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(54) Title: METHOD FOR TREATING PROSTATE CANCER

(57) Abstract: The invention provides a method for treating prostate cancer in a subject comprising administering to the subject an effective amount of a selective inhibitor of one or more of CDK8 and CDK19. In some embodiments the inhibitor inhibits CDK19. In some embodiments, the inhibitor inhibits CDK8 at a Kd of lower than 200 nM and/or inhibits CDK19 at a Kd of lower than 100 nM. In some embodiments, the prostate cancer is androgen independent. In some embodiments, the prostate cancer is androgen independent due to one or more of androgen receptor gene amplification, androgen receptor gene mutation, ligand-independent transactivation of androgen receptor and activation of intracellular androgen synthesis. In some embodiments, the inhibitor inhibits increased activity of NF-κB. In some embodiments, the inhibitor does not inhibit increased basal levels of NF-κB. In some embodiments, inhibition of one or more genes by AR is not inhibited.

METHOD FOR TREATING PROSTATE CANCER

BACKGROUND OF THE INVENTION

Field of the invention

The invention relates to the treatment of cancer. More particularly, the invention relates to the treatment of prostate cancer.

Summary of the related art

As the most common malignancy in US males, prostate cancer remains a challenging disease. In contrast to other human cancers, it is exquisitely dependent on androgenic steroids that exert their biological effects through the androgen receptor (AR)[1, 2].

The classical model for AR activation involves a conformational change induced by ligand binding, enhanced nuclear translocation, and binding to the androgen-responsive elements in the proximal promoters or distal enhancers of target genes to regulate transcription[3]. AR-regulated genes are essential for prostate tumor cell growth, invasion and metastasis[2, 3]. More importantly, recent studies indicate that AR binding dynamics to chromatin vary in prostate cancer cells, depending on cellular context, producing different effects on gene expression in different cases[4-6]. Therefore, it is critically important to fully understand the molecular mechanisms of AR-mediated transcription, especially those that can be targeted by new drugs.

The first line treatments for patients with advanced prostate cancer are androgen–deprivation therapies that suppress the AR signaling by either inhibiting the androgen-synthetic pathway or antagonizing AR function[2]. Despite strong responses to androgen–deprivation therapies, patients often relapse with a more aggressive, therapy-resistant form of the disease referred to as castration refractory prostate cancer (CRPC)[7, 8]. Recent studies showed that most of CRPC tumor cells continue to utilize their endogenous androgen signaling system to drive their growth through restoration of AR function[9-11]. The mechanisms of AR reactivation include AR gene amplification, ligand-independent transactivation of AR, or activation of intracellular androgen synthesis[12-14]. Novel anti-androgen therapeutic agents are being developed to treat CRPC, including a new potent testosterone-synthesis inhibitor (abiraterone)[15, 16] and a high-affinity anti-AR drug (MDV-3100, a.k.a. enzalutamide)[17, 18]. Although clinical studies showed that these drugs confer survival advantage[13, 19-21], the CRPC still remains far from being cured and requires new effective treatments after the acquisition of resistance to these drugs. All the

existing methods for blocking androgen signaling rely on inhibiting the production of the ligand or the ligand-receptor association, which can be overcome in cancers by multiple mechanisms of AR reactivation. Several novel anti-AR drugs have recently been developed to block the AR signaling by inducing AR protein degradation[22-24]. Recent studies have indicated, however, that AR not only induces certain cancer-promoting genes but also represses other genes that are involved in androgen synthesis, DNA synthesis and proliferation[25]. Activation of the latter genes by blocking all the effects of AR or by inducing AR degradation may stimulate the transition of PCa cells from an androgen-dependent (AD) to an androgen-independent (AI) state.

There is therefore, a need to develop a strategy targeting other molecules that potentiate AR-mediated transcription to block the hyperactive androgen signaling and to extend the effectiveness of hormone therapies in prostate cancer patients. In particular, there is a need to develop a strategy for inhibiting AR-mediated induction of transcription but not the repression of transcription by AR.

BRIEF SUMMARY OF THE INVENTION

The invention provides a new strategy targeting other molecules that potentiate AR-mediated transcription to block the hyperactive androgen signaling and to extend the effectiveness of hormone therapies in prostate cancer patients. The instant inventors have surprisingly discovered a novel method for inhibition of AR signaling that functions independently of ligand-AR interaction, and which is based on the inhibition of two closely related transcription-regulating serine/threonine kinases, CDK8 and CDK19.

In contrast to better-known members of the CDK family[26], the closely related CDK8 and CDK19 regulate transcription but not cell cycle progression, and their depletion does not inhibit the growth of normal cells[27] or many tumor cells[28, 29]. CDK8 and CDK19 are the two isoforms of a component of the transcription-regulating Mediator complex[30] but can also act outside of the Mediator[31, 32]. Early studies depicted CDK8 as a transcriptional co-repressor based on its negative regulation of the general transcription initiation factor IIH[33] and a group of transcriptional activators[34]. However, a series of recent reports demonstrated that CDK8 serves as a positive transcription regulator in multiple signaling pathways with biomedical relevance, including the p53 pathway[35], Wnt/β-catenin pathway[36], the serum response network[28], the TGFβ signaling pathway[35], as well as Thyroid hormone Receptor[37] and Sterol-Regulatory Element Binding Protein[38]-dependent transcription. In regard to cancer, CDK8 has been recognized as an oncogene in melanoma and colorectal cancers[36, 39] and it was recently implicated in the cancer stem cell phenotype[40]. In contrast to CDK8, its vertebrate paralog CDK19 has been poorly studied because it is not expressed as highly as CDK8 in most tissues. However, CDK19 is expressed in normal prostate[41]. High CDK8 and CDK19 expression levels were also found to be predictive markers of poor relapse-free survival in breast cancers and in platinum-treated ovarian cancers[29]. Furthermore, CDK8 was shown to be a mediator of damage-induced tumor-promoting paracrine activities of normal tissues, colon carcinoma and fibrosarcoma cells[29]. However, there was no prior evidence linking CDK8 or CDK19 with AR activity or androgen-independent growth of prostate cancers.

The invention provides a method for treating prostate cancer in a subject comprising administering to the subject an effective amount of a selective inhibitor of one or more of CDK8 and CDK19. In some embodiments the inhibitor inhibits CDK19. In some embodiments, the

inhibitor inhibits CDK8 at a Kd of lower than 200 nM and/or inhibits CDK19 at a Kd of lower than 100 nM.

In some embodiments, the prostate cancer is androgen independent. In some embodiments, the prostate cancer is androgen independent due to one or more of androgen receptor gene amplification, androgen receptor gene mutation, ligand-independent transactivation of androgen receptor and activation of intracellular androgen synthesis.

In some embodiments, the inhibitor inhibits increased activity of NF-κB. In some embodiments, the inhibitor does not inhibit increased basal levels of NF-κB.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows that CDK19 protein is expressed at higher levels in AR-positive (LNCaP, LN3, C42, CWR22rv1) prostate cancer cells compared to AR-negative (DU145 and PC3) prostate cancer cell lines, or to fibrosarcoma (HT1080), human embryonic kidney (HEK293) and colon carcinoma (HCT116) cells.

Figure 1B shows that CDK19 protein is expressed at higher levels in in AR-positive (LNCaP, LN3, C42, CWR22rv1) prostate cancer cells compared to AR-negative (PC3) prostate cancer cells, non-malignant prostate epithelial cells (RWPE-1), fibrosarcoma (HT1080), human embryonic kidney (HEK293) and colon carcinoma (HCT116) cells.

Figure 1C shows that CDK19 RNA is expressed at higher levels in in AR-positive (LNCaP, LN3, C42, CWR22rv1) prostate cancer cells compared to AR-negative (PC3) prostate cancer cells, non-malignant prostate epithelial cells (RWPE-1), fibrosarcoma (HT1080), human embryonic kidney (HEK293) and colon carcinoma (HCT116) cells.

Figure 1D shows that androgen treatment down-regulates CDK8 protein, whereas androgen depletion up-regulates CDK19 and CDK8 protein expression in LNCaP cells.

Figure 2A shows that treatment of androgen-dependent LNCaP cells with Senexin A significantly inhibits androgen-stimulated transcriptional activation of several androgen-responsive genes such as PSA (KLK3), KLK2, TMPRSS2 and PGC under either androgen-supplemented or androgen-deprived conditions.

Figure 2B shows that treatment of androgen-dependent LNCaP cells with Senexin B significantly inhibits androgen-stimulated transcriptional activation of several androgen-inducible genes such as PSA (KLK3), KLK2, SGK1, KLF5 and PGC under androgen-supplemented conditions.

Figure 2C shows that treatment of androgen-dependent LNCaP cells with Senexin B does not interfere with the inhibition of several androgen-inhibited genes such as AR, OPRK1, STXBP6 and CDK8.

Figure 3 shows that pretreatment of androgen-deprived LNCaP cells by Senexin B (at 1 μM and 4 μM) for one hour significantly inhibits androgen-stimulated transcription of several androgen-responsive genes such as PSA (KLK3), KLK2, TMPRSS2 and PGC.

Figure 4A shows that in HEK293 cells that express both CDK8 and CDK19 and overexpress full-length wild-type AR, Senexin A (1 μM and 5 μM) significantly inhibits the activation of an androgen-responsive construct (firefly luciferase reporter under PSA gene promoter) in the presence of R1881 but not in androgen-free media.

Figure 4B shows that in HEK293 cells that express both CDK8 and CDK19 and overexpress full-length wild-type AR, Senexin A (1 μM and 5 μM) significantly inhibits the activation of another androgen-responsive construct (firefly luciferase reporter under PGC gene promoter) in the presence of R1881 but not in androgen-free media.

Figure 5 shows that in HEK293 cells that express both CDK8 and CDK19 and overexpress full-length wild-type AR, Senexin B significantly inhibits the activation of an androgen-responsive construct (firefly luciferase reporter under PSA gene promoter) in the presence of R1881 but not in androgen-free media.

Figure 6A shows that LNCaP derivatives LN3 and C4-2 and CWR22 derivative CWR22rv1 androgen-independent prostate cancer cells grow well under androgen-depleted conditions (in CSS media), but this androgen-independent growth was strongly inhibited by Senexin B.

Figure 6B shows that 5 μM Senexin B strongly inhibits the growth of LNCaP derivative LN3 and significantly inhibits the growth of CWR22 derivative CWR22rv1 androgen-independent prostate cancer cells under androgen-depleted conditions (in CSS media), and that 10 μM MDV3100 (enzalutamide) weakly inhibits the growth of LNCaP-LN3 cells and does not inhibit the growth of CWR22rv1 cells under the same androgen-depleted conditions.

Figure 6C shows that LNCaP derivatives LN3 and C4-2 and CWR22 derivative CWR22rv1 androgen-independent prostate cancer cells highly express AR-dependent genes, PSA and KLK2 compared to the androgen-dependent parental LNCaP cells after 3-day androgen

deprivation (AD3), and that Senexin B down-regulates the expression of PSA and KLK2 in all three androgen-independent-prostate cancer cell lines grown in the absence of androgen.

Figure 6D shows that 5 μM Senexin B strongly down-regulates the expression of PSA and KLK2 in LNCaP-LN3 and CWR22rv1 androgen-independent-prostate cancer cell lines grown in the absence of androgen, whereas 10 μM MDV3100 (enzalutamide) weakly down-regulates the expression of PSA and does not down-regulate the expression of KLK2 in LNCaP-LN3 cells and does not down-regulate the expression of either PSA or KLK2 in CWR22rv1 cells under the same androgen-depleted conditions.

Figure 7 shows that the growth of PC-3 cells in androgen-depleted CSS media is inhibited by Senexin B.

Figure 8 shows effects of Senexin B treatment on the tumor volume growth curve of LN3 xenografts in nude mice.

Figure 9 shows effects of Senexin B treatment on mouse body weights.

Figure 10 shows effects of Senexin B treatment on final tumor weights.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides a method for treating prostate cancer in a subject comprising administering to the subject an effective amount of a selective inhibitor of one or more of CDK8 and CDK19. In some embodiments the inhibitor inhibits CDK19. In some embodiments, the inhibitor inhibits CDK8 at a Kd of lower than 200 nM and/or inhibits CDK19 at a Kd of lower than 100 nM. For purposes of the invention, "specific inhibitors of CDK8/19" are small molecule compounds that inhibit CDK8 or CDK8 and CDK19 to a greater extent than they inhibit certain other CDKs. In some embodiments, such compounds further inhibit CDK8 to a greater extent than CDK9. In preferred embodiments, such greater extent is at least 2-fold more than CDK9. Compounds that are useful in the invention are described in co-pending US Patent Publications 20120071477 and 20120071477 and PCT Publication WO2013/116786. Extent of inhibition is measured by the assays taught in co-pending PCT Publication WO2013/116786.

In some embodiments, the prostate cancer is androgen independent. In some embodiments, the prostate cancer is androgen independent due to one or more of androgen receptor gene amplification, androgen receptor gene mutation, ligand-independent transactivation of androgen receptor and activation of intracellular androgen synthesis.

In some embodiments, the inhibitor inhibits induced activity of NF-κB. In some embodiments, the inhibitor does not inhibit increased basal levels of NF-κB. The term "induced NFκB transcriptional activity" means that the transcriptional function performed by NFκB is performed at greater than basal NFκB transcriptional activity level. The term "basal NFκB transcriptional activity" means the level of transcriptional function performed by NFκB in a cell under normal conditions, i.e., in the absence of the disease or disorder. In some embodiments, the amount of active NFκB in the nucleus of the cells is not increased, but rather only the level of NFκB activity is increased.

The term "treating" means reducing or eliminating at least some of the signs or symptoms of the disease. The term "subject" includes a human. The terms "administering", "administration" and the like are further discussed below.

In some embodiments, a compound according to the invention is administered as a pharmaceutical formulation including a physiologically acceptable carrier. The term "physiologically acceptable" generally refers to a material that does not interfere with the effectiveness of the compound and that is compatible with the health of the subject. The term

"carrier" encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microspheres, liposomal encapsulation, or other material well known in the art for use in physiologically acceptable formulations. It will be understood that the characteristics of the carrier, excipient, or diluent will depend on the route of administration for a particular application. The preparation of physiologically acceptable formulations containing these materials is described in, e.g., Remington's Pharmaceutical Sciences, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, Pa., 1990. The active compound is included in the physiologically acceptable carrier or diluent in an amount sufficient to deliver to a patient a prophylactically or therapeutically effective amount without causing serious toxic effects in the patient treated. The term an "effective amount" or a "sufficient amount" generally refers to an amount sufficient to affect a reduction or elimination of at least one symptom or sign of the disease or disorder.

In the methods according to the invention, administration of a compound according to the invention can be by any suitable route, including, without limitation, parenteral, oral, intratumoral, sublingual, transdermal, topical, intranasal, aerosol, intraocular, intratracheal, intrarectal, mucosal, vaginal, by dermal patch or in eye drop or mouthwash form. Administration of the compound or pharmaceutical formulation can be carried out using known procedures at dosages and for periods of time effective to reduce symptoms or surrogate markers of the disease.

The following examples are intended to further illustrate certain embodiments supporting the invention and are not intended to limit the scope of the invention.

Example 1

CDK8 and CDK19 expression in prostate cancer

Figure 1A shows immunoblotting of CDK8, CDK19, AR and GAPDH (normalization standard) in HT1080 (fibrosarcoma), HEK-293 (embryonic kidney), MDA-MB-231 (breast carcinoma), HCT116 (colon carcinoma) and prostate cancer cell lines LNCaP (androgen-dependent), androgen-independent LNCaP derivatives C4-2 and LN3 and androgen-independent prostate cancer cell lines CWR22rv1, DU145 and PC-3. The following primary antibodies were used for immunoblotting: goat-anti-CDK8 (Santa Cruz, sc-1521), rabbit-anti-CDK19 (Sigma, HPA007053), rabbit-anti-AR (Santa Cruz, sc-13062) and mouse-anti-GAPDH (Santa Cruz, sc-32233). While CDK8 shows similar expression levels in all the cell lines, with significantly

lower expression only in MDA-MB-231, CDK19 is almost undetectable in HT1080 and HCT116 cells but is expressed in all the prostate cancer lines that express AR.

Figure 1B shows immunoblotting of CDK8, CDK19, AR, nucleolin and GAPDH (the two latter are normalization standards) in HT1080 (fibrosarcoma), HEK-293 (embryonic kidney), HCT116 (colon carcinoma), RWPE-1 (immortal but untransformed prostate epithelial cells) and prostate cancer cell lines LNCaP (androgen-dependent), androgen-independent LNCaP derivatives C4-2 and LN3 and androgen-independent prostate cancer cell lines CWR22rv1 and PC-3. While CDK8 shows similar expression levels in all the cell lines, CDK19 is strongly overexpressed in those prostate cancer lines that express AR relative to all the other cell lines. Hence, elevated CDK19 expression is associated with AR-expressing prostate cancer cells.

Figure 1C shows qPCR analysis of mRNA expression of CDK8 and CDK19 in the same cell lines that were used for immunoblotting analysis in Figure 1B. The qPCR results agree with the results of immunoblotting, with CDK8 showing similar RNA expression in all the cell lines (with the highest levels observed in PC3 cells), whereas CDK19 shows much higher RNA expression in AR-expressing prostate cancer cells than in any other cell lines.

Figure 1D shows the expression of CDK8, CDK19 and α-tubulin (normalization standard, Sigma, T5168) in androgen-dependent LNCaP cells cultured in complete media supplemented with fetal bovine serum (FBS) or in charcoal-stripped serum (CSS) media (androgen-deprived, AD) or in CSS media supplemented with 100 pM androgen agonist R1881 (also known as methyltrienolone) for the indicated number of days. This analysis shows that androgen treatment downregulates CDK8 whereas androgen deprivation up-regulates CDK8 and CDK19 proteins in LNCaP cells (Fig. 1D), indicating that CDK8 and CDK19 expression is regulated via AR.

Example 2

Effects of CDK8/19 inhibitors on AR activity

To test the role of CDK8/19 in AR activity, we have used selective small-molecule inhibitors of CDK8/19 developed by Senex Biotechnology, Inc. (Senex) and termed Senexin A (a.k.a. SNX2-1-53) and Senexin B (a.k.a. SNX2-1-165). Senexin A has been described in a recent article[29] and Senexin B in PCT Publication WO2013/116786. These small molecules selectively bind to the ATP pockets of CDK8/19 to inhibit their kinase activity. Senexin B

inhibits CDK8/19 kinase activity at lower Kd (140 nM for CDK8 and 80 nM for CDK19) and possesses higher water solubility (as high as 50 mM) compared to Senexin A.

The effects of Senexin A (5 μM) on the expression of the indicated androgen-responsive genes in LNCaP cells cultured in normal culture media for 3d (FBS) or in androgen-deprived (CSS) media for 5d (AD, androgen deprivation) or in androgen-supplemented media (500 pM R1881) for 24hr after 5-day androgen-deprivation (AD→+A) were evaluated. Treatment of androgen-dependent LNCaP cells with Senexin A significantly inhibited androgen-stimulated transcriptional activation of several androgen-responsive genes such as PSA (KLK3), KLK2, TMPRSS2 and PGC under either androgen-supplemented or androgen-deprived conditions (Figure 2A).

The effects of Senexin B (1 μM and 4 μM) on the expression of the indicated androgen-responsive genes in LNCaP cells cultured in androgen-deprived (CSS) media for 5d (A-) or in androgen-supplemented media (500 pM R1881) for 24hr after 5-day androgen-deprivation (A+) were also evaluated. Treatment of androgen-dependent LNCaP cells with Senexin B significantly inhibited androgen-stimulated transcriptional activation of several androgen-responsive genes such as PSA (KLK3), KLK2, TMPRSS2, SGK1, KLF5 and PGC under androgen-supplemented conditions (Figure 2B). On the other hand, treatment of the same cells with Senexin B did not interfere with the inhibition of several androgen-inhibited genes such as AR, OPRK1 or STXBP6 (Figure 2C). Androgen addition also inhibited the expression of CDK8 (but not of CDK19), and Senexin B did not interfere with this inhibition (Figure 2C). Hence, CDK8/19 inhibition has an especially beneficial effect of inhibiting only the induction but not the repression of gene expression by androgen.

The effect of Senexin B on the expression of androgen-responsive genes in LNCaP cells cultured in CSS media for 3d [R1881(-)] or in androgen-supplemented media (500 pM R1881) for 24 hr after 2-day androgen-deprivation [R1881(+)] was measured. Senexin B was added 1 hr before R1881 treatment and maintained in culture until RNA sample collection. Gene expression was measured by qPCR, with housekeeping gene RPL13A as normalization standard (*: $p<0.05$ between Senexin B and DMSO). Pretreatment of androgen-deprived LNCaP cells by Senexin B significantly inhibited androgen-stimulated transcription of these genes (Figure 3), suggesting that CDK8/19 positively regulate androgen signaling in prostate cancer cells.

To confirm the role of CDK8/19 in AR activation, we analyzed the inhibitory effects of Senexin A and Senexin B by a promoter activity assay in HEK293 cells that express both CDK8 and CDK19 (Figure 1A). When full-length wild-type AR was overexpressed in HEK-293 cells, either Senexin A or Senexin B significantly inhibited the activation of an androgen-responsive construct (firefly luciferase reporter under PSA gene promoter) in the presence of R1881 but not in androgen-free media (Figure 4A and Figure 5). Similar results were observed with another androgen-responsive promoter (PGC) (Figure 4B). These results indicate that CDK8/19 positively regulates AR function.

Example 3

Effects of CDK8/19 inhibitors on cell growth and ARG expression in androgen-independent prostate cancer cells in androgen-depleted media

In most of CRPC patients, prostate cancer tumor cells restore their AR activities despite low-androgen environment or presence of AR antagonists. We tested whether a CDK8/19 inhibitor Senexin B inhibits androgen-independent growth in several androgen-independent prostate cancer cell lines that were derived from castration-relapse or metastatic xenografts of parental androgen-dependent prostate cancer cell lines, including LNCaP derivatives LN3 and C4-2 and CWR22 derivative CWR22rv1. The effect of Senexin B on the growth of AR-expressing androgen-independent prostate cancer cells in androgen-free media was measured. 2 x 10^5 prostate cancer cells were seeded in CSS media with different concentrations of Senexin B and cultured for the indicated number of days before the total cell number was counted (n=4). These androgen-independent prostate cancer cells grow well under androgen-depleted conditions (in CSS media), but this androgen-independent growth was strongly inhibited by Senexin B (Figure 6A).

We have also analyzed the growth of androgen-independent cell lines, LNCaP derivative LN3 and CWR22 derivative CWR22rv1, in CSS media, in the absence or in the presence of Senexin B or androgen antagonis MDV3100 (enzalutamide). $2x10^5$ cells were seeded in CSS media with vehicle (DMSO) control, 5 μM Senexin B or 10 μM MDV3100 and cultured for indicated time before total cell number was counted (n=3). Figure 6B shows that Senexin B strongly inhibited the growth of LNCaP-LN3 and significantly inhibited the growth of CWR22rv1 cells, whereas MDV3100 weakly inhibited the growth of LNCaP-LN3 cells and does not inhibit the growth of CWR22rv1 cells under the same androgen-depleted conditions.

Endogenous AR activities in these cells were estimated by qPCR analysis of mRNA expression of AR-dependent genes, KLK3 (PSA) and KLK2. The effect of Senexin B on the expression of KLK2 and KLK3 (PSA) in androgen-independent prostate cancer cells was measured. Figure 6C shows basal gene expression in LNCaP and androgen-independent prostate cancer cell lines under 3-day androgen-deprivation conditions (AD3), and gene expression in cells cultured in CSS media (2d) and treated with Senexin B or vehicle control for 24 hours. *: $p<0.05$ between Senexin B and DMSO.

All three androgen-independent prostate cancer cell lines showed much higher expression of these genes compared to the androgen-dependent parental LNCaP cells after 3-day androgen deprivation (AD3, Figure 6C). Strikingly, Senexin B down-regulated the expression of PSA and KLK2 in all three androgen-independent-prostate cancer cell lines grown in the absence of androgen (Figure 6C) as effectively as it inhibits androgen-stimulated PSA/KLK2 expression in LNCaP cells (Figure 3). Figure 6D compares the effects of 5 μM Senexin B and 10 μM MDV3100 (enzalutamide) on the expression of PSA and KLK2 in LNCaP-LN3 and CWR22rv1 cells grown in the absence of androgen. Senexin B strongly down-regulates PSA and KLK2 expression in both androgen-independent-prostate cancer cell lines, whereas MDV3100 weakly down-regulates the expression of PSA and does not down-regulate the expression of KLK2 in LNCaP-LN3 cells and does not down-regulate the expression of either PSA or KLK2 in CWR22rv1 cells under the same androgen-depleted conditions.

These results suggest that Senexin B suppresses ligand-independent AR signaling in androgen-independent prostate cancer cells, which is required by these cells to proliferate in a low-androgen environment. The observation that Senexin B is able to inhibit cell growth and downregulate expression of androgen-regulated genes in CWR22rv1 cells is of special interest since constitutive androgen signaling in this cell line is rendered by a truncated AR[42]. This truncated form is resistant to current anti-androgen drugs designed for targeting the ligand-binding domain of AR because the C-terminal truncation deletes the ligand-binding domain and makes it ligand-independent. Hence CDK8/19 may also play an important role in active transcription mediated by activated ARs (full-length, mutated or truncated) in androgen-independent-prostate cancer cells.

We have also tested if Senexin B inhibits the growth of an androgen-independent-prostate cancer cell line PC-3, which does not express AR (Fig. 1A), and which has developed the

androgen-independent phenotype through an AR-independent mechanism. PC-3 cell growth was previously shown to be inhibited by the inhibition of transcription factor NFκB[43-45], and CDK8/19 inhibition was discovered by Senex to decrease the induction of NFκB transcriptional activity (PCT Publication WO2013/040153). The effect of Senexin B on PC-3 prostate cancer cell growth in androgen-free media was measured. 2 x 10^5 PC-3 cells were seeded in CSS media with different concentrations of Senexin B and cultured for the indicated number of days before the total cell number was counted (n=4). *: $p<0.05$ between Senexin B and DMSO. As shown in Figure 7, the growth of PC-3 cells in androgen-depleted CSS media was inhibited by Senexin B. Hence, CDK8/19 inhibition inhibits the androgen-independent growth of androgen-independent prostate cancer cells that have developed androgen independence through different mechanisms.

Example 4

CDK8/19 inhibitor Senexin B inhibits the in vivo xenograft growth of LNCaP-LN3 cells in nude mice

6-8 week-old nude male mice (Jackson Laboratory) were subcutaneously injected with 2 million LN-CaP LN3 (LN3) prostate cancer cells in the right flank, with Matrigel. Visible tumors formed ~14 days after injection. Mice with similar tumor volumes were then randomized into two groups and treated for 2 weeks (5 days per week) with daily i.p. injections of 40 mg/kg Senexin B or an equal volume of vehicle solution. The tumor size was measured by caliper 3 times per week and calculated by the equation length*width*width*0.5. As shown in Figure 8, Senexin B treatment dramatically inhibits tumor growth of LN3 cells in male nude mice relative to mice treated with vehicle control. Senexin B treatment had no effects on body weight of the hosts (Figure 9) and treated mice looked as healthy as the mice in the vehicle control group. At the end of the experiment, mice from each group were sacrificed to determine final tumor weight. As shown in Figure 10, the weights of tumors that developed in Senexin B-treated mice were significantly lower than the weights of tumors from the control group, consistent with the difference observed from tumor volume measurement in Figure 8. In summary, the data suggest that inhibition of CDK8/19 kinase activity would be a potential therapeutic method to block the tumor growth of advanced prostate cancer cells.

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What is claimed is:

1. A method for treating prostate cancer in a subject comprising administering to the subject an effective amount of a selective inhibitor of one or more of CDK8 and CDK19.

2. The method according to claim 1, wherein the inhibitor inhibits CDK19.

3. The method according to claim 1, wherein the inhibitor inhibits CDK8.

4. The method according to claim 1, wherein the inhibitor inhibits CDK8 and CDK19.

5. The method according to claim 1, wherein the prostate cancer is androgen independent.

6. The method according to claim 5, wherein the prostate cancer is androgen independent due to one or more of androgen receptor gene amplification, androgen receptor gene mutation, ligand-independent transactivation of androgen receptor and activation of intracellular androgen synthesis.

7. The method according to claim 1, wherein the inhibitor inhibits increased activity of NF-κB.

8. The method according to claim 5, wherein the inhibitor inhibits increased activity of NF-κB.

9. The method according to claim 1, wherein one or more genes is inhibited by AR.

10. The method according to claim 9, wherein the inhibition of one or more genes by AR is not inhibited.

11. The method according claim 9 or 10, wherein the one or more genes inhibited by AR includes CDK8.

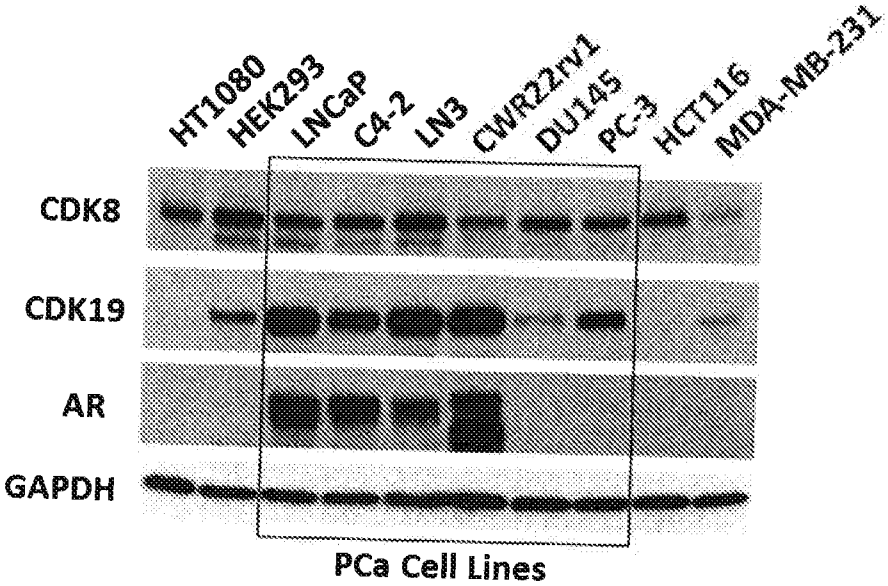

HT1080
HEK293
LNCaP
C4-2
LN3
CWR22rv1
DU145
PC-3
HCT116
MDA-MB-231
CDK8
CDK19
AR
GAPDH
PCa Cell Lines

Fig. 1A

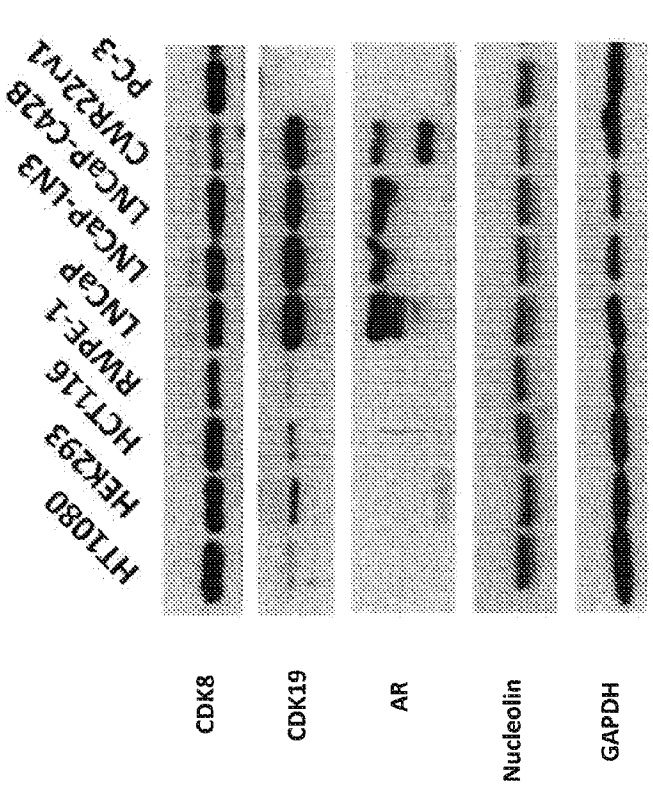


Figure 1B

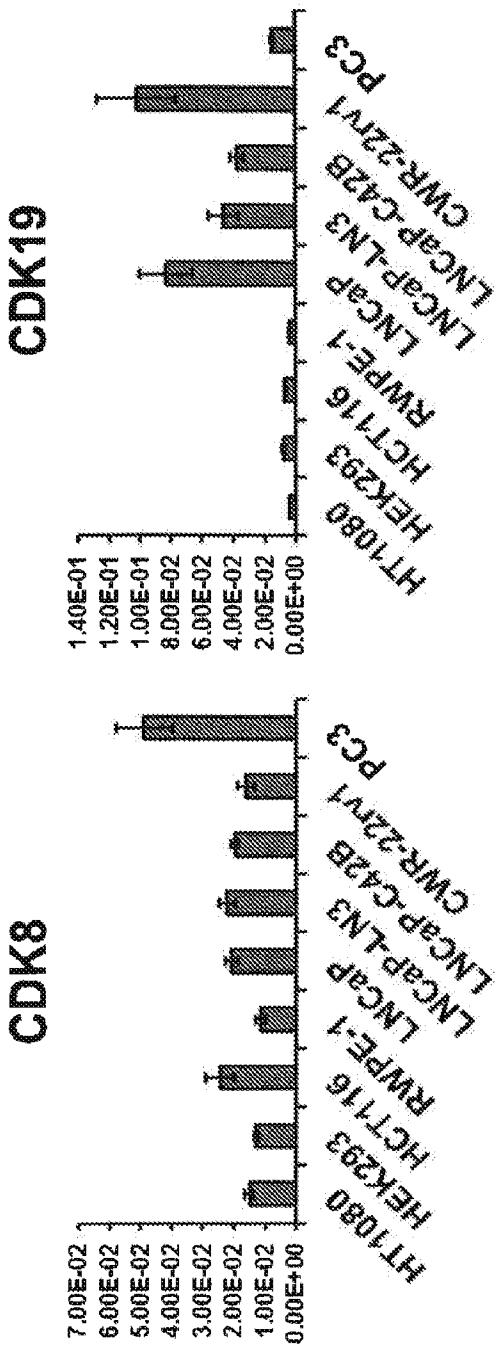


Figure 1C

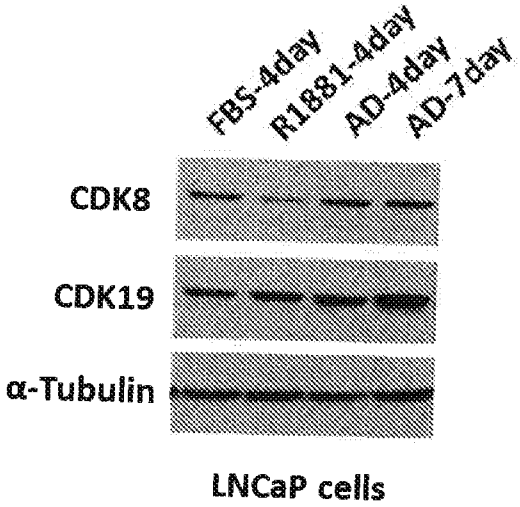

FBS-4day
R1881-4day
AD-4day
AD-7day
CDK8
CDK19
α-Tubulin
LNCaP cells

Fig. 1D

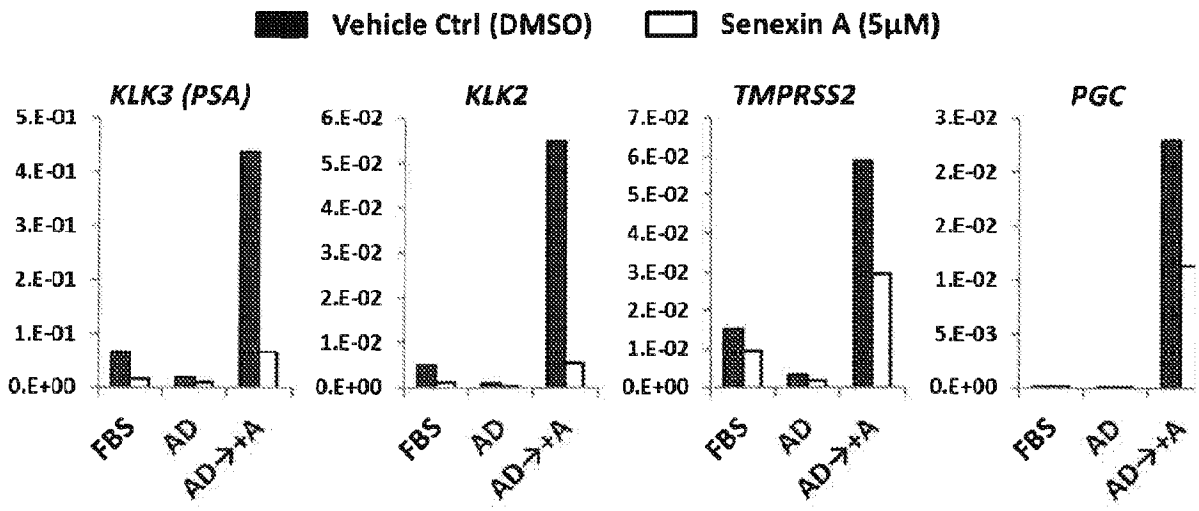

Vehicle Ctrl (DMSO)
Senexin A (5μM)
KLK3 (PSA)
KLK2
TMPRSS2
PGC
5.E-01
4.E-01
3.E-01
2.E-01
1.E-01
0.E+00
6.E-02
5.E-02
4.E-02
3.E-02
2.E-02
1.E-02
0.E+00
7.E-02
6.E-02
5.E-02
4.E-02
3.E-02
2.E-02
1.E-02
0.E+00
3.E-02
2.E-02
2.E-02
1.E-02
5.E-03
0.E+00
FBS
AD
AD→+A

Fig. 2A

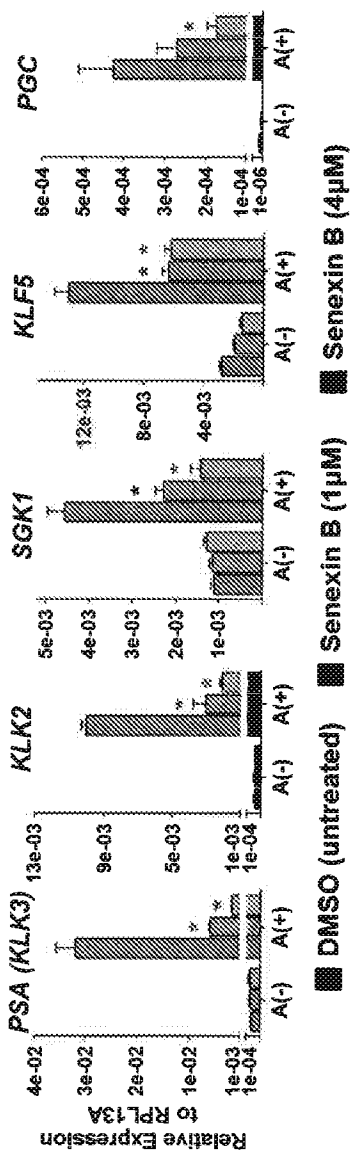


Figure 2B

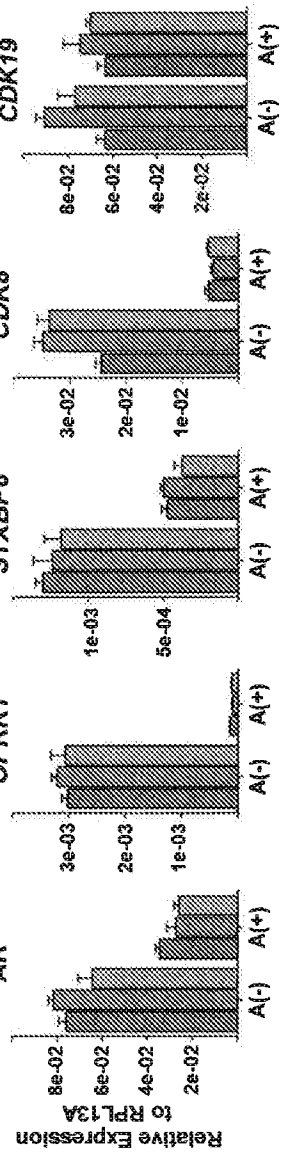


Figure 2C

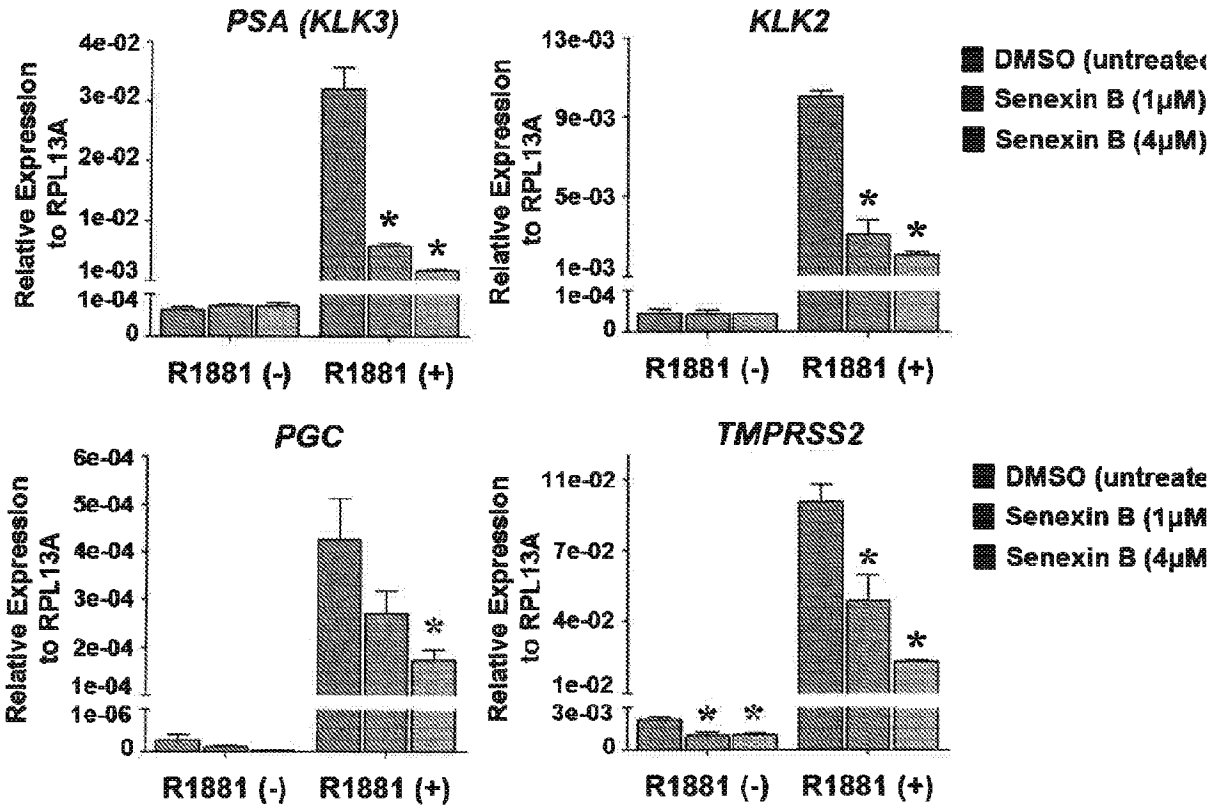

PSA (KLK3)
KLK2
PGC
TMPRSS2
Relative Expression to RPL13A
R1881 (-)
R1881 (+)
DMSO (untreated
Senexin B (1μM)
Senexin B (4μM)
4e-02
3e-02
2e-02
1e-02
1e-03
1e-04
0
13e-03
9e-03
5e-03
6e-04
5e-04
4e-04
3e-04
2e-04
1e-06
11e-02
7e-02
4e-02
3e-03

Fig. 3

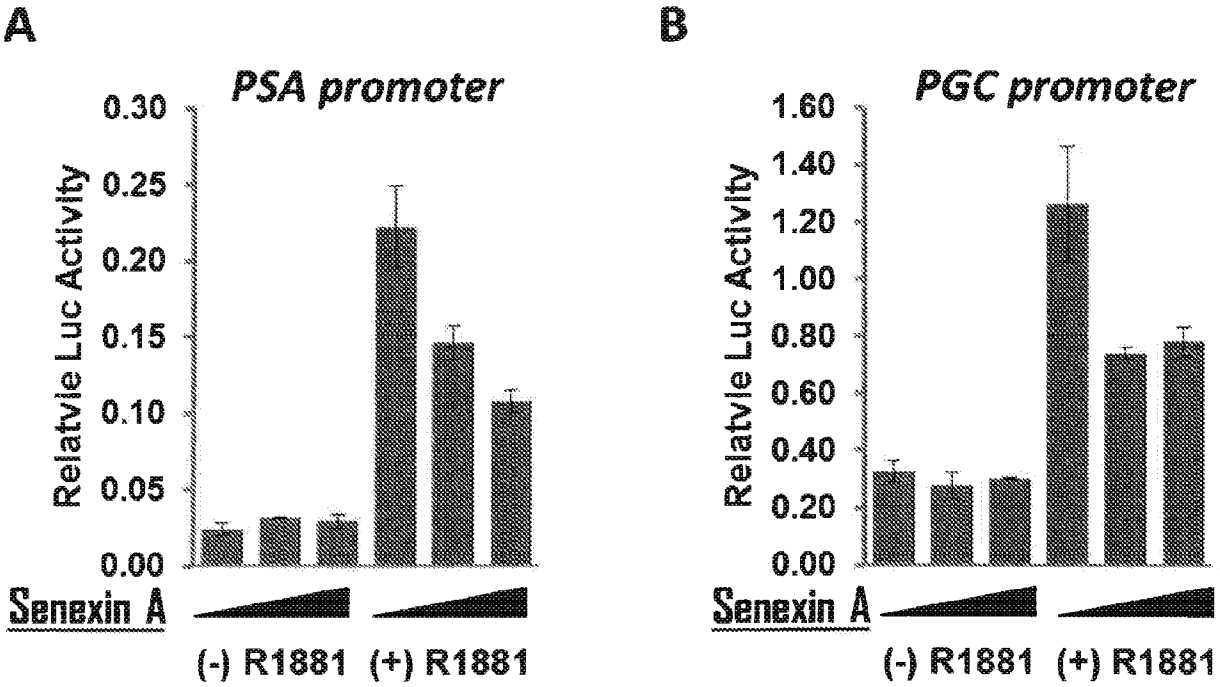

A
PSA promoter
Relatvie Luc Activity
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Senexin A
(-) R1881
(+) R1881
B
PGC promoter
Relatvie Luc Activity
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Senexin A
(-) R1881
(+) R1881

Fig. 4

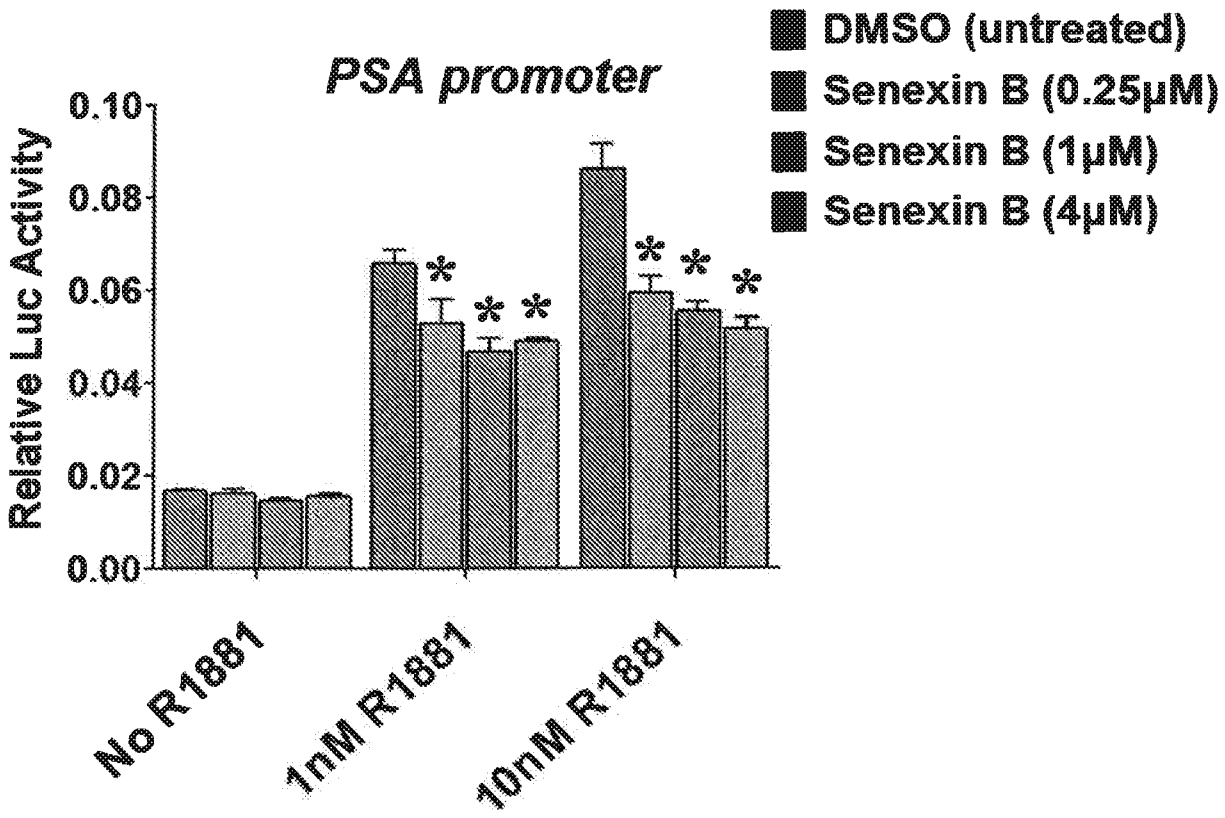

PSA promoter
DMSO (untreated)
Senexin B (0.25μM)
Senexin B (1μM)
Senexin B (4μM)
Relative Luc Activity
0.10
0.08
0.06
0.04
0.02
0.00
*
No R1881
1nM R1881
10nM R1881

Fig. 5

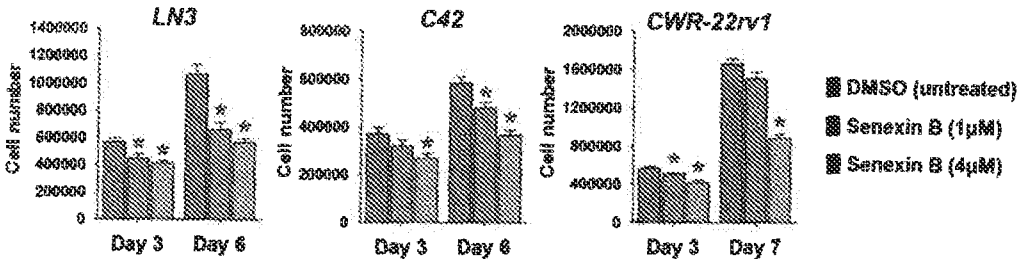
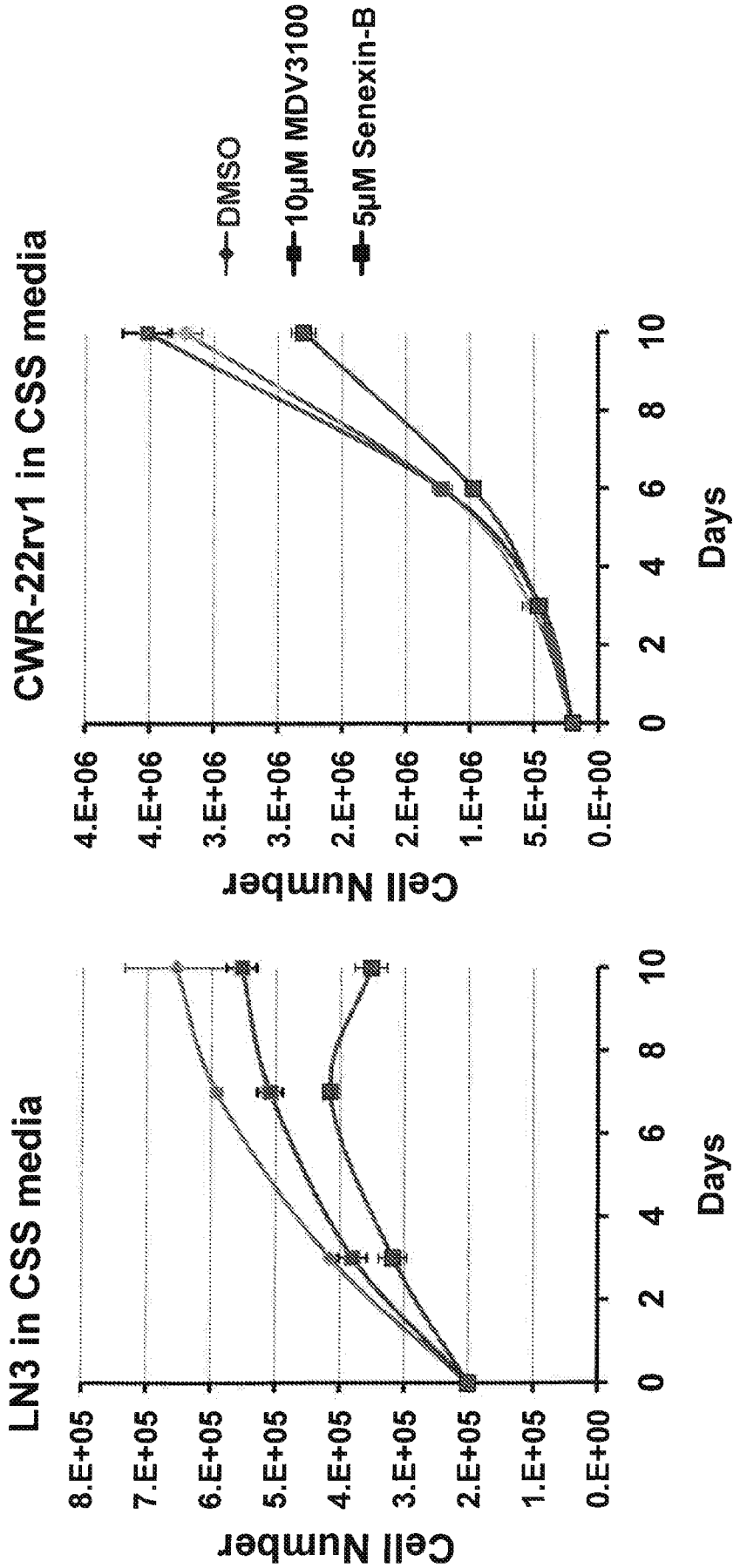
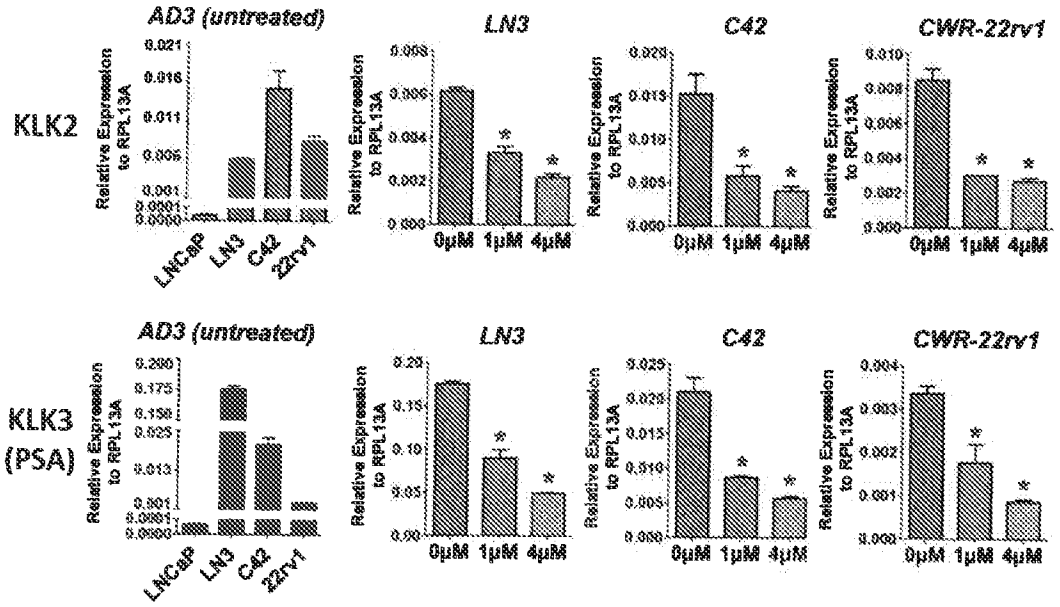

LN3
C42
CWR-22rv1
Cell number
Day 3
Day 6
Day 7
DMSO (untreated)
Senexin B (1μM)
Senexin B (4μM)

Fig. 6A

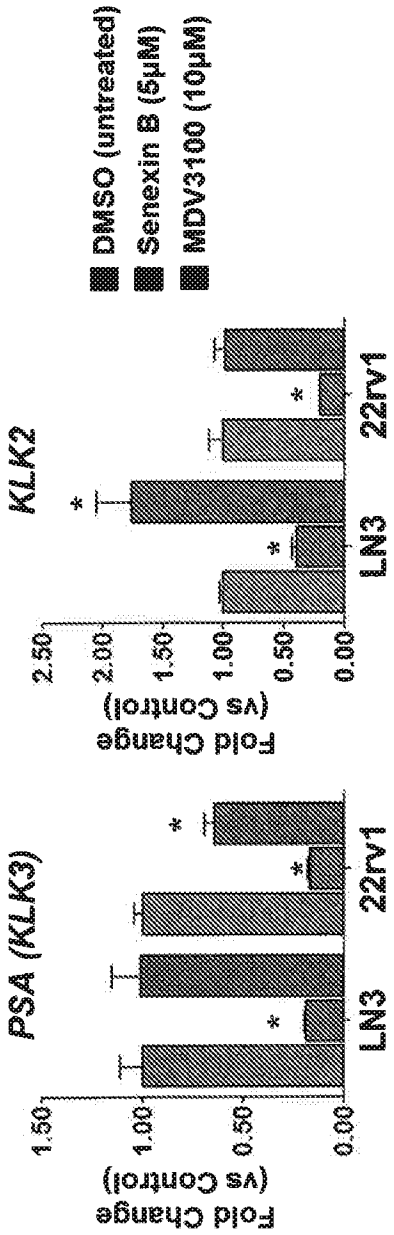
Figure 6B


LN3 in CSS media
Cell Number
8.E+05
7.E+05
6.E+05
5.E+05
4.E+05
3.E+05
2.E+05
1.E+05
0.E+00
0
2
4
6
8
10
Days
CWR-22rv1 in CSS media
Cell Number
4.E+06
4.E+06
3.E+06
3.E+06
2.E+06
2.E+06
1.E+06
5.E+05
0.E+00
0
2
4
6
8
10
Days
DMSO
10μM MDV3100
5μM Senexin-B


KLK2
KLK3 (PSA)
AD3 (untreated)
LN3
C42
CWR-22rv1
Relative Expression to RPL13A
LNCaP
LN3
C42
22rv1
0μM
1μM
4μM

Fig, 6C

Figure 6D


DMSO (untreated)
Senexin B (5μM)
MDV3100 (10μM)
PSA (KLK3)
Fold Change
(vs Control)
1.50
1.00
0.50
0.00
LN3
22rv1
KLK2
Fold Change
(vs Control)
2.50
2.00
1.50
1.00
0.50
0.00
LN3
22rv1

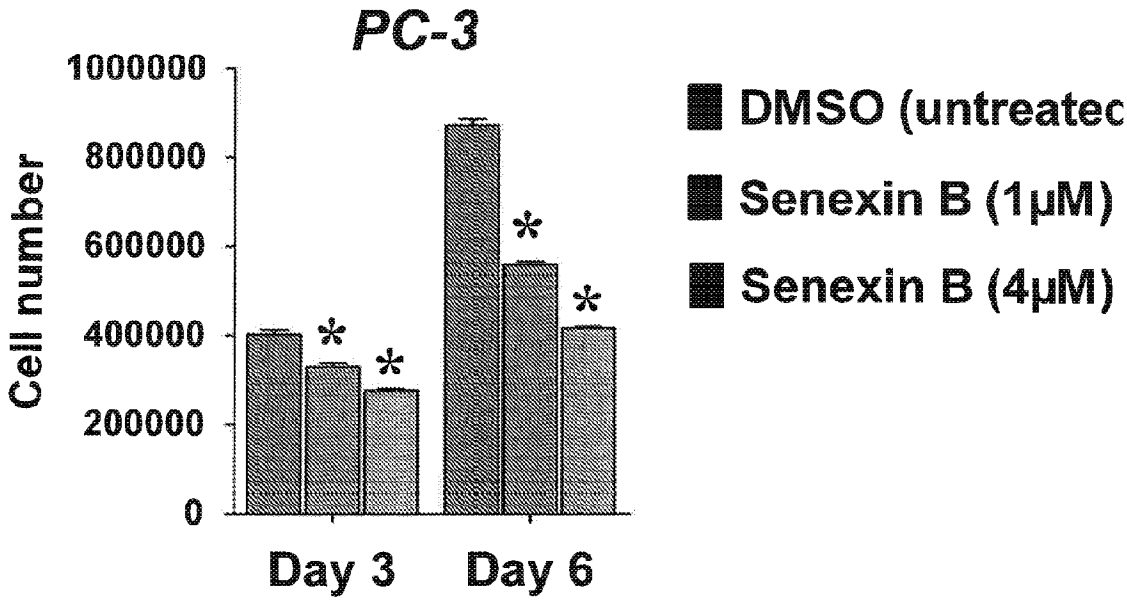

PC-3
1000000
800000
600000
400000
200000
0
Cell number
Day 3
Day 6
*
*
*
*
DMSO (untreatec
Senexin B (1μM)
Senexin B (4μM)

Fig. 7

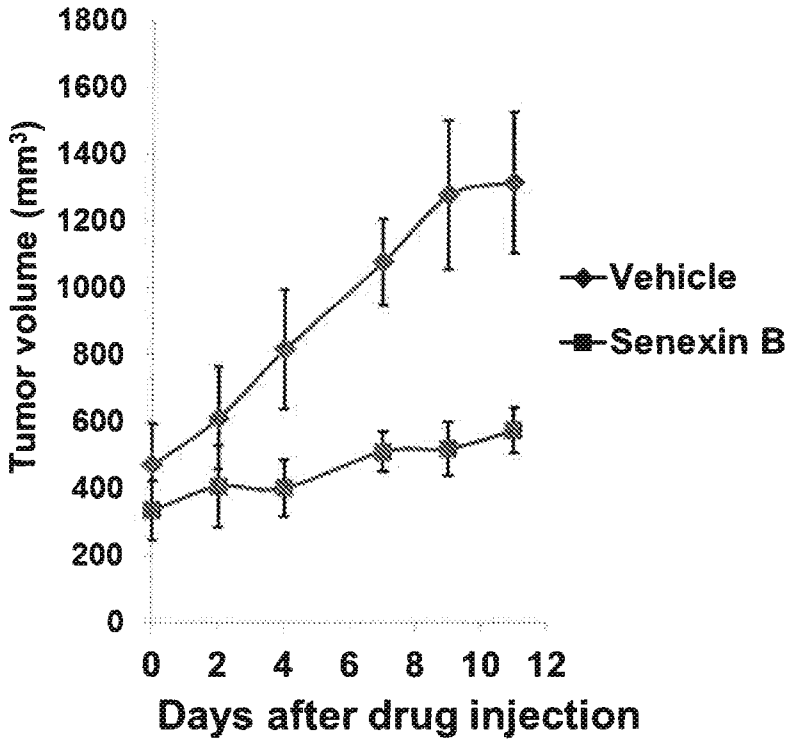

1800
1600
1400
1200
1000
800
600
400
200
0
Tumor volume (mm3)
0 2 4 6 8 10 12
Days after drug injection
Vehicle
Senexin B

FIGURE 8

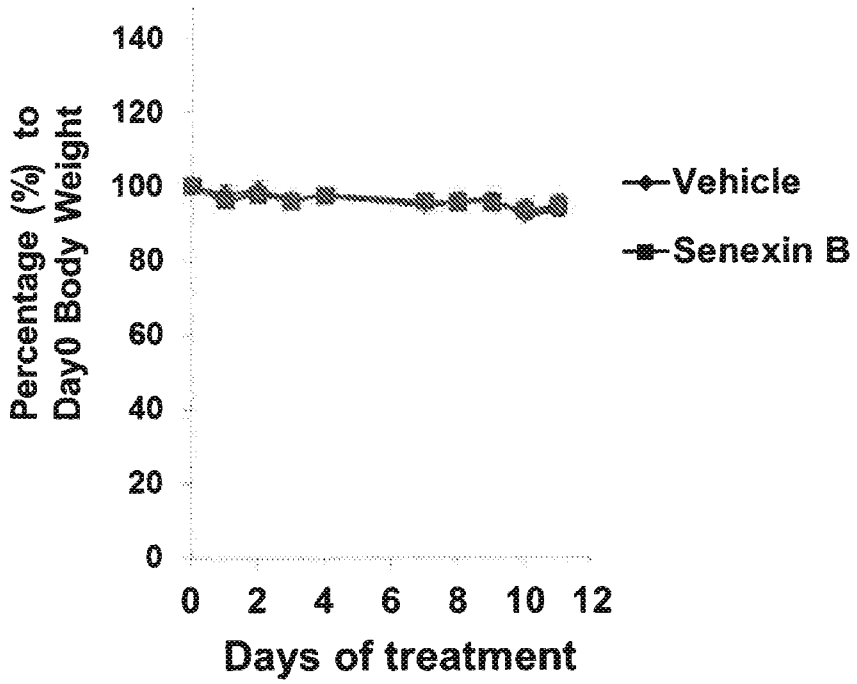

140
120
100
80
60
40
20
0
Percentage (%) to Day0 Body Weight
0 2 4 6 8 10 12
Days of treatment
Vehicle
Senexin B

FIGURE 9

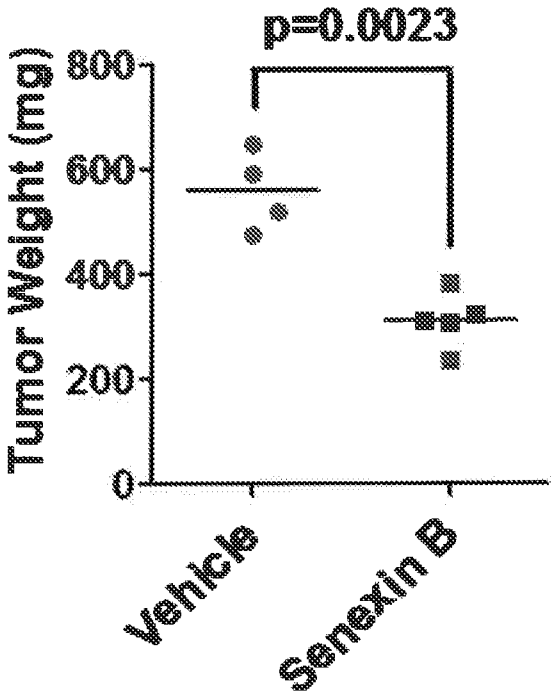


FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/067990

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/517 A61K31/5377 C07D239/94
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	D. C. PORTER ET AL: "Cyclin-dependent kinase 8 mediates chemotherapy-induced tumor-promoting paracrine activities", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 109, no. 34, 21 August 2012 (2012-08-21), pages 13799-13804, XP055056523, ISSN: 0027-8424, DOI: 10.1073/pnas.1206906109 page 13800 - page 13804 -----	1-11
Y	US 2012/071477 A1 (PORTER DONALD C [US] ET AL) 22 March 2012 (2012-03-22) cited in the application page 4 - page 14 -----	1-11

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2014	04/02/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kling, Isabelle

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/067990

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012071477 A1	22-03-2012	NONE	