementation of Nexrutine® would benefit PCA patients undergoing prostatectomy or RT. The 6–8 weeks period after diagnosis and before beginning treatment was effectively used to administer Nexrutine® to PCA patients with either Gleason score >6 or Prostate Specific Antigen (PSA) >10ng/ml. 9 patients receiving RT and 12 patients undergoing surgery were enrolled in this trial. Indeed, oral administration of Nexrutine® (500 mg *tid*) one to two months prior to radiation/surgery or with radiation decreased PSA in 81 % of patients with no signs of grade 3 toxicity (59). The trial also established the safety of Nexrutine®. This was the first clinical study, which tested the tolerance and efficacy of Nexrutine® in cancer patients. These studies have strengthened the potential use of Nexrutine® in combination with existing therapy to maximize clinical benefits for patients. Along these lines, given that reactivation of (AR) signaling is critical in the development of CRPC, studies to establish

the potential of Nexrutine® alone and in combination with FDA approved androgen antagonists and androgen synthesis inhibitors are warranted (60). Given that androgen deprivation therapy (ADT) is an important treatment component of the armamentarium to treat patients with intermediate-to-high risk disease as well as to treat those men with recurrent PCA, use of non toxic alternatives will have substantial benefit in delaying the progression (2).

Antitumorigenic effects of Nexrutine® in other tumor models

Recent studies from our lab unraveled the benefits of Nexrutine® against pancreatic cancer (49, 50). Similarly, Nexrutine® decreased both NF-κB and STAT3 levels and transcriptional activity in pancreatic cancer cell lines (50). Disrupting the cross talk between NF-κB and STAT3, ensured inhibition of the feedback loop. Further, Nexrutine® was shown to inhibit NF-κB mediated transactivation of COX-2, which resulted in decreased expression of COX-2 (50). Inhibition of STAT3 reduced the elevated levels of ROS and autophagy (a survival mechanism) in pancreatic cancer cells (49). In a COX-2 over-expressing BK5–COX-2 preclinical model of pancreatic cancer, Nexrutine® intervention reduced fibrosis (50). Fibrosis or desmoplasia produced through tumor-stromal interactions impedes drug delivery leading to therapeutic resistance (61). Along these lines, Nexrutine®-mediated disruption of pancreatic desmoplasia makes it a particularly attractive adjuvant for conventional therapy.

Further Nexrutine® inhibited the survival of several melanoma cell lines by modulating their oxidative stress levels (Hambright *et al.*, Oncotarget, in press). Supporting studies from other groups have demonstrated the chemopreventive potential in breast and skin cancer (45, 46). Interestingly, decreased cell viability and increased apoptosis in SkBr3 cells and induction of autophagy in MDA-MB231 ER negative breast cancer cells were reported (45). Nexrutine® also decreased COX-2 and PPARγ in breast cancer cells (45). Nexrutine® had negligible cytotoxic effect on primary murine keratinocytes (46). Using well established mouse two-stage carcinogenesis model, Kumar *et al.* showed reduced tumor incidence and associated decrease in the levels of COX-2 and NF-κB (46). In addition, Nexrutine® exhibited anti-tumorigenic activities in a multiple myeloma (MM) cells *in vitro* and in a preclinical model *in vivo*. Nexrutine® exposure reduced cell viability through apoptosis and inhibition of mTOR activation in murine 5TGM1 and human RPMI 8226 MM cells (62). Furthermore, Nexrutine® administration reduced overall tumor burden in a MM preclinical mouse model (62). That study also suggested potential for combining Nexrutine® with autophagy inhibitors for enhanced therapeutic benefit. Taken together, these preclinical observations suggest the potential utility of Nexrutine® as a secondary and/or tertiary chemopreventive agent for management of not only prostate but also other inflammation-associated malignancies.

Conclusions, future directions and challenges

In summary, Nexrutine® has been observed to have positive impact in inhibiting carcinogenesis pathways and biological processes involved not only in solid tumors but also in hematological malignancies (Figure 1B). These benefits have been reported to be

potentially through both autophagy and apoptosis, perhaps in a cell type contextual manner as well as due to modulation of key inflammatory signaling pathways. It is well established that chronic inflammation and associated COX-2 overexpression is an early event in the pathogenesis of “inflammation-related” cancers (63). COX-2 is transcriptionally regulated by STAT3, NF-κB and CREB; therefore, it is possible that Nexrutine® suppresses COX-2 by down regulation of these transcription factors in tumor cells (64, 65). Suppression of this signaling not only inhibits tumor cell growth it could potentially sensitize cancer cells to conventional treatment. However, such concepts have not been tested. Furthermore, although epidemiological studies showed reduced cancer risk in people who regularly take NSAIDs, its long-term use has been associated with gastrointestinal or cardiovascular side effects (66, 67). In this scenario Nexrutine® could provide an alternative strategy for its anti-inflammatory use. Imbalance of pro- and anti-inflammatory cytokines and increased oxidative stress is associated with negative energy balance in which energy consumed (caloric intake) is greater than the energy expended (caloric expenditure) (68).This negative energy contributes to obesity, one of the risk factors for number of malignancies including PCA (69). Accordingly, given the ability of Nexrutine® to modulate inflammatory molecules, its preventative benefits in modulating obesity possibly as an exercise mimetic is an interesting hypothesis to test. In addition, the potential of Nexrutine® to alter adverse effects associated with cancer and cancer treatment including fatigue also need to be evaluated. However, further research involving long-term pharmacokinetic studies to establish the toxicity profile of Nexrutine® and biomarker-driven trials are warranted to determine the full beneficial impact of Nexrutine®.

Even though herbal extracts have shown promising results in preclinical models, the transition from ‘bench to bedside’ is hindered by their lack of bioavailability, difficulties in setting quality control parameters and more importantly the varied effect observed during different stages of disease or at different doses. In the case of Nexrutine® we used the solid support matrix in UPLC, which can cause adsorptive sample loss, thus limiting the fractionation of natural extracts containing diverse bioactive molecules. Hence, use of a high throughput counter current chromatography (CCC) fractionation method that excludes the solid support matrix can help elaborate the composition of Nexrutine® (70, 71). Quantitative composition activity relationship (QCAR), a newly identified approach based on algorithms to correlate the chemical composition of an extract to its biological activity could help in designing the quality control and standardization protocols for Nexrutine® thus alleviating some concerns of variation due to its natural origin (72, 73).

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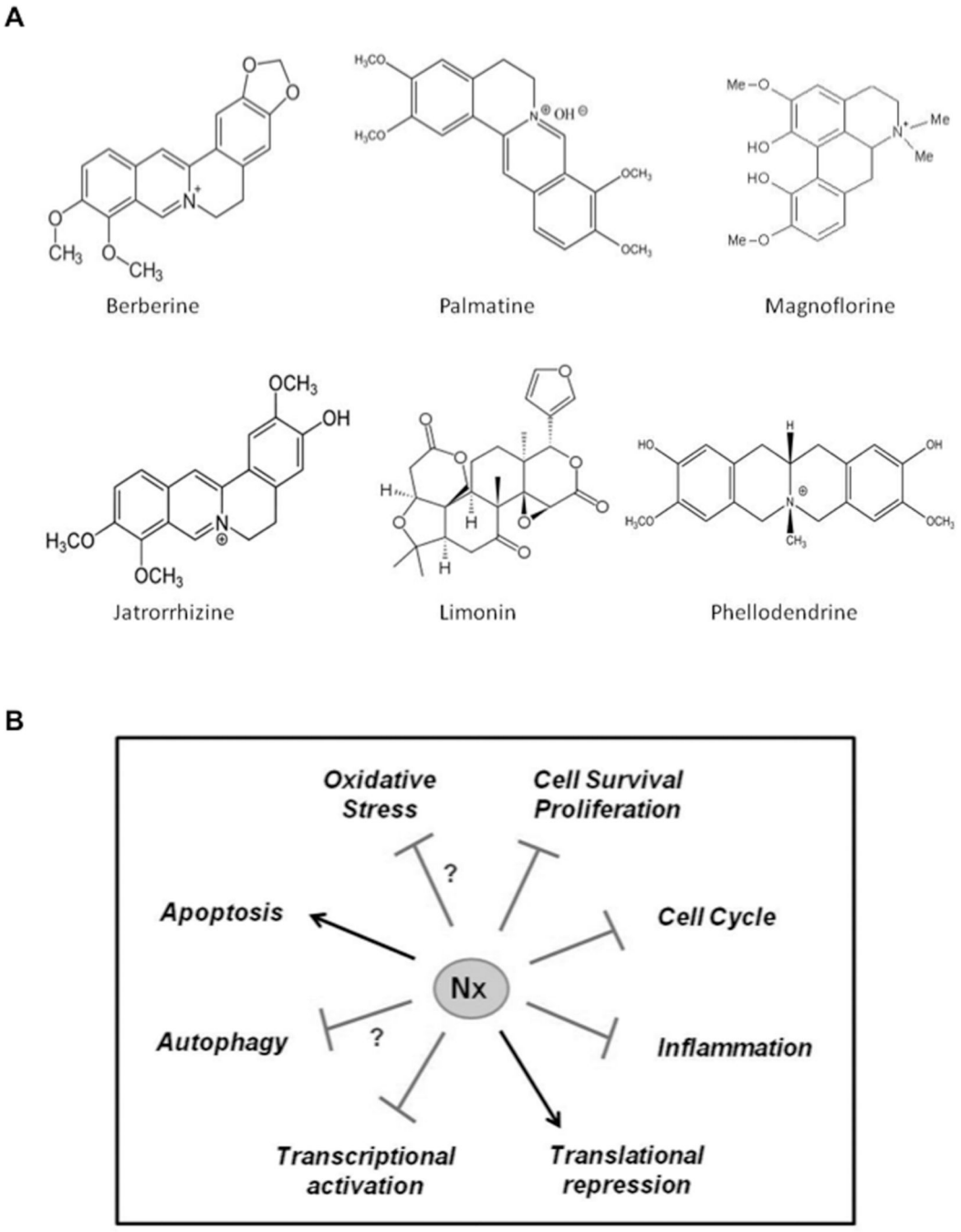

Figure 1.

A) Major phytoconstituents of *Phellodendron amurense* bark extract.

B) Model depicting the molecular mechanism of action of Nx based on current data.

Table 1

Change in treatment landscape for CRPC in the past four years. Adapted from www.cancer.gov and Yin *et al.*, *IJMS* (2012).

Category	Drug Name	Approved
Androgen Receptor Antagonist	Enzalutamide	Aug, 2012
Cyp17 Inhibitor	Abiraterone	Dec, 2012
Microtubule inhibitor	Cabazitaxel	June, 2010
Vaccine against PAP antigen	Sipuleucel-T	Aug, 2010
RANKL Antibody	Denosumab	Sept, 2011
Radium 223- Ca^{2+} mimetic	Alpharadin	May, 2013