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(54) **Title**: COMPOSITIONS AND USES OF COMBINATIONS OF DIM-RELATED INDOLES AND SELECTED ANTI-ANDROGEN COMPOUNDS

(57) **Abstract**: The present invention relates to compositions and kits comprising Diindolymethane (DIM)-related indoles and selected Anti-Androgen Compounds described herein including Diarylthiohydantoin compounds, and methods of co-administration and/or co-formulation of DIM-related indoles with the selected Anti-Androgen Compounds such as Diarylthiohydantoin compounds. The present invention also relates to such compositions, kits and methods for treating androgen and Androgen Receptor (AR)-related conditions. Among androgen and AR-related conditions benefited is prostate cancer.

COMPOSITIONS AND USES OF COMBINATIONS OF DIM-RELATED INDOLES AND SELECTED ANTI-ANDROGEN COMPOUNDS

PRIORITY BENEFIT

[0001] This application claims the benefit of U.S. provisional application No. 61/879,013, filed on September 17, 2013, which is incorporated herein by reference in its entirety.

1. FIELD OF THE INVENTION

[0002] The present invention relates to compositions and kits comprising Diindolymethane (DIM)-related indoles and selected Anti-Androgen Compounds described herein including Diarylthiohydantoin compounds, and methods of co-administration and/or co-formulation of DIM-related indoles with the selected Anti-Androgen Compounds such as Diarylthiohydantoin compounds. The present invention also relates to such compositions, kits and methods for treating androgen and Androgen Receptor (AR)-related conditions. Among androgen and AR-related conditions benefited is prostate cancer.

2. BACKGROUND OF THE INVENTION

2.1. Androgen Receptor (AR)-related conditions

[0003] Androgen, the male steroid hormone, is responsible for male sexual differentiation and development, as well as the maintenance and support of sexual tissues in the adult. Androgens are important for the development and progression of age-associated pathologies in adults, including male pattern baldness, benign prostatic hyperplasia and prostate cancer (PC) in men, and polycystic ovarian syndrome (PCOS) and hirsutism in women. Androgen action is exerted through the androgen receptor (AR), a 110-kDa member of the steroid receptor family of transcription factors. The physiological ligands for the AR are testosterone and dihydrotestosterone (DHT). The later has at least 10-fold stronger binding affinity.

[0004] AR is a nuclear hormone receptor, which is activated by binding of androgen ligands. Upon androgen binding, AR dissociates from the cytoplasmic chaperone protein HSP90, self-dimerizes and translocates to the nucleus. The AR interacts with coactivators and

chromatin modifying enzymes, and it also binds to androgen-response elements (ARE) within the genome. These ARE regulate the expression of genes relevant to prostatic growth and function. Additionally, ARE has been linked to cancer development and progression. Among the numerous genes regulated by the AR, AR activation has been shown to upregulate the expression of prostate specific antigen (PSA), cyclin-dependent kinases (cdk) 1, 2 and 4, cyclin A and B, along with many other genes. Importantly, AR mutation, amplification, and overexpression AR variants often occur and are involved in the development of androgen-independent growth and resistance to antiandrogen therapy in prostate cancer.

[0005] Prostate cancer (PC) is the second most prevalent cause of death in men in the USA and Europe. The dependence of PC on androgens and the AR has been recognized for decades. The current state of PC therapy is unsatisfactory. When detected early, with the tumor strictly confined to the prostate gland, PC can be often controlled by implantation of radioactive seeds, or by surgical prostatectomy, which often results in incontinence and impotence. Locally advanced prostate cancer can often be reasonably controlled when in the pelvis and can be encompassed into a single port of an external radiation beam. For advanced PC however, the standard treatment is AR blockade, usually in combination with Leutinizing Hormone Releasing Hormone (LHRH) agonists, which suppress both adrenal and testicular testosterone. The rationale of this approach is that early prostate cancer invariably depends on androgens for growth. The mechanism of action of clinically utilized antiandrogens is thought to involve blockade of the AR by direct binding and/or by interference with binding of the AR to the ARE of DNA or inhibition of the production of androgens. Current antiandrogen therapies are associated with emergence of resistance to the clinical response in a significant proportion of patients over a one to two year period. The molecular basis for antiandrogen resistance and therapeutic failure is complex and poorly understood. As such, there is an urgent need to develop more effective therapeutic modalities which extend the period of therapeutic efficacy of antiandrogen use for both PC and for other disorders affected by AR activity.

2.2. Small Molecule Antiandrogens Are a Large and Diverse Class of Compounds

[0006] Antiandrogens are classified as steroidal or nonsteroidal based on their respective chemical structures. The major antiandrogens in clinical use worldwide are the

nonsteroidal bicalutamide, flutamide and nilutamide and the steroidal cyproterone acetate (CPA). Bicalutamide (Casodex) is the most extensively studied nonsteroidal antiandrogen. However Bicalutamide has now been shown to possess both AR blocking activity (antagonism) as well as AR activating activity (agonism) explaining in part the short clinical response associated with its use (see, e.g., U.S. Patent No. 7,709,517, at col. 1).

[0007] In addition, a new class of antiandrogens working specifically through the inhibition of the CYP 17 enzyme has been developed. These compounds reduce the activity of the AR by reducing systemic and tumor associated production of androgens and associated ligand activation of the AR. This class of newly developed antiandrogens includes Abiraterone acetate, and Orteronel (TAK-700). However, CYP 17 inhibitors do not overcome the propensity of the AR to undergo ligand-independent activation through intracellular kinase activity in PC. Also, a class of compounds which downregulate the production of the AR protein and block its translocation to the nucleus are under development. This class of antiandrogens is exemplified by Orteronel (TAK-700).

[0008] Recently more potent and purely antagonistic antiandrogens—imidazolidine derivatives which have a substituted alkyl group in 3- position such as Diarylhydantoin and Diarylthiohydantoin compounds—have been developed, which are exemplified by Enzalutamide (MDV3100 [Xtandi®]) and ARN-509. These compounds have been described in various publications including U.S. Patent No. 8,183,274, U.S. Patent No. 7,709,517, U.S. Patent No. 8,470,829, U.S. Patent No. 8,445,507, U.S. Patent No. 8,110,594, U.S. Patent Publication No. 2012/0295944, U.S. Patent Publication No. 20130034501, U.S. Patent Publication No. 20130072511, U.S. Patent Publication No. 20110306615 and Int. Patent Publication No. WO 2006/028226, which are incorporated by reference herein in their entireties. In addition, antiandrogen bicyclic 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compounds, such as AZD-3514, have been developed, as described in U.S. Patent Publication No. 2013/0203714.

[0009] While Diarylthiohydantoins appear to be better tolerated than castration in men with PC, clinical resistance and disease progression has been noted even with the use of Enzalutamide, the most specific and potent antiandrogen currently in use (Yin L, Hu Q, Hartmann RW., Recent progress in pharmaceutical therapies for castration-resistant prostate cancer. Int J Mol Sci. 2013 Jul 4;14(7):13958-78. doi: 10.3390/ijms140713958.). The

development of enzalutamide resistance is in part mediated through increased expression of AR splice variants, which are constitutively active (Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM., Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res. 2013 Jan 15;73(2):483-9. doi: 10.1158/0008-5472.CAN-12-3630. Epub 2012 Nov 1). This is becoming a major challenge for the treatment of Castration Resistant Prostate Cancer (CRPC) and metastatic CRPC (mCRPC).

2.3. Biologic activities of Diindolylmethane (DIM) and related indoles.

[0010] Cruciferous vegetables contain a family of plant protective compounds called glucobrassicins which give rise to active compounds with the indole molecular ring, exemplified by the stable dimer 3,3'-diindolylmethane (DIM). DIM is the linear, dimer molecule formed from the condensation of two molecules of precursor Indole-3-carbinol (I3C). DIM, its precursor I3C and closely related indole compounds of natural and synthetic origin are now known to possess therapeutic activities involving both the Estrogen Receptor (ER) and AR.

[0011] DIM in pure crystalline form is highly insoluble in both water and oil conferring limited oral and topical bioavailability and necessitating absorption-enhancing formulations. Previous experimental work has described the use of DIM and the related trimeric derivative of I3C, 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane (LTR), in specialized formulations (see U.S. Patent No. 6,086,915, which is incorporated by reference herein in its entirety). Enhanced absorption of DIM from microencapsulated DIM provides for better oral bioavailability and higher tissue levels, but prostate tissue was not studied (Anderton MJ, Manson MM, Verschoyle R, Gescher A, Steward WP, Williams ML, Mager DE. Physiological modeling of formulated and crystalline 3,3'-diindolylmethane pharmacokinetics following oral administration in mice. Drug Metab Dispos. 2004 Jun;32(6):632-8). More recent patent applications describe the formulation of DIM in cod liver oil (U.S. Patent Application No. 20130039979) and the formulation of DIM in block co-polymers (U.S. Patent Application No. 30130065933) to enhance oral bioavailability. Improved formulation of DIM with a solubilizing agent is also described for improved topical administration (U.S. Patent Application No. 20090062244).

[0012] Laboratory studies suggest that DIM-related activity includes the inhibition of prostate cancer cell growth *in vitro* and prostate cancer progression in animal models. In

translational research studies, DIM from microencapsulated DIM has been shown to possess cancer inhibitory activity resulting in growth arrest and control of PC cells *in vitro* and of tumor metastasis in transplanted prostate cancer (Singh-Gupta V, Banerjee S, Yunker CK, Rakowski JT, Joiner MC, Konski AA, Sarkar FH, Hillman GG. B-DIM impairs radiation-induced survival pathways independently of androgen receptor expression and augments radiation efficacy in prostate cancer. Cancer Lett. 2012 May 1;318(1):86-92. doi: 10.1016/j.canlet.2011.12.006.). Previous in vitro studies reported in Le et al. (Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells. J Biol Chem. 2003 Jun 6;278(23):21136-45.) and U.S. Patent Publication No. 2005/0058600 describe DIM and related indoles as a distinct class of antiandrogens possessing similar molecular size and interaction with the AR as seen with bicalutamide. As described above, it is now known that bicalutamide possesses both antagonistic and agonistic activity towards the AR (see, e.g., U.S. Patent No. 7,709,517). It is not yet known whether DIM functions like bicalutamide *in vivo* with both AR antagonist and agonist activity. It is known, however, that the action of DIM is associated with increased production and activity of IL-6 (Xue L, Pestka JJ, Li M, Firestone GL, Bjeldanes LF. 3,3'-Diindolylmethane stimulates murine immune function in vitro and in vivo. J Nutr Biochem. 2008 May;19(5):336-44). Increased activity of IL-6 is known to result in ligand independent activation of the AR, tumor survival, and disease progression in PC and CRPC (Azevedo A, Cunha V, Teixeira AL, Medeiros R. IL-6/IL-6R as a potential key signaling pathway in prostate cancer development. World J Clin Oncol. 2011 Dec 10;2(12):384-96). Microencapsulated, absorption-enhanced DIM (BR-DIM®) has been used in a phase I dose-escalation study in men with non-metastatic CRPC, and found to be well tolerated and to have modest PSA lowering activity (Heath EI, Heilbrun LK, Li J, Vaishampayan U, Harper F, Pemberton P, Sarkar FH, A phase I dose-escalation study of oral BR-DIM (BioResponse 3,3'- Diindolylmethane) in castrate-resistant, non-metastatic prostate cancer. Am J Transl Res. 2010 Jul 23;2(4):402-11).

[0013] Based on the failure of even the most specific and potent antiandrogens to achieve durable remission and disease free survival in many cases of PC, novel therapy is needed that addresses the molecular basis of antiandrogen resistance. Finding combinations of agents which may overcome or delay the phenomena of antiandrogen resistance will provide an important therapeutic advance for PC, as well as for other AR related disorders. Such

combinatorial therapy will also benefit from specialized formulations and methods of antiandrogen administration that overcome pharmacokinetic and pharmacodynamic limitations of currently available antiandrogens.

3. SUMMARY OF THE INVENTION

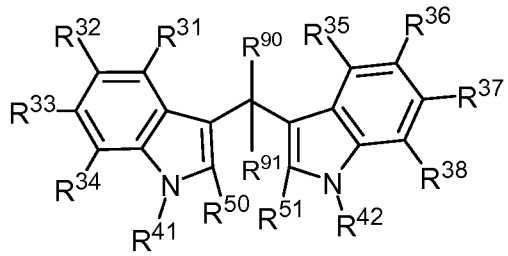
[0014] In one aspect, provided herein are methods of treating an androgen or Androgen Receptor-driven disorder in a subject in need thereof, comprising administering to the subject (i) an amount of a DIM-related indole, and (ii) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

[0015] In another aspect, provided herein are pharmaceutical fixed dosage compositions comprising a combination of: (i) an amount of a DIM-related indole, and (ii) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof. In certain embodiments, provided herein are pharmaceutical compositions comprising a therapeutically effective amount of a combination of: (i) a DIM-related indole, and (ii) an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

[0016] In yet another aspect, provided herein are kits comprising in one or more containers: (a) an amount of a DIM-related indole, and (b) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compounds is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof. In some embodiments of the kits, the DIM-related indole and the Anti-Androgen Compound are in separate containers. In other embodiments, the DIM related indole and the Anti-Androgen compound are in the same container. In specific embodiments, the DIM related indole and the Anti-Androgen compound are in the same container, in a fixed dose combination.

[0017] In certain of the methods, compositions and kits provided herein, the DIM-related indole is selected from the group consisting of:

a compound of formula I:



(I)

wherein R^{32} and R^{36} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and ethoxycarbonyl groups,

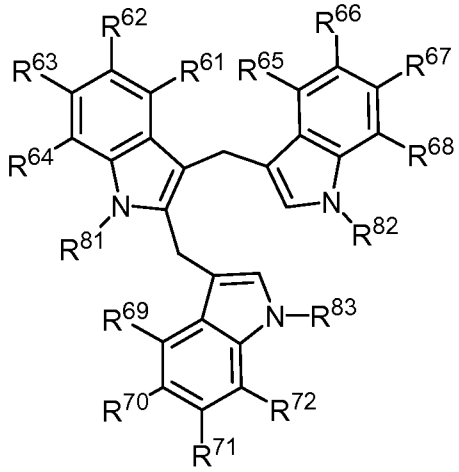
R^{33} and R^{37} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy,

R^{31}, R^{34}, R^{35}, R^{38}, R^{41}, and R^{42} are hydrogen,

R^{50}, R^{51} are either hydrogen or methyl, or phenyl, and

R^{90}, R^{91} are hydrogen;

a compound of formula II:

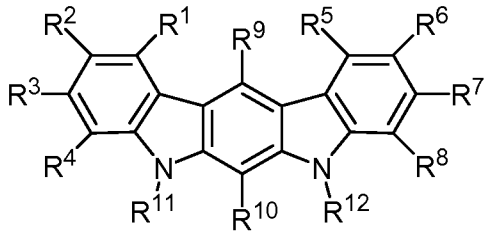


(II)

wherein R^{62}, R^{63}, R^{66}, R^{67}, R^{70}, and R^{71} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and

R^{61}, R^{64}, R^{65}, R^{68}, R^{69}, R^{72}, R^{81}, R^{82}, and R^{83} are hydrogen;

a compound of formula (III):



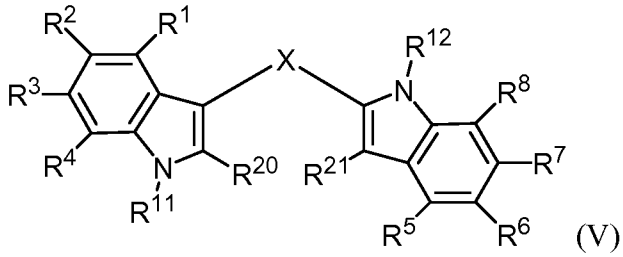
(III)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are substituents independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_5-C_{20} aryl, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxycarbonyl, halocarbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C_1-C_{24} alkyl)-substituted carbamoyl, di-(C_1-C_{24} alkyl)-substituted carbamoyl, mono-substituted arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C_1-C_{24} alkyl)-substituted amino, methoxy, mono- and di-(C_5-C_{20} aryl)-substituted amino, C_2-C_{24} alkylamido, C_6-C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, phenyl, sulfo, sulfonato, C_1-C_{24} alkylsulfanyl, arylsulfanyl, C_1-C_{24} alkylsulfinyl, C_5-C_{20} arylsulfinyl, C_1-C_{24} alkylsulfonyl, C_5-C_{20} arylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, and combinations thereof, and further wherein any two adjacent (ortho) substituents may be linked to form a cyclic structure selected from five-membered rings, six-membered rings, and fused five-membered and/or six-membered rings, wherein the cyclic structure is aromatic, alicyclic, heteroaromatic, or heteroalicyclic, and has zero to 4 non-hydrogen substituents and zero to 3 heteroatoms, and

R^{11} and R^{12} are independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkoxycarbonyl, amino-substituted C_1-C_{24} alkyl, (C_1-C_{24} alkylamino)-substituted C_1-C_{24} alkyl, and di-(C_1-C_{24} alkyl)amino-substituted C_1-C_{24} alkyl,

with the provisos that at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} is other than hydrogen, and when R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are selected from hydrogen, halo, alkyl and alkoxy, then R^{11} and R^{12} are other than hydrogen and alkyl; and

a compound of formula (V):



(V)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^{11}, R^{12}, and X are defined as for compounds of formula (III), and

R^{20} and R^{21} are defined as for R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 as for compounds of formula III.

[0018] In certain embodiments, the compositions, kits and methods described herein employ structurally-related, synthetically-derived, substituted diindolylmethane compounds. Preferred DIM-related compounds for use in the methods and compositions of the invention include, but are not limited to, hydoxylated DIMs, methoxylated DIMs, 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane (LTR), hydroxylated LTRs, methoxylated LTRs, 5,5'-dimethylDIM (5-Me-DIM), 2,2'-dimethylDIM (2-Me-DIM), 5,5'-dichloroDIM (5-Cl-DIM), imidazolelyl-3,3'-diindolylmethane, nitro-substituted imidazolelyl-3,3'-diindolylmethanes, 2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo-[2,3-b]carbazole, 6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole and 2,10-dicarbethoxy-6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole, and 2,6-dicarbethoxy-3,3'-dimethyl-13,14-diindolylmethane and indole-3-carbinol. In preferred embodiments, DIM or the DIM-related indole is suspended as microparticles in a starch carrier matrix. In some embodiments, the DIM-related indole is DIM. In one embodiment, DIM is processed DIM. In other embodiments, the DIM-related indole is I3C.

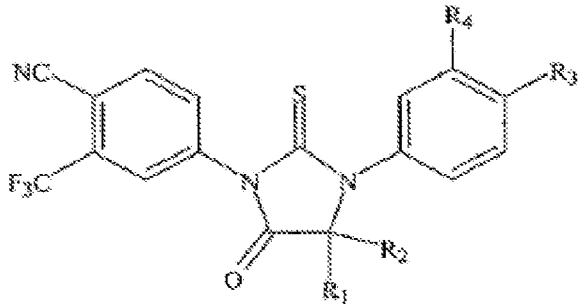
[0019] In some embodiments of the methods described herein, DIM-related indole is administered orally. In other embodiments, DIM-related indole is administered parenterally or intra-arterially. In other embodiments, DIM-related indole is injected directly into prostate gland tissue. In particular embodiments, DIM-related indole is injected directly into prostate gland tissue using ultrasound guidance. In other particular embodiments, DIM-related indole is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter. In certain embodiments, the amount of the DIM-related indole administered to the subject is 1 to 20 mg per kg, 3 to 10 mg or 1 to 5 mg per kg of the subject's weight per day.

In some embodiments, the amount of the processed DIM administered to the subject is 150-900 mg per day. In specific embodiments, the amount of the processed DIM administered to the subject is less than 625 mg, less than 600 mg, equal to or less than 450 mg, equal to or less than 375 mg, equal to or less than 300 mg, or equal to or less than 225 mg per day. In some embodiments, the DIM-related indole is administered parenterally or intra-arterially at 50 to 5,000 mg per dose, 100 to 3,000 mg per dose; equal to or less than 1,000 mg per dose, equal to or less than 500 mg per dose, or equal to or less than 250 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

[0020] In certain embodiments of the compositions described herein, the amount of the processed DIM in the composition is 75-225 mg; or the amount of the processed DIM is less than 375 mg, less than 300 mg, equal to or less than 225 mg, equal to or less than 160 mg ,or equal to or less than 75 mg (e.g., in compositions formulated for oral administration). In certain embodiments of the kits described herein, the amount of the processed DIM is 75-225 mg per dose; or the amount of the processed DIM is less than 375 mg, less than 300 mg, equal to or less than 225 mg, equal to or less than 160 mg, or equal to or less than 75 mg per dose (e.g., in units formulated for oral administration).

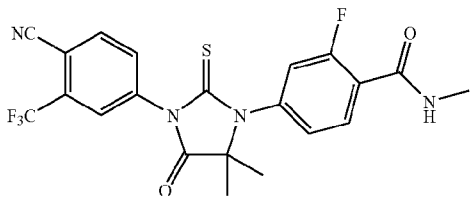
[0021] In certain embodiments of the compositions described herein, the amount of the DIM-related indole is 50 to 5,000 mg or 100 to 3,000 mg; or the amount of the DIM-related indole is equal to or less than 1,000 mg, equal to or less than 500 mg, or equal to or less than 250 mg (e.g., in compositions formulated for parenteral or intra-arterial administration). In certain embodiments of the kits described herein, the amount of the DIM-related indole is 50 to 5,000 mg per dose or 100 to 3,000 mg per dose; or equal to or less than 1,000 mg per dose, equal to or less than 500 mg per dose, or equal to or less than 250 mg per dose (e.g., in units formulated for parenteral or intra-arterial administration).

[0022] In certain embodiments of the methods, compositions and kits provided herein, the Anti-Androgen Compound is a Diarylhydantoin compound. In specific embodiments, the diarylthiohydantoin compound has the formula:

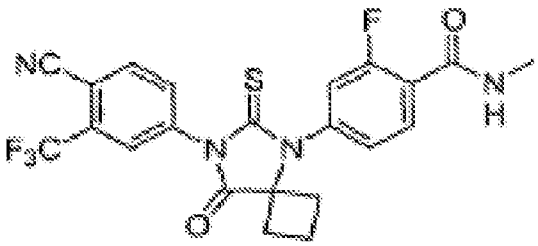


wherein Ri and R2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,
wherein R3 is selected from the group consisting of carbamoyl, alkyl carbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and
wherein R4 is hydrogen or fluorine.

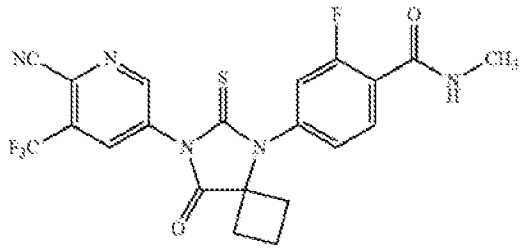
[0023] In one embodiment, the Diarylthiohydantoin compound is 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide ("Enzalutamide") having the following formula:



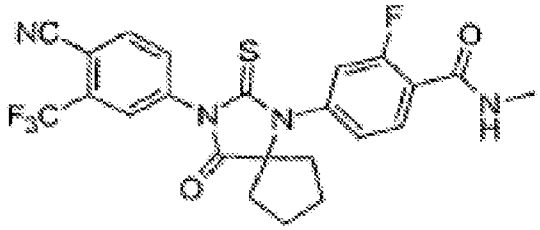
[0024] In one embodiment, the Diarylhydantoin compound has the formula:



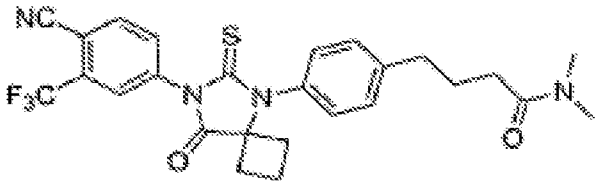
[0025] In another embodiment, the diarylthiohydantoin compound is 4-[7-(6-cyano-5-trifluoromethylpyridin-3-yl)-8-oxo-6-thioxo-5,7-diazaspir- o[3,4]oct-5-yl]-2-fluoro-N-methylbenzamide ("ARN 509") having the following formula:



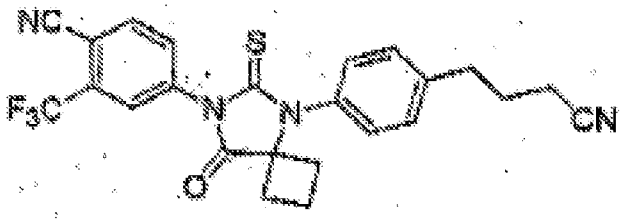
[0026] In another embodiment, the Diarylthiohydantoin compound has the formula:



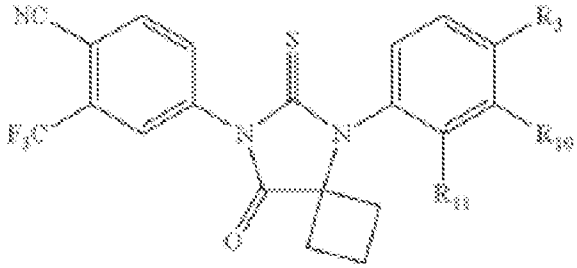
[0027] In yet another embodiment, the Diarylthiohydantoin compound has the formula:



[0028] In one embodiment, the Diarylthiohydantoin compound has the formula:



[0029] In certain embodiments, the diarylthiohydantoin compound has the formula:

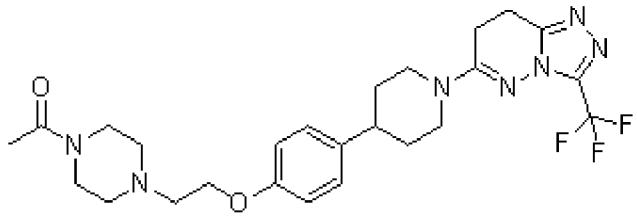


wherein R3 is selected from the group consisting of hydroxy, methylcarbamoyl, methylcarbamoylpropyl, methylcarbamoylethyl, methylcarbamoylmethyl, methylsulfonecarbamoylpropyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, carbamoylmethyl, carbamoylethyl, carboxymethyl,

methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoylpropyl, carboxypropyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, hydroxyethylcarbamoylethyl, and hydroxyethoxycarbonylethyl, and

wherein R10 and R11 are both H or, respectively, F and H, or H and F. In some of these embodiments, R10 and R11 are, respectively, F and H. In some of these embodiments, R3 is methylcarbamoyl. In some of these embodiments, R3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, and alkylcarbamoylalkyl.

[0030] In certain embodiments, the Anti-Androgen Compound is the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, and wherein the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound is -(4-(2-(4-(1-(3-(trifluoromethyl)-7,8-dihydro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)phenoxy)ethyl)piperazin-1-yl)ethanone ("AZD-3514") having the following formula:

[0031] 

[0032] In certain embodiments of the methods described herein, the Anti-Androgen Compound is administered orally. In other embodiments, the Anti-Androgen Compound is administered parenterally or intra-arterially. In some embodiments, the Anti-Androgen Compound is injected directly into prostate gland tissue. In a specific embodiment, the Anti-Androgen Compound is injected directly into prostate gland tissue using ultrasound guidance. In yet another specific embodiment, the Anti-Androgen Compound is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter.

[0033] In certain embodiments of the methods described herein, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) administered (e.g., orally) to the subject is 1 to 20 mg per kg, 2 to 6 mg per kg, or 1 to 2 mg per kg of the subject's weight per day. In specific embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) administered (e.g., orally) to the subject is 40 to 240 mg per day, 40 to 160 mg per day, 40 to 150 mg per

day, or 80 to 120 mg per day. In other specific embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) administered (e.g., orally) to the subject is less than 240 mg per day, less than 200 mg per day, less than 160 mg per day, equal to or less than 120 mg per day, equal to or less than 100 mg per day, equal to or less than 80 mg per day, or equal to or less than 50 mg per day.

[0034] In certain embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) administered parenterally or intra-arterially to the subject is 50 to 5,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks. In specific embodiments, the amount the Anti-Androgen Compound (e.g., Enzalutamide) administered to the subject is 100 to 2,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

[0035] In certain embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) administered parenterally or intra-arterially to the subject is less than 1000 mg per dose, less than 500 mg per dose, less than 250 mg per dose, less than 160 mg per dose, less than 120 mg per dose, or less than 100 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

[0036] In certain embodiments of the composition or the kits described herein, the Anti-Androgen Compound is formulated for oral administration. In other embodiments, the Anti-Androgen Compound is formulated for parenteral or intra-arterial administration. In specific embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) in the composition (e.g., for oral administration such as pill, tablet or capsule) is 40 to 250 mg, or 40 to 160 mg. In other specific embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) in the composition (e.g., for oral administration such as pill tablet or capsule) is less than 160 mg, equal to or less than 100 mg, equal to or less than 120 mg, equal to or less than 80 mg, equal to or less than 50 mg, or equal to or less than 40 mg. In some embodiments, the amount of the Anti-Androgen Compound (e.g., Enzalutamide) in one unit of the kit is 40 to 160 mg; or the amount of the Enzalutamide in one unit of the kit is less than 160 mg, equal to or less than 120 mg, equal to or less than 100 mg, equal to or less than 80 mg, equal to or less than 50 mg, or equal to or less than 40 mg (e.g., formulated for oral administration). In certain embodiments of the compositions and kits described herein, the amount of the Anti-Androgen Compound is 50 to 5,000 mg or 100 to 2,000 mg per composition or kit unit (e.g., formulated

for intra-arterial or parenteral administration). In other embodiments of the compositions and kits described herein, the amount of the Anti-Androgen Compound is less than 1000 mg, less than 500 mg, less than 250 mg, less than 160 mg, or less than 100 mg per composition or kit unit (e.g., formulated for intra-arterial or parenteral administration).

[0037] In some embodiments, the androgen or Androgen Receptor-driven disorder treated in accordance with the methods described herein and using the combination compositions and kits described herein is a disorder characterized by an increased expression of Androgen Receptor (AR) and/or an increased expression of AR splice variants. In certain embodiments, the androgen or Androgen Receptor-driven disorder is prostate cancer, prostatic intraepithelial neoplasia (PIN), or benign prostatic hyperplasia (BPH). In particular embodiments, the androgen or Androgen Receptor-driven disorder is prostate cancer. In one embodiment, the prostate cancer is castrate resistant prostate cancer (CRPC), e.g., metastatic CRPC (mCRPC) or non-metastatic CRPC. In other specific embodiments, the prostate cancer is hormone sensitive prostate cancer. In one embodiment, the androgen or Androgen Receptor-driven disorder is PIN. In one embodiment, the androgen or Androgen Receptor-driven disorder is BPH.

[0038] In some embodiments, methods, compositions and kits described herein are effective for treating one or more of the disorders listed above. In some embodiments, treatment using combination compositions described herein is more effective than treatment with the Anti-Androgen Compound alone and/or treatment with the DIM-related compound alone. The effectiveness for treating the disease can be demonstrated by an improvement in one or more symptoms or parameters of the disorder, e.g., as demonstrated by any test known in the art to assess such symptom or parameter. In some embodiments, treatment using combination compositions described herein is effective, or more effective than treatment with the Anti-Androgen Compound alone and/or treatment with the DIM-related compound alone, to: reduce tumor volume; slow progression of tumor growth; delay or prevent development of metastasis; normalize tissue biopsy; improve quality of life or reduce side effects associated with the treatment using the Anti-Androgen Compound; delay or prevent the development of resistance to the Anti-Androgen Compound; overcome the development of resistance to the Anti-Androgen Compound; decrease the amount of prostate specific antigen (PSA); increase progression-free survival, increase the period of stable disease, or increase overall survival. In

specific embodiments, treatment using combination compositions described herein shows efficacy (e.g., is more effective than treatment with the Anti-Androgen Compound alone and/or treatment with the DIM-related compound alone) in any one, two, three, four or five parameters/criteria listed in the preceding sentence.

[0039] In certain embodiments, the subject treated with compositions and methods described herein is resistant to an Anti-Androgen Compound. In some embodiments, the subject has been treated with an Anti-Androgen Compound. In particular embodiments, the subject is resistant to a Diarylthiohydantoin compound.

[0040] In certain embodiments of the methods described herein, the DIM-related indole is administered before administering the Anti-Androgen Compound.. In other embodiments, the DIM-related indole is administered after administering the Anti-Androgen Compound. In yet other embodiments, the DIM-related indole is administered simultaneously with the Anti-Androgen Compound. In some embodiments, the DIM-related indole and the Anti-Androgen Compound are co-formulated into a single pharmaceutical composition or delivery vehicle. In specific embodiments, the DIM-related indole and the Anti-Androgen Compound are co-formulated into one pill, tablet or capsule. In other embodiments, the DIM-related indole and the Anti-Androgen Compound are co-formulated into one pill, tablet or capsule. In some embodiments, the DIM-related indole and/or the Anti-Androgen Compound are administered once daily. In other embodiments, the DIM-related indole and/or the Anti-Androgen Compound are administered twice daily. In some embodiments, the Anti-Androgen Compound is administered once daily and the DIM-related indole is administered twice daily.

[0041] In preferred embodiments, the subject treated in accordance with the methods described herein and using the compositions and kits described herein is a human.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0042] **Fig. 1: Synergistic effects of BR-DIM and enzalutamide (MDV3100) on LNCaP and 22RV1 cell proliferation.** (A) and (B) The results from MTT showed that BR-DIM and enzalutamide treatment inhibited androgen-dependent prostate cancer cells: LNCaP and androgen-independent prostate cancer cells: 22RV1 cell proliferation. (C) and (D) Isobologram analysis of concentration-effect data showed synergistic effects of BR-DIM and enzalutamide on LNCaP and 22RV1 cell proliferation (N=6,**, $p<0.01$).

[0043] **Fig. 2: Overexpression of AR variants in prostate cancer cell lines.** (A) and (B) Expression of AR variants was increased in AR positive prostate cancer cell lines including LNCaP, C4-2B, VcaP and 22RV1 cells compared with immortalized Non-neoplastic human prostatic epithelial cells: RWPE1 and PZ-HPV-7.

[0044] **Fig. 3: Androgen deprivation induced expression of AR and AR variants, stem cell signatures and EMT phenotype in prostate cancer cell lines.** Total RNA was extracted from C4-2B and 22Rv1 cells cultured in charcoal stripped serum (FBS) or FBS for 2 weeks. The results from real time PCR showed increased expression of AR and AR variants concomitant with up-regulation of stem cell markers and mesenchymal markers in C4-2B cells (A) and 22Rv1 cells (B). (N-cad: n-cadherin, vim: vimentin).

[0045] **Fig. 4: Expression of AR and AR variants were increased in sphere forming cells from castration-resistant prostate cancer cells but not in androgen sensitive cell line. DIM decreased expression of AR and AR variants in prostate cancer cells.** (A) Sphere-forming cells from LNCaP cells, androgen sensitive cell line, displayed increased expression of stem cell markers and decreased expression of AR and AR variants. (B) Sphere-forming cells from 22Rv1 cells, castration-resistant cell line, displayed increased expression of AR and AR variants concomitant with increased expression of stem cell markers. (C) BR-DIM treatment led to decreased expression of AR and AR variants as well as stem cell markers in 22Rv1 sphere forming cells. (D) Western blot showed that BR-DIM treatment decreased the expression of AR, AR variant, and PSA in LNCaP cells. BR-DIM treatment also decreased expression of PSA in 22RV1 cells, suggesting that BR-DIM might prevent AR and AR variants from nuclear translocation.

[0046] **Fig. 5: Expression of AR variants and Lin28B was increased in patients' tumor tissues.** (A) and (B) The results from real time PCR showing that AR variants expression is increased in patients' tumor. (C) and (D). upregulation of Lin28B was found in prostate cancer (PCa) tissues compared to normal tissues (Normal: n=83, tumor: n=124, TumorG6: Tumor with Gleason grade 6, n=42, TumorG7: n=45, TumorG8,9: n=37).

[0047] **Fig. 6: The expression of miR-27b, miR-124 and miR-320 family were decreased in human prostate cancer tissue specimens.** (A) and (B) Expression of miR-124 and miR-27b was lower in prostate cancer tissues from patients with higher Gleason grade tumor than those from normal tissues. (C) – (F) The levels of miR-320 family was

downregulated in prostate cancer tissues from patients with higher Gleason grade tumor than those from normal tissues. (N: Normal: n=72, TG6: Tumor with Gleason grade 6, n=30, TG7: n=46, TG8,9: n=34).

[0048] **Fig. 7: The relationship between AR variants and expression of Lin28B and in PCa tissue specimens.** (A) and (B) AR3 and AR132b were positively correlated with expression of Lin28B in prostate cancer tissues with Gleason grade 8 and 9.

[0049] **Fig. 8: The expression of miR-27b, miR-124 and miR-320 family were upregulated in human prostate cancer tissue specimens by BR-DIM treatment.** (A) and (B) Expression of miR-124 and miR-27b was upregulated in prostate cancer tissues from patient treated by BR-DIM compared with untreated patients. (C) – (F) Expression of miR-320 family was upregulated in prostate cancer tissues from patient treated by BR-DIM compared with untreated patients. (patients: n=72, BR-DIM treatment group, n=11).

[0050] **Fig. 9: BR-DIM treatment down-regulated Lin28B expression in prostate cancer patient tissue specimens.** Total RNA obtained from FFPE PCa tissues from patients and used for determining mRNA expression using real time RT-PCR. Relative mRNA levels were normalized to beta-actin. BR-DIM treatment significantly down-regulated the expression of Lin28B.

[0051] **Fig. 10: Synergistic effects of BR-DIM and enzalutamide (MDV3100) on LNCaP cell proliferation.** (A) The results from MTT showed that BR-DIM and enzalutamide treatment inhibited LNCaP cell proliferation. (B) Isobologram analysis of concentration-effect data showed synergistic effects of BR-DIM and enzalutamide on LNCaP cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating synergy from co-administration of BR-DIM and enzalutamide.

[0052] **Fig. 11: Synergistic effects of BR-DIM and enzalutamide (MDV3100) on LNCaP cell proliferation.** (A) The results from MTT showed that BR-DIM and enzalutamide treatment inhibited LNCaP cell proliferation. (B) Isobologram analysis of concentration-effect. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating synergy from co-administration of BR-DIM and enzalutamide.

[0053] **Fig. 12: Synergistic effects of BR-DIM and enzalutamide (MDV3100) on C4-2B cell proliferation.** (A) The results from MTT showed that BR-DIM and enzalutamide treatment inhibited C4-2B cell proliferation. (B) Isobologram analysis of concentration-effect data showed synergistic effects of BR-DIM and enzalutamide on C4-2B cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating synergy from co-administration of BR-DIM and enzalutamide.

[0054] **Fig. 13: Synergistic effects of BR-DIM and enzalutamide (MDV3100) on C4-2B cell proliferation.** (A) The results from MTT showed that BR-DIM and enzalutamide treatment inhibited C4-2B cell proliferation. (B) Isobologram analysis of concentration-effect data showed synergistic effects of BR-DIM and enzalutamide on C4-2B cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating synergy from co-administration of BR-DIM and enzalutamide.

[0055] **Fig. 14: Synergistic effects of BR-DIM and ARN-509 on C4-2B cell proliferation.** (A) The results from MTT showed that BR-DIM and ARN-509 treatment inhibited C4-2B cell proliferation. (B) Isobologram analysis of concentration-effect data showed synergistic effects of BR-DIM and ARN-509 on C4-2B cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating synergy from co-administration of BR-DIM and ARN-509.

[0056] **Fig. 15: Lack of synergistic effect of Casodex and enzalutamide (MDV3100) on LNCaP cell proliferation.** (A) The results from MTT showed that Casodex and enzalutamide co-treatment did not exhibit synergistic inhibitory effect on LNCaP cell proliferation. (B) Isobologram analysis of concentration-effect data showed lack of synergistic effects of Casodex and enzalutamide on LNCaP cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating lack of synergy from co-administration of Casodex and enzalutamide.

[0057] **Fig. 16: Lack of synergistic effect of Casodex and enzalutamide (MDV3100) on C4-2B cell proliferation.** (A) The results from MTT showed that Casodex and

enzalutamide co-treatment did not exhibit synergistic inhibitory effect on C4-2B cell proliferation. (B) Isobologram analysis of concentration-effect data showed lack of synergistic effects of Casodex and enzalutamide on C4-2B cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating lack of synergy from co-administration of Casodex and enzalutamide.

[0058] **Fig. 17: Lack of synergistic effect of Casodex and ARN-509 on C4-2B cell proliferation.** (A) The results from MTT showed that Casodex and ARN-509 co-treatment did not exhibit synergistic inhibitory effect on C4-2B cell proliferation. (B) Isobologram analysis of concentration-effect data showed lack of synergistic effects of Casodex and ARN-509 on C4-2B cell proliferation. (C) A summary table showing Combination Indexes (CI values) at 50%, 75% and 90% Effective Dose level (i.e., ED50, ED75 and ED90, respectively) demonstrating lack of synergy from co-administration of Casodex and ARN-509.

5. DETAILED DESCRIPTION OF THE INVENTION

[0059] In one aspect, the invention provides for compositions and kits comprising a DIM-related indole and a non-steroidal anti-androgen compound that induces or is known to induce expression or overexpression of Androgen Receptor (AR) and/or AR splice variant(s). The invention also provides methods for treating an androgen or Androgen Receptor-related disorder (such as an androgen or Androgen Receptor-driven disorder). In certain embodiments, the invention provides for treating an androgen or Androgen Receptor-related disorder which is characterized by increased expression of AR and/or AR variants (such as AR splice variants), for example, increased expression of AR and/or AR variants in the tissue affected by the disorder relative to normal tissue. In certain embodiments, the invention provides for treating an androgen or Androgen Receptor-related disorder which is characterized by the expression of AR variants, such as AR3, AR132b and/or AR122b. The androgen or Androgen Receptor-related disorders include, without limitation, prostate cancer, prostatic intraepithelial neoplasia (PIN), benign prostatic hyperplasia (BPH), male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism. In certain embodiments, provided herein are methods for treating an androgen or Androgen Receptor-related disorder in a subject subject (e.g., a human subject) by administering to the subject: (i) a DIM-related indole and (ii) a non-steroidal anti-androgen compound that induces or is known to induce expression or overexpression of Androgen

Receptor (AR) and/or AR splice variant(s). In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject (i) a DIM-related indole and (ii) a non-steroidal anti-androgen compound that is known to induce expression or overexpression of Androgen Receptor (AR) and/or AR splice variant(s).

[0060] In some aspects, the invention provides compositions and kits comprising (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Selected Anti-Androgen Compound described in Section 5.2 below, or a pharmaceutically acceptable salt, prodrug or solvate thereof. The invention also provides methods for treating an androgen or Androgen Receptor-related disorder (e.g., prostate cancer, benign prostatic hyperplasia, male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism) in a subject by administering to the subject: (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Selected Anti-Androgen Compound described in Section 5.2 below, or a pharmaceutically acceptable salt, prodrug or solvate thereof. In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Selected Anti-Androgen Compound described in Section 5.2 below, or a pharmaceutically acceptable salt, prodrug or solvate thereof. In certain embodiments, provided herein are methods of treating an androgen or Androgen Receptor-related disorder (e.g., prostate cancer, benign prostatic hyperplasia, male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism) in a subject in need thereof comprising the steps of (a) administering a DIM-related indole (e.g., DIM or I3C) to the subject, and (b) administering a Selected Anti-Androgen Compound described in Section 5.2 below, or a pharmaceutically acceptable salt, prodrug or solvate thereof, to the subject. In some embodiments, step (a) is performed before step (b). Inother embodiments, step (a) is performed after step (b). In yet other embodiments, step (a) and step (b) are performed simultaneously. In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Selected Anti-Androgen Compound described in Section 5.2 below, or a pharmaceutically acceptable salt, prodrug or solvate thereof.

[0061] In some aspects, the invention provides compositions and kits comprising (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylhydantoin compound, a

Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt, prodrug or solvate thereof. The invention also provides methods for treating an androgen or Androgen Receptor-related disorder (e.g., prostate cancer, prostatic intraepithelial neoplasia (PIN), benign prostatic hyperplasia, male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism) in a subject by administering to the subject: (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt, prodrug or solvate thereof. In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt, prodrug or solvate thereof.

[0062] In a particular aspect, the invention provides compositions and kits comprising (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylthiohydantoin compound (e.g., enzalutamide or ARN-509), or a pharmaceutically acceptable salt, prodrug or solvate thereof.. The invention also provides methods for treating an androgen or Androgen Receptor-related disorder (e.g., prostate cancer, prostatic intraepithelial neoplasia (PIN), benign prostatic hyperplasia, male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism) in a subject by administering to the subject: (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylthiohydantoin compound (e.g., enzalutamide or ARN-509), or a pharmaceutically acceptable salt, prodrug or solvate thereof. In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject (i) a DIM-related indole (e.g., DIM or I3C) and (ii) a Diarylthiohydantoin compound (e.g., enzalutamide or ARN-509), or a pharmaceutically acceptable salt, prodrug or solvate thereof.

[0063] In other particular embodiments, the invention provides compositions and kits comprising a DIM-related indole (e.g., DIM or I3C) and a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound (e.g., AZD-3514), or a pharmaceutically acceptable salt, prodrug or solvate thereof. The invention also provides methods for treating an androgen or Androgen Receptor-related disorder (e.g., prostate cancer, benign prostatic hyperplasia, male-pattern

baldness, polycystic ovarian syndrome (PCOS) or hirsutism) in a subject by administering to the subject a DIM-related indole (e.g., DIM or I3C) and a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound (e.g., AZD-3514), or a pharmaceutically acceptable salt, prodrug or solvate thereof. In specific embodiments, the invention provides methods for treating prostate cancer (e.g., castrate resistant prostate cancer) in a subject by administering to the subject a DIM-related indole (e.g., DIM or I3C) and a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound (e.g., AZD-3514), or a pharmaceutically acceptable salt, prodrug or solvate thereof.

[0064] In some embodiments of the invention, a DIM-related compound is co-administered with any of the Selected Anti-androgen Compounds described herein (e.g., a Diarylthiohydantoin compound), either concomitantly or sequentially. In other embodiments of the invention, a DIM-related compound is co-formulated with any of the Selected Anti-Androgen Compounds described herein (e.g., a Diarylthiohydantoin compound) (i.e., the agents are combined into one composition or delivery vehicle before administration).

[0065] The invention is based on the finding by the inventors that co-administration of a DIM-related indole with a Diarylthiohydantoin compound results in the enhanced growth arrest of prostate cancer (PC) cells. In particular, the inventors have unexpectedly found that co-administration of a DIM-related indole (DIM) with a Diarylthiohydantoin compound (enzalutamide) shows synergistic activity in inducing growth arrest of PC cells (see Examples). In addition, the inventors have found that a DIM-related indole results in transcriptional inactivation of Androgen Receptor (AR) and AR splice variants in PC cells (see Examples). It is known that the emergence of AR splice variants during Diarylhydantoin therapy is associated with resistance to treatment (Li et al., 2013, Cancer Res. 73(2):483-9). The data presented in this application showing that: (i) a DIM-related indole and a Diarylthiohydantoin compound synergize in inducing growth arrest of PC cells, and (ii) a DIM-related indole inhibits the expression of AR splice variants in PC cells, suggest that co-administration of a DIM-related indole with an anti-androgen compound that induces expression or overexpression of AR splice variants (such as a Diarylthiohydantoin compound) can result in an improved therapeutic response in vivo. In addition, the data presented herein suggest that co-administration of a DIM-related indole with an anti-androgen compound that induces expression or overexpression of AR splice variants (e.g., a Diarylthiohydantoin) can delay or prevent the development of resistance to treatment with such anti-androgens or overcome

resistance to treatment with such anti-androgens. Furthermore, since co-administration of a DIM-related indole with a Diarylthiohydantoin compound resulted in unexpectedly synergistic activity in inhibiting the growth of PC cells, co-administration or co-formulation of these agents can decrease the respective doses of these agents required to achieve therapeutic effect relative to administration of any of these two agents alone, which may in turn reduce side effects associated with the use of higher doses of these agents in patients. Based on the data presented herein, the inventors anticipate that a more sustained response to treatment of prostate cancer, as well as prostatic intraepithelial neoplasia (PIN), and improvement in the overall therapeutic response can be achieved by using the combination of a DIM-related indole and a Diarylhydantoin-related compound, compared to that seen with Diarylhydantoin (Enzalutamide) monotherapy.

[0066] In specific embodiments, disclosed herein are uses of the combination of DIM-related indoles and Selected Anti-Androgen Compounds described in Section 5.2 (e.g., enzalutamide or ARN-509), wherein the combination is more therapeutically effective than the use of the Selected Anti-Androgen Compounds alone and/or the use of the DIM-related indoles alone (e.g., in the treatment of prostate cancer such as castrate resistant prostate cancer). Without being bound by any particular mechanism of action, it is contemplated that the combined administration utilizes unexpected activity from the DIM or DIM-related indole to improve the level of growth arrest and cancer cell death induced by anti-androgens described herein. In addition, and also without being bound by any particular mechanism of action, it is contemplated that the effects of DIM or a DIM-related indole are in part due to transcriptional inactivation of both wild-type AR and AR splice variants which are constitutively active and whose expression is increased in enzalutamide resistant PC. The object of combined use of DIM or DIM-related indoles co-administered with Selected Anti-Androgen Compounds described in Section 5.2 is to increase the efficacy of treatment over the use of the Selected Anti-Androgen Compounds described in Section 5.2 alone. Importantly, combined administration and administration of co-formulations of a DIM-related compound and a Selected Anti-Androgen Compound (such as compounds described in Section 5.2) is expected to result in a synergistically greater tumoral growth arrest and therapeutic response than when DIM or Selected Anti-Androgen Compound are not used together. This is attributed to unique interactions of DIM-related indoles with Selected Anti-Androgen Compounds described in

Section 5.2, in particular, Diarylthiohydantoin compounds. The combined administration of DIM or DIM-related indoles with Selected Anti-Androgen Compounds described in Section 5.2 is also directed to forestalling and/or reversing treatment resistance to such anti-androgen compounds which emerges during chronic use of such anti-androgen compounds alone.

[0067] In some embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen Compounds described in Section 5.2 (e.g., enzalutamide or ARN-509) is more effective than the use of either agent alone (e.g., the use of the anti-androgen compound alone). In specific embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen Compounds described in Section 5.2 (e.g., enzalutamide or ARN-509) is at least or more than 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5 or 5 times more effective than the use of either agent alone (e.g., the use of the anti-androgen compound alone). In other specific embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 (e.g., enzalutamide or ARN-509) is effective to delay the development of resistance, prevent the development of resistance, or overcome resistance to such Anti-Androgen Compounds. In other specific embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 (e.g., enzalutamide or ARN-509) is effective to reduce the dose of either agent required for efficacy (relative to the dose required for efficacy when used alone), e.g., at least or more than 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 4.5 or 5 times. Efficacy can be shown by an improvement in one or more parameters or symptoms of the disorder or disease being treated. The improvement can be manifested in an alteration in a parameter of a disorder or disease in a therapeutic direction, or in stabilization of a parameter that would otherwise be deteriorating in a non-therapeutic direction.

[0068] The combination therapy described herein can be effective to improve one, two, three or more of the parameters or symptoms of a disease or disorder. The combination therapy described herein can be more effective than the use of the described agents alone in improving at least one, at least two, or at least three of the parameters or symptoms of a disease or disorder.

[0069] It is contemplated that treatment of disorders described herein (e.g., prostate cancer) using the combination of a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 can be effective, for example, to: inhibit the progression,

spread and/or duration of a disease or disorder; reduce or ameliorate the severity of a disease or disorder; ameliorate one or more symptoms of a disease or disorder; reduce the duration of one or more symptom of a disease or disorder; increase overall survival; prolong progression-free survival; reduce tumor size; prevent or delay the growth of a tumor; prevent, delay or reduce the development of metastasis; prevent or delay the spread of metastasis; prevent, delay or overcome the development of resistance to treatment; improve the quality of life (e.g., by reducing side effects due to treatment); and/or prevent recurrence of a disease or disorder in a subject. Specifically in relation to treating benign prostatic hyperplasia (BPH), using the combination of a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 can be effective, for example, to inhibit symptom severity and/or progression of the disorder. Amelioration of symptoms of BPH may be manifested in reduction in the standard Lower Urinary Tract Symptom Score (LUTS). Specifically in relation to treating prostatic intraepithelial neoplasia (PIN), the effectiveness of a combination of a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 can be manifested, for example, by showing more normal results on repeat transrectal prostate needle biopsy and/or reduction in the LUTS score after the administration of the combination (e.g., relative to results or score obtained prior to the administration, or relative to average results or score in patients with PIN).

[0070] In specific embodiments, the treatment with the combination of a DIM-related indole and a Selected Anti-Androgen Compound decreases PSA levels in the treated subject by, or by more than, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98%.

[0071] In certain embodiments, the compositions, kits and methods of the invention involve the oral use of DIM or a DIM-related compound administered simultaneously with a Selected Anti-Androgen Compound described in Section 5.2. In a preferred embodiment, the Selected Anti-Androgen Compound is a Diarylthiohydantoin compound, such as Enzalutamide, ARN-509 or a compound closely related (e.g., structurally and/or functionally similar) to Enzalutamide or ARN-509. In some embodiments, DIM or a DIM-related compound is combined in a single tablet, pill or capsule with Enzalutamide or ARN-509 and administered once or twice daily. In one embodiment, the anti-androgen compound for use in combination compositions, kits and methods described herein is AZD-3514. In a particular embodiment, DIM or a DIM-related compound is combined in a single tablet, pill or capsule with AZD-3514. In a particular embodiment of the methods described herein, (i) a Selected Anti-

Androgen Compound described in Section 5.2 and (ii) a DIM-related indole are combined in the same delivery vehicle and administered simultaneously.

[0072] In certain embodiments, 40-160 mg of Enzalutamide is taken orally once daily and 75-350 mg DIM from BioResponse DIM (BR-DIM [B-DIM]) is taken orally twice daily. In some of these embodiments, (i) less than 160 mg, 120 mg or less than 120 mg, 80 mg or less than 80 mg, or 40 mg of Enzalutamide is taken once daily, and (ii) 225 mg or less than 225 mg, 200 mg or less than 200 mg, 150 mg or less than 150 mg, 100 mg or less than 100 mg, or 75 mg DIM from BioResponse DIM (BR-DIM [B-DIM]) is taken twice daily.

[0073] In particular embodiments, a pharmaceutical composition comprising (i) less than less than 80 mg, less than 60 mg or 40 mg of Enzalutamide, and (ii) less than 225 mg, 200 mg or less than 200 mg, 150 mg or less than 150 mg, 100 mg or less than 100 mg, or 75 mg of DIM (e.g., BR-DIM [B-DIM]), is formulated for twice daily administration.

[0074] In an alternative embodiment, DIM or a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 are co-formulated in a suspension or drug-eluting microsphere for direct administration into prostate gland tissue. In this embodiment, microparticles of the Selected Anti-Androgen Compound described in Section 5.2, such as Enzalutamide or ARN-509, are embedded into microspheres using techniques well known in the art or described herein. DIM or DIM-related indole can be similarly embedded into microspheres using techniques well known in the art or described herein. In a further embodiment, DIM or a DIM-related indole and a Selected Anti-Androgen Compound described in Section 5.2 are co-formulated in a drug eluting microsphere and administered intra-arterially or injected directly into diseased tissue, including injection into prostate gland tissue using ultrasound guidance. In some of the preferred embodiments, such co-formulated microsphere comprises Enzalutamide or ARN-509.

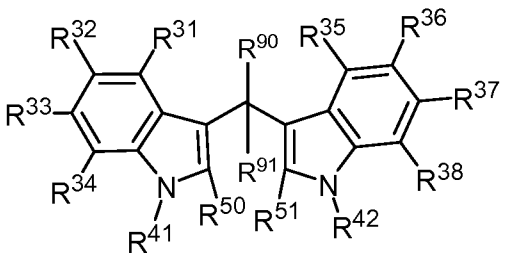
[0075] In certain embodiments of the invention, the subject is a human. Targeted human diseases include, without limitation, prostate cancer, benign prostatic hypertrophy and male-pattern baldness in men. In some of the therapeutic applications, disclosed are microspheres comprising a DIM-related indole (e.g., DIM) and a Selected Anti-Androgen Compound described in Section 5.2 for use during prostate gland arterial embolization procedures accomplished using angiographically directed arterial catheters and microcatheters. In an alternative mode of practice, microspheres comprising a DIM-related indole (e.g., DIM)

and a Selected Anti-Androgen Compound described in Section 5.2 are injected directly into prostate using either ultrasound guidance, direct vision, radiologic guidance, or other various operative and non-operative approaches and techniques. In some of the specific embodiments, microspheres comprising a DIM-related indole (e.g., DIM) and a Selected Anti-Androgen Compound described in Section 5.2 are injected into metastatic PC tumors in a tissue, including without limitation, bone and brain, using any technique known in the art or described herein.

5.1. Diindolylmethane Related Indoles

[0076] The DIM-related indoles or DIM compounds useful in the methods and compositions of the invention include DIM (3,3'-diindolylmethane) and the related linear DIM trimer (2-(indol-3-ylmethyl)-3,3'-diindolylmethane [also written: 2 (Indol-3-ylmethyl)-indol-3-yl]indol-3-ylmethane] (LTR), and Indole-3-Carbinol (I3C). As used herein, "DIM-related compound", "DIM-related indole", and "DIM derivative" are used interchangeably, and refer to both natural metabolites and analogs of DIM, and also to "structurally-related, synthetically-derived, substituted diindolylmethane compounds" and "synthetic derivatives of DIM", such as those disclosed herein and known in the art. As used herein, "cruciferous-related indoles" encompasses the terms "DIM-related compound", "DIM-related indole", and "DIM derivative". One of ordinary skill in the art will recognize that in any of the pharmaceutical compositions or methods of the invention where DIM is used, a DIM-related compound, including a structurally-related, synthetically-derived, substituted diindolylmethane compound or synthetic derivative of DIM, can be used.

[0077] The chemical structure of a DIM is as follows (where each of the R groups is H):



(I)

[0078] In particular embodiments, the DIM-related indole is a compound of formula I, wherein R^{42}, R^{51}, R^{35}, R^{36}, R^{37}, R^{38}, R^{90}, R^{41}, R^{50}, R^{31}, R^{32}, R^{33}, R^{34} and R^{91} individually and independently, are hydrogen or a substituent selected from the group consisting of a halogen, a

hydroxyl, a nitro, —OR^{100}, —CN, —$NR^{100}R^{101}$, —$NR^{100}R^{101}R^{102+}$, —COR^{100}, CF_3, —S(O)nR^{100} (n=0-2), —$SO_2NR^{100}R^{101}$, —$CONR^{100}R^{101}$, —$NR^{100}COR^{101}$, —$NR^{100}C(O)NR^{101}R^{102}$, —$P(O)(OR^{100})_n$ (n=1-2), optionally substituted alkyl, halovinyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroaryl, or optionally substituted cycloalkyl or cycloakenyl, all of one to ten carbons and optionally containing 1-3 heteroatoms O or N, wherein R^{100}, R^{101} and R^{102} are optionally substituted alkyl, alkenyl, alkynl, aryl, heteroalkyl, heteroaryl of one to ten carbons, and R^{90} and R^{91} may further be O to create a ketone. In particular embodiments, the compound includes at least one such substituent, preferably at a position other than, or in addition to R^{42} and R^{41}, the linear or branched alkyl or alkoxy group is one to five carbons, and/or the halogen is selected from the group consisting of chlorine, iodine, bromine and fluorine.

[0079] In certain embodiments, an active hydroxylated or methyoxylated metabolite of DIM, *i.e.*, a compound of formula I, wherein R^{32}, R^{33}, R^{36}, and R^{37} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and R^{31}, R^{34}, R^{35}, R^{38}, R^{41}, R^{42}, R^{50}, and R^{51} are hydrogen, is utilized. In particular embodiments, the DIM-related indole is a mono- or di-hydroxylated DIM derivatives at carbon positions 2, 4-7 and 2', and 4'-7', including each of [2, 4, 5, 6 or 7]-monohydroxy-DIM or [2', 4', 5', 6' or 7']-monohydroxy-DIM (*e.g.* 2-hydroxy-DIM, 4-hydroxy-DIM, etc.); and each of [2, 4, 5, 6 or 7], [2, 4, 5, 6 or 7]-dihydroxy-DIM, [2', 4', 5', 6' or 7'], [2', 4', 5', 6' or 7']-dihydroxy-DIM, or [2, 4, 5, 6 or 7], [2', 4', 5', 6' or 7']-dihydroxy-DIM (*e.g.* 2,4-dihydroxy-DIM, 2,5-dihydroxy-DIM etc, 2,2'-dihydroxy-DIM, 2,4'-dihydroxy-DIM etc.); particularly bilaterally symmetrical species, such as 2,2'-dihydroxy-DIM.

[0080] In particular embodiments, the indolyl moieties are symmetrically substituted, wherein each moiety is similarly mono-, di-, tri-, para-, etc. substituted. In other particular embodiments, R^{42}, R^{51}, R^{35}, R^{37}, R^{38}, R^{90}, R^{41}, R^{50}, R^{31}, R^{33}, R^{34} and R^{91} are hydrogen, and R^{36} and R^{32} are a halogen selected from the group consisting of chlorine, iodine, bromine and fluorine. Representative compounds include, but are not limited to, 3,3'-diindolylmethane, 5,5'-dichloro-diindolylmethane; 5,5'-dibromo-diindolylmethane; and 5,5'-difluoro-diindolylmethane. Additional preferred such DIM derivatives include compounds wherein R^{42}, R^{51}, R^{35}, R^{37}, R^{38}, R^{90}, R^{41}, R^{50}, R^{31}, R^{33}, R^{34} and R^{91} are hydrogen, and R^{36} and R^{32} are an alkyl or alkoxyl having from one to ten carbons, and most preferably one to five carbons.

Representative compounds include, but are not limited to, 5,5'-dimethyl-diindolylmethane, 5,5'-diethyl-diindolylmethane, 5,5'-dipropyl-diindolylmethane, 5,5'-dibutyl-diindolylmethane, 5,5'-dipentyl-diindolylmethane, 5,5'-dimethoxy-diindolylmethane, 5,5'-diethoxy-diindolylmethane, 5,5'-dipropyloxy-diindolylmethane, 5,5'-dibutyloxy-diindolylmethane, and 5,5'-diamyloxy-diindolylmethane.

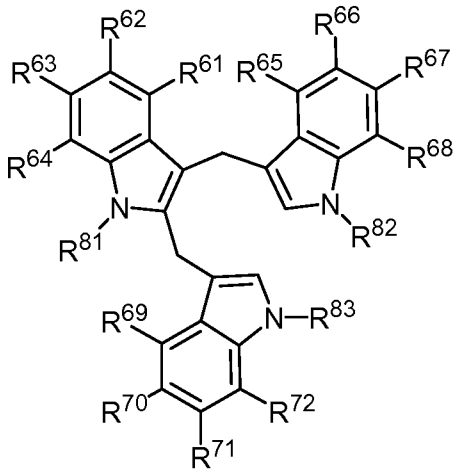
[0081] Additional preferred DIM derivatives include compounds wherein R^{51}, R^{35}, R^{36}, R^{37}, R^{38}, R^{90}, R^{50}, R^{31}, R^{32}, R^{33}, R^{34} and R^{91} are hydrogen, and R^{42} and R^{41} are an alkyl or alkoxyl having from one to ten carbons, and most preferably one to five carbons. Representative compounds include, but are not limited to, N,N'-dimethyl-diindolylmethane, N,N'-diethyl-diindolylmethane, N,N'-dipropyl-diindolylmethane, N,N'-dibutyl-diindolylmethane, and N,N'-dipentyl-diindolylmethane. In yet another embodiment, R^{42}, R^{35}, R^{36}, R^{37}, R^{38}, R^{90}, R^{41}, R^{31}, R^{32}, R^{33}, R^{34} and R^{91} are hydrogen, and R^{51} and R^{50} are alkyl of one to ten carbons, and most preferably one to five carbons. Representative compounds include, but are not limited to, 2,2'-dimethyl-diindolylmethane, 2,2'-diethyl-diindolylmethane, 2,2'-dipropyl-diindolylmethane, 2,2'-dibutyl-diindolylmethane, and 2,2'-dipentyl-diindolylmethane. In another embodiment, R^{42}, R^{51}, R^{35}, R^{37}, R^{38}, R^{90}, R^{41}, R^{50}, R^{31}, R^{33}, R^{34} and R^{91} are hydrogen, and R^{36} and R^{32} are nitro.

[0082] In an alternative embodiment, active DIM derivatives with R_{32} and R_{36} substituents made up of ethoxycarbonyl groups, and R_{50}, R_{51} are either hydrogen or methyl, are utilized.

[0083] In another embodiment, active substituted DIM derivatives including methylated and chlorinated compounds, exemplified by those that include 5,5'-dimethylDIM (5-Me-DIM), 2,2'-dimethylDIM (2-Me-DIM), and 5,5'-dichloroDIM (5-Cl-DIM) are described in U.S. Patent Application Publication No. 20020115708 by Safe, published August 22, 2002, incorporated herein by reference in its entirety, are utilized in the present invention. In another embodiment, active DIM derivatives include imidazolelyl-3,3'-diindolylmethane, including nitro substituted imidazolelyl-3,3'-diindolylmethanes, and additional DIM-related compounds described in U.S. Patent Application Publication No. 2004/0043965 by Jong, Ling, published March 4, 2004, incorporated herein by reference in its entirety, are utilized. In a further embodiment, active DIM derivatives described in US Patent # 6,656,963, US Patent

#6,369,095 and U.S. Patent Application Publication No. 20060229355 by Bjeldanes et al., published October 12, 2006, incorporated herein by reference in its entirety, are utilized.

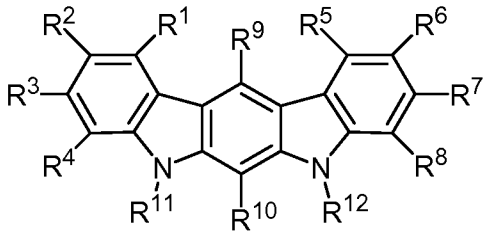
[0084] The chemical structure of LTR is as follows (where each of the R groups is H):



(II)

[0085] In certain embodiments, an active hydroxylated or methyoxylated metabolite of LTR, *i.e.*, a compound of formula II, wherein R^{62}, R^{63}, R^{66}, R^{67}, R^{70}, and R_{71} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and R^{61}, R^{64}, R^{65}, R^{68}, R^{69}, R^{72}, R^{81}, R^{82}, and R^{83} are hydrogen, is utilized.

[0086] In certain embodiments, a DIM related compound has formula (III):



[0087] (III)

[0088] wherein:

[0089] R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are substituents independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_5-C_{20} aryl, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxycarbonyl, halocarbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C_1-C_{24} alkyl)-substituted carbamoyl, di-(C_1-C_{24} alkyl)-substituted carbamoyl, mono-substituted arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C_1-C_{24} alkyl)-substituted amino, mono- and di-(C_5-C_{20} aryl)-substituted amino, C_2-C_{24}

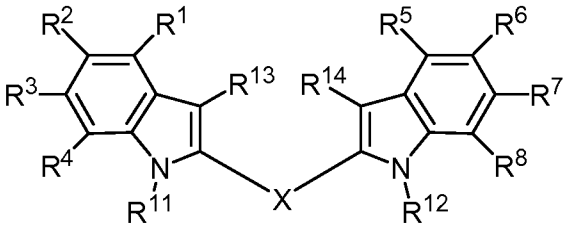
alkylamido, C_6-C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C_1-C_{24} alkylsulfanyl, arylsulfanyl, C_1-C_{24} alkylsulfinyl, C_5-C_{20} arylsulfinyl, C_1-C_{24} alkylsulfonyl, C_5-C_{20} arylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, and combinations thereof, and further wherein any two adjacent (ortho) substituents may be linked to form a cyclic structure selected from five-membered rings, six-membered rings, and fused five-membered and/or six-membered rings, wherein the cyclic structure is aromatic, alicyclic, heteroaromatic, or heteroalicyclic, and has zero to 4 non-hydrogen substituents and zero to 3 heteroatoms; and

[0090] R^{11} and R^{12} are independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkoxycarbonyl, amino-substituted C_1-C_{24} alkyl, (C_1-C_{24} alkylamino)-substituted C_1-C_{24} alkyl, and di-(C_1-C_{24} alkyl)amino-substituted C_1-C_{24} alkyl,

[0091] with the provisos that: at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} is other than hydrogen; and when R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are selected from hydrogen, halo, alkyl and alkoxy, then R^{11} and R^{12} are other than hydrogen and alkyl.

[0092] A preferred embodiment includes the use of 2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo-[2,3-b]carbazole (SRI13668 (SRI Inc., Menlo Park, CA)). Additional preferred embodiments include the use of 6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole and 2,10-dicarbethoxy-6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole (SRI Inc., Menlo Park, CA).

[0093] In another embodiment, a DIM related compound has formula (IV):

[0094] 

(IV)

[0095] wherein:

[0096] R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are substituents independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_5-C_{20} aryl, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxycarbonyl, halocarbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C_1-C_{24} alkyl)-substituted carbamoyl, di-(C_1-C_{24} alkyl)-substituted carbamoyl, mono-

substituted arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C_1-C_{24} alkyl)-substituted amino, mono- and di-(C_5-C_{20} aryl)-substituted amino, C_2-C_{24} alkylamido, C_5-C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, sulfo, sulfonato, C_1-C_{24} alkylsulfanyl, arylsulfanyl, C_1-C_{24} alkylsulfinyl, C_5-C_{20} arylsulfinyl, C_1-C_{24} alkylsulfonyl, C_5-C_{20} arylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, and combinations thereof, and further wherein any two adjacent (ortho) substituents may be linked to form a cyclic structure selected from five-membered rings, six-membered rings, and fused five-membered and/or six-membered rings, wherein the cyclic structure is aromatic, alicyclic, heteroaromatic, or heteroalicyclic, and has zero to 4 non-hydrogen substituents and zero to 3 heteroatoms, with the proviso that one but not both of R^2 and R^6 is amino, mono-substituted amino, or di-substituted amino;

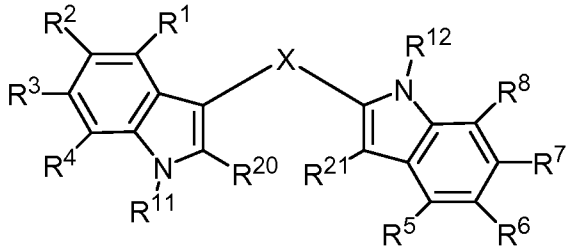
[0097] R^{11} and R^{12} are independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkoxycarbonyl, amino-substituted C_1-C_{24} alkyl, (C_1-C_{24} alkylamino)-substituted C_1-C_{24} alkyl, and di-(C_1-C_{24} alkyl)amino-substituted C_1-C_{24} alkyl;

[0098] R^{13} and R^{14} are defined as for R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8, with the proviso that at least one of R^{13} and R^{14} is other than hydrogen; and

[0099] X is O, S, arylene, heteroarylene, $CR^{15}R^{16}$ or NR^{17} wherein R^{15} and R^{16} are hydrogen, C_1-C_6 alkyl, or together form $=CR^{18}R^{19}$ where R^{18} and R^{19} are hydrogen or C_1-C_6 alkyl, and R^{17} is as defined for R^{11} and R^{12}.

[00100] A preferred embodiment includes the use of 2,6-dicarbethoxy-3,3'-dimethyl-13,14-diindolylmethane (SRI Inc., Menlo Park, CA).

[00101] In another embodiment, a DIM related compounds has formula (V):



[00102] (V)

[00103] wherein:

[00104] R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^{11}, R^{12}, and X are defined as for compounds of formula (III); and

[00105] R^{20} and R^{21} are defined as for R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8.

[00106] In yet another embodiment, the DIM-related indole is an indole-3-carbinol tetrameric derivative (Brandi *et al.*, 2003, Cancer Res. 63:4028-4036). In a further embodiment the DIM-related indole is an indole-3-carbinol derivative described as an anti-tumor agent (Weng JR, Tsai CH, Kulp SK, Wang D, Lin CH, Yang HC, Ma Y, Sargeant A, Chiu CF, Tsai MH, Chen CS. A potent indole-3-carbinol derived antitumor agent with pleiotropic effects on multiple signaling pathways in prostate cancer cells. Cancer Res. 2007 Aug 15;67(16):7815-24).

[00107] Substituted DIM analogs are readily prepared by condensation of formaldehyde with commercially available substituted indoles. Precursor compounds can be synthesized by dimethylformamide condensation of a suitable substituted indole to form a substituted indole-3-aldehyde. Suitable substituted indoles include indoles having substituents at R^{42}, R^{51}, R^{35}, R^{36}, R^{37} and R^{38} positions. These include, but are not limited to 5-methoxy, 5-chloro, 5-bromo, 5-fluoro, 5'-methyl, 5-nitro, n-methyl and 2-methyl indoles. The substituted indole 3-aldehyde product is treated with a suitable alcohol such as methanol and solid sodium borohydride to reduce the aldehyde moiety to give substituted I3Cs. Substituted DIMs are prepared by condensing the substituted indole-3-carbinol products. This may be achieved, for example, by treatment with a phosphate buffer having a pH of around 5.5 – 7.4.

[00108] In a preferred embodiment, a DIM-related compound is processed DIM. The method of making processed DIM (i.e., DIM with enhanced oral availability) is described in Example 1.

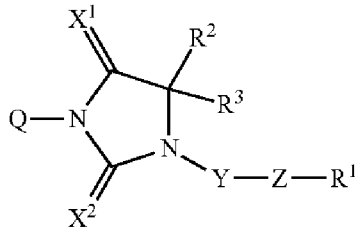
5.2. The Selected Anti-Androgen Compounds

[00109] In addition to DIM-related indoles, the present invention contemplates use of Selected Anti-Androgen Compounds in methods, compositions and kits of the invention.

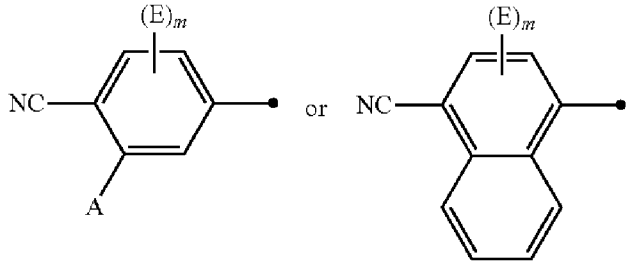
[00110] The term "Selected Anti-Androgen Compound(s)" encompasses compounds of the following Formulas A, B, C, D and E:

[00111] Formula A:

(I)



wherein Q is



wherein

A is a hydrogen atom, a halogen atom, —ORa or a C_{1-4} alkyl group which may be substituted by one or more halogen atoms;

E is independently selected from a C_{1-6} alkyl group;

m is selected from integers from 0 to 3;

R^2 and R^3 are independently selected from a C_{1-6} alkyl group;

X^1 and X^2 are independently selected from O and S,

Y is selected from an arylene group and a divalent 5- or 6-membered monocyclic or 8- to 10-membered condensed heterocyclic group, wherein the arylene group and the heterocyclic group may be substituted by 1 to 3 substituents independently selected from E^1,

E^1 is independently selected from a hydroxyl group, a halogen atom, a C_{1-4} alkyl group, a cyano group, a C_{1-4} alkoxy group, a carbamoyl group, a C_{1-4} alkylcarbamoyl group, a di(C_{1-4} alkyl)carbamoyl group, an amino group, a C_{1-4} alkylamino group, a di(C_{1-4} alkyl)amino group, a sulfamoyl group, a C_{1-4} alkylsulfamoyl group and a di(C_{1-4} alkyl)sulfamoyl group;

Z is —CON(—Ra)—, —CO—, —C(═NH)—, —SO_2—, —SO_2N(—Ra)—, —SO_2N(—R^1)—, —N(—Ra)CO—, —N(—Ra)CON(—Rb)—, —N(—COR^1)CO—, —N(—Ra)SO_2—, —N(SO_2R^1)SO_2—, —N(—Ra)— or —N(—Ra)SO_2N(—Rb)—;

R^1 is a hydrogen atom, a hydroxyl group, a C_{1-6} alkyl group which may be substituted by one or more substituents selected from B, a heterocyclic group which may be substituted by one or more substituents selected from B, an aryl group which may be substituted by one or more substituents selected from B, or a C_{3-8} cycloalkyl group which may be substituted by one or more substituents selected from B;

B is independently selected from a C_{1-4} alkyl group (except in the case where R^1 is a C_{1-6} alkyl group), a halogen atom, a hydroxyl group, a cyano group, —$CONRa^1Rb^1$, —N(—Ra)CORb, —NRa^1Rb^1, —N(—Ra)SO_2Rb, —$SO_2NRa^1Rb^1$, —SO_2Ra, —COORa, —ORa, an aryl group, a heterocyclic group, and a C_{3-8} cycloalkyl group (wherein the aryl group, the heterocyclic group, the heteroaryl group, and the cycloalkyl group may be substituted by one or more substituents selected from a C_{1-4} alkyl group or a Cl_{1-4} alkoxy group, a halogen atom, and a hydroxyl group);

Ra and Rb are independently selected from a hydrogen atom, and a C_{1-6} alkyl group wherein the alkyl group may be substituted by one or more substituents selected from a hydroxyl group, a C_{1-6} alkoxy group, a halogen atom, an aryl group and a heterocyclic group;

Ra^1 and Rb^1 are independently selected from a hydrogen atom and a C_{1-6} alkyl group wherein the alkyl group may be substituted by one or more substituents selected from a hydroxyl group, a C_{1-6} alkoxy group, a halogen atom, an aryl group and a heterocyclic group; or Ra^1 and Rb^1 together with a nitrogen atom to which they bind may form a nitrogen-containing heterocyclic group;

provided that when Y is a heterocyclic group and X^1 and X^2 are O, m is not 0;

and that when Y is an arylene group,

Z is not —CON(—Ra)— or —CO—; and

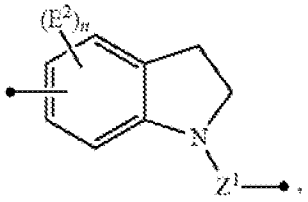
—Z—R^1 is not an arylsulphonyl group, an amino group, a C_{1-6} alkylamino group or a di(C_{1-6} alkyl)amino group, or a salt, or prodrug thereof.

[00112] Compounds encompassed by Formula A and the substitutuents of Formula A are defined as in Tachibana et al., US 8,470,829 and/or US 2011/0306615, which are incorporated by reference herein in their entirety (and, the disclosures of the compounds

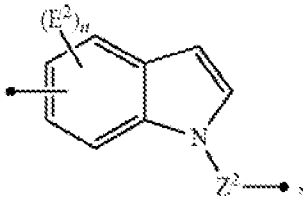
encompassed by Formula A throughout these patent publications (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula A may be synthesized as described in Tachibana et al., US 8,470,829 and/or US 2011/0306615. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula A disclosed or exemplified in Tachibana et al., US 8,470,829 and/or US 2011/0306615, or is selected from a group comprising the exemplified compounds of Formula A as detailed in Tachibana et al., US 8,470,829 and/or US 2011/030661.

[00113] Further, in some embodiments, the Selected Anti-Androgen compound of Formula (A) is a compound wherein –Y-Z- is selected from the following YZ^1 to YZ^7:

YZ^1



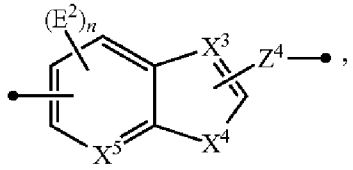
YZ^2



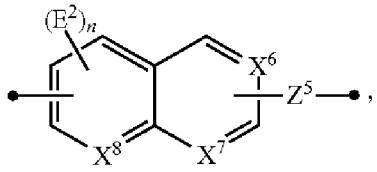
YZ^3



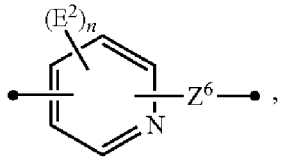
YZ^4



YZ^5

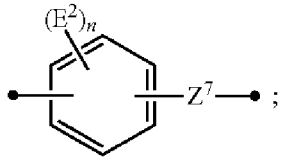


YZ^6



and

YZ^7



wherein n is selected from integers from 0 to 3;

E^2 is independently selected from a hydroxyl group, a halogen atom, a C_{1-4} alkyl group, a cyano group, a C_{1-4} alkoxy group, a carbamoyl group, a C_{1-4} alkylcarbamoyl group, a di(C_{1-4} alkyl)carbamoyl group, an amino group, a C_{1-4} alkylamino group, a di(C_{1-4} alkyl)amino group, a sulfamoyl group, a C_{1-4} alkylsulfamoyl group and a di(C_{1-4} alkyl)sulfamoyl group;

X^3, X^S, X^6, X^7 and X^8 are independently selected from CH and N, provided that X^6, X^7 and X^8 are not CH at the same time;

X^4 is —CH_2—, —S—, —O— or —N(—W)—, provided that X^4 is not —CH_2— when both X^3 and X^5 are CH;

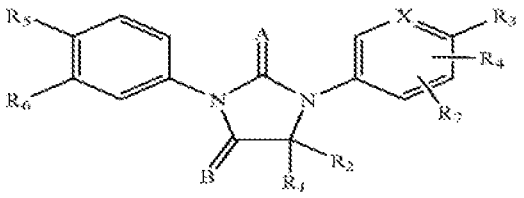
W is a hydrogen atom, a C_{1-6} alkyl group, a C_{1-6} alkoxy group, —SO_2Ra, —SO_2NRa^{1}Rb^1 or —CORa;

Z^1, Z^2, Z^3, Z^4, Z^5, Z^6 and Z^7 are —CON(—Ra)—, —CO—, —C(=NH)—, —SO_2—, —SO_2N(—Ra)—, —SO_2N(—R^1)—, —N(—Ra)CO—, —N(—Ra)CON(—Rb)—, —N(—COR^1)CO—, —N(—Ra)SO_2—, —N(—SO_2R^1)SO_2—, —N(—Ra)— or —N(—Ra)SO_2N(—Rb)—;

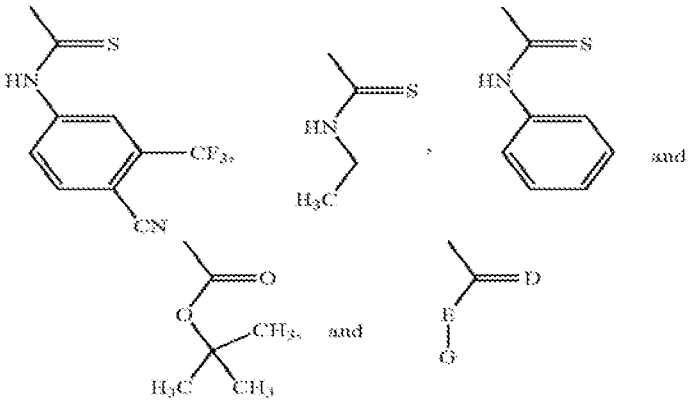
provided that m is not 0 when both of X^1 and X^2 above are O and —Y—Z— is any of YZ^1 to YZ^6.

[00114] Compounds encompassed by the compounds of the formulas presented above and the substitutuents of such compounds are defined as in Tachibana et al., US 8,470,829 and/or US 2011/0306615, which are incorporated by reference herein in their entireties (and, the disclosures of the compounds encompassed by such formulas throughout these patent publications (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of such formulas may be synthesized as described in Tachibana et al., US 8,470,829 and/or US 2011/0306615. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of one of the formulas described in the preceding paragraph disclosed or exemplified in Tachibana et al., US 8,470,829 and/or US 2011/0306615, or is selected from a group of such compounds as detailed in Tachibana et al., US 8,470,829 and/or US 2011/030661.

[00115] Formula B:

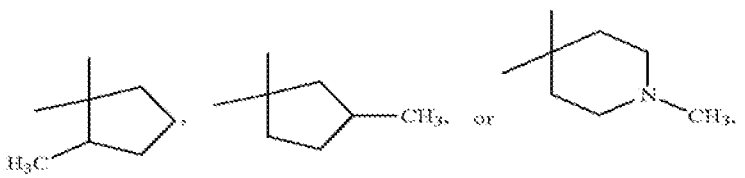


wherein R5 is CN or NO_2 or SO2R11, wherein R6 is CF3, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, halogenated alkyl, halogenated alkenyl, halogenated akynyl, halogen, wherein A is sulfur (S) or oxygen (O), wherein B is O or S or NR8, wherein R8 is selected from the group consisting of H, methyl, aryl, substituted aryl, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heterocyclic aromatic or non-aromatic, substituted heterocyclic aromatic or non-aromatic, cycloalkyl, substituted cycloalkyl, SO2R11, NR11R12, (CO)OR11, (CO)NR11R12, (CO)R11, (CS)R11, (CS)NR11R12, (CS)OR11,



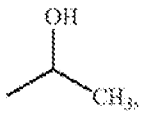
wherein D is S or O and E is N or O and G is alkyl, aryl, substituted alkyl or substituted aryl; or D is S or O and E-G together are C1-C4 lower alkyl,

wherein R1 and R2 are independently alkyl, haloalkyl, hydrogen, aryl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, halogenated alkenyl, halogenated akynyl, arylalkyl, arylalkenyl, arylalkynyl, heterocylic aromatic or non-aromatic, substituted heterocyclic aromatic or non-aromatic, cycloalkyl, substituted cycloalkyl, or R1 and R2 are connected to form a cycle which can be heterocyclic, substituted heterocyclic, cycloalkyl, substituted cycloalkyl,



wherein X is carbon or nitrogen and can be at any position in the ring, and

wherein R3, R4, and R7 are independently selected from the group consisting of hydrogen, halogen, methyl, methoxy, formyl, haloacetoxy, trifluoromethyl, cyano, nitro, hydroxyl, phenyl, amino, methylcarbamoyl, methylcarbamoyl-substituted alkyl, dimethylcarbamoyl-substituted alkyl, methoxycarbonyl, acetamido, methanesulfonamino, carbamoyl-substituted alkyl, methanesulfonyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, hydroxyethylcarbamoyl-substituted alkyl, hydroxyl-substituted alkyl, hydroxyl-substituted alkenyl, carbamoyl-substituted alkenyl, methoxycarbonyl-substituted alkyl, cyano-substituted alkyl,



aryl, substituted aryl, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, halogenated alkenyl, halogenated alkynyl, SO2R11, NR11R12, NR12(CO)OR11, NH(CO)NR11R12, NR12 (CO)R11, O(CO)R11, O(CO)OR11, O(CS)R11, NR12 (CS)R11, NH(CS)NR11R12, NR12 (CS)OR11, arylalkyl, arylalkenyl, arylalkynyl, heterocyclic aromatic or non-aromatic, substituted heterocyclic aromatic or non-aromatic, cycloalkyl, substituted cycloalkyl, haloalkyl, methylsulfonecarbamoyl-substituted alkyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, methoxycarbonyl, acetamido, methanesulfonamido, carbamoyl-substituted alkyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoyl-substituted alkyl, carboxy-substituted alkyl, 4-(1,1-dimethylethoxy)carbonyl)-1-piperazinyl, hydroxyethylcarbamoyl-substituted alkyl, hydroxyethoxycarbonyl-substituted alkyl, 3-cyano-4-trifluoromethylphenylcarbamoyl.

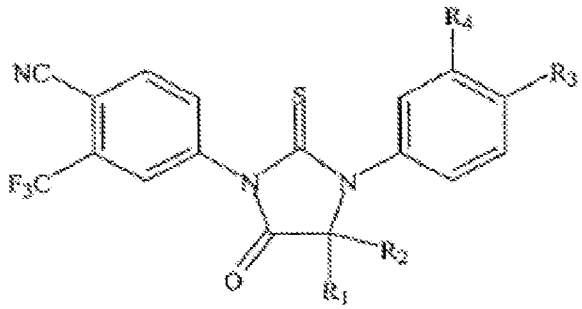
wherein R11 and R12 are independently hydrogen, aryl, aralkyl, substituted aralkyl, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, halogenated alkyl, halogenated alkenyl, halogenated alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heterocyclic aromatic or non-aromatic, substituted heterocyclic aromatic or non-aromatic, cycloalkyl, or substituted cycloalkyl, or R11 and R12 can be connected to form a cycle which can be heterocyclic aromatic or non-aromatic, substituted heterocyclic aromatic, cycloalkyl, or substituted cycloalkyl.

[00116] Compounds encompassed by Formula B and the substitutuents of Formula B are defined as in Sawyers et al., US 7,709,517, which is incorporated by reference herein in its

entirety (and, the disclosures of the compounds encompassed by Formula B throughout this patent (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula B may be synthesized as described in Sawyers et al., US 7,709,517. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula B disclosed or exemplified in Sawyers et al., US 7,709,517, or is selected from a group comprising the exemplified compounds of Formula A as detailed in Sawyers et al., US 7,709,517.

[00117] Compounds encompassed by Formula B are also described in Sawyers et al., US 8,183,274, US 2012/0295944 and US 2013/0034501, which are incorporated by reference herein in their entireties (and, the disclosures of the compounds encompassed by Formula B throughout these patent publications (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula B may be synthesized as described in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula B disclosed or exemplified in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501, or is selected from a group comprising the exemplified compounds of Formula B as detailed in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501.

[00118] Formula C:



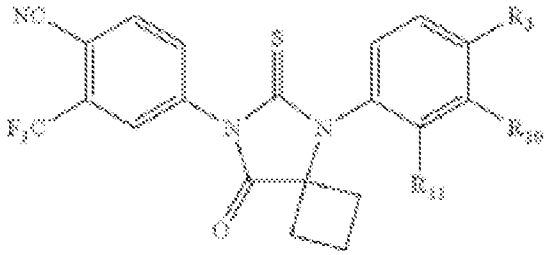
wherein Ri and R2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,
wherein R3 is selected from the group consisting of carbamoyl, alkyl carbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and
wherein R4 is hydrogen or fluorine.

[00119] Compounds encompassed by Formula C and the substitutuents of Formula C are defined as in Jung et al., WO 2007/127010, which is incorporated by reference herein in its entirety (and, the disclosures of the compounds encompassed by Formula C throughout this patent publication (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula C may be synthesized as described in Jung et al., WO 2007/127010. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula C disclosed or exemplified in Jung et al., WO 2007/127010, or is selected from a group comprising the exemplified compounds of Formula C as detailed in Jung et al., WO 2007/127010.

[00120] Compounds encompassed by Formula C and the substitutuents of Formula C can also be defined as in Jung et al., USP 8,110,594, which is incorporated by reference herein in its entirety (and, the disclosures of the compounds encompassed by Formula C throughout this patent publication (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula C may be synthesized as described in Jung et al., USP 8,110,594. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula C disclosed or exemplified in Jung et al., USP 8,110,594, or is selected from a group comprising the exemplified compounds of Formula C as detailed in Jung et al., USP 8,110,594.

[00121] Compounds encompassed by Formula C are also described in Sawyers et al., US 8,183,274, US 2012/0295944 and US 2013/0034501, and Jung et al., US 8,110,594, US 8,445,507 and US 2013/0072511, which are incorporated by reference herein in their entireties (and, the disclosures of the compounds encompassed by Formula C throughout these patent publications (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula C may be synthesized as described in Sawyers et al., US 8,183,274, US 2012/0295944 and/or US 2013/0034501, or Jung et al., US 8,110,594, US 8,445,507 and/or US 2013/0072511. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula C disclosed or exemplified in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501, and/or Jung et al., US 8,110,594, US 8,445,507 or US 2013/0072511, or is selected from a group comprising the exemplified compounds of Formula C as detailed in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501, and/or Jung et al., US 8,110,594, US 8,445,507 or US 2013/0072511.

[00122] Formula D:



wherein R3 is selected from the group consisting of hydroxy, methylcarbamoyl, methylcarbamoylpropyl, methylcarbamoylethyl, methylcarbamoylmethyl, methylsulfonecarbamoylpropyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, carbamoylmethyl, carbamoylethyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoylpropyl, carboxypropyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, hydroxyethylcarbamoylethyl, and hydroxyethoxycarbonylethyl, and

wherein R10 and R11 are both H or, respectively, F and H, or H and F.

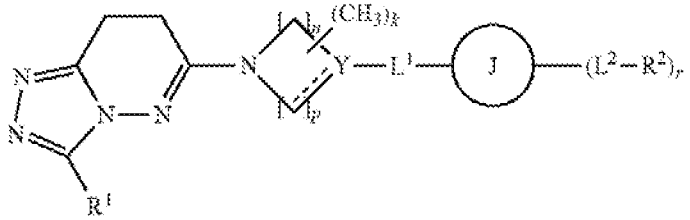
[00123] Compounds encompassed by Formula D and the substitutuents of Formula D are defined as in Sawyers et al., WO 2006/124118, which is incorporated by reference herein in its entirety (and, the disclosures of the compounds encompassed by Formula D throughout this patent publication (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula D may be synthesized as described in Sawyers et al., WO 2006/124118. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula D disclosed or exemplified in Sawyers et al., WO 2006/124118, or is selected from a group comprising the exemplified compounds of Formula D as detailed in Sawyers et al., WO 2006/124118.

[00124] Compounds encompassed by Formula D and the substitutuents of Formula D can also be defined as in Sawyers et al., USP 8,183,274, which is incorporated by reference herein in its entirety (and, the disclosures of the compounds encompassed by Formula D throughout this patent publication (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula D may be synthesized as described in Sawyers et al., USP 8,183,274 In specific embodiments, an Anti-Androgen

Compound of the invention is a compound of Formula D disclosed or exemplified in Sawyers et al., USP 8,183,274, or is selected from a group comprising the exemplified compounds of Formula D as detailed in Sawyers et al., USP 8,183,274.

[00125] Compounds encompassed by Formula D are also described in Sawyers et al., US 8,183,274, US 2012/0295944 and US 2013/0034501, which are incorporated by reference herein in their entireties (and, the disclosures of the compounds encompassed by Formula D throughout these patent publications (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula D may be synthesized as described in Sawyers et al., US 8,183,274, US 2012/0295944 and/or US 2013/0034501. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula D disclosed or exemplified in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501, or is selected from a group comprising the exemplified compounds of Formula D as detailed in Sawyers et al., US 8,183,274, US 2012/0295944 or US 2013/0034501.

[00126] Formula E:



wherein

R^1 represents haloC_{1-4}alkyl;

k represents 0, 1 or 2;

n and p independently represent 1 or 2;

Y represents N, C, CH or COH;

⋯ represents a single bond when Y is N, CH or COH;

═ represents a double bond when Y is C, L^1 is a direct bond and J is indolyl or pyrrolopyridinyl;

L^1 represents a direct bond, —$(CR^3R^4)_t$—, —$(CR^3R^4)_v$—O—$(CR^3R^4)_v$—, N(R^5)—$(CH_2)_q$—, —S—, —S(O)— or —$S(O)_2$—;

R^3 and R^4, identically or differently on each occurrence, represent hydrogen or methyl;

R^5 represents hydrogen or methyl;

q, identically or differently on each occurrence, represents 0, 1, 2, 3 or 4;

t represents 1, 2 or 3;

v, identically or differently on each occurrence, represents 0, 1 or 2;

J represents:

aryl;

C_{3-6}cycloalkyl;

a monocyclic 4, 5, 6 or 7 membered heterocyclic ring which comprises 1, 2 or 3 heteroatoms independently selected from O, N or S;

a 5 or 6 membered monocyclic heteroaryl ring which comprises 1, 2, 3 or 4 heteroatoms independently selected from O, N or S; or

a 9 or 10 membered bicyclic heteroaryl ring system which comprises 1, 2, 3, 4 or 5 heteroatoms independently selected from O, N or S;

L^2 represents a direct bond, —$(CR^3R^4)_t$—, —C(O)N(R^5)—$(CH_2)_q$—, —C(O)N(R^5)—$(CH_2)_q$—$S(O)_2$—, —NR^5C(O)—$(CH_2)_q$—, —C(O)—$(CH_2)_q$—, —O—$(CR^9R^{10})_q$—, —O—$(CR^3R^4)_q$—NR^5—$(CH_2)_q$—, —O—$(CR^3R^4)_q$—C(O)NR^5—$(CH_2)_q$—, —S—, —S(O)— or —$S(O)_2$—;

R^9 and R^{10}, identically or differently on each occurrence, represent hydrogen, methyl, ethyl, isopropyl, cyclopropyl or methoxymethyl;

R^2 represents:

halo, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, carboxy, C_{1-6}alkoxy, cyano, oxo, fluoroC_{1-6}alkyl, hydroxy, amino, N—C_{1-4}alkylamino or N,N-di-C_{1-4}alkylamino;

aryl, wherein the aryl ring is optionally substituted with 1, 2 or 3 substituents selected from R^6;

a monocyclic 4, 5, 6 or 7 membered heterocyclic ring which comprises 1, 2 or 3 heteroatoms independently selected from O, N or S and wherein the heterocyclic ring is optionally substituted with 1, 2 or 3 substituents selected from R^6;

a monocyclic 5 or 6 membered heteroaryl ring which comprises 1, 2, 3 or 4 heteroatoms independently selected from O, N or S and wherein the heteroaryl ring is optionally substituted with 1, 2 or 3 substituents selected from R^6; or

a 9 or 10 membered bicyclic heteroaryl ring system which comprises 1, 2, 3, 4 or 5 heteroatoms independently selected from O, N or S and wherein the heterocyclic ring is optionally substituted with 1, 2 or 3 substituents selected from R^6;

R^6 represents amino, halo, C_{1-6}alkyl, C_{3-6}cycloalkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, C_{1-6}alkoxy, C_{2-6}alkanoyl, C_{1-6}alkoxyC_{1-6}alkyl, carboxy, cyano, oxo, fluoroC_{1-6}alkyl, hydroxy, hydroxyC_{1-6}alkyl, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, oxetan-3-ylcarbonyl, N—C_{1-4}alkylamino, N,N-di-C_{1-4}alkylamino or C(O)NR^7R^8 wherein R^7 and R^8 independently represent hydrogen or methyl; and

r represents 0, 1, 2 or 3.

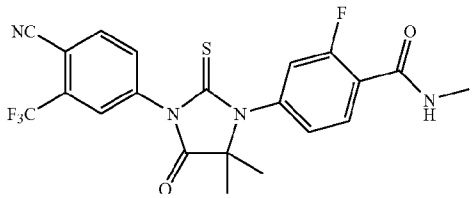
[00127] Compounds encompassed by Formula E and the substitutuents of Formula E are defined as in Bradbury et al., US 2013/0203714, which is incorporated by reference herein in its entirety (and, the disclosures of the compounds encompassed by Formula E throughout this patent publication (e.g., in the claims) are specifically incorporated by reference herein in their entireties). The compounds of Formula E may be synthesized as described in Bradbury et al., US 2013/0203714. In specific embodiments, an Anti-Androgen Compound of the invention is a compound of Formula E disclosed or exemplified in Bradbury et al., US 2013/0203714, or is selected from a group comprising the exemplified compounds of Formula E as detailed in Bradbury et al., US 2013/0203714.

[00128] In certain embodiments, the Selected Anti-Androgen Compound used in the compoisitions, kits and methods provided herein is compound of Formula A (described above in this section). In certain embodiments, the Selected Anti-Androgen Compound used in the compoisitions, kits and methods provided herein is compound of Formula B (described above in this section). In certain embodiments, the Selected Anti-Androgen Compound used in the compoisitions, kits and methods provided herein is compound of Formula C (described above in this section). In certain embodiments, the Selected Anti-Androgen Compound used in the compoisitions, kits and methods provided herein is compound of Formula D (described above in this section). In certain embodiments, the Selected Anti-Androgen Compound used in the compoisitions, kits and methods provided herein is compound of Formula E (described above in this section).

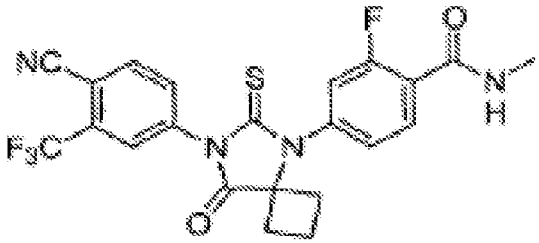
[00129] In certain embodiments, a pharmaceutically acceptable salt, a solvate (e.g., hydrate), or a prodrug of a Selected Anti-Androgen Compound, e.g., a compound of Formula A, compound of Formula B, compound of Formula C, compound of Formula D or compound of Formula E, can be used in the compositions, kits or methods described herein. In some embodiments, a salt, a solvate (e.g., hydrate), or a prodrug of a compound of Formula A, compound of Formula B, compound of Formula C, compound of Formula D or compound of Formula E is the salt, solvate (e.g., hydrate), or prodrug of such compound disclosed in the patents and patent publications referenced in this section or known in the art.

[00130] Specific examples of some of the Selected Anti-Androgen Compounds that can be used in the compositions, kits and methods provided herein include, without limitation, the following compounds:

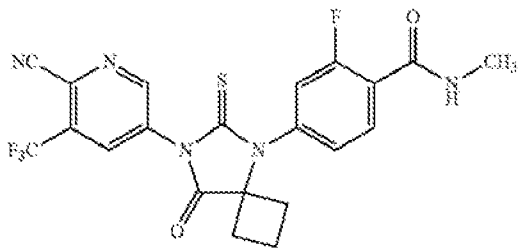
[00131] (1): 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide ("Enzalutamide") having the following formula:



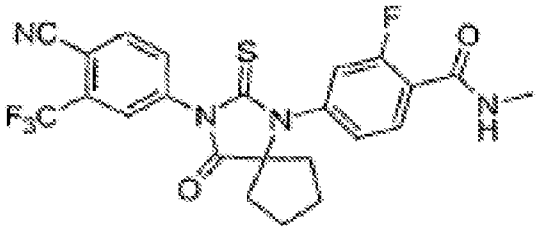
[00132] (2):



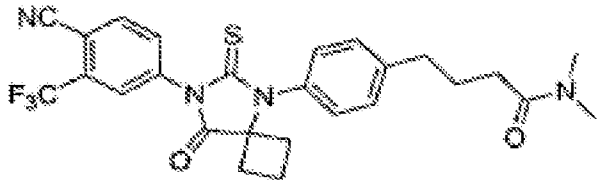
[00133] (3): 4-[7-(6-cyano-5-trifluoromethylpyridin-3-yl)-8-oxo-6-thioxo-5,7-diazaspir- o[3,4]oct-5-yl]-2-fluoro-N-methylbenzamide ("ARN 509") having the following formula:



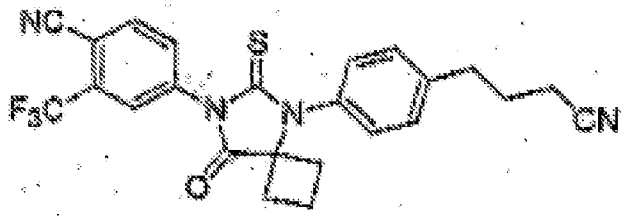
[00134] (4):



[00135] (5):

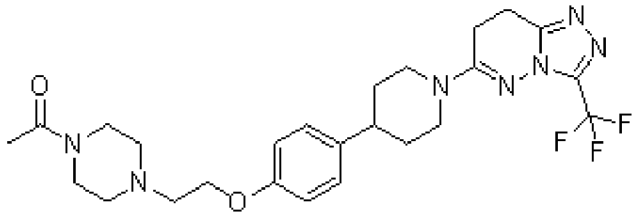


[00136] (6):



[00137] Compounds (1)-(6) above are described in Sawyers et al., US 8,183,274, US 2012/0295944 and US 2013/0034501, and/or Jung et al., WO 2007/127010, US 8,110,594, US 8,445,507 and US 2013/0072511, which are incorporated by reference herein in their entireties. The compounds (1)-(6) may be synthesized as described in Sawyers et al., US 8,183,274, US 2012/0295944 and US 2013/0034501, or Jung et al., WO 2007/127010, US 8,110,594, US 8,445,507 and US 2013/0072511.

[00138] Another example of the Selected Anti-Androgen Compound that can be used in the compositions, kits and methods provided herein is AZD-3514 having the formula:



[00139] 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compounds such as AZD-3514 are described in Bradbury et al., US 2013/0203714, which is incorporated by reference herein in its entirety. The 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compounds may be synthesized as described in Bradbury et al., US 2013/0203714.

[00140] In certain embodiments, the Selected Anti-Androgen Compound that can be used in the compositions, kits and methods provided herein is a compound of Formula A, Formula B, Formula C, Formula D and/or Formula E, which exhibits Androgen Receptor (AR) antagonistic activity and minimal or no AR agonistic activity. In specific embodiments, the Selected Anti-Androgen Compound that can be used in the compositions, kits and methods

provided herein is a compound of Formula A, Formula B, Formula C, Formula D and/or Formula E, which exhibits strong Androgen Receptor (AR) antagonistic activity and no detectable AR agonistic activity.

[00141] The terms "Diarylhydantoin-related compounds" and "Diarylhydantoin compounds" as used herein encompasses compounds of Formulas B, C and D. The term "Diarylthiohydantoin compounds" encompasses compounds of Formulas C and D.

[00142] The Diarylthiohydantoin compounds useful in the compositions, kits and methods of the invention include, without limitation, 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide ("Enzalutamide") and 4-[7-(6-cyano-5-trifluoromethylpyridin-3-yl)-8-oxo-6-thioxo-5,7-diazaspir- o[3,4]oct-5-yl]-2-fluoro-N-methylbenzamide ("ARN-509"). The 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compounds useful in the compositions, kits and methods of the invention include, without limitation, 1-(4-(2-(4-(1-(3-(trifluoromethyl)-7,8-dihydro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)phenoxy)ethyl)piperazin-1-yl)ethanone ("AZD-3514").

5.3. Patient Identification and Selection

[00143] The subject, or patient, to be treated using the methods of the invention is an animal, *e.g.*, a mammal, e.g., a human, a cow, a dog, a cat, a goat, a horse, a sheep or a pig, In a preferred embodiment, the patient is a human, and can be a fetus, child, or adult. In a preferred embodiment, the subject is a human male. In another embodiment, the subject is a human female. In some embodiments, the subject is 18 to 55 years old. In other embodiments, the subject is more than 55 years old.

[00144] In certain embodiment, the subject has (e.g., has been diagnosed with) an androgen or Androgen Receptor-driven disease or disorder. In certain embodiments, an increased expression (e.g., overexpression) of AR and/or AR variants has been detected in a tissue or fluid sample of the subject. In one embodiment, expression (e.g., increased expression relative to normal) of AR variants, e.g., AR3, AR132b or AR122b, has been detected in a tissue or fluid sample of the subject. In specific embodiments, the subject has (e.g., has been diagnosed with) prostate cancer, benign prostatic hyperplasia, male-pattern baldness, polycystic ovarian syndrome (PCOS) or hirsutism. In one embodiment, the subject has prostate cancer.

[00145] In some embodiments, the subject is a human male who has (e.g., has been diagnosed with) prostate cancer. In one embodiment, the subject has (e.g., has been diagnosed with) castrate-resistance prostate cancer (or hormone refractory prostate cancer). In another embodiment, the subject has (e.g., has been diagnosed with) castrate sensitive prostate cancer (or hormone sensitive prostate cancer, such as androgen sensitive prostate cancer). In one embodiment, the subject has (e.g., has been diagnosed with) non-metastatic prostate cancer. In another embodiment, the subject has (e.g., has been diagnosed with) a metastatic prostate cancer. In one embodiment, the subject has non-metastatic castrate-resistance prostate cancer. In another embodiment, the subject has metastatic castrate resistant prostate cancer.

[00146] The diagnosis of prostate cancer can be made by any method known in the art, e.g., prostate biopsy, physical examination, measurement of PSA levels or evaluation of symptoms. In one embodiment, the diagnosis is made by prostate biopsy.

[00147] Response to treatment can be documented by any method know in the art, e.g., by repeat prostate biopsy of the same zone of the gland. Such repeat prostate biopsy can show normalization of the histology upon treatment using the combination compositions described herein.

[00148] In particular embodiments, the subject has (e.g., has been diagnosed with) a localized early stage of prostate cancer, such as PIN. PIN can be diagnosed, e.g., using prostatic needle biopsy.

[00149] In other particular embodiments, the subject has (e.g., has been diagnosed with) an advanced prostate cancer.

[00150] In one embodiment, the subject has (e.g., has been diagnosed with) stage T1 prostate cancer in the four-stage TNM (Timor/Nodes/Metastasis) system. In one embodiment, the subject has (e.g., has been diagnosed with) stage T2 prostate cancer in the four-stage TNM system. In one embodiment, the subject has (e.g., has been diagnosed with) stage T3 prostate cancer in the four-stage TNM system. In one embodiment, the subject has (e.g., has been diagnosed with) stage T4 prostate cancer in the four-stage TNM system. In one embodiment, the subject has (e.g., has been diagnosed with) the grade of the prostate cancer tumor on a Gleason score of 2 to 4. In one embodiment, the subject has (e.g., has been diagnosed with) the grade of the prostate cancer tumor on a Gleason score of 5 to 7. In one embodiment, the

subject has (e.g., has been diagnosed with) the grade of the prostate cancer tumor on a Gleason score of 8 to 10.

[00151] In one embodiment, the subject has (e.g., has been diagnosed with) benign prostatic hyperplasia. In one embodiment, the subject has (e.g., has been diagnosed with) male-pattern baldness. In one embodiment, the subject has (e.g., has been diagnosed with) PCOS. In one embodiment, the subject has (e.g., has been diagnosed with) hirsutism. In one embodiment, the subject has (e.g., has been diagnosed with) PIN.

[00152] In a particular embodiment, the subject has an increased level of PSA, e.g., as shown by a test. For example, the level can be increased in the subject relative to the level known to be normal, or relative to the level of PSA in the subject in prior tests (before the subject was diagnosed with the disease). In some embodiments, administration of the combination described herein results in a decrase of PSA levels (e.g., more than the decrease in PSA after administration of Anti-Androgen alone or DIM alone).

[00153] In certain embodiments, the subject has been previously (i.e., before starting treatment with the combination described herein) administered an antiandrogen compound (e.g., a steroidal antiandrogen compound or a non-steroidal antiandrogen compound). In particular embodiments, the subject has been previously administered a Selected Anti-Androgen Compound (i.e., one of the Selected Anti-Androgen Compounds described in Section 5.2, above). In one embodiment, the subject has been previously administered a Diarylthiohydantoin compound, e.g., enzalutamide or ARN-509. In yet another embodiment, the subject has not been previously treated with an antiandrogen compound, or has not been previously treated with a Selected Anti-Androgen Compound (i.e., one of the Selected Anti-Androgen Compounds described in Section 5.2, above). In one embodiment, the subject has not been previously administered a Diarylthiohydantoin compound, e.g., enzalutamide or ARN-509.

[00154] In a particular embodiment, the subject has lost responsiveness to or developed resistance to an antiandrogen compound (before starting treatment with the combination described herein). In one embodiment, the subject has developed resistance to a steroidal antiandrogen compound. In another embodiment, the subject has developed resistance to a non-steroidal antiandrogen compound. In particular embodiments, the subject has developed resistance to a Selected Anti-Androgen Compound (i.e., one of the Selected Anti-Androgen

Compounds described in Section 5.2, above). In one embodiment, the subject has developed resistance to administered a Diarylthiohydantoin compound, e.g., enzalutamide or ARN-509.

[00155] In specific embodiments, the subject has been previously (i.e., before starting treatment with the combination described herein) treated with a chemotherapeutic agent (e.g., docetaxel). In yet another embodiment, the subject has not been previously treated with a chemotherapeutic agent (e.g., docetaxel).

[00156] In some embodiments, the subject has never had hormone therapy (prior to the combination treatment described herein). In other embodiment, the subject has had hormone therapy.

[00157] In particular embodiments, the subject has advanced prostate cancer (e.g., an advanced stage of prostate cancer). In other embodiment, the subject has localized prostate cancer.

5.4. Dosage and Administration of DIM-related indoles and Selected Anti-Androgen Compounds

[00158] A DIM-related compound and a Selected Anti-Androgen Compound may be administered by any means and at any dosage, as described below. The actual administered amount of these compounds may be decided by a supervising physician or veterinarian and may depend on multiple factors, such as, the age, condition, file history, etc., of the subject, or patient, in question.

[00159] In certain embodiments, a therapeutically effective amount of a DIM-related indole (e.g., DIM or I3C) is used in the compositions, kits and methods described herein. In certain embodiments, a therapeutically effective amount of a Selected Anti-Androgen Compound (e.g., Enzalutamide, ARN-509 or AZD-3514) is used in the compositions, kits and methods described herein. In some embodiments, a therapeutically effective amount of a DIM-related indole (e.g., DIM or I3C) and a therapeutically effective amount of a Selected Anti-Androgen Compound (e.g., Enzalutamide, ARN-509 or AZD-3514) is used in the compositions, kits and methods described herein. In some embodiments, a therapeutically effective amount is an amount that is effective to treat an impairment at a certain daily frequency of administration (e.g., once a day or twice a day). In some embodiments, where a combination of a Selected Anti-Androgen Compound and a DIM-related indole is used in the compositions, kits and methods described herein, the therapeutically effective amount of a

Selected Anti-Androgen Compound is less than the therapeutically effective amount of such compound when it is used alone (i.e., without a DIM-related indole).

[00160] A DIM-related indole and a Selected Anti-Androgen Compound can be administered to a patient concomitantly or sequentially. For example, these drugs can be administered at the same time, or within a certain number of minutes or hours of each other (e.g., 30 minutes, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 18 hours or 24 hours). In some embodiments, DIM-related indoles are co-administered with a Selected Anti-Androgen Compound without co-formulation (in two separate compositions). In other embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen Compound is a fixed dose combination. For example, a DIM-related indole and a Selected Anti-Androgen Compound can be formulated in one composition, such as a pill, a tablet or a capsule.

[00161] In certain embodiments, a DIM-related indole is administered intravenously, parenterally, intraarterially, subcutaneously, orally or injected directly into a tissue (e.g., into the prostate gland tissue), or by any other method known in the art or described herein. In one preferred embodiment, a DIM-related indole is administered orally. In another embodiment, a DIM-related indole is injected directly into prostate gland tissue using ultrasound guidance. In yet another embodiment, a DIM-related indole is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter.

[00162] In certain embodiments, a Selected Anti-Androgen Compound is administered intravenously, parenterally, intraarterially, subcutaneously, orally or directly into a tissue (e.g., into the prostate gland), or by any other method known in the art or described herein. In one preferred embodiment, a Selected Anti-Androgen Compound is administered orally. In another embodiment, a Selected Anti-Androgen Compound is injected directly into prostate gland tissue using ultrasound guidance. In yet another embodiment, a Selected Anti-Androgen Compound is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter. In another embodiment, a Selected Anti-Androgen Compound is administered intra-arterially or parenterally.

[00163] Oral administration for a DIM-related indole and the Selected Anti-Androgen Compound is the most preferred route.

[00164] In one embodiment, a DIM-related indole and a Selected Anti-Androgen Compound are administered using the same route of administration, such as any of the routes of administration described herein or known in the art. In some of these embodiments, a DIM-related indole and a Selected Anti-Androgen Compound are administered in the same fixed dosage composition. In other embodiments, a DIM-related indole and a Selected Anti-Androgen Compound are administered in different compositions.

[00165] A preferred embodiment is the oral administration of DIM or synthetic derivatives in an acceptable formulation for oral administration. Preferably, the diindolylmethane used in the invention has been processed to enhance bioavailability, as is described in U.S. Patent No. 6,086,915; however any suitable preparation of diindolylmethane or of a structurally-related, synthetically-derived, substituted diindolylmethane, can be used in the methods and compositions of the invention. Also one of the preferred embodiments is the oral administration of Selected Anti-Androgen Compounds (e.g., Enzalutamide, ARN-509 or AZD-3514). In preferred embodiments, unit doses of liquid filled gelatin capsules comprising a Selected Anti-Androgen Compound are administered which contain the Selected Anti-Androgen Compound as a solution in caprylocaproyl polyoxylglycerides. Optionally, inactive ingredients include pharmaceutical excipients and may include butylated hydroxanisol, sorbitol sorbitan, glycerin, purified water and titanium dioxide.

[00166] In methods involving the oral use of one or more DIM-related indoles, *e.g.*, DIM, and one or more Selected Anti-Androgen Compounds, the oral delivery of indole is facilitated and accomplished according to formulations and methods described in U.S. Patent No. 6,086,915, incorporated by reference herein in its entirety. In one embodiment, DIM, or a DIM-related indole, is processed with phosphatidyl choline. Alternatively, oral bioavailability of DIM-related indoles can be improved using other means including particle size reduction, complexation with phosphatidylcholine, and formation into rapidly dissolving particles and nanoparticles.

[00167] DIM is administered in a dose from 25-600 mg/day or 25- 500 mg/day orally. DIM may be administered once per day, or two or more times per day. Preferably the dose is administered 2-3 times per day. Most preferably, the DIM is administered in a formulation designed for enhanced oral absorption, *e.g.*, microencapsulated with TPGS (as described in U.S. Patent No. 6,086,915). This formulation (25-30% DIM by weight) is administered in a

dose of 100 – 2000 mg/day, or more preferably at a dose of 300-600 mg, providing 75 –150 mgs of actual DIM, given orally twice per day. As an alternative to DIM, the closely related linear trimer (LTR) may be employed in methods and doses described for DIM. As a further alternative, the orally active DIM derivatives described in U.S. Patent Application Publication No. 2004/0043965 may be employed in the present invention. Structurally-related, synthetically-derived, substituted diindolylmethane's, as described by Jong (U.S. Patent No. 6,800,655 and Patent Application Publication No. 2004/0043965) are administered according to the present invention in an acceptable formulation for oral administration in a dose of 10-400 mg/day. Preferably, these substituted diindolylmethanes are administered in an absorption-enhanced formulation at a dose of 50 to 250 mg/day.

[00168] For oral use, DIM can be used, preferably formulated for enhanced absorption in a daily dose of 0.5-12 mg/kg per day. For intravenous use, DIM can be used, preferably formulated as an intravenous suspension or emulsion, in a daily dose of 0.5-15 mg/kg per day.

[00169] In certain embodiments, Enzalutamide may be administered in a dose of 40 mg to 240 mg or 40 mg to 160 mg/daily, for example, orally (e.g., once a day). In certain embodiments, equal to or less than 160 mg, equal to or less than 120 mg, equal to or less than 90 mg, or equal to or less than 80 mg of Enzalutamide is administered daily (e.g., once a day, orally), when administration of Enzalutamide is combined with a DIM-related indole (e.g., BR-DIM).

[00170] In certain embodiments, ARN-509 may be administered in a dose of 40 mg to 240 mg, or 40 mg to 160 mg/daily, for example, orally (e.g., once a day). In certain embodiments, equal to or less than 160 mg, equal to or less than 120 mg, equal to or less than 90 mg, or equal to or less than 80 mg of ARN-509 is administered daily (e.g., once a day, orally), when administration of ARN-509 is combined with a DIM-related indole (e.g., BR-DIM).

[00171] Co-administration or co-formulation of a DIM-related compound with a Selected Anti-Androgen Compound may be effective to reduce the dose of a DIM-related indole to be administered to a subject. For example, when co-administered or co-formulated with a Selected Anti-Androgen Compound, DIM or a DIM-related indole may be administered orally in a dose that is equal to or less than 450 mg/day, 425 mg/day, 400 mg/day, 375 mg/day, 350 mg/day, 325 mg/day, 300 mg/day, 275 mg/day, 250 mg/day, 225 mg/day, 200 mg/day, 175

mg/day, 150 mg/day, 125 mg/day, 100 mg/day, 75 mg/day, or 50 mg/day. For example, DIM may be administered in the amount from 25 mg to 450 mg, from 50 mg to 400 mg, from 75 mg to 375 mg, from 75 mg to 350 mg, from 75 mg to 300 mg, from 75 mg to 250 mg, from 75 mg to 225 mg, from 75 mg to 150 mg per day when used in combination with (e.g., co-administered with or co-formulated with) a Selected Anti-Androgen Compound.

[00172] In certain embodiments, certain combinations of DIM-related indoles, *e.g.*, DIM, and one or Selected Anti-Androgen Compounds in intra-arterial delivery systems, parenteral delivery systems, oral delivery systems, or by simultaneous delivery by multiple routes provides therapeutic efficacy are believed to provide more than the additive efficacy of each agent used alone at maximal dose. Therefore, methods involving combined use of a DIM-related indole and a Selected Anti-Androgen Compounds at less than their maximal doses increase both the safety and efficacy of DIM-related indoles and Selected Anti-Androgen Compounds. Improved efficacy may result in a shorter duration of required therapy than with individual agents used alone. Combined use can allow a reduction in dose or shortening of the period of high dose treatment. Combined use can improve the long term therapeutic result, and durability of clinical response, including maintaining stable disease. Combined use with lowered dose and duration of use can minimize toxicity. In addition, combined use with lowered dose supports long-term use with lessened toxicity or free of toxicity.

[00173] In some embodiments, a period of combination treatment with a DIM-related indole and a Selected Anti-Androgen compound, is followed by a period of administration, or continued use, of the DIM-related indole alone (without the Selected Anti-Androgen Compound).

[00174] Regarding periods of treatment, a subject can be treated with the combination of a DIM-related indole and a Selected Anti-Androgen compound for 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years or more than 5 years. In certain embodiments, the subject is treated with the combination of a DIM-related indole and a Selected Anti-Androgen compound for more than: 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, or more than 5 years. In some embodiments, the subject is treated with the combination of a DIM-

related indole and a Selected Anti-Androgen compound, without developing resistance to the Selected Anti-Androgen compound, for more than: 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, or more than 5 years. In particular embodiments, the subject is treated with the combination of a DIM-related indole and a Selected Anti-Androgen compound, without developing resistance to the Selected Anti-Androgen compound, for more than 3 months, more than 6 months, more than 1 year or more than 2 years. In some embodiments, the subject is treated with the amount of a DIM-related indole and an amount of a Selected Anti-Androgen compound, wherein the amount of the DIM-related indole and/or the amount of the Selected Anti-Androgen compound is less than the amount known to be therapeutically effective when administered alone (e.g., when the amount of enzalutamide is less than 160 mg daily), for more than: 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, or more than 5 years (e.g., without developing resistance to the Anti-Androgen, without manifestation of toxicity or appearance of side effects associated with treatement with the Anti-Androgen, and/or with improvement in one or more symptoms or parameters of the disease). In certain embodiments, the clinical response (which can be manifested in improvement or stabilization of one or more parameters or symptoms of the disease) to the combination of a DIM-related indole and a Selected Anti-Androgen compound is observed for more than: 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, or more than 5 years. In specific embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen compound stabilizes the disease being treated (e.g., stops or substantially stops progression of the disease) for more than: 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, or more than 5 years. In certain embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen compound achieves efficacy (which can be manifested in improvement or stabilization of one or more parameters or symptoms of the disease) in less than: 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year. In specific

embodiments, the combination of a DIM-related indole and a Selected Anti-Androgen compound achieves efficacy (which can be manifested in improvement or stabilization of one or more parameters or symptoms of the disease) in less than 2 weeks, less than 1 month or less than 3 months.

[00175] Further, co-administration or co-formulation of Selected Anti-Androgen Compound with a DIM-related compound may be effective to reduce the dose of a Selected Anti-Androgen Compound to be administered to a subject. For example, when co-administered or co-formulated with a DIM or a DIM-related indole, a Selected Anti-Androgen Compound may be administered orally in a dose that is equal to or less than 160 mg/day, 150 mg/day, 140 mg/day, 130 mg/day, 120 mg/day, 110 mg/day, 100 mg/day, 90 mg/day, 80 mg/day, 70 mg/day, 60 mg/day, 50 mg/day, 40 mg/day, 30 mg/day, or 20 mg/day. For example, a Selected Anti-Androgen Compound may be administered in the amount from 20 mg to 160 mg, from 40 mg to 160 mg, from 20 mg to 150 mg, from 40 mg to 150 mg, from 20 mg to 120 mg, from 40 mg to 120 mg, from 20 mg to 100 mg, from 40 mg to 100 mg, from 20 mg to 80 mg, or from 40 mg to 80 mg, or from 20 mg to 40 mg per day when used in combination with (e.g., co-administered with or co-formulated with) a DIM-related indole.

[00176] In some embodiments, DIM or a structurally-related, synthetically-derived, substituted diindolylmethane can be administered in the form of a sterile, injectable suspension. The suspension can be injected directly into the tissue (e.g., prostate gland tissue). Injection of the tissue can be followed post-operatively by oral use of DIM in an acceptable formulation and at an effective dose. Such a suspension consists of, for example, microcrystalline DIM or structurally-related, synthetically-derived, substituted diindolylmethanes (0.2 – 2% wt/volume) in a suspension of physiologic salts, and pH adjusters. Particle sizes of DIM or structurally-related, substituted diindolylmethane crystals in suspensions are from 50 to 500 microns in average diameter. pH adjusters such as NaOH are added to bring the pH to 7.5-8. Preferably, 1-2 cc of suspension containing 10-20 mg of DIM or a substituted diindolylmethane, depending on the size of the tissue is injected directly into the tissue. In alternative embodiments for direct injection into tissue, DIM analogues, including imidazolelyl-3,3'-diindolylmethane, nitro substituted imidazolelyl-3,3'-diindolylmethanes and DIM-related compounds described in U.S. Patent Application Publication No. 2004/0043965 by Jong, Ling, published March 4, 2004, can be used in manufacture of the sterile suspension. Preferably, 1-2 cc of suspension containing

0.001 mg/kg to 100 mg per kg of structurally-related, synthetically-derived, substituted diindolylmethane as described by Jong can be administered by direct injection into the tissue. A sterile, injectable suspension may also be formulated to contain a Selected Anti-Androgen Compound, in addition to DIM using techniques known in the art.

[00177] In another alternative embodiment, injectable emulsions of DIM or a structurally-related, synthetically-derived, substituted diindolylmethane can be formulated to overcome the low solubility of DIM in both water and lipid. A specialized micro-emulsion utilizes phospholipids to optimize the solubility of DIM and related compounds and improve the stability of the emulsion. Preferably, 1-3 cc of a sterile, injectable emulsion containing 20-200 mg of DIM or substituted diindolylmethane is injected into the tissue. An injectable emulsion may also be formulated to contain a Selected Anti-Androgen Compound, in addition to DIM using techniques known in the art.

[00178] In another alternative embodiment, DIM or a structurally-related, synthetically-derived, substituted diindolylmethane can be incorporated within bio-compatible, stable microspheres (e.g., for use during prostate gland arterial embolization). In one preferred use, DIM and/or a Selected Anti-Androgen Compounds are included in the production of hydrophilic, non-resorbable, microspheres produced from an acrylic polymer and impregnated with porcine gelatin. Examples of production techniques for DIM-impregnated microspheres (e.g., for controlled, targeted embolization of prostate gland) are described in U.S. Patent No. 5,635,215 and U.S. Patent Application Publication No. 2003/0211165 by Vogel *et al.*, published November 13, 2003, both of which are herein expressly incorporated by reference in their entireties. Dose ranges for administration of DIM and structurally-related, synthetically-derived, substituted diindolylmethanes, when used in microspheres as extended-release drug delivery devices, are from 25-2000 mgs per treatment. When used in conjunction with Selected Anti-Androgen Compounds impregnated microspheres, the dose range for administration of DIM or a DIM-related compound is 50-1000 mgs and the Selected Anti-Androgen Compounds dose is from 50 – 1000 mgs per embolization treatment.

[00179] In particular embodiments, absorption-enhanced DIM or a structurally-related, synthetically-derived, substituted diindolylmethane is administered in conjunction with Enzalutamide, ARN-509 or AZD-3514. In one embodiment, effective doses of DIM would be the same as used when DIM is administered alone.

5.5. Combination Therapy

[00180] The methods and compositions of the present invention can also be useful in combination with other therapeutic agents and therapeutic modalities which may be used for the treatment of the diseases described herein. For example, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) chemotherapy, radiaton therapy or surgery, as appropriate. In another example, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) another antiandrogen or an antiestrogen compound.

[00181] In certain embodiments, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) a CYP 17 lyase inhibitor or a CYP 17, 20 lyase inhibitor. In some embodiments, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) a drug that inhibits 17 alpha-hydroxylase/C17,20 lyase (CYP17A1) enzyme. In one embodiment, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) abiraterone (Zytiga®). In a specific embodiment, abiraterone is used in the methods and compositions described herein in the amount of 500 to 1250 mg (or any amount in between, e.g., 1000 mg) per dose (e.g., orally once daily). In one embodiment, abiraterone is used in the methods and compositions described herein in the amount of 1000 mg per dose (e.g., orally once daily). In one embodiment, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) orteronel (TAK-700). In one embodiment, the methods and compositions described herein can be used in combination with (simultaneously with, before or after) galeterone (TOK-001).

5.6. Pharmaceutical Compositions

[00182] The pharmaceutical compositions according to the present invention preferably comprise one or more pharmaceutically acceptable carriers and the active constituents. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[00183] It will be appreciated that the amounts of the compounds described herein required for said treatment will vary according to the route of administration, the disorder to be treated, the condition, age, and file history of the subject, the galenic formulation of the pharmaceutical composition, etc.

[00184] Therapeutic formulations include those suitable for parenteral (including intramuscular and intravenous), topical, oral, vaginal, rectal or intradermal administration. Thus, the pharmaceutical composition may be formulated as tablets, pills, syrups, capsules, suppositories, formulations for transdermal application, powders, especially lyophilized powders for reconstitution with a carrier for intravenous administration, etc.

[00185] Preferably, the DIM or a DIM-related compound used in the invention has been processed to enhance bioavailability, as is described in U.S. Patent No. 6,086,915. DIM or LTR processed in this manner is referred to as "processed DIM" and "processed LTR", respectively. However, any suitable preparation of DIM or a DIM-related compound can be used in the methods, kits and compositions of the invention. Optionally, additional absorption enhancing agents active with DIM-related indole such as grapefruit extracts or extract of black pepper providing extracts of piperine can be included.

[00186] The following is a list of ingredients useful for formulating processed DIM or a DIM-related compound:

1. About 10 to about 40 percent by weight of DIM or a DIM-related compound.

2. About 10 to about 40 percent by weight of the following, alone or in combination: vitamin E succinate polyethylene glycol 1000; vitamin E succinate Polyethylene glycols with polyethylene glycol (with a molecular weight range of 400-2000); other polyethylene glycol esters such as those formed by fatty acids such as oleic acid or stearic acid; polyvinylpyrrolidones; polyvinylpolypyrrolidones; Poloxamer 188, Tweens; or Spans.

3. About 5 to about 20 percent by weight of the following, alone or in combination: phosphatidyl choline (derived from soy lecithin and supplied as Phospholipon 50G from Rhone Poulenc Rorer); dioleoyl phosphatidylcholine; phoshatidylglycerol; dioleoylphosphatidylglycerol; dimyristoylphosphatidylcholine; dipalmitoylphosphatidylcholine; phosphatidylethalolamines; phosphatidylserines; or sphingomyelins; or other sources of phospholipids as those from purified Milk Fat Globule Membrane; glycerolesters; poly glycerol esters; or ethoxylated castor oil.

4. About 15 to about 30 percent by weight of the following, alone or in combination: hexanol; ethanol; butanol; heptanol; 2-methyl-1-pentanol; various ketone solvents that would be acceptable in foods such as methyl ethyl ketone, acetone and others; propylene glycol; and certain ester solvents such as ethyl acetate.

5. About 20 to about 40 percent by weight of the following, alone or in combination: modified starch such as Capsul™ Starch from National Starch, Inc.; methylcellulose; hydroxypropyl methylcellulose; hydroxyethylcellulose; hydroxypropylethylcellulose; pectin; gum arabic; gelatin; or other polymeric matrix-forming preparation known to those skilled in the art, soluble in water and, suitable for spray drying.

6. About 0.5 to about 35 percent by weight of the following, alone or in combination: aerosil 200; or any other flow enhancing excipient from silica, or related salt, known to those skilled in the art.

[00187] The following is a detailed method of formulating processed DIM:

1. 6.75 kg of TPGS is heated just beyond its melting point with constant stirring in a heated vessel ("First vessel").

2. 9.38 kg of hexanol and 9.83 kg of jet milled DIM is added to the first vessel and the mixture stirred to a uniform suspension at 70°C. 1.4 kg of phosphatidyl choline is then added.

3. In a second larger vessel, 185 L of water and 10.7 kg of starch are thoroughly mixed with a Cowles blade. This mixture is neutralized to pH 7 with a small amount of sodium carbonate and then heated to 75°C and stirred for 1 hour.

4. The contents of the first vessel is added to the starch mixture in the second larger vessel and thoroughly mixed with a homogenizing rotor/stator type mixer at moderate speed for 15 minutes.

5. The mixture from step 4 is spray dried with a small amount (approximately .5%) of hydrophilic silica to provide a free flowing powder of finely dispersed microparticles containing the co-precipitated TPGS, phosphatidyl choline and DIM in an amorphous, non-crystalline structure.

6. The flowable powder product is collected and stored in evacuated foil sacks, after de-aerating and flushing with nitrogen.

7. Analysis of presence of unchanged dietary ingredient, reveals about 30 to about 33 percent by weight of DIM.

[00188] The procedure of making processed DIM may be summarized as follows:

(a) heating one or more solubilizing emulsifiers selected from the group consisting of vitamin E succinate polyethylene glycol 1000, polyvinylpyrrolidone, polyoxyethylene stearate, sodium cholate, deoxycholate and taurocholate;

(b) adding to the product of step (a) a solvent and a surfactant phospholipid selected from the group consisting of phosphatidyl choline, dioleoyl phosphatidyl choline, phosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoylphosphatidylcholine, dipalitoylphosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin to produce a solution;

(c) dissolving in the solution of step (b) LTR and/or DIM;

(d) adding to the solution of step (c) a solution containing an encapsulator;

(e) mixing the solution produced in step (d) to produce a microdispersion with a particle size of 5 microns or less; and

(f) spray drying the resulting mixture to leave a solid hydrophobic phytochemical composition.

[00189] In general, a suitable (therapeutically effective) amount of DIM or LTR is 50-500 mg per day. DIM is preferably administered in an absorption enhancing formulation, as described in U.S. Patent No. 6,086,915, at 50-200 mg per day, more preferably 400 – 800 mg per day, as a suspension of microparticles in a starch carrier matrix. The LTR is preferably administered in an absorption enhancing formulation at 50-200 mg per day, more preferably 200-800 mg per day, as a suspension of microparticles in a starch carrier matrix. The actually administered amounts of phytochemical may be decided by a supervising physician or veterinarian.

[00190] Any suitable preparation of Selected Anti-Androgen Compounds can be used in the methods, kits and compositions of the invention. Suitable preparations of Selected Anti-Androgen Compounds are known in the art. For example, reference is made to Tachibana et al., US 8,470,829 and US 2011/0306615, Sawyers et al, WO 2006/124118, US 7,709,517, US 8,183,274, US 2012/0295944 and US 2013/0034501; Jung et al., WO 2007/127010, US 8,110,594, US 8,445,507 and US 2013/0072511, and Bradbury et al., US 2013/0203714 for descriptions of suitable preparations of respective compounds disclosed therein, which are

incorporated by reference herein in their entireties. Examples of suitable dosages and routes of administration of Anti-Androgen Compounds are also provided in Tachibana et al., US 8,470,829 and US 2011/0306615, Sawyers et al, WO 2006/124118, US 7,709,517, US 8,183,274, US 2012/0295944 and US 2013/0034501; Jung et al., WO 2007/127010, US 8,110,594, US 8,445,507 and US 2013/0072511, and Bradbury et al., US 2013/0203714 for respective compounds disclosed therein, and such disclosures are incorporated by reference herein in their entireties.

[00191] In general, a suitable (e.g., therapeutically effective) amount of Enzalutamide may be 40-500 mg per day. Enzalutamide is preferably administered at 40-160 mg per day, more preferably 80-120 mg per day. A suitable (e.g., therapeutically effective) amount of ARN-509 may be 40-500 mg per day. ARN-509 is preferably administered at 80-250 mg per day, more preferably 90-120 mg per day. The actually administered amounts of these compounds or another Selected Anti-Androgen Compound may be decided by a supervising physician or veterinarian.

[00192] Pharmaceutical compositions according to the present invention preferably comprise one or more pharmaceutically acceptable carriers and the active constituents, *e.g.*, a DIM-related indole alone, or a DIM-related indole and one or more Selected Anti-Androgen Compounds. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[00193] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatin, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

[00194] Therapeutic formulations suitable for oral administration, *e.g.*, tablets and pills, may be obtained by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by mixing DIM or a structurally-related, synthetically derived, substituted diindolylmethane (and optionally, a Selected Anti-Androgen

Compound described herein), and compressing this mixture in a suitable apparatus into tablets having a suitable size. Prior to the mixing, the DIM or structurally-related DIM (and optionally, a Selected Anti-Androgen Compound described herein) may be mixed with a binder, a lubricant, an inert diluent and/or a disintegrating agent.

[00195] In a preferred embodiment, one or more of the DIM-related indoles is co-formulated with one or more of the Selected Anti-Androgen Compounds in gelatin or soft-gelatin capsules as a solution in caprylocaproyl polyoxylglycerides. Optionally, inactive ingredients for capsules of co-formulated DIM-related indole and Anti-Androgen include excipients including butylated hydroxanisol, sorbitol sorbitan, glycerin, purified water and titanium dioxide.

[00196] In a preferred embodiment, DIM or a structurally-related, synthetically derived, substituted diindolylmethane is mixed with a binder, such as microcrystalline cellulose, and a surfactant, such as sodium lauryl sulphate until a homogeneous mixture is obtained. Subsequently, another binder, such as polyvidone, is transferred to the mixture under stirring with a small amount of added water. This mixture is passed through granulating sieves and dried by desiccation before compression into tablets in a standard tableting apparatus. Optionally, one or more of the Selected Anti-Androgen Compounds described herein can be added into the mixture.

[00197] A tablet may be coated or uncoated. An uncoated tablet may be scored. A coated tablet may be coated with sugar, shellac, film or other enteric coating agents.

[00198] Therapeutic formulations suitable for parenteral administration include sterile solutions or suspensions of the active constituents. An aqueous or oily carrier may be used. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Formulations for parenteral administration also include a lyophilized powder comprising phytochemical that is to be reconstituted by dissolving in a pharmaceutically acceptable carrier that dissolves said phytochemical.

[00199] When the pharmaceutical composition is a capsule, it may contain a liquid carrier, such as a fatty oil, *e.g.*, cacao butter.

[00200] Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium

chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

[00201] In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald *et al.*, 1980, Surgery 88:507; Saudek *et al.*, 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy *et al.*, 1985, Science 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, J. Neurosurg. 71:105).

[00202] For use in tissue injection, stable, biocompatible microspheres, easily injected through needles and angiography catheters, are preferred. Microspheres with diameters from 10 to about 300 micrometers in diameter are most preferred. Due to the poor water solubility of DIM and structurally-related, synthetically-derived, substituted diindolylmethanes, a method for preparation of biodegradable polymeric drug delivery devices using relatively low temperatures and non-aqueous solutions is useful. Techniques for manufacture of microspheres appropriate for the physicochemical characteristics of DIM, LTR, and synthetic DIM-related drugs are described in U.S. Patent No. 5,718,921, which is incorporated by reference herein in its entirety. Manufacture of appropriate microspheres with desirable sustained release characteristics containing DIM or a structurally-related, synthetically-derived, substituted diindolylmethane, and/or EGFR-antagonist is further described in U.S. Patent Application Publication No. 2003/0211165 by Vogel *et al.*, published November 13, 2003, which is incorporated herein by reference in its entirety. Alternatively, embolic compositions comprising macromers having a backbone of a polymer having units with a 1,2-diol and/or 1,3-diol structure which incorporate DIM or a structurally-related, synthetically-derived, substituted diindolylmethane, and/or EGFR-antagonists can be made according to U.S. Patent Application Publication No. 2003/0223956 by Goupil *et al.*, published December 4, 2003, which is incorporated herein by reference in its entirety. In some embodiments, one or more of

the Selected Anti-Androgen Compounds described herein is embedded into microspheres for use in tissue injections, either alone or together with a DIM-related indole, using techniques known in the art.

[00203] In other embodiments, a controlled release formulation comprising biodegradable polymer microspheres or microparticles wherein DIM or a structurally-related, synthetically-derived, substituted diindolylmethane (and, optionally, one or more of the Selected Anti-Androgen Compounds described herein) is suspended in a polymer matrix, the polymer matrix being formed from at least two highly water soluble biodegradable polymers, and the microspheres being coated with a (d, 1 lactide-glycolide) copolymer is preferred. The selection of the particular (d, l lactide-glycolide) copolymer will depend in a large part on how long a period the microsphere is intended to release the active ingredient. For example, a (d, 1 lactide-glycolide) copolymer made from about 80% lactic acid and 20% glycolic acid is very stable and would provide a microsphere suitable for release of DIM, LTR, and synthetic DIM-related drugs over a period of weeks.

[00204] Other controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533).

[00205] In one embodiment of the pharmaceutical composition according to the invention, two or more active constituents are comprised as separate entities.

[00206] Pharmaceutical/Nutraceutical Compositions:

[00207] Pharmaceutica/Nutraceutical Dosage Forms for DIM-related indoles: Multi-application DIM-related indole containing particles are manufactured by various techniques including spray drying, spray cooling, selective precipitation, crystallization and other particle forming methods. The resulting particles are used in the manufacture of the following dosage forms, some of which are described in U.S. Patent No. 6,086,915, incorporated by reference herein in its entirety.

[00208] I. Spray Dried Microencapsulated solid dispersions

1. TPGS/phosphospholipid spray-dried particles. Production of absorption-enhanced DIM-related indole particle formation is provided in U.S. Patent No. 6,086,915.
2. Liquid emulsions using TPGS/phosphospholipid spray-dried particles. Production of emulsions for oral use utilizes absorption-enhanced

DIM-related indole particle formation is provided in the U.S. Patent No. 6,086,915.

3. Flavored DIM granules for oral use (Chocolate, Orange "sprinkles"). Production of flavored granules for oral use utilizes absorption-enhanced DIM-related indole particles (DIM/TPGS) as provided in U.S. Patent No. 6,086,915. Production steps include dry mixing DIM/TPGS particles with maltodextrin granules, addition of flavoring particles and granulation using a standard fluid bed granulator.

[00209] II. Spray Dried Polymer based solid dispersions

[00210] Production techniques for DIM-related indoles may utilize those described in U.S. Patent Application No. 20030072801, entitled "Pharmaceutical compositions comprising drug and concentration-enhancing polymers," herein incorporated by reference in its entirety. In particular production involves the following dissolution enhancing polymers, used with and without lipid stabilizers:

1. Polymer included: Hydroxy Propyl Methylcellulose
2. Polymer : Hydroxy Propyl Cellulose

[00211] III. Cyclodextrin Based Formulations

[00212] Examples of manufacturing techniques are described in U.S. Patent No. 4,877,778 and U.S. Patent Applications Nos. 20040053888; 20030073665; and 20020068720, each of which is herein incorporated by reference in its entirety. Using cyclodextrin loading production techniques to incorporate DIM-related indoles the following final formulations are produced:

1. Dry particle complex for oral use
2. Intravenous emulsion
3. Parenteral emulsion

[00213] IV. Nanoparticle-Based dispersions

[00214] Examples of manufacturing techniques are described in U.S. Patent Nos. 6,288,040; 6,165,988; 6,117,454; and U.S. Patent Application Publication No. 20030032601; each of which is incorporated by reference in its entirety. Using nanoparticle production techniques to incorporate DIM-related indoles the following final formulations are produced:

1. Dry particle complex for oral use.

2. Intravenous emulsion
3. Parenteral emulsion

[00215] V. Liposome based formulations

[00216] Examples of manufacturing techniques are described in U.S. Patent Nos. 4,906,476; 5,006,343; and U.S. Patent Application Publication No. 20030108597. Using liposome production techniques to incorporate DIM-related indoles the following final formulations are produced:

1. Dry particle complex for oral use
2. Intravenous emulsion
3. Parenteral emulsion

[00217] Food Bar Products are produced according to the present invention according to known manufacturing and baking practices. Detailed of food bar composition and manufacturing techniques useful with DIM, DIM-Related Indoles, are specified in U.S. Patent Application Publication Nos. 20030068419 entitled "Food bar compositions" and 20020168448 entitled "Nutritional food bar for sustained energy".

[00218] Drink Mix Products are produced according to the present invention according to known manufacturing practices. Detailed drink mix composition and manufacturing techniques useful with DIM, DIM-Related Indoles, are specified in U.S. Patent No. 6,599,553 by Kealey *et al.*, entitled "Dry drink mix and chocolate flavored drink made therefrom". In certain embodiments, DIM is incorporated in fortified foods, such as drink mixes and food bars, during food production using a particulate form of DIM that is formulated for enhanced absorption (BioResponse-DIM [BioResponse, LLC, Boulder, CO]). Typically, the DIM is provided in a dose of 10-75 mg/serving (40-300 mg/serving of BioResponse-DIM).

6. Kits

[00219] The invention also provides a pharmaceutical pack (e.g., blister pack) or kit comprising one or more containers filled with one or more of the ingredients for practicing the methods of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00220] A kit of the invention can comprise, in one or more containers, a DIM-related indole and a Selected Anti-Androgen Compound (e.g., a Diarylthiohydantoin compound). In a specific embodiment, a DIM-related indole is DIM (e.g., processed DIM) or I3C. In specific embodiments, a Selected Anti-Androgen Compound is Enzalutamide, ARN-509 or AZD-3514. In one embodiment, a DIM-related indole and a Selected Anti-Androgen Compound are in one container in the kit (in the same delivery vehicle). In one embodiment, a DIM-related indole and a Selected Anti-Androgen Compound are together in one pill, capsule or tablet in the kit (e.g., in a blister pack). The kit may comprise multiple capsules, tablets or pills. In other embodiments, a DIM-related indole and a Selected Anti-Androgen Compound are in two separate containers of the kit.

[00221] In a specific embodiment, the DIM-related indole and/or the Selected Anti-Androgen Compound are formulated for oral administration, e.g., co-formulated in a single pill, tablet or capsule. In a specific embodiment, the DIM-related indole and/or the Selected Anti-Androgen Compound are formulated for parenteral or intra-arterial administration, e.g., co-formulated. In a particular embodiment, the DIM-related indole and/or the Selected Anti-Androgen Compound are formulated in a suspension of drug-eluting microspheres for direct administration into a tissue or intra-arterially, e.g., co-formulated in a single microsphere suspension.

[00222] The kit can comprise any dosage of a DIM-related indole and a Selected Anti-Androgen Compound described herein.

[00223] In certain embodiments, the kit can comprise one or more containers, utensils and/or instructions. A utensil can comprise item(s) to administer the drug, such as a syringe, needle, or a fluid container cup,. A container can contain one or multiple doses of a DIM-related indole and/or a Selected Anti-Androgen Compound (e.g., multiple doses in single dose units). The kit may further contain instructions for administration of the compounds of the invention, e.g., instructions regarding dosages, frequency of administration, indications, mode of administration, counter-indications, etc. For example, the instructions may indicate that a DIM-related-indole and/or a Selected Anti-Androgen Compound is to be taken once daily or twice daily. The instructions may also indicate whether a DIM-related indole should be taken simultaneously with a Selected Anti-Androgen Compound (if these compounds are not co-formulated), or whether a DIM-related indole should be taken within, before or after 0.25 h, 0.5

h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 4.5 h, 5 h, 5.5 h or 6 h of administration of a Selected Anti-Androgen Compound.

[00224] The invention is further explained by the following illustrative examples:

7. EXAMPLES:

7.1. Example 1: Manufacture of processed DIM for enhanced oral bioavailability

[00225] In general, a suitable (therapeutically effective) amount of diindolylmethane is preferably administered in an absorption enhancing formulation, as described in U.S. Patent No. 6,086,915, at 100 - 2000 mg per day as a suspension of microparticles in a starch carrier matrix. The actually administered amounts of Diindolylmethane may be decided by a supervising physician.

[00226] Preparation of processed Diindolylmethane was accomplished according to the steps outlined in U.S. Patent No. 6,086,915, herein incorporated by reference in its entirety. Briefly, this included mixture of about 10-40% by final weight of either diindolylmethane with about 10-40% by final weight of vitamin E polyethylene glycol 1000 succinate (Vitamin-E-TPGS, Eastman Chemical), 2-20% by final weight, phosphatidyl choline (Phospholipon 50G, Rhone Poulenc) and 15-30% by final weight hexanol. This mixture was made homogeneous by mixing. The homogeneous mixture of indoles and other oil soluble substituents listed above was added to a solution of modified starch in water (Capsul Starch from National Starch, Inc.). The starch component forms from 30-70% of the final dry weight of the product. The well dispersed final combined mixture was then subjected to spray drying. The resultant product was a fine powder containing either diindolylmethane contained within the starch particles.

[00227] Alternatively, the starch component described above as Capsul Starch from National Starch, Inc., can instead be made using Maltodextrin NF (Maltrin M100, GPC), 20–30 % of the final dry weight, together with Gum Arabic USP/NF (TIC Pretested, TIC Gums), 20–30 % of the final dry weight, together making 40-70% of the final dry weight. Additionally, fumed silica (Aerosil 200, Degussa), 1-2% of the final weight, can be added during the spray drying process as a flow aid.

7.2. Example 2: Manufacture of capsules containing a DIM-related indole and, optionally, a Selected Anti-Androgen Compound

[00228] Capsules containing 150 - 300 mg of processed diindolylmethane, as produced according to the steps described in Example 1, were made by mixing the processed diindolylmethane with microcrystalline cellulose (or rice flour excipient) and placing the mixed powder into opaque gelatin capsules.

[00229] Alternatively, in some of the embodiments in which a DIM-related indole is co-formulated with a Selected Anti-Androgen Compound, capsules containing processed diindolylmethane (DIM), as produced according to the steps described in Example 1, are made by mixing the processed DIM with a Selected Anti-Androgen Compound, such as a Diarylthiohydantoin, according to the following per capsule amounts. The ingredients are mixed according to the following amounts and capsules are filled using standard machinery:

Component	**Preferred Capsule Weight Of Active (mg)**	**Range of Capsule Weight for Active (mg)**
Processed DIM	75	(30-100)
Enzalutamide	40	(20-60)
ARN-509	(30-50)	(20-70)
AZD-3514	(20-100)	(10-250)

7.3. Example 3: Manufacture of stable microspheres containing a DIM-related indole and, optionally, a Selected Anti-Androgen Compound

[00230] Due to the poor water solubility of DIM, a production technique for stable, biocompatible microspheres containing DIM is developed based on production technology described in U.S. Patent No. 5,718,921, herein expressly incorporated by reference in its entirety. This process uses a polyanhydride polymer which is dissolved in a volatile organic solvent, in which the DIM or structurally-related, synthetically-derived, substituted diindolylmethane is dispersed and co-dissolved in the polymer solution. The mixture is suspended in an organic oil, and the organic solvent is extracted into the oil, creating microspheres. The method enables the preparation of DIM containing microspheres from a variety of biodegradable polymers, including hydrophobic polyanhydrides such as (pCPP:SA, 50:50) and CPP copolymerized with dodecanedoic acid (DD), (pCPP:DD, 20:80) and (pCPP:DD, 50:50).

[00231] For the preparation of DIM microspheres, four grams of the polymer, pCPP:SA, 20:80, mw=16000, is dissolved in 20 ml methylene chloride, to which is added 1 gram of

microcrystalline DIM and suspended in the polymer solution using a mechanical stirrer. The mixture is then dropped into silicon oil (Dow Chemical Company, Midland, Mich.) that contains between approximately 1.0 and 20% of Span™ or another surfactant or emulsifying agent. Span™ emulsifiers are preferred. This is then stirred at a set stirring rate. Stirring is done using an overhead stirrer type RZR50 ("CAFRAMA", Wiarton, Ont.) and a three-blade impeller. After 1 hour, petroleum ether is introduced and stirring is continued for another hour. The microspheres are isolated by filtration, washed with petroleum ether, dried overnight in a lyophilizer (Labconco, Freeze Dryer 8), are sieved (U.S. Standard Sieve Series, Newark, Wire Cloth Company, Newark, N.J.) and are stored at less than 0°C.

[00232] This process of manufacture will yield DIM impregnated microspheres with diameters of from 50 to 1000 microns. The recovery in this production process can be limited to 50% due to some polymer precipitating on the stirrer. The DIM microspheres are sieved to remove microspheres with diameters greater than 500 microns to result in microspheres with a final size distribution of 50-500 microns. The smaller microspheres are then sieved to remove those with diameters less than 50 microns, leaving microspheres with diameters between 500 and 500 microns. The resulting 2 grams of microspheres are again dried in a lyophilizer, autoclaved to sterilize, and resuspended at a concentration of 1.0 ml of microspheres in 5 ml of sterile physiological saline. The 5 ml suspension of microspheres will contain approximately 250 mgs of DIM and the 10 ml suspension of microspheres will contain approximately 500 mgs of DIM, adequate for therapeutic use in a single procedure (e.g., tissue injection procedure).

[00233] In some of the embodiments in which a DIM-related indole is co-formulated with a Selected Anti-Androgen Compound, the Selected Anti-Androgen Compound can also be dispersed and co-dissolved in the polymer solution together with the DIM-related compound, as described above. Alternatively, a Selected Anti-Androgen Compound can be embedded into microspheres, either alone or together with a DIM-related compound, using any technique known in the art.

7.4. Example 4: Manufacture of an injectable emulsion formulation of a DIM-related indole and, optionally, a Selected Anti-Androgen Compound

[00234] Stable microemulsions of DIM, designed for intravenous use or tissue injection, are developed to provide a convenient means of administering DIM to achieve high tissue

concentrations of DIM quickly and at a predictable time. In addition, microemulsions of a DIM-related indole can be used in conjunction with a Selected Anti-Androgen Compound, or can be co-formulated to contain both a DIM-related indole and a Selected Anti-Androgen Compound.

[00235] The low solubility of DIM in both water and lipid requires development of a specialized micro-emulsion that utilizes phospholipids to optimize the solubility of DIM and improve the stability of the microemulsion. To prepare the micro-emulsion Ethyl oleate (EO), Phosphatidyl Choline (PC) (from egg yolk), and calcein, are purchased from Sigma-Aldrich, Inc (St. Louis, Mo.). Distearoyl-phosphatidylethanolamin-N-poly(ethyleneglycol) 2000 (DSPE-PEG) is purchased from Avanti Polar Lipids (Alabaster, Ala.).

[00236] Using a modification of the method of Yu *et al.* (Yu *et al.*, 1993, Int. J. Pharm. 89:139-146), the microemulsion is manufactured as follows: 160 grams of EO and 60 grams of PC are dissolved in 1 liter pure ethanol. 24 grams of microcrystalline DIM (mean particle size 0.25 micron) is added and dissolved in this "oily phase". 20 grams of DSPEG-PEG is then dissolved in 500 cc of USP water (Aqueous phase). The oily ethanolic solution (oily phase) with the dissolved DIM is then slowly added into the DSPE-PEG solution (aqueous phase) under moderate magnetic stirring. The aqueous phase immediately turns milky with opalescence as the result of the microemulsion produced. The microemulsion is then subjected to low pressure at 360 mm Hg and maintained at 50°C. The low pressure is used to concentrate the emulsion through removal of the ethanol and a portion of the water. Using an infrared absorption assay to determine the DIM content of the microemulsion, a final concentration of DIM of 7.5 mg/ml is established. Sodium hydroxide is added to increase the pH to the 5.0-7.5 range.

[00237] Using this manufacturing technique emulsions of DIM are prepared and subjected to stability testing to demonstrate that the particle size within the emulsion remained between 150 and 200 nm. The production technique results in a micro-emulsion with % weight ranges of the components in the following preferred ranges:

Component	Approx %Weight
DIM	0.05-0.1
Lipids (EO:PC:DSPE-PEG; 8:3:1)	45 – 28
Water	50 – 70
Ethanol	1 – 2

[00238] Alternatively, an ethanol-free production method can be utilized to produce a stable micro-emulsion of DIM or DIM derivatives and analogues, using Lipofundin MCT B (Braun Melsungen AG, Melsungen, Germany), a preformed basic emulsion, and high pressure homogenization of microcrystalline DIM. This method utilizes jet-milled DIM, with particle size reduced to 0.1 micron average diameter (performed by Micron Technologies, Inc., Exton, Pa.). Using this technique 700 mg of 0.1 micron diameter DIM crystals are homogenized in 100 cc Lipofundin using equipment and methods as described (Akkar *et al.*, 2003, Eur J Pharm Biopharm. 55:305-12). This results in a stable lipid-based micro-emulsion with particle size less than 200 nm and a DIM content of 7 mg/cc of the emulsion.

[00239] In some of the embodiments in which a DIM-related indole and a Selected Anti-Androgen Compound are co-formulated, a micro-emulsion containing a DIM-related indole and a Selected Anti-Androgen Compound, is made by preparing DIM as described above and further adding a Selected Anti-Androgen Compound, such as a Diarylthiohydantoin, into the micro-emulsion, or by any other method known in the art.

7.5. Example 5: Sterile Liposome-encapsulated DIM for Oral, Rectal, and/or Intravenous Use and, optionally, a Selected Anti-Androgen Compound

[00240] Liposomes are microscopic vesicles composed of a phospholipid bilayer that encapsulate active agents for specialized delivery to specific tissues. Manufacturing techniques for DIM Liposomes are developed based on the published liposome manufacturing techniques as described in U.S. Patent Nos. 4,906,476; 5,006,343; and U.S. Patent Application Publication No. 20030108597, each of which is incorporated by reference herein in its entirety.

[00241] Liposomes are formulated utilizing N-(carbonyl-methoxy-polyethylene glycol 2000)-1,2disteaoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (MPGEG-DSPE) (2-4 mg/ml); fully hydrogenated soy phosphatidylcholine (HSPC) (2-11 mg/ml); and cholesterol (1-

4 mg/ml). Each 30 ml vial produced contains 30-60 mg of DIM-related indole at a concentration of 1-2 mg/ml.

[00242] DIM Liposomes are preferably administered intravenously or per rectum if intravenous access is not possible.

[00243] In some of the embodiments in which a DIM-related indole and a Selected Anti-Androgen Compound are co-formulated (i.e., formulated into one composition), a liposome containing both a DIM-related indole and a Selected Anti-Androgen Compound can be formulated using methods well known in the art. In other embodiments, a liposome containing a Selected Anti-Androgen Compound alone (i.e., without the DIM-related indole) and a liposome containing a DIM-related indole alone (i.e., without the Anti-Androgen) are formulated separately and are administered either contemporaneously or sequentially.

7.6. Example 6: Manufacture of Cyclodextrin complex formulations with DIM-related indoles for improved bio-delivery

[00244] *Introduction*: As poorly soluble drug agents, DIM-related indoles, in general, require solubility enhancing formulation steps which are bio-compatible for parenteral and improved oral drug delivery. Parenteral formulations for intramuscular, intravenous, and pulmonary aerosol delivery benefit from complexation with various cyclodextrins (alpha-cyclodextrin, beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin, and sulfobutylether-beta-cyclodextrin).

[00245] *Methods*: Specific formulations and formulation steps are developed utilizing cyclodextrins. Formulations are based on methods and observations that molecules containing indole rings successfully complex with cyclodextrins providing a subsequent solubility advantage over the indole alone (Cao *et al.*, 2000, Chemosphere 40:1411-6). Therefore, prototype formulation utilizing microcrystalline DIM, compatible solvent systems, with and without lipid stabilizers are manufactured using spray drying technology. Dry particle products are appropriate for suspension in aqueous vehicles for intramuscular or intravenous drug delivery.

[00246] Preferred cyclodextrins utilized are:

1. β-cyclodextrin, which is generally more applicable for the complexation of hydrophobic molecules. It is anticipated that 2 molecules of β-cyclodextrin will be needed per molecule of DIM.

2. Hydroxypropyl β-cyclodextrin, which is known to be very soluble, on the order of 30% and more.
3. Sulfobutyl β-cyclodextrin (trade-name Captisol®). Captisol has a molecular weight of ~2200 mg/mmol so at 2:1 cyclodextrin to DIM, the amount of DIM that can be put into solution will be considerably higher than the known solubility of DIM.

[00247] Sample preparations to be undertaken: Pharmaceutically acceptable solvents will be utilized to form solutions for spray drying with Hydroxypropyl β-cyclodextrin and Sulfobutyl β-cyclodextrin. Complexes of each of the β-cyclodextrins with DIM will be prepared with a slight excess of the cyclodextrin and spray dried to produce approximately 10 to 20 grams of each formulation. Further formulation suitable for intravenous, intramuscular and pulmonary aerosol use will utilize published manufacturing techniques (Steckel *et al.*, 2004, Int J Pharm. 278:187-95).

[00248] Testing of prepared DIM-cyclodextrin formulations: Prepared samples will be analyzed as to amorphous crystal structure and stability using standard techniques (Rodriguez-Spong *et al.*, 2004, Adv Drug Deliv Rev. 56:241-74). Testing of prepared formulations will include release testing of DIM in simulated gastric acid. *In vivo* release and bioavailability testing in animal and human models will utilize plasma DIM assays as described in U.S. Patent Application Publication No. 20030096855.

[00249] *Conclusions*: Cyclodextrin DIM Spray-dried formulations are expected to demonstrate shelf stability, form stable suspensions in 5% dextrose solutions for intravenous administration, and stable suspensions in 0.9% NaCl for intramuscular, parenteral administration.

[00250] In some of the embodiments in which a DIM-related indole and a Selected Anti-Androgen Compound are co-formulated, a DIM-cyclodextrin formulation of the DIM-related indole can be used in the combination composition.

7.7. Example 7: Combination of a DIM-related Indole and a Selected Anti-Androgen Compound Shows Synergistic Activity in Inhibiting the Growth of Prostate Cancer Cells. Combination of a DIM-related Indole and a Selected Anti-Androgen Compound Produces Synergistic Growth Inhibition in Prostate Cancer (PCa) Cell Lines Relevant to Castrate Resistant PCa.

[00251] This example shows that a DIM-related indole and a Diarylhydantoin-related compound exhibit synergistic effect in inhibiting the growth of prostate cancer cells.

[00252] This example shows that a DIM-related indole and a Diarylhydantoin-related compound exhibit synergistic interaction, inhibiting the growth of prostate cancer cells. Synergistic growth inhibition was observed in LNCAP, 22RV1, and C4-2B PCa cells during 72 hour culture. Importantly, the synergistic growth inhibition was observed in both 22RV1 prostate cancer cell and C4-2B prostate cancer cells which possess androgen independent growth reflective of Castrate Resistant Prostate Cancer (CRPCa) driven by AR splice variants which occur in clinical PCa. Specifically the AR3 AR variant (also known as AR-V7) is present in 22RV1 prostate cancer cell and C4-2B PCa cells and drives PCa progression (Liu et al., 2014, "Mechanisms of the androgen receptor splicing in prostate cancer cells," Oncogene 33(24):3140-50, doi: 10.1038/onc.2013.284).

[00253] Using LNCaP, 22RV1 and C4-2B cell lines the MTT assay was performed according to established methods. This study utilized methods described in Hao et al., 2012, "In vitro and in vivo prostate cancer metastasis and chemoresistance can be modulated by expression of either CD44 or CD147," PLoS ONE 7:e40716. Briefly, the LNCaP, 22RV1 and C4-2B Prostate Cancer ("PC" or "PCa") cells were seeded in 96-well plates and incubated for 24 h, and then the cells were treated with BR-DIM (i.e., processed DIM), MDV3100 (enzalutamide), ARN-509, or combination of the two drugs (i.e., BR-DIM and MDV3100, or BR-DIM and ARN-509) for 72 hours. Control cells were treated with DMSO as a vehicle control. Then, the cells were incubated with 0.5mg/ml MTT for 2-3 hours at 37 °C and 5% CO2. After incubation, the medium was removed and DMSO was added into each well, and mixed well with shaking for 10 minutes. The spectrophotometric absorbance was determined by using Ultra Multifunctional Microplate Reader (Tecan, Durham, NC) at 560nm (with lower absorbance indicating growth inhibition and cell death). The results are shown in Fig. 1, showing unexpectedly synergistic activity of BR-DIM and enzalutamide in inhibiting the growth of PC cells. In particular, the results in Fig. 1 show unexpected synergistic activity of BR-DIM in combination with enzalutamide demonstrating greater than additive growth inhibitory activity in LNCaP and 22Rv1 Prostate Cancer (PCa) cell lines. Figures 1A and 1B show the inhibition of cell proliferation of the combination of DIM from BR-DIM and Enzalutamide (MDV3100) treatment in LNCaP and 22RV1 cells as indicated by the MTT

assay. Synergistic interaction is indicated in the Isobologram charts in Figures 1C and 1D, showing results at the Estimated Dose of 90% (ED90) below the line of additivity.

[00254] In further evaluation of these results (representing the average of 3 experiments in LCaP cells and 3 experiments in 22RV1 cells (N=6), Combination Indexes (CI) were calculated for all combination conditions according to methods previously described (Zhao et al., 2010, "Comparison of methods for evaluating drug-drug interaction," Front Biosci (Elite Ed) 2:241-9). A CI result of less than 1 indicates synergistic interaction where the documented growth inhibition is greater that the additive growth inhibition of DIM plus MDV3100.

[00255] At IC 50%, 75% and 90% Effective Dose levels, the following chart summarizes the CI results where CI values of less than 1.0 indicate synergistic growth inhibitory interaction derived from the data in Fig. 1. ED50=50% Effective Dose level; ED75=75% Effective Dose level; and ED90=90% Effective Dose level.

[00256] Summary of Combination Indexes (CI's) Demonstrating Synergy from Co-Administration of BR-DIM and Enzalutamide (MDV3100) in Cultured PCa Cell Lines:

PCa Cell Line	CI at ED50	CI at ED75	CI at ED90	Figure Reference
LNCaP	1.3481	**0.93447***	**0.71045***	Figure 1
22RV1	1.31605	1.06578	**0.87073***	

*CI results of less than 1 indicate synergistic combined effect

[00257] The obtained CI values presented above show that BR-DIM and enzalutamide exhibit synergistic effect in inhibiting the growth of PC cells.

[00258] As illustrated in further experiments shown in Fig. 2, the present results are significant regarding the combined activity of DIM related indoles and Diarylhydantoin AR antagonists since the LNCaP and 22RV1 PCa cells lines express elevated levels of AR splice variants compared with non-cancerous prostate epithelial cell lines RWPE1 and PZ-HPV-7. The presence of AR variants such as AR3 (AR-V7) in both prostate cancer cell lines and during Diarylhydantoin AR antagonist clinical treatment is known to contribute to PCa resistance to growth inhibition from therapeutic agents (see Antonarakis et al., 2014, "AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer," N Engl J Med. 371:1028-1038, DOI: 10.1056/NEJMoa1315815).

[00259] In order to confirm the observation of synergistic growth inhibition from combined exposure to DIM and Diarylhydantoin AR antagonists, further experiments were

conducted with C4-2B PCa cell line which, like the 22Rv1 PCa cell line and as shown in Figures 2 and 3, expresses elevated levels of AR variants. AR variants are further elevated in these cell lines following deprivation of Androgens in the culture media. This is shown in Fig. 3 in the charcoal filtered media condition (CS-FBS) which recreates the *in vivo* condition of Androgen Deprivation Therapy. Thus, the use of both 22Rv1 and C4-2B cell lines is highly relevant to the clinical condition of Castrate Resistant Prostate Cancer (CRPC).

[00260] Using LNCaP and C4-2B cell lines the growth inhibition indicating MTT assay was again performed according to described established methods following 72 hr. culture of PCa cells with DIM (BR-DIM), Enzalutamide (MDV3100) or the combination of BR-DIM and MDV3100, and in addition with DIM (BR-DIM), ARN-509, or the combination of BR-DIM and ARN-509. The results are shown in additional Figures 10-14, which all demonstrate synergistic interaction for increased growth inhibition. Using the data from Figures 10-14, Combination Indexes (CI's) were calculated by methods described earlier and results are presented in the following tables:

[00261] Summary of Additional Assays Indicating Synergy of BR-DIM and Enzalutamide (MDV3100) in Cultured PCa Cell Lines:

PCa Cell Line	CI at ED50	CI at ED75	CI at ED90	Figure Reference
LNCaP	1.244	**0.990**	**0.805**	Figure 10
	1.382	1.068	**0.834**	Figure 11
C4-2B	1.188	1.021	**0.887**	Figure 12
	1.221	**0.956**	**0.770**	Figure 13

[00262] Summary of Assay Indicating Synergy of BR-DIM and ARN-509 in Cultured PCa Cell Lines:

PCa Cell Line	CI at ED50	CI at ED75	CI at ED90	Figure Reference
C4-2B	**0.957**	**0.446**	**0.213**	Figure 14

[00263] The obtained CI values presented above which are less than a value of 1 show that BR-DIM and both enzalutamide (MDV3100) and ARN-509 exhibit a synergistic, greater than additive effect in inhibiting the growth of PCa cells. Furthermore, the demonstration of synergistic growth inhibition was demonstrated in PCa cell lines known to possess AR variants

including AV3 (AR-V7) known to be associated with CRPCa. The presence of AR3 (AR-V7) in CRPCa specimens was subsequently shown to be specifically reduced by exposure to DIM providing direct evidence which explains in part the observed synergistic growth inhibition of the combined treatment with MDV3100 and ARN-509 (see results presented in Example 8).

[00264] Demonstration of synergistic increases in proliferation inhibition with the combination of a DIM-related indole and a Diarylthiohydantoin compound indicate that combined use may offer treatment advantage in men with prostate cancer such as CRPC (Castrate Resistant Prostate Cancer). In particular, demonstration of synergistic increases in growth inhibition with the combination of a DIM-related indole and a Diarylthiohydantoin compound indicate that combined use can offer treatment advantage in men with prostate cancer, including metastatic, CRPCa, and chemotherapy resistant PCa. In addition, synergistic growth inhibition indicates an unexpected discovery providing a new clinical intervention for treatment resistance to AR antagonist monotherapy using MDV3100 and ARN-509 (regarding therapy with MDV3100 or ARN-509 see Nakazawa et al., 2014, "Androgen Receptor Splice Variants in the Era of Enzalutamide and Abiraterone," Horm Cancer 07/22/2104, DOI: 10.1007/s12672-014-0190-1).

7.8. Example 8: A DIM-related Indole Reduces the Expression of AR Variants, and Prostate Cancer Cells Show Increased Expression of AR Variants. DIM Reduces the Expression of AR Variants associated with CRPCa in PCa Cells as well as reducing BioMarkers in PCa cells associated with AR Variants and clinical CRPCa.

[00265] This example shows that (i) prostate cancer cells show increased expression of AR splice variants relative to normal prostate cells, and (ii) a DIM-related indole transcriptionally inactivates, and thus, reduces the expression of AR and AR splice variants in prostate cancer cells.

[00266] Cell culture experimental results showing changes in AR splice variants with BR-DIM:

[00267] First, the expression of AR splice variants among several prostate cancer cell lines compared to normal prostate epithelial cell lines (RWPE1 and PZ-HPV-7 cells) was determined, and the results are shown in Fig. 2 documenting differential expression of AR splice variants among these cell lines. Fig. 2 shows that AR variants increased in prostate cancer cell lines compared with immortalized Non-neoplastic human prostatic epithelial cells:

RWPE1 and PZ-HPV-7. In particular, it was found that prostate cancer cell lines have increased expression of AR splice variants, AR 3 (AR-V7) and AR 132b relative to normal, non-neoplastic human prostatic epithelial cells.

[00268] It is known that the emergence of splice variants (ARV's) of the AR during Diarylhydantoin therapy is associated with resistance to clinical response (Li Y, Chan SC, Brand LJ, Hwang TH, Silverstein KA, Dehm SM. Androgen receptor splice variants mediate enzalutamide resistance in castration-resistant prostate cancer cell lines. Cancer Res. 2013 Jan 15;73(2):483-9. doi: 10.1158/0008-5472.CAN-12-3630. Epub 2012 Nov 1). Thus, experiments were conducted to test whether androgen deprivation results in upregulation of AR splice variants, and whether such upregulation of the AR splice variants correlates with tumor cell aggressiveness (as manifested, e.g., in induction of expression of EMT and cancer stem cell markers). In this experiment, C4-2B and 22Rv1 cells were cultured with normal serum (FBS) or the charcoal serum (charcoal filtered androgen-free serum or CS-FBS) to reduce any trace amount of androgens, and the expression of AR, AR splice variants (AR3 (AR-V7), AR132b, AR122b), and various cancer stem cell markers was measured. The results of this experiment are documented in Fig. 3. The results show that androgen deprivation induced increased expression of AR and AR splice variants, stem cell signatures and EMT phenotype in prostate cancer cell lines, which are belived to be resposible for castration resistant and tumor metastasis and relapses. In particular, the results show that androgen deprivation induced increased expression of AR and AR splice variants -- AR3 (AR-V7), AR132b, AR122b.

[00269] Androgen Receptor (AR) blockade induces oxidative stress in prostate cancer cells inducing inflammatory cell signaling and acquisition of epithelial-mesenchymal-transition (EMT) changes. Activation of EMT induces less differentiated prostate cell status which is associated with emergence of castrate resistance and promotion of AR splice variants. Results presented in Figures 4C and 4D demonstrate the activity of DIM as reflected in changes in the stem cell signatures associated with the Embryonal Mesenchymal Transition (EMT) phenotype and the suppressive effects on AR splice variant levels were investigated in PCa cell lines. Increased EMT biomarkers are associated with increased AR splice variant levels and activity relevant to CRPCa. EMT biomarkers including Lin28B, nanog, Oct4, and Sox2 are associated and contribute to castration resistance, tumor matastasis, AR variant activity. Figure 4B shows

that AR, AR variants (AR3 (AR-V7), AR132b, AR122b) and EMT cell markers (Lin28B, nanog, Oct4, and Sox2) are all elevated in 22RV1 sphere cells which are castrate resistant.

[00270] The results show that increased expression of AR and AR splice variants was concomitant with up-regulation of stem cell markers and mesenchimal markers in prostate cancer cells. These results demonstrate that androgen deprivation therapy increases expression of AR and AR splice variants, and that the expression of AR splice variants is associated with cancer cell aggressiveness.

[00271] The expression of AR and AR splice variants, and the effect of DIM (such as BR-DIM) on the expression of AR, AR splice variants and PSA, was tested in parental LNCaP and 22Rv1 cells as well as sphere forming cells derived from LNCaP and 22Rv1 cells. The expression of AR, AR splice variants and PSA was assessed using real time PCR and/or Western Blot, and the results of this experiment are presented in Fig. 4. As Figures 4C and 4D illustrate, BR-DIM treatment reduces the expression and protein levels of AR and AR splice variants in 22 Rv1 PCa cells. The materials and methods used in this experiment are described below:

[00272] Real time RT-PCR: The cells were grown in 10% FBS or 10% Charcoal stripped FBS and then total RNA was isolated from these cells. Real time RT-PCR was used to determine expression of EMT and stem cell markers according to method of Kong D, Banerjee S, Ahmad A et al. Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. PLoS ONE 2010;5:e12445., and Kong D, Heath E, Chen W et al. Loss of let-7 up-regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatures that are attenuated by BR-DIM. PLoS ONE 2012;7:e33729..

[00273] Western Blot: Cells were seeded in dishes and after 24 h of incubation, the cells were treated with BR-DIM for 3 days or single cell suspensions were plated on ultra low adherent wells of 60 mml dishes (Corning) in DMEM/F12 (Invitrogen) supplemented with B27 and N2 (Invitrogen). Single cell status was confirmed under microscope. Fresh medium was added every 3-4 days. After 3 days of incubation, cells were treated with BR-DIM for 3 days. Cell lysates were prepared from these treated cells and then Western Blot was performed according to established methods (Kong D, Banerjee S, Ahmad A et al. Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. PLoS ONE 2010;5:e12445., and Kong D, Heath E, Chen W et al. Loss of let-7 up-

regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatures that are attenuated by BR-DIM. PLoS ONE 2012;7:e33729.).

[00274] The results presented in Fig. 4, A and B, show that the expression of AR and AR splice variants was increased in sphere forming cells from castration-resistant prostate cancer cells (22RV1) but not in an androgen sensitive cell line (LNCaP). This indicates that demonstration of synergistic inhibition of cell growth by the combination of DIM and either Enzalutamide or ARN-509 in 22Rv1 cells as presented in Example 7 is relevant to the clinical condition of resistance to monotherapy with either Enzalutamide or ARN-509 where splice variants contribute. Importantly, the results presented in Fig. 4, C, show that BR-DIM treatment led to decreased expression of AR and AR splice in 22Rv1 sphere forming cells, and results presented in Fig. 4, D, show that BR-DIM treatment decreased the expression of AR, AR variants (AR-V) and PSA in both LNCaP cells and 22RV1 cells. Overall, the results presented in Fig. 4 show that BR-DIM treatment decreased expression of AR and AR variants, EMT and stem cell markers, which when used on combination with enzalutamide is expected to solve the problem of enzalutamide resistant tumors. Since resistance to AR receptor antagonist treatment, including resistance to MDV3100 (Enzalutamide) is associated with EMT and stem cell markers, reversal of the EMT phenotype by DIM as demonstrated provides a mechanism to counter EMT changes encountered in AR antagonist treated PCa. These results provide a evidence of cellular changes in CRPCa induced by DIM in addition to the reductin in AR splice variant levels. The unexpected finding of synergistic inihibition of PCa cell growth with the combination of DIM and MD3100 or ARN-509, particularly in the 22Rv1 cell line, indicates multiple unanticipated interactions from the combination providing a basis for therapeutic benefit in AR antagonist resistant PCa.

[00275] Unexpected findings from all of the above experiments make it clear that DIM (such as BR-DIM) reduces the expression of AR splice variants which is associated with enzalutamide resistance. These findings make it also clear that DIM reduces the expression of AR splice variants and reverses cellular changes associated with EMT which is associated with CRPCa and clinical resistance to AR antagonist drugs, including resistance to Diarylhydantoin-related compounds.

[00276] These results show that the use of a DIM-related indole reduces the amount of AR slice variants, and thus can be effective in the treatment of conditions in which AR splice

variants are overexpressed. The results further show that the use of a DIM-related indole in combination with a Diarylhydantoin-related compound achieves synergistic inhibition of cancer cell growth (via, e.g., induction of apoptosis/cell cycle arrest). Thus, the use of a combination of a DIM-related compound and a Diarylhydantoin-related compound can achieve a synergistic inhibition of tumor growth, and/or prevent, delay or overcome development of resistance to a Diarylhydantoin-related compound. In particular, reduction of the expression of AR splice variants by BR-DIM in CRPC patients is expected to result in arrest of tumor growth and clinical improvement when used in combination with a Diarylhydantoin-related compound.

[00277] Subsequently, in separate experiment, the expression of AR splice variants and EMT markers were assessed in human prostate cancer tissue specimens of different, increasing severity Gleason grades (higher Gleason grades G6-G9) compared to normal prostate tissue. This included determination of relative levels of AR splice variant AR3 (AR-V7) and AR132b. In addition, the expression of Lin28B, a marker of EMT and tumor cell aggressiveness, was also determined. The results presented in Fig. 5 clearly show that the expression of AR variants (AR3, AR132B) are typically higher in higher Gleason grade of the tumor and Gleason Grade is further associated with higher expression of the EMT MicroRNA and EMT biomarker, Lin28B.

[00278] In conclusion, demonstration of synergistic increases in inhibition of prostate cancer cell growth with the combination of a DIM-related indole and a Diarylhydantoin-related compound in the 22Rv1 cell line presented in Example 7 occurs despite the proven presence of AR receptor splice variants and increased EMT activity. Evidence presented in Fig. 4 shows that DIM reduces levels of AR variants (specifically AR3 (AR-V7)) and markers of EMT, known to be associated with clinical resistance to AR antagonist drugs, including resistance to Diarylhydantoin-related compounds including Enzalutamide in men with CRPC (see Antonarakis et al., 2014, "AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer," N Engl J Med. 371:1028-1038, DOI: 10.1056/NEJMoa1315815).

[00279] The data presented in Fig. 5 show that the expression of AR splice variants is higher in higher Gleason grade tumors (as compared to histologically normal adjacent prostate tissue). Importantly, a strong correlation was demonstrated between increased levels of Lin28B and severity of the associated PCa. The data presented in Fig 6. show that higher Gleason Grade of PCa tumor tissue is associated with supression and lower levels of specific

tumor supressive MicroRNA's (miR's) in addtion to changes in Lin28B. MicroRNA's are regulatory, non-coding small RNA molecules which function by controlling sensitivity of PCa to AR related growth signals. Lower levels of miR-124 are associated with PCa cell proliferation and miR-124 specifically targets the AR to inhibit its over activity (see Shi et al., 2013, "Tumor suppressive miR-124 targets androgen receptor and inhibits proliferation of prostate cancer cells," Oncogene 32(35):4130-8, doi: 10.1038/onc.2012.425). Similarly, miR-27b is lower and specifically contributes to CRPC (Sun et al., 2010, "Castration-resistant prostate cancer–related microRNAs," 2010 Genitourinary Cancers Symposium, Abstract 150, published on Meeting Library, ASCO University webpage).

[00280] Demonstration of synergistic increases in inhibition of prostate cancer cell growth with the combination of a DIM-related indole and a Diarylhydantoin-related compound in relevant cell lines indicates that this combined use offers treatment advantage in men with prostate cancer, and specifically, in men with CRPC.

7.9. Example 9: Clinical Data Derived from Before and After BR-DIM treatment of men diagnosed with Prostate Cancer. Prostate Tissue Data Obtained from Before and After BR-DIM Treatment of Men with Prostate Cancer Show Changes Consistent with Increased Responsiveness to AR Antagonists.

[00281] From the clinical trial study (from prostate biopsy tissue obtained under clinical trial study), it was found that AR variants (as presented in Fig. 5, A and B) and stem cell markers (of EMT), including Lin28B (as presented in Fig. 5C), were increased in prostate cancer patient tumor tissues compared with normal tissues (the results presented in Figure 5 are described in Example 8). In particular, AR variants and stem cell markers of EMT were increased in the baseline biopsy in prostate cancer patient tumor tissues compared with adjacent normal prostate tissues. Importantly, the AR variant and Lin28B levels increased most in the highest Gleason Grade tumors (G6-G9). Moreover, as demonstrated in Fig. 7, Lin28B expression positively correlated with increased expression of AR variants such as AR3 and AR132b in prostate cancer tissues with higher Gleason grade tumors (with a highly statistically significant correlation). Notably, miRNAs including miR-27b and miR-124, which inhibit the expression of AR and AR variants and miRNAs such as the miR-320 family, which down-regulates expression of Lin28B, were down-regulated in prostate cancer patient tissues (see Fig. 6). The data presented in Fig 7., clearly show that AR variant AR3 and AR132b are present in increased amounts in PCa tissue in direct association with increases in Lin28B, the

MircoRNA cellular marker for EMT in PCa. This tissue analysis provides a basis for establishing the relevance and impact of the observed activity of DIM administered to men with prostate cancer and followed by before and after DIM treatment prostate tissue examination.

[00282] BR-DIM treatment increased the expression of miR-27b, miR-124 and miR-320 (Fig. 8) and repressed Lin28B expression (Fig. 9). In particular, BR-DIM treatment of PCa patients increased the expression of miR-27b, miR-124 and miR-320 (Fig. 8) and repressed Lin28B expression (Fig. 9) in tumor tissue when pre-treatment (Patient) and post treatment (Patient+BR-DIM) were compared. These data indicate that a DIM-related indole, and in particular, DIM, will be successful, in combination with a Diarylhydantoin-related compound, in particular enzalutamide, in the treatment of men with prostate cancer (such as naive men with prostate cancer) for the problem of CRPC and drug (e.g., enzalutamide) resistance. Further, these data indicate that a DIM-related indole, and in particular, DIM, will be successful to modify the control over the AR to inhibit AR variant production and to inhibit EMT which supports AR variant production. Since increased AR variant levels are responsible for resistance to Enzalutamide and ARN-509, the data further support the observed synergistic interaction showing that a DIM-related indole such as DIM in combination with a Diarylhydantoin-related compound, in particular enzalutamide or ARN-509, provide an improved method in the treatment of men with prostate cancer (such as treatment naive men with prostate cancer), for the problem of CRPC, and for drug (e.g., enzalutamide, ARN-509) treatment resistance in prostate cancer.

[00283] Methods used in the study presented in Figs. 6-9:

[00284] Patients and tumor tissue collection: After obtaining institutional review board approval, pre-treatment PCa tissues and matched adjacent normal tissues were obtained from retrospective Biospecimen Core of Karmanos Cancer Institute (KCI) from patients who underwent radical prostatectomy from 2004-2010 at KCI. Also obtained were PCa tissue specimens from on-going clinical trial of BR-DIM intervention prior to radical prostatectomy of newly diagnosed PCa patients at KCI and Henry Ford Health System (HFHS), Detroit, Michigan. Formalin-fixed, paraffin-embedded (FFPE) tissues were cut for miRNA and mRNA analysis. Patients' clinical characteristics were obtained from the hospital database. Pathological features were ascertained from microscopic evaluation of tumor slides by

pathologists both at KCI and at HFHS. Gleason score was obtained in each case from the database.

[00285] Real-time RT-PCR: To determine the mRNA or miRNA levels in prostate cancer patient tissues, the total RNA was isolated from FFPE tissues using miRNeasy FFPE Kit (Qiagen) according to the manufacturer's instruction. The DNA was removed using an RNase-free NDase kit (Qiagen). For determination of the mRNA levels, 1 μg of RNA was reverse transcribed into coda using a High Capacity RNA-to-coda Kit (Applied bios stems, Foster, CA) according to the manufacturer's instruction. Real time PCR was used to quantify mRNA expression by using SIR® Green RT-PCR Reagents (Applied bios stems). The relative amount of mRNA was normalized to the expression of beta-actin. For testing the miRNA levels, 20 ng of RNA were reverse transcribed into cDNA using a Universal cDNA Synthesis Kit (Exiqon, Woburn, MA) according to the manufacturer's instruction. Real time PCR was performed using specific miRNA primers (Exiqon) to quantify miRNA expression by using SYBR® Green RT-PCR.

7.10. Example 10: The Effect of the Combination of a DIM-related Indole and a Selected Anti-Androgen Compound on the Growth of Prostate Cancer Cells

[00286] To determine the effect of combinations of various DIM-related compounds with various Selected Anti-Androgen Compounds on the cell proliferation, apoptosis and PSA levels of prostate cancer cells, the effect of treatment using the following compounds and combinations of compounds on LNCaP, C4-2B, and 22RV1 cells is evaluated in MTT, apoptosis and/or PSA assays: DIM alone, I3C alone, BR-DIM alone, Enzalutamide alone, ARN-509 alone, AZD-3514 alone, DIM plus Enzalutamide, I3C plus Enzalutamide, BR-DIM plus Enzalutamide, DIM plus ARN-509, I3C plus ARN-509, BR-DIM plus ARN-509, DIM plus AZD-3514, I3C plus AZD-3514, and BR-DIM plus AZD-3514.

[00287] Apoptosis assay: Apoptosis will be assayed by using Dead Cell Apoptosis Kit with Annexin V Alexa Fluor® 488 & Propidium Iodide (PI) - for Flow Cytometry (life technology) according to manufacture' instruction. Briefly, LNCaP and 22RV1 cells treated with the drug mentioned above and then harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS). Re-centrifuge the washed cells, discard the supernatant and resuspend the cells in 1X annexin-binding buffer. Determine the cell density and dilute in 1X annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to

have 100 μL per assay. Add 5 μL Alexa Fluor® 488 annexin V and 1 μL 100 μg/mL PI working solution to each 100 μL of cell suspension. Incubate the cells at room temperature for 15 minutes. After the incubation period, add 400 μL 1X annexin-binding buffer, mix gently, and keep the samples on ice. Analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm and 575 nm (or equivalent) using 488 nm excitation.

[00288] PSA assay: PSA levels will be determined according to the method described earlier by using Human PSA ELISA kit (ANOGEN). Briefly, LNCaP and 22RV1 cells were grown in six-well plates in complete RPMI 1640. When cells were 60% confluent, the cells were washed with serum-free medium and maintained in complete RPMI 1640 with or without treatment for 24 and 48 hours. The conditioned medium was then collected and the protein concentration in the conditioned medium was quantified. The conditioned medium with equal amounts of protein for each sample was subjected to PSA detection using Human PSA ELISA Kit (Anogen, Mississauga, Ontario, Canada) according to the manufacturer's protocol.

7.11. Example 11: Treating Prostate Cancer Tumors in an In Vivo Model of Prostate Cancer Using a DIM-related Indole and a Selected Anti-Androgen Compound

[00289] To determine the clinical effect of the combination of a DIM-related indole with a Selected Anti-Androgen Compound, an in vivo animal model of prostate cancer is used, and the clinical effect in groups of animals treated with DIM (e.g., BR-DIM) alone, Enzalutamide alone, and DIM (e.g., BR-DIM) plus Enzalutamide is determined. In other experiments, the clinical effect in groups of animals treated with DIM (e.g., BR-DIM) alone, ARN-509 alone, and DIM (e.g., BR-DIM) plus ARN-509 can be determined. In yet other experiments, the clinical effect in groups of animals treated with DIM (e.g., BR-DIM) alone, AZD-3514 alone, and DIM (e.g., BR-DIM) plus AZD-3514 can be determined. In some of the above-described experiments, DIM can be substituted by I3C.

[00290] To compare the tumorigenicity and to test the effects of the above-listed drugs and combinations of drugs on aggressiveness of tumor cells, mice will be castrated and injected orthotopically with C4-2B and 22RV1 cells (1X106 cells) suspended in serum-free RPMI medium, following our published procedure (Raffoul JJ, Wang Y, Kucuk O, Forman JD, Sarkar FH, Hillman GG. Genistein inhibits radiation-induced activation of NF-kappaB in prostate cancer cells promoting apoptosis and G2/M cell cycle arrest. BMC Cancer

2006;6:107. and Raffoul JJ, Banerjee S, Che M et al. Soy isoflavones enhance radiotherapy in a metastatic prostate cancer model. Int J Cancer 2007;120:2491-8). The experimental group will consist of 8 groups of 8 mice in each group (total 64 animals). Thirty days after tumor cell implantation, tumor will be formed and at that time point, the treatment will be started. The mice will be treated, e.g., with 5 mg of BR-DIM, enzalutamide and combination of BR-DIM with enzalutamide administered daily by gavage or maintained as untreated control. All mice will be euthanized, and the tumors will be neatly excised free of any extraneous adhering tissue. Total protein and RNA will be extracted from tumor tissues and prepared for Western blot analysis or real-time RT-PCR following our published procedures. Immunohistochemical (IHC) staining will also be performed using formalin-fixed tumor tissue sections and evaluate tumor cell morphology, rate of mitosis, growth pattern, necrosis, cystic change, and inflammatory cellular response. Routine IHC studies will be done to assess the expression of E-cadherin, vimentin, Lin28B, Nanog, TWIST1, CD44 and EZH2 following our published procedures.

7.12. Example 12: Treating Prostate Cancer in Humans Using a DIM-related indole and a Selected Anti-Androgen Compound

[00291] Testing combined use of BR-DIM with Enzalutamide (or another combination of a DIM-related indole and a Selected Anti-Androgen Compound) in humans: An Open Label Study Determining Safety and Tolerability of Enzalutamide (formerly MDV3100) in Combination With absorption-enhanced, microencapsulated DIM (BioResponse-DIM, BR-DIM) in Prostate Cancer Patients is conducted with community Urologists. Subjects diagnosed with cancer of the prostate and already prescribed and tolerating Enzalutamide but with evidence of rising PSA and/or advancing disease will be offered additional BioResponse DIM as a prescribed supplement. PSA levels, Pain Scores, and Imaging studies will be done with appropriate methods.

[00292] The expectation of this study is that the combination of Enzalutamide (MDV3100) with BR-DIM in metastatic castrate resistant prostate cancer (mCRPC) will overcome resistance to prior hormonal therapy with abiraterone acetate (AA) (secondary resistance) or primary resistance to Enzalutamide. The intent is to demonstrate rescue of patients from the development of primary or secondary resistance to AR targeted therapies using Enzalutamide (MDV3100) by adding combined DIM from BR-DIM.

[00293] Methods: The primary endpoint will be to determine effects of combined use on rising PSA. Secondary endpoint of this open label study will be to assess the safety, tolerability, and highest tolerated dose of BR-DIM in combination with the standard dose of Enzalutamide. After informed consent, patients under the care of community urologists with progressive mCRPC will be invited to receive marketed BR-DIM at no cost and treated with 120-160 mg per day of Ezalutamide in combination with DIM from BR-DIM at 200-600 mg/day. Patients with mCRPC will be sought who have experienced PSA failure with AA showing intrinsic resistance (<3 months treatment) or acquired resistance (>6 months treatment). BR-DIM dose will be advanced if BR-DIM is well tolerated and PSA is rising. Response to combined treatment with Ezalutamide and BR-DIM will be assessed by monthly PSA testing, PET imaging, AR allelic status, circulating tumor cells, and tumor biopsies as medically indicated.

7.13. Example 13: The Synergistic Activity Observed Using a DIM-related Indole in Combination with a Diarylhydantoin-related Compound, such as Enzalutamide or ARN-509, Cannot be Achieved when the DIM-related Indole is replaced with a Non-DIM-related AR antagonist

[00294] To determine whether the observed synergistic growth inhibition observed in PCa cell culture and MTT assay was unique to the combination of a DIM-related indole and selected AR antagonists of the Diarylhydantoin class, further experiments were undertaken with bicalutamide (Casodex), a less selective AR antagonist with known agonist activity. These experiments included testing for growth inhibitory activity of the combination of Enzalutamide (MDV3100) with Casodex, and ARN-509 with Casodex. The results from these studies are presented and in Figures 15-17. In addition, the following charts summarize the Combination Index (CI) results for these experiments.

[00295] Summary of Additional PCa Growth Inhibition Assays Indicating Lack of Synergy of Enzalutamide (MDV3100) combined with Bicalutamide (Casodex) in Cultured PCa Cell Lines:

PCa Cell Line	CI at ED50	CI at ED75	CI at ED90	Figure Reference
LNCaP	1.122	1.201	1.128	Figure 15
C4-2B	1.063	1.181	1.318	Figure 16

[00296] Summary of an Additional PCa Growth Inhibition Assay Indicating Lack of Synergy of ARN-509 combined with Bicalutamide (Casodex) in Cultured PCa Cell Lines:

PCa Cell Line	CI at ED50	CI at ED75	CI at ED90	Figure Reference
C4-2B	1.094	1.073	1.399	Figure 17

[00297] The results show a notable lack of synergistic interaction between Bicalutamide (Casodex) and either Enzalutamide or ARN-509 as indicated by CI's greater than one. The lack of synergistic interaction with Bicalutamide (Casodex) indicates unexpected and distinct differences from DIM-related indoles despite the fact that both Casodex and DIM have been shown to possess similar behavior displacing Testosterone from the AR in an *in vitro* study ("Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells," J Biol Chem. 2003, 278(23):21136-45). In conclusion, the failure of Casodex to demonstrate measurable synergistic growth inhibition in combination with MDV3100 or ARN-509 despite some similarities to DIM indicates that the unexpected finding of synergistic growth inhibition with the combination of DIM and MDV3100 and ARN-509 represents a unique and unpredictable discovery.

[00298] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.

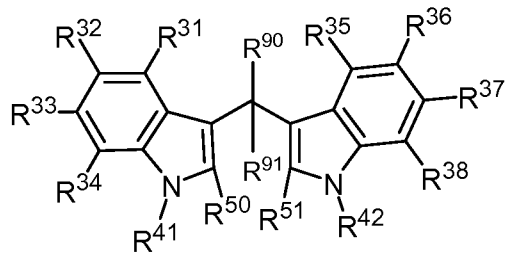
[00299] Various references such as patents, patent applications, and publications are cited herein, the disclosures of which are hereby incorporated by reference herein in their entireties.

WHAT IS CLAIMED IS:

1. A method of treating an androgen or Androgen Receptor-driven disorder in a subject in need thereof, comprising administering to the subject (i) an amount of a DIM-related indole, and (ii) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein the DIM-related indole is selected from the group consisting of:

a compound of formula I:



(I)

wherein R^{32} and R^{36} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and ethoxycarbonyl groups,

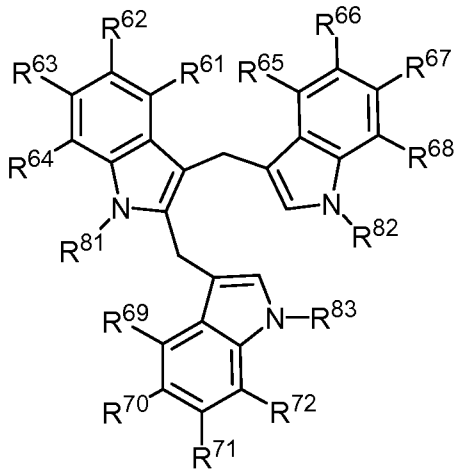
R^{33} and R^{37} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy,

R^{31}, R^{34}, R^{35}, R^{38}, R^{41}, and R^{42} are hydrogen,

R^{50}, R^{51} are either hydrogen or methyl, or phenyl, and

R^{90}, R^{91} are hydrogen;

a compound of formula II:

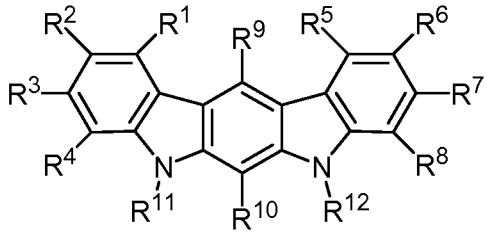


(II)

wherein R^{62}, R^{63}, R^{66}, R^{67}, R^{70}, and R^{71} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and

R^{61}, R^{64}, R^{65}, R^{68}, R^{69}, R^{72}, R^{81}, R^{82}, and R^{83} are hydrogen;

a compound of formula (III):



(III)

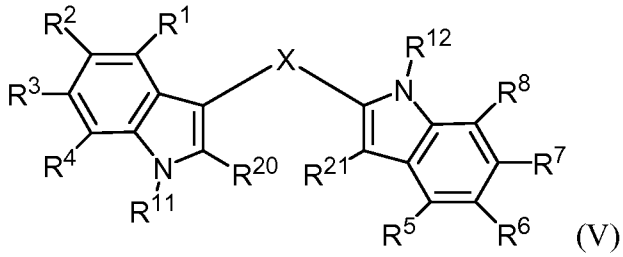
wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are substituents independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_5-C_{20} aryl, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxycarbonyl, halocarbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C_1-C_{24} alkyl)-substituted carbamoyl, di-(C_1-C_{24} alkyl)-substituted carbamoyl, mono-substituted arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C_1-C_{24} alkyl)-substituted amino, methoxy, mono- and di-(C_5-C_{20} aryl)-substituted amino, C_2-C_{24} alkylamido, C_6-C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, phenyl, sulfo, sulfonato, C_1-C_{24} alkylsulfanyl, arylsulfanyl, C_1-C_{24} alkylsulfinyl, C_5-C_{20} arylsulfinyl, C_1-C_{24} alkylsulfonyl, C_5-C_{20} arylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, and combinations thereof, and

further wherein any two adjacent (ortho) substituents may be linked to form a cyclic structure selected from five-membered rings, six-membered rings, and fused five-membered and/or six-membered rings, wherein the cyclic structure is aromatic, alicyclic, heteroaromatic, or heteroalicyclic, and has zero to 4 non-hydrogen substituents and zero to 3 heteroatoms, and

R^{11} and R^{12} are independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkoxycarbonyl, amino-substituted C_1-C_{24} alkyl, (C_1-C_{24} alkylamino)-substituted C_1-C_{24} alkyl, and di-(C_1-C_{24} alkyl)amino-substituted C_1-C_{24} alkyl,

with the provisos that at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} is other than hydrogen, and when R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are selected from hydrogen, halo, alkyl and alkoxy, then R^{11} and R^{12} are other than hydrogen and alkyl; and

a compound of formula (V):



(V)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^{11}, R^{12}, and X are defined as for compounds of formula (III), and

R^{20} and R^{21} are defined as for R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 as for compounds of formula III.

3. The method of claim 1, wherein the DIM-related indole is selected from the group consisting of 3,3' diindolylmethane (DIM), hydoxylated DIMs, methoxylated DIMs, 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane (LTR), hydroxylated LTRs, methoxylated LTRs, 5,5'-dimethylDIM (5-Me-DIM), 2,2'-dimethylDIM (2-Me-DIM), 5,5'-dichloroDIM (5-Cl-DIM), imidazolelyl-3,3'-diindolylmethane, nitro-substituted imidazolelyl-3,3'-diindolylmethanes, 2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo-[2,3-b]carbazole, 6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole, 2,10-dicarbethoxy-6-

ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole, 2,6-dicarbethoxy-3,3'-dimethyl-13,14-diindolylmethane, and indole-3-carbinol (I3C).

4. The method of claim 3, wherein the DIM-related indole is DIM.

5. The method of claim 3, wherein the DIM-related indole is I3C.

6. The method of claim 4, wherein the DIM is processed DIM.

7. The method of any one of claims 1-6, wherein the DIM-related indole is suspended as microparticles in a starch carrier matrix.

8. The method of any one of claims 1-6, wherein the DIM-related indole is microencapsulated with phosphatidylcholine (PC), complexed with PC, or made into rapidly dissolving microparticles and nanoparticles.

9. The method of any one of claims 1-8, wherein the DIM-related indole is administered orally.

10. The method of any one of claims 1-8, wherein the DIM-related indole is administered parenterally or intra-arterially.

11. The method of any one of claims 1-8, wherein the DIM-related indole is injected directly into prostate gland tissue.

12. The method of claim 11, wherein the DIM-related indole is injected directly into prostate gland tissue using ultrasound guidance.

13. The method of any one of claims 1-8, wherein the DIM-related indole is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter.

14. The method of any one of claims 1-13, wherein the amount of the DIM-related indole administered to the subject is 1 to 20 mg per kg of the subject's weight per day.

15. The method of claim 14, wherein the amount of the DIM related indole administered to the subject is 3 to 10 mg or 1 to 5 mg per kg of the subject's weight per day.

16. The method of claim 6, wherein the amount of the processed DIM administered to the subject is 150-900 mg per day.

17. The method of claim 6, wherein the amount of the processed DIM administered to the subject is less than 625 mg per day, less than 450 mg per day, equal to or less than 375 mg per day, equal to or less than 300 mg per day, or equal to or less than 225 mg per day.

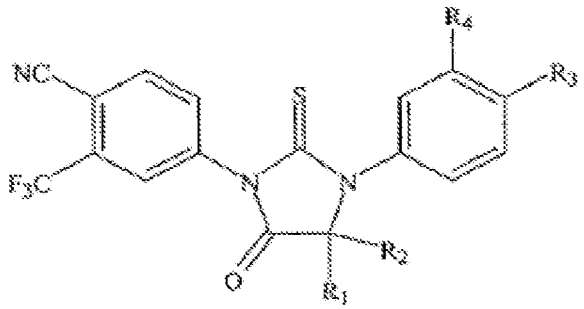
18. The method of claim 10, wherein the amount of the DIM-related indole is 50 to 5,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

19. The method of claim 10, wherein the amount of the DIM-related indole is 100 to 3,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

20. The method of claim 10, wherein the amount of the DIM-related indole is equal to or less than 1,000 mg per dose, equal to or less than 500 mg per dose, or equal to or less than 250 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

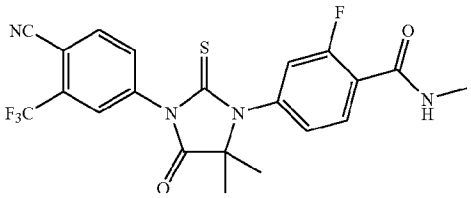
21. The method of any one of claims 1-20, wherein the Anti-Androgen Compound is a diarylthiohydantoin compound.

22. The method of claim 21, wherein the diarylthiohydantoin compound has the formula:

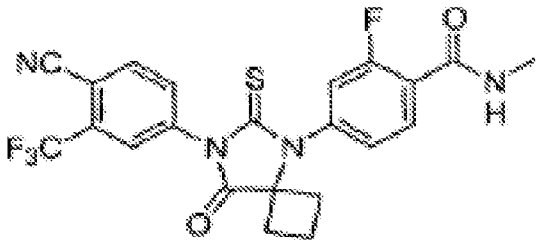


wherein Ri and R2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,
wherein R3 is selected from the group consisting of carbamoyl, alkyl carbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and
wherein R4 is hydrogen or fluorine.

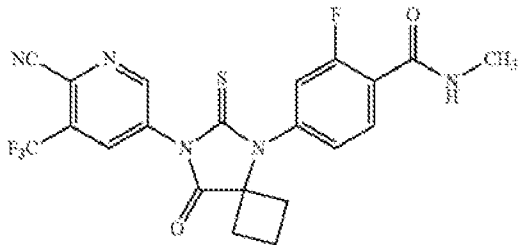
23. The method of claim 22, wherein the diarylthiohydantoin compound is 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide ("Enzalutamide") having the following formula:



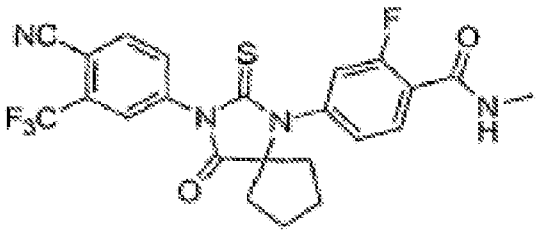
24. The method of claim 22, wherein the Diarylhydantoin compound has the formula:



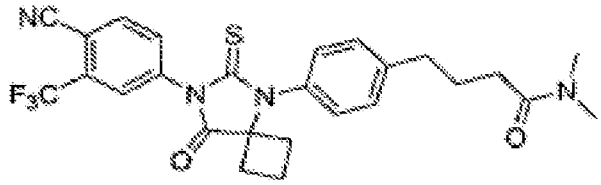
25. The method of claim 22, wherein the diarylthiohydantoin compound is 4-[7-(6-cyano-5-trifluoromethylpyridin-3-yl)-8-oxo-6-thioxo-5,7-diazaspir- o[3,4]oct-5-yl]-2-fluoro-N-methylbenzamide ("ARN 509") having the following formula:



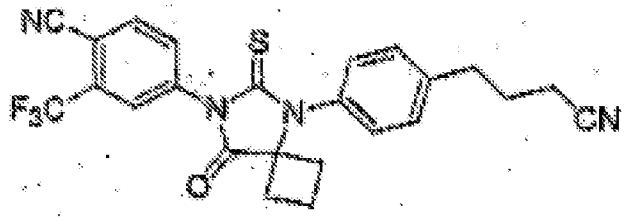
26. The method of claim 22, wherein the diarylthiohydantoin compound has the formula:



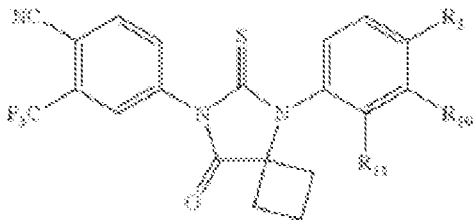
27. The method of claim 22, wherein the diarylthiohydantoin compound has the formula:



28. The method of claim 22, wherein the diarylthiohydantoin compound has the formula:



29. The method of claim 21, wherein the diarylthiohydantoin compound has the formula:



wherein R3 is selected from the group consisting of hydroxy, methylcarbamoyl, methylcarbamoylpropyl, methylcarbamoylethyl, methylcarbamoylmethyl, methylsulfonecarbamoylpropyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, carbamoylmethyl, carbamoylethyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoylpropyl, carboxypropyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, hydroxyethylcarbamoylethyl, and hydroxyethoxycarbonylethyl, and

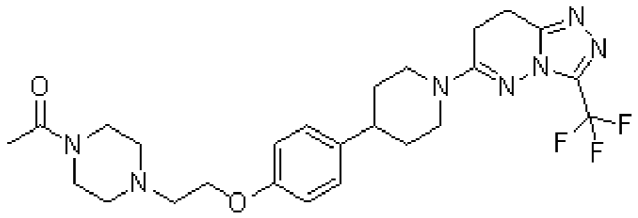
wherein R10 and R11 are both H or, respectively, F and H, or H and F.

30. The method of claim 29, wherein R10 and R11 are, respectively, F and H.

31. The method of claim 29 or 30, wherein R3 is methylcarbamoyl.

32. The method of claim 29, wherein R3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, and alkylcarbamoylalkyl.

33. The method of any one of claims 1-20, wherein the Anti-Androgen Compound is the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, and wherein the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound is 1-(4-(2-(4-(1-(3-(trifluoromethyl)-7,8-dihydro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)phenoxy)ethyl)piperazin-1-yl)ethanone ("AZD-3514") having the following formula:



34. The method of any one of claims 1-33, wherein the Anti-Androgen Compound is administered orally.

35. The method of any one of claims 1-33, wherein the Anti-Androgen Compound is administered parenterally or intra-arterially.

36. The method of any one of claims 1-33, wherein the Anti-Androgen Compound is injected directly into prostate gland tissue.

37. The method of claim 36, wherein the Anti-Androgen Compound is injected directly into prostate gland tissue using ultrasound guidance.

38. The method of any one of claims 1-33, wherein the Anti-Androgen Compound is administered by prostate gland arterial embolization procedure using a catheter or a microcatheter.

39. The method of any one of claims 1-38, wherein the amount of the Anti-Androgen Compound administered to the subject is 1 to 20 mg per kg, 2 to 6 mg per kg, or 1 to 2 mg per kg of the subject's weight per day.

40. The method of claim 23, wherein the amount of the Enzalutamide administered to the subject is 40 to 240 mg per day, 40 to 160 mg per day, or 80 to 120 mg per day.

41. The method of claim 23, wherein the amount of the Enzalutamide administered to the subject is less than 240 mg per day, less than 160 mg per day, equal to or less than 120 mg per day, equal to or less than 100 mg per day, equal to or less than 80 mg per day, or equal to or less than 50 mg per day.

42. The method of claim 35, wherein the amount of the Anti-Androgen Compound administered to the subject is 50 to 5,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

43. The method of claim 42, wherein the amount the Anti-Androgen Compound administered to the subject is 100 to 2,000 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

44. The method of claim 35, wherein the amount of the Anti-Androgen Compound administered to the subject is less than 1000 mg per dose, less than 500 mg per dose, less than 250 mg per dose, less than 160 mg per dose, less than 120 mg per dose, or less than 100 mg per dose, wherein the dose is administered every 1, 2, 3, 4, 5, 6, 7, 8 or more weeks.

45. The method of any one of claims 1-44, wherein the androgen or Androgen Receptor-driven disorder is a disorder characterized by an increased expression of Androgen Receptor (AR) and/or an increased expression of AR splice variants.

46. The method of any one of claims 1-44, wherein the androgen or Androgen Receptor-driven disorder is prostate cancer, prostatic intraepithelial neoplasia, or benign prostatic hyperplasia.

47. The method of claim 46, wherein the androgen or Androgen Receptor-driven disorder is prostate cancer.

48. The method of claim 47, wherein the prostate cancer is castrate resistant prostate cancer (CRPC).

49. The method of claim 48, wherein the prostate cancer is metastatic CRPC (mCRPC).

50. The method of claim 48, wherein the prostate cancer is non-metastatic CRPC.

51. The method of claim 47, wherein the prostate cancer is hormone sensitive prostate cancer.

52. The method of any one of claims 1-51, which is effective for treating the disorder.

53. The method of any one of claims 1-52, which is more effective for treating the disorder than treatment with the Anti-Androgen Compound alone and/or treatment with the DIM-related compound alone.

54. The method of claim 52 or 53, wherein the effectiveness for treating the disease is demonstrated by an improvement in one or more symptoms or parameters of the disorder.

55. The method of claim 52 or 53, which is more effective than treatment with the Anti-Androgen Compound alone and/or treatment with the DIM-related compound alone to: reduce tumor volume; slow progression of tumor growth; delay or prevent development of metastasis; normalize repeat tissue biopsy; improve quality of life or reduce side effects associated with the treatment using the Anti-Androgen Compound; delay or prevent the development of resistance to the Anti-Androgen Compound; overcome the development of resistance to the Anti-Androgen Compound; decrease the amount of prostate specific antigen (PSA); increase the period of stable disease; increase progression-free survival, or increase overall survival.

56. The method of any one of claims 1-55, wherein the subject is resistant to an Anti-Androgen Compound.

57. The method of any one of claims 1-56, wherein the subject has been treated with an Anti-Androgen Compound.

58. The method of claim 56 or 57, wherein the subject is resistant to a Diarylthiohydantoin compound.

59. The method of any one of claim 1-58, wherein the DIM-related indole is administered before administering the Anti-Androgen Compound..

60. The method of any one of claims 1-58, wherein the DIM-related indole is administered after administering the Anti-Androgen Compound.

61. The method of any one of claim 1-58, wherein the DIM-related indole is administered simultaneously with the Anti-Androgen Compound..

62. The method of any one of claims 1-58, wherein the DIM-related indole and the Anti-Androgen Compound are co-formulated into a single pharmaceutical composition or delivery vehicle.

63. The method of claim 62, wherein the DIM-related indole and the Anti-Androgen Compound are co-formulated into one pill, tablet or capsule.

64. The method of any one of claims 1-63, wherein the DIM-related indole and/or the Anti-Androgen Compound are administered once daily.

65. The method of any one of claims 1-63, wherein the DIM-related indole and/or the Anti-Androgen Compound are administered twice daily.

66. The method of any one of claims 1-65, wherein the subject is a human.

67. A pharmaceutical fixed dosage composition comprising a combination of: (i) an amount of a DIM-related indole, and (ii) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

68. A pharmaceutical composition comprising a therapeutically effective amount of a combination of: (i) a DIM-related indole, and (ii) an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

69. A kit comprising in one or more containers: (a) an amount of a DIM-related indole, and (b) an amount of an Anti-Androgen Compound, wherein the Anti-Androgen Compound is a Diarylhydantoin compound, a Diarylthiohydantoin compound or a 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, or a pharmaceutically acceptable salt thereof.

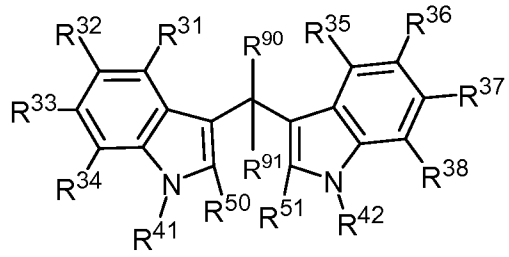
70. The kit of claim 69, wherein the DIM-related indole and the Anti-Androgen Compound are in separate containers.

71. The kit of claim 69, wherein the DIM related indole and the Anti-Androgen compound are in the same container.

72. The kit of claim 71, wherein the DIM-related indole and the Anti-Androgen compound are in a fixed dose combination.

73. The composition or the kit of any one of claims 67-72, wherein the DIM-related indole is selected from the group consisting of:

a compound of formula I:



(I)

wherein R^{32} and R^{36} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and ethoxycarbonyl groups,

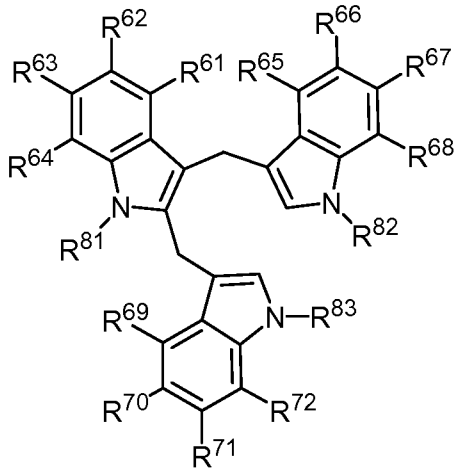
R^{33} and R^{37} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy,

R^{31}, R^{34}, R^{35}, R^{38}, R^{41}, and R^{42} are hydrogen,

R^{50}, R^{51} are either hydrogen or methyl, or phenyl, and

R^{90}, R^{91} are hydrogen;

a compound of formula II:

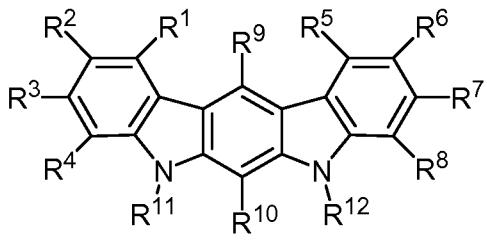


(II)

wherein R^{62}, R^{63}, R^{66}, R^{67}, R^{70}, and R^{71} are substituents independently selected from the group consisting of hydrogen, hydroxyl, and methoxy, and

R^{61}, R^{64}, R^{65}, R^{68}, R^{69}, R^{72}, R^{81}, R^{82}, and R^{83} are hydrogen;

a compound of formula (III):



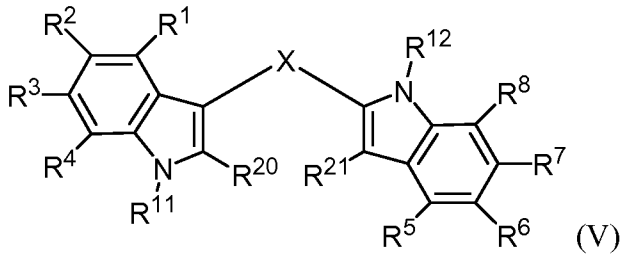
(III)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, and R^{10} are substituents independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkenyl, C_2-C_{24} alkynyl, C_5-C_{20} aryl, C_6-C_{24} alkaryl, C_6-C_{24} aralkyl, halo, hydroxyl, sulfhydryl, C_1-C_{24} alkoxy, C_2-C_{24} alkenyloxy, C_2-C_{24} alkynyloxy, C_5-C_{20} aryloxy, acyl, acyloxy, C_2-C_{24} alkoxycarbonyl, C_6-C_{20} aryloxycarbonyl, halocarbonyl, C_2-C_{24} alkylcarbonato, C_6-C_{20} arylcarbonato, carboxy, carboxylato, carbamoyl, mono-(C_1-C_{24} alkyl)-substituted carbamoyl, di-(C_1-C_{24} alkyl)-substituted carbamoyl, mono-substituted arylcarbamoyl, thiocarbamoyl, carbamido, cyano, isocyano, cyanato, isocyanato, isothiocyanato, azido, formyl, thioformyl, amino, mono- and di-(C_1-C_{24} alkyl)-substituted amino, methoxy, mono- and di-(C_5-C_{20} aryl)-substituted amino, C_2-C_{24} alkylamido, C_6-C_{20} arylamido, imino, alkylimino, arylimino, nitro, nitroso, phenyl, sulfo, sulfonato, C_1-C_{24} alkylsulfanyl, arylsulfanyl, C_1-C_{24} alkylsulfinyl, C_5-C_{20} arylsulfinyl, C_1-C_{24} alkylsulfonyl, C_5-C_{20} arylsulfonyl, phosphono, phosphonato, phosphinato, phospho, phosphino, and combinations thereof, and further wherein any two adjacent (ortho) substituents may be linked to form a cyclic structure selected from five-membered rings, six-membered rings, and fused five-membered and/or six-membered rings, wherein the cyclic structure is aromatic, alicyclic, heteroaromatic, or heteroalicyclic, and has zero to 4 non-hydrogen substituents and zero to 3 heteroatoms, and

R^{11} and R^{12} are independently selected from the group consisting of hydrogen, C_1-C_{24} alkyl, C_2-C_{24} alkoxycarbonyl, amino-substituted C_1-C_{24} alkyl, (C_1-C_{24} alkylamino)-substituted C_1-C_{24} alkyl, and di-(C_1-C_{24} alkyl)amino-substituted C_1-C_{24} alkyl,

with the provisos that at least one of R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11} and R^{12} is other than hydrogen, and when R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 are selected from hydrogen, halo, alkyl and alkoxy, then R^{11} and R^{12} are other than hydrogen and alkyl; and

a compound of formula (V):



(V)

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^{11}, R^{12}, and X are defined as for compounds of formula (III), and

R^{20} and R^{21} are defined as for R^1, R^2, R^3, R^4, R^5, R^6, R^7, and R^8 as for compounds of formula III.

74. The composition or the kit of any one of claims 67-72, wherein the DIM-related indole is selected from the group consisting of 3,3' diindolylmethane (DIM), hydoxylated DIMs, methoxylated DIMs, 2-(Indol-3-ylmethyl)-3,3'-diindolylmethane (LTR), hydroxylated LTRs, methoxylated LTRs, 5,5'-dimethylDIM (5-Me-DIM), 2,2'-dimethylDIM (2-Me-DIM), 5,5'-dichloroDIM (5-Cl-DIM), imidazolelyl-3,3'-diindolylmethane, nitro-substituted imidazolelyl-3,3'-diindolylmethanes, 2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo-[2,3-b]carbazole, 6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole, 2,10-dicarbethoxy-6-ethoxycarbonyloxy-5,7-dihydro-indolo-[2,3-b]carbazole, 2,6-dicarbethoxy-3,3'-dimethyl-13,14-diindolylmethane, and indole-3-carbinol (I3C).

75. The composition or the kit of claim 74, wherein the DIM-related indole is DIM.

76. The composition or the kit of claim 74, wherein the DIM-related indole is I3C.

77. The composition or the kit of claim 75, wherein the DIM is processed DIM.

78. The composition or the kit of any one of claims 67-77, wherein the DIM-related indole is suspended as microparticles in a starch carrier matrix.

79. The composition or the kit of any one of claims 67-77, wherein the DIM-related indole is microencapsulated with phosphatidylcholine (PC), complexed with PC, or made into rapidly dissolving microparticles and nanoparticles.

80. The composition or the kit of any one of claims 67-79, wherein the DIM-related indole is formulated for oral administration.

81. The composition or the kit of any one of claims 67-79, wherein the DIM-related indole is formulated for parenteral or intra-arterial administration.

82. The composition of claim 77, wherein the amount of the processed DIM is 75-225 mg.

83. The composition of claim 77, wherein the amount of the processed DIM is less than 375 mg, less than 300 mg, less than 225 mg, equal or less than 150 mg or equal or less than 75 mg.

84. The kit of claim 77, wherein the amount of the processed DIM is 75-225 mg per dose.

85. The kit of claim 77, wherein the amount of the processed DIM is less than 375 mg, less than 300 mg, less than 225 mg, equal or less than 150 mg or equal or less than 75 mg per dose.

86. The composition of claim 81, wherein the amount of the DIM-related indole is 50 to 5,000 mg or 100 to 3,000 mg.

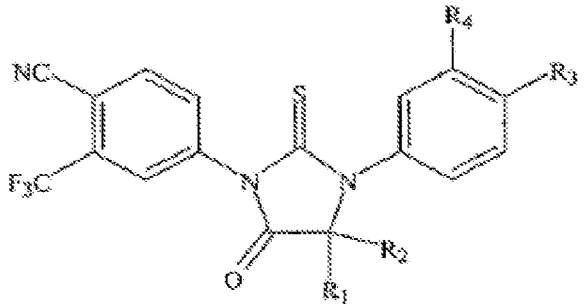
87. The composition of claim 81, wherein the amount of the DIM-related indole is equal to or less than 1,000 mg, equal to or less than 500 mg, or equal to or less than 250 mg.

88. The kit of claim 81, wherein the amount of the DIM-related indole is 50 to 5,000 mg per dose or 100 to 3,000 mg per dose.

89. The kit of claim 81, wherein the amount of the DIM-related indole is equal to or less than 1,000 mg per dose, equal to or less than 500 mg per dose, or equal to or less than 250 mg per dose.

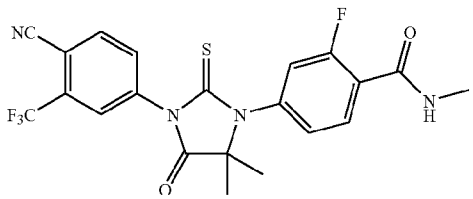
90. The composition or the kit of any one of claims 67-89, wherein the Anti-Androgen Compound is a diarylthiohydantoin compound.

91. The composition or the kit of claim 90, wherein the diarylthiohydantoin compound has the formula:

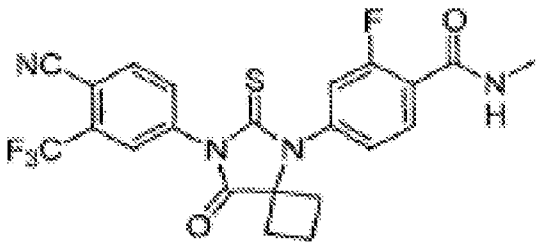


wherein R1 and R2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,
wherein R3 is selected from the group consisting of carbamoyl, alkyl carbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and
wherein R4 is hydrogen or fluorine.

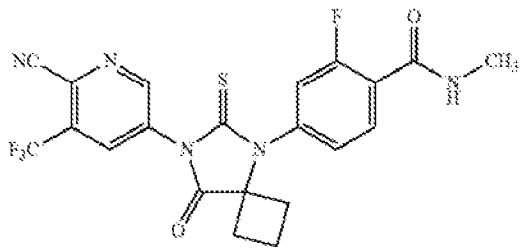
92. The composition or the kit of claim 91, wherein the diarylthiohydantoin compound is 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide ("Enzalutamide") having the following formula:



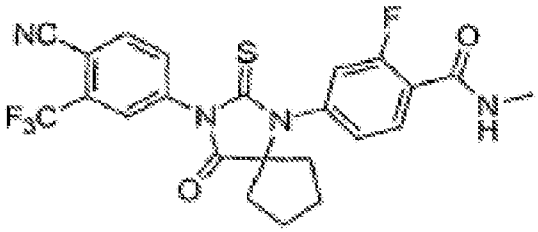
93. The composition or the kit of claim 91, wherein the Diarylhydantoin compound has the formula:



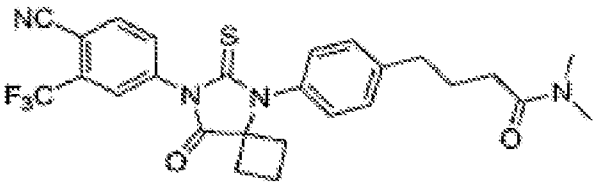
94. The composition or the kit of claim 91, wherein the diarylthiohydantoin compound is 4-[7-(6-cyano-5-trifluoromethylpyridin-3-yl)-8-oxo-6-thioxo-5,7-diazaspiro[3,4]oct-5-yl]-2-fluoro-N-methylbenzamide ("ARN 509") having the following formula:



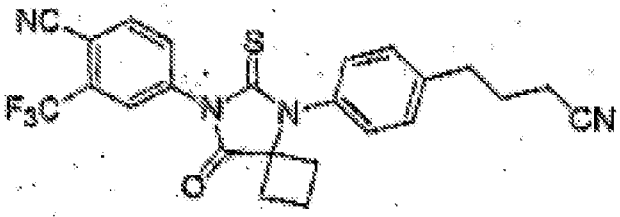
95. The composition or the kit of claim 91, wherein the diarylthiohydantoin compound has the formula:



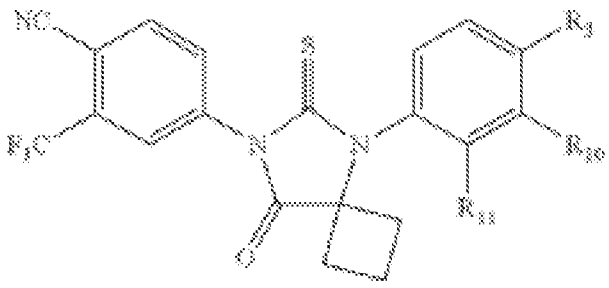
96. The composition or the kit of claim 91, wherein the diarylthiohydantoin compound has the formula:



97. The composition or the kit of claim 91, wherein the diarylthiohydantoin compound has the formula:



98. The composition or the kit of claim 80, wherein the diarylthiohydantoin compound has the formula:



wherein R3 is selected from the group consisting of hydroxy, methylcarbamoyl, methylcarbamoylpropyl, methylcarbamoylethyl, methylcarbamoylmethyl, methylsulfonecarbamoylpropyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, carbamoylmethyl, carbamoylethyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoylpropyl, carboxypropyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, hydroxyethylcarbamoylethyl, and hydroxyethoxycarbonylethyl, and

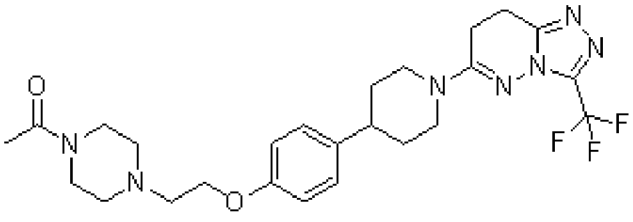
wherein R10 and R11 are both H or, respectively, F and H, or H and F.

99. The composition or the kit of claim 98, wherein R10 and R11 are, respectively, F and H.

100. The composition or the kit of claim 98 or 99, wherein R3 is methylcarbamoyl.

101. The composition or the kit of claim 98, wherein R3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, and alkylcarbamoylalkyl.

102. The composition or the kit of any one of claims 67-89, wherein the Anti-Androgen Compound is the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound, and wherein the 7,8-dihydro[1,2,4]triazolo[4,3-b]pyradizine compound is 1-(4-(2-(4-(1-(3-(trifluoromethyl)-7,8-dihydro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)phenoxy)ethyl)piperazin-1-yl)ethanone ("AZD-3514") having the following formula:



103. The composition or the kit of any one of claims 67-102, wherein the Anti-Androgen Compound is formulated for oral administration.

104. The composition or the kit of any one of claims 67-102, wherein the Anti-Androgen Compound is formulated for parenteral or intra-arterial administration.

105. The composition of claim 92, wherein the amount of the Enzalutamide is 40 to 160 mg.

106. The composition of claim 92, wherein the amount of the Enzalutamide is less than 160 mg, equal to or less than 100 mg, equal to or less than 120 mg, equal to or less than 80 mg, equal to or less than 50 mg, or equal to or less than 40 mg.

107. The kit of claim 92, wherein the amount of the Enzalutamide is 40 to 160 mg per dose.

108. The kit of claim 92, wherein the amount of the Enzalutamide is less than 160 mg per dose, equal to or less than 120 mg per dose, equal to or less than 100 mg per dose, equal to or less than 80 mg per dose, equal to or less than 50 mg per dose, or equal to or less than 40 mg per dose.

109. The composition of claim 104, wherein the amount of the Anti-Androgen Compound is 50 to 5,000 mg or 100 to 2,000 mg.

110. The composition of claim 104, wherein the amount of the Anti-Androgen Compound is less than 1000 mg, less than 500 mg, less than 250 mg, less than 160 mg, or less than 100 mg.

111. The kit of claim 104, wherein the amount of the Anti-Androgen Compound is 50 to 5,000 mg per dose or 100 to 2,000 mg per dose.

112. The kit of claim 104, wherein the amount of the Anti-Androgen Compound is less than 1000 mg per dose, less than 500 mg per dose, less than 250 mg per dose, less than 160 mg per dose, or less than 100 mg per dose.

113. The composition or kit of any one of claims 67-112, wherein the combination of the DIM-related indole and the Anti-Androgen Compound is effective for treating an androgen or Androgen Receptor-driven disorder in a subject.

114. The composition or kit of claim 113, which is more effective for treating the androgen or Androgen Receptor-driven disorder than treatment with the amount of the Anti-Androgen Compound alone and/or treatment with the amount of the DIM-related compound alone.

115. The composition or kit of claim 113 or 114, wherein the androgen or Androgen Receptor-driven disorder is a disorder characterized by an increased expression of Androgen Receptor (AR) and/or an increased expression of AR splice variants.

116. The composition or kit of any one of claims 113-115, wherein the androgen or Androgen Receptor-driven disorder is prostate cancer or benign prostatic hyperplasia.

117. The composition or kit of claim 116, wherein the androgen or Androgen Receptor-driven disorder is prostate cancer.

118. The composition or kit of claim 117, wherein the prostate cancer is castrate resistant prostate cancer (CRPC).

119. The composition or kit of claim 118, wherein the prostate cancer is metastatic CRPC (mCRPC).

120. The composition or kit of claim 118, wherein the prostate cancer is non-metastatic CRPC.

121. The composition or kit of claim 117, wherein the prostate cancer is hormone sensitive prostate cancer.

122. The composition or kit of any one of claims 113-119, wherein the effectiveness for treating the disease is demonstrated by an improvement in one or more symptoms or parameters of the disease.

123. The composition or kit of any one of claims 113-122, which is more effective than the amount of the Anti-Androgen Compound alone or the amount of the DIM-related compound alone to: reduce tumor volume; slow progression of tumor growth; delay or prevent development of metastasis; improve quality of life or reduce side effects associated with the treatment using the Anti-Androgen Compound; delay or prevent the development of resistance to the Anti-Androgen Compound; overcome the development of resistance to the Anti-Androgen Compound; decrease the amount of prostate specific antigen (PSA); increase progression-free survival, or increase overall survival.

124. The composition or kit of any one of claims 113-123, wherein the subject is resistant to an Anti-Androgen Compound.

125. The composition or kit of any one of claims 113-124, wherein the subject has been treated with an Anti-Androgen Compound.

126. The composition or kit of any one of claims 113-125, wherein the subject is resistant to a Diarylthiohydantoin compound.

127. The composition or kit of any one of claims 113-126, wherein the subject is human.

128. The composition or kit of any one of claims 67-127, wherein the DIM-related indole and the Anti-Androgen Compound are co-formulated into one pill, tablet or capsule.

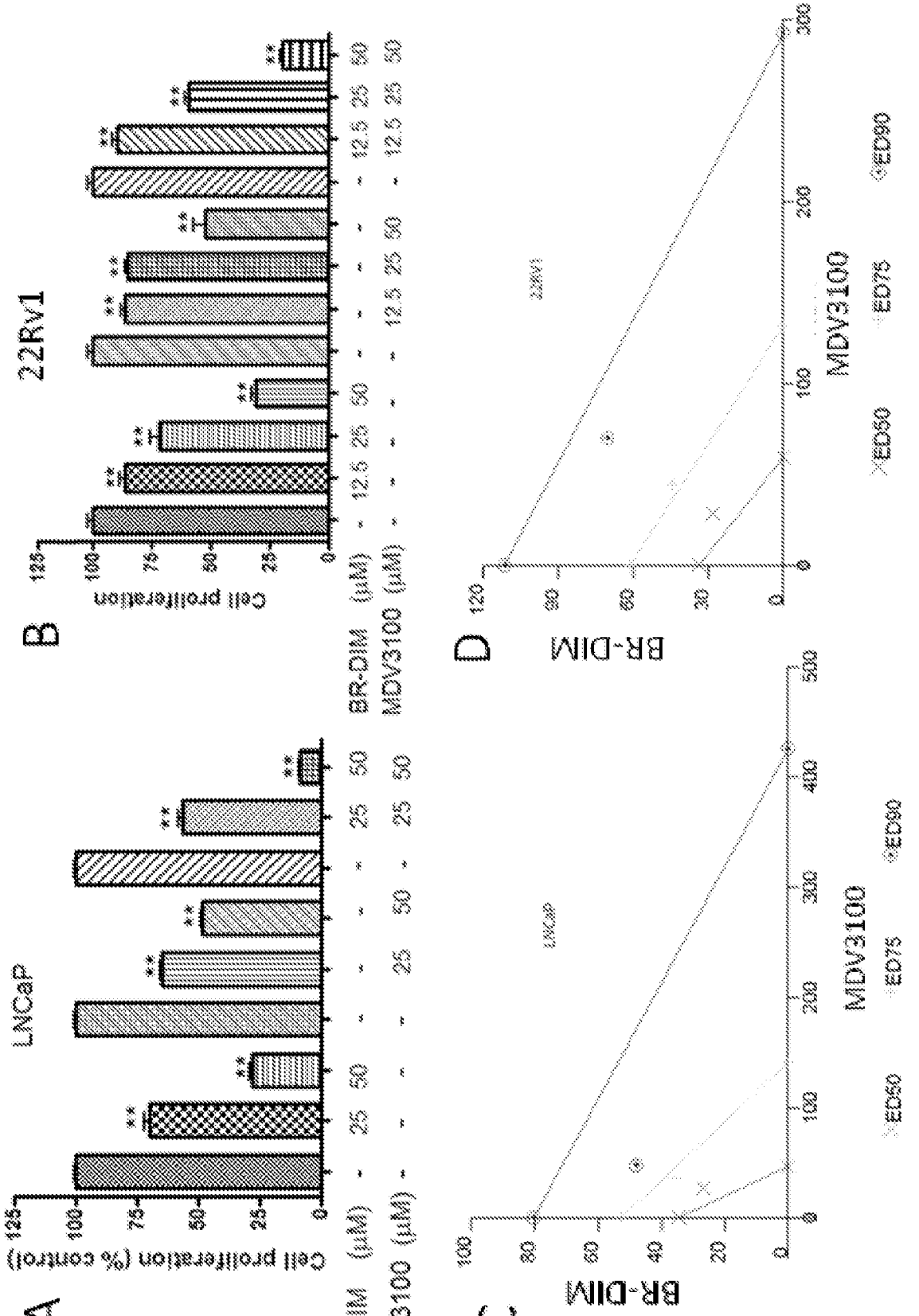

A
LNCaP
Cell proliferation (% control)
BR-DIM (μM)
MDV3100 (μM)
B
22Rv1
Cell proliferation
BR-DIM (μM)
MDV3100 (μM)
C
LNCaP
BR-DIM
MDV3100
ED50
ED75
ED90
D
22Rv1
BR-DIM
MDV3100
ED50
ED75
ED90

Fig. 1

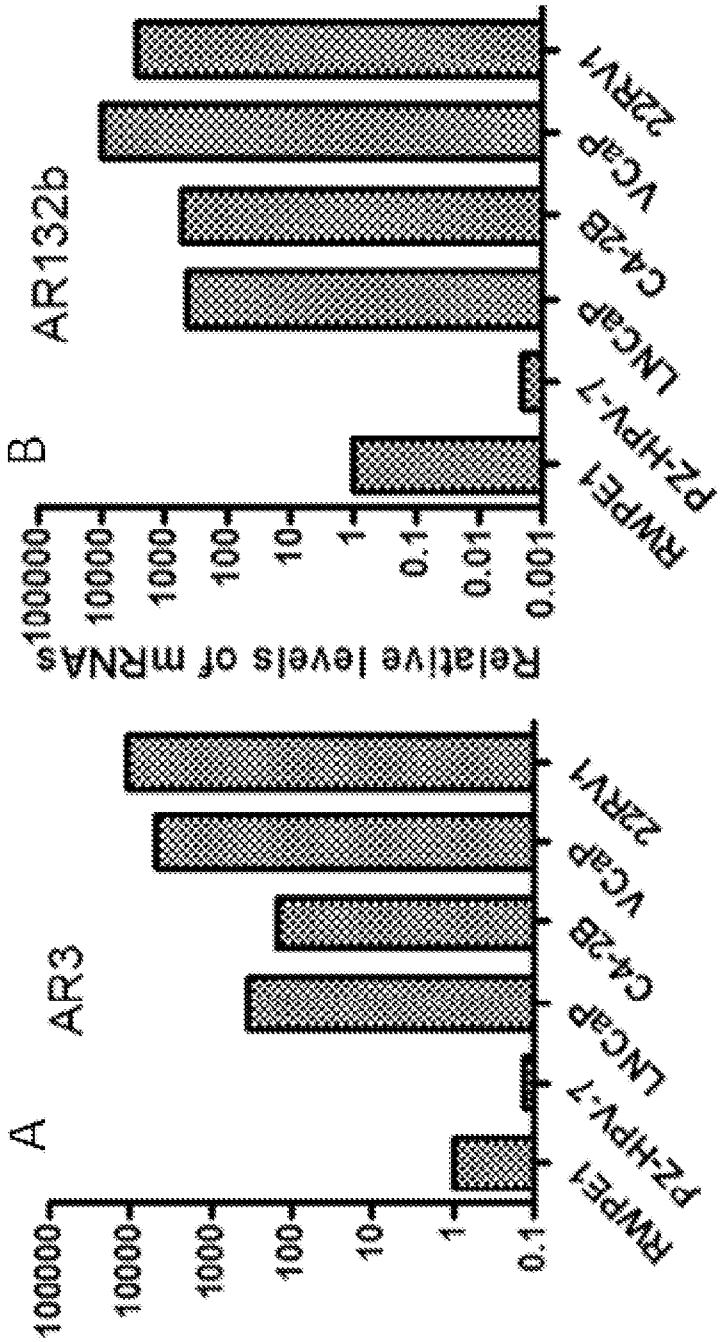

A
AR3
B
AR132b
Relative levels of mRNAs
100000
10000
1000
100
10
1
0.1
0.01
0.001
RWPE1
PZ-HPV-7
LNCaP
C4-2B
VCaP
22RV1

Fig. 2

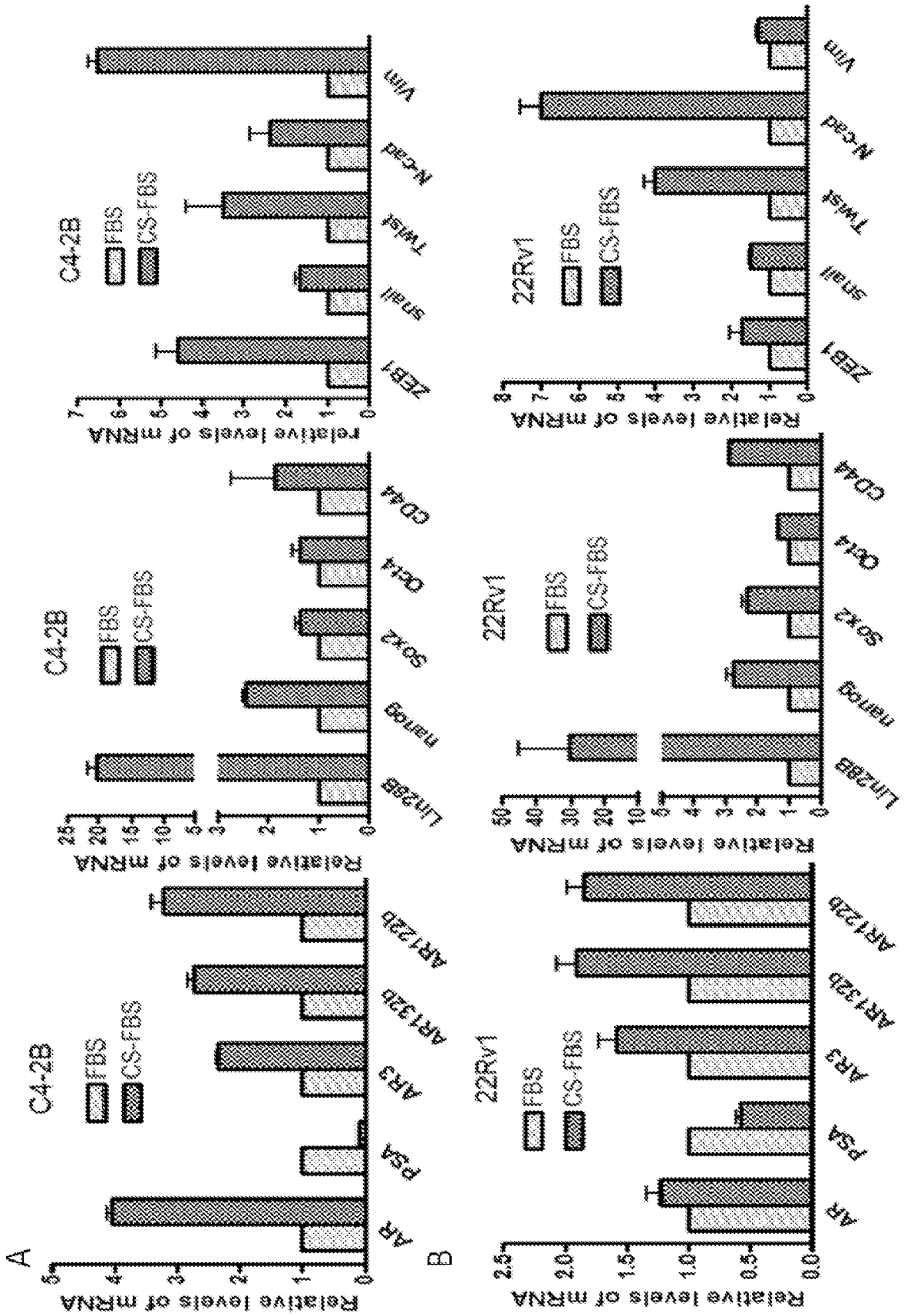

A
B
C4-2B
22Rv1
FBS
CS-FBS
Relative levels of mRNA
AR
PSA
AR3
AR1/2b
AR1/2b
Lin28B
nanog
Sox2
Oct4
CD44
ZEB1
Snail
Twist
N-Cad
Vim

Fig. 3

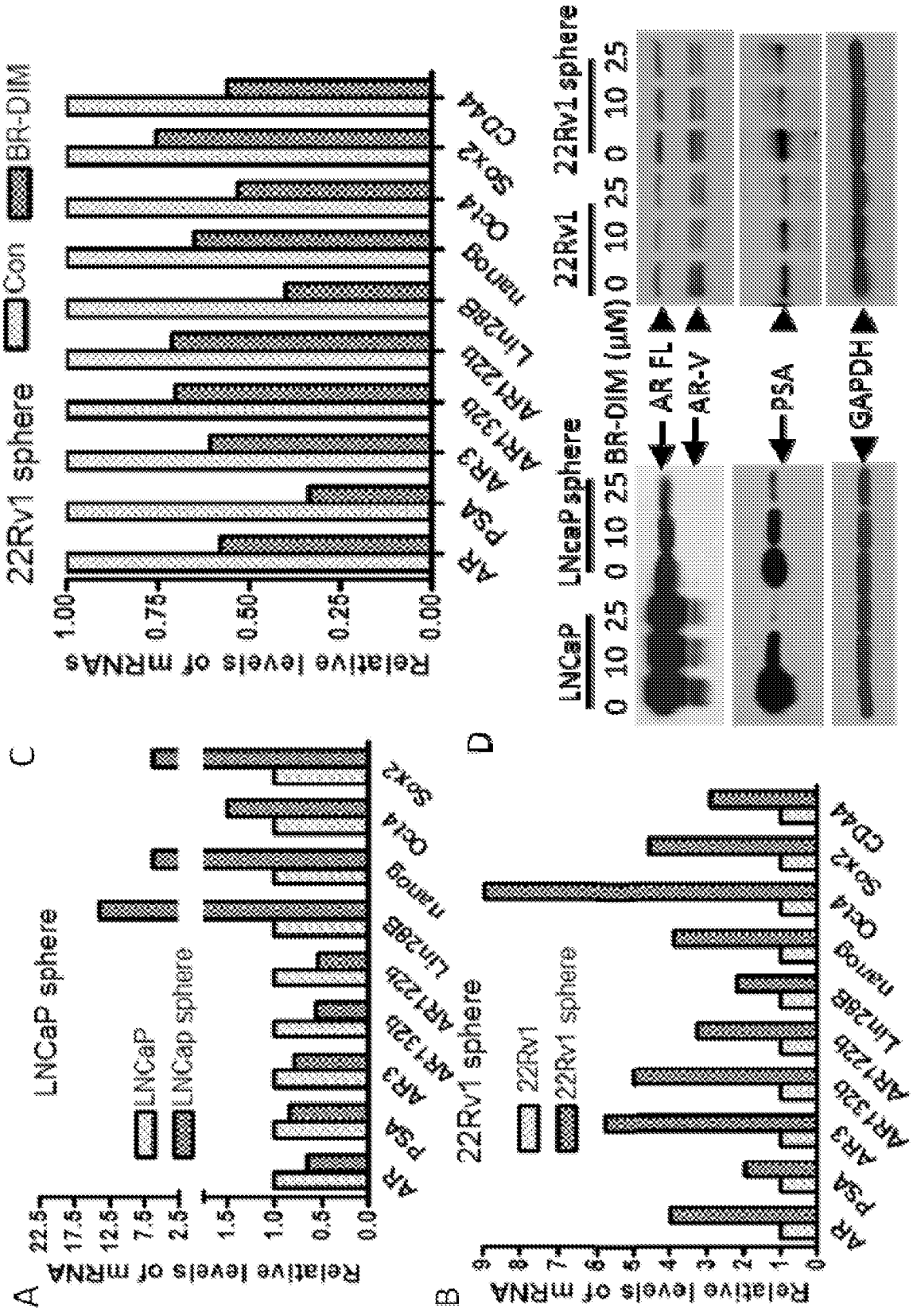

A
LNCaP sphere
LNCaP
LNCap sphere
Relative levels of mRNA
22.5
17.5
12.5
7.5
2.5
1.5
1.0
0.5
0.0
AR
PSA
AR3
AR1/2b
AR1/3b
Lin28B
nanog
Oct4
Sox2
B
22Rv1 sphere
22Rv1
22Rv1 sphere
Relative levels of mRNA
9
8
7
6
5
4
3
2
1
0
AR
PSA
AR3
AR1/3b
AR1/2b
Lin28B
nanog
Oct4
Sox2
CD44
C
22Rv1 sphere
Con
BR-DIM
Relative levels of mRNAs
1.00
0.75
0.50
0.25
0.00
AR
PSA
AR3
AR1/3b
AR1/2b
Lin28B
nanog
Oct4
Sox2
CD44
D
LNCaP
LNCaP sphere
22Rv1
22Rv1 sphere
0 10 25 0 10 25 BR-DIM (μM) 0 10 25 0 10 25
AR FL
AR-V
PSA
GAPDH

Fig. 4

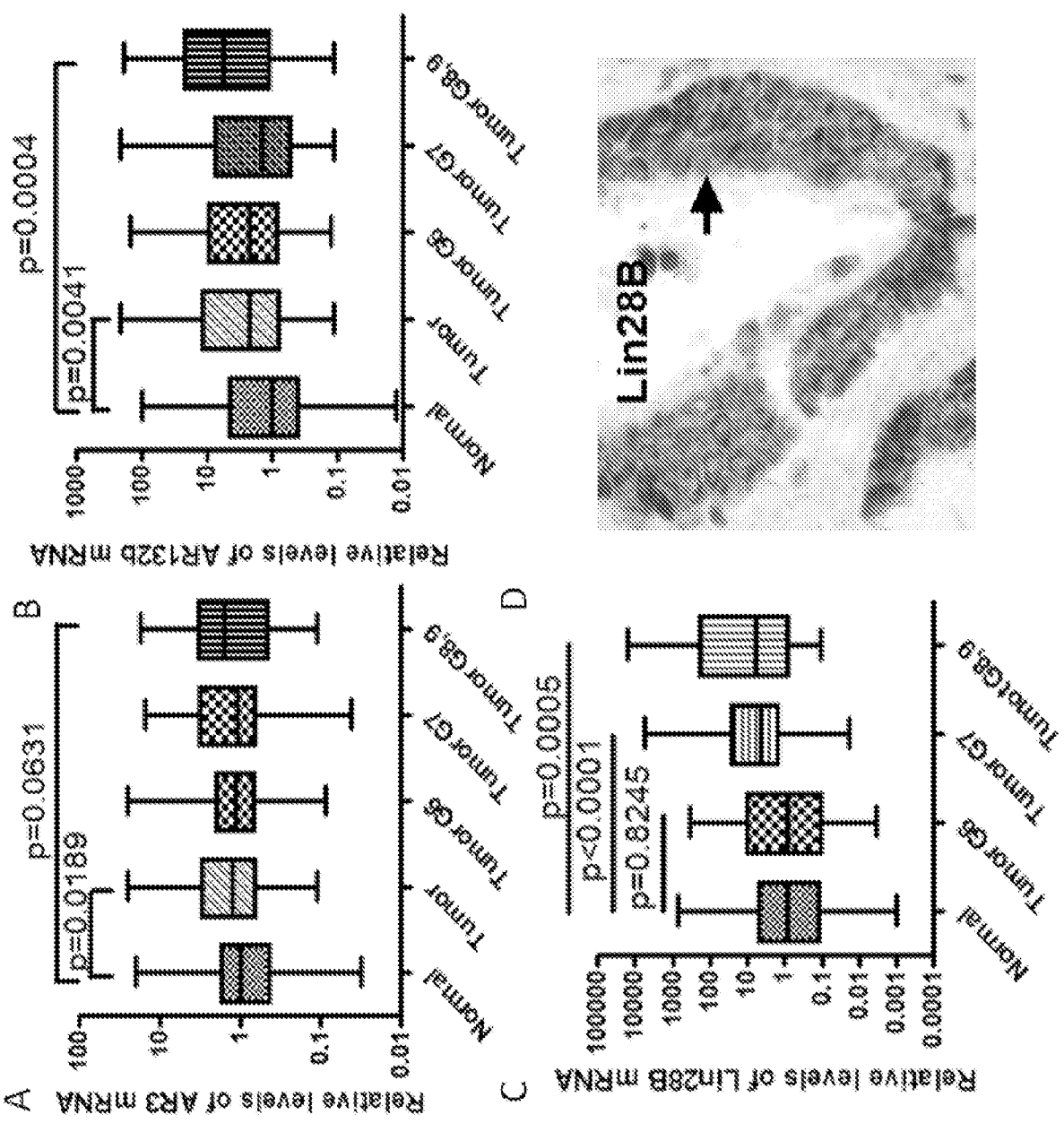

A
Relative levels of AR3 mRNA
p=0.0631
p=0.0189
Normal
Tumor
Tumor GB
Tumor GT
Tumor GB,9
B
Relative levels of AR132b mRNA
p=0.0004
p=0.0041
C
Relative levels of Lin28B mRNA
p=0.0005
p<0.0001
p=0.8245
D
Lin28B

Fig. 5

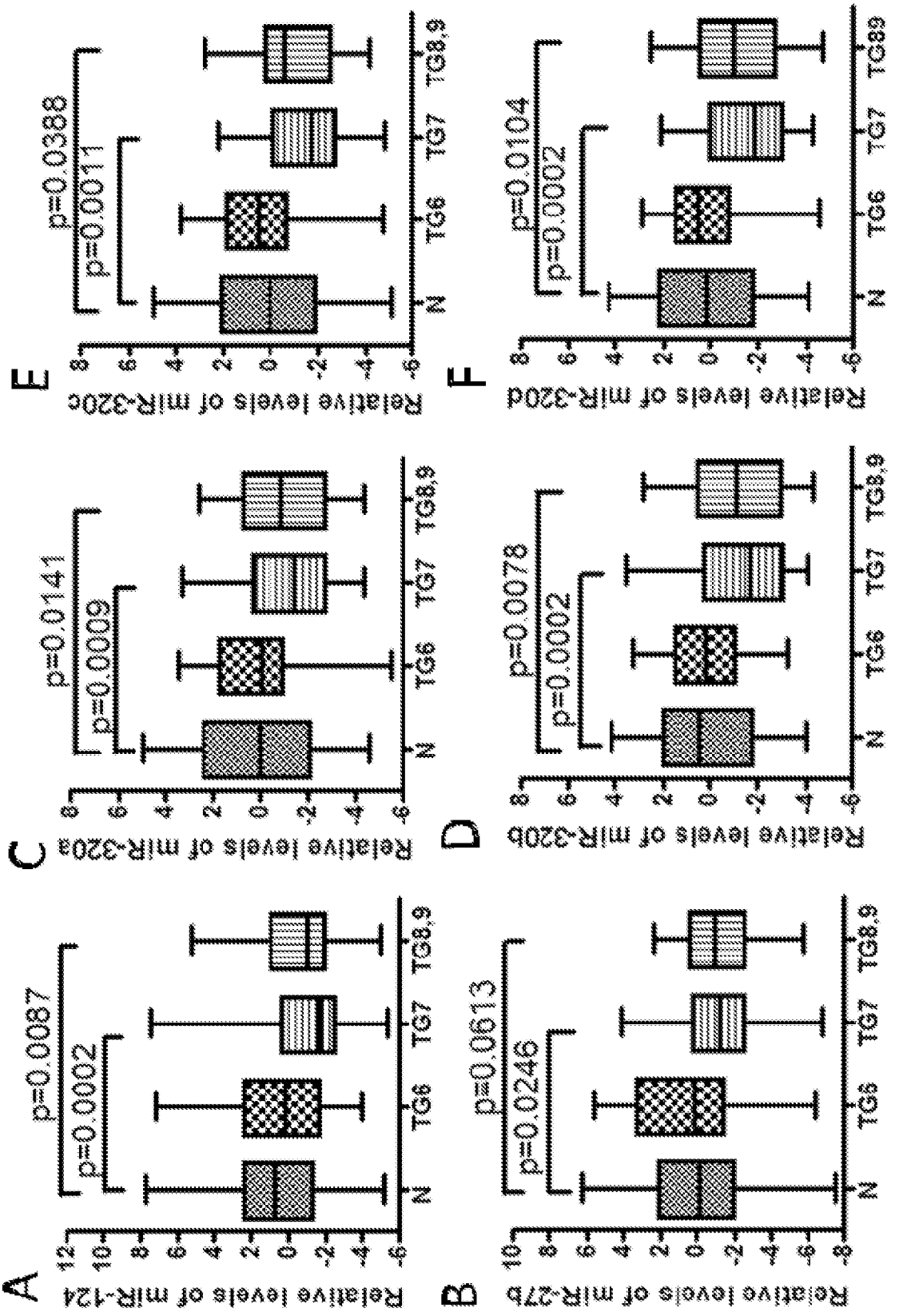

A
Relative levels of miR-124
p=0.0087
p=0.0002
B
Relative levels of miR-27b
p=0.0613
p=0.0246
C
Relative levels of miR-320a
p=0.0141
p=0.0009
D
Relative levels of miR-320b
p=0.0078
p=0.0002
E
Relative levels of miR-320c
p=0.0388
p=0.0011
F
Relative levels of miR-320d
p=0.0104
p=0.0002
N
TG6
TG7
TG8,9

Fig. 6

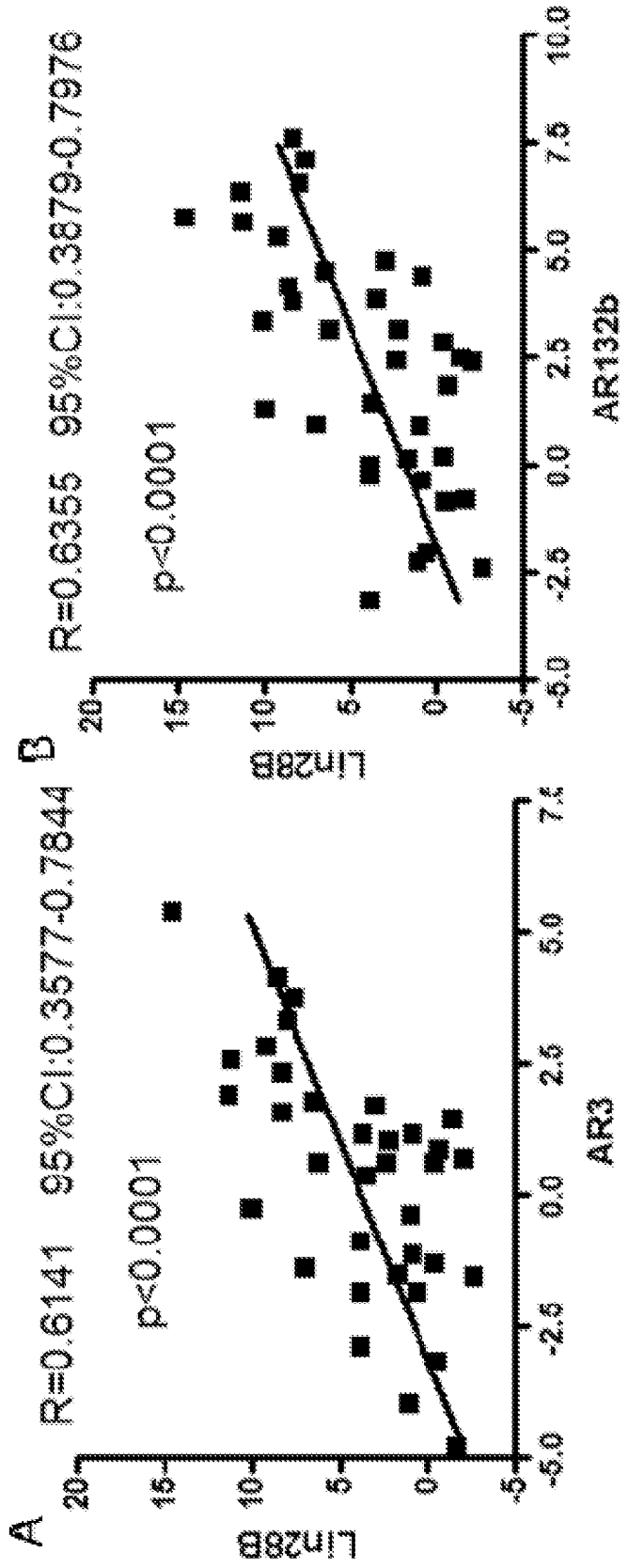

A
R=0.6141 95%CI:0.3577-0.7844
p<0.0001
Lin28B
AR3
B
R=0.6355 95%CI:0.3879-0.7976
p<0.0001
Lin28B
AR132b

Fig. 7

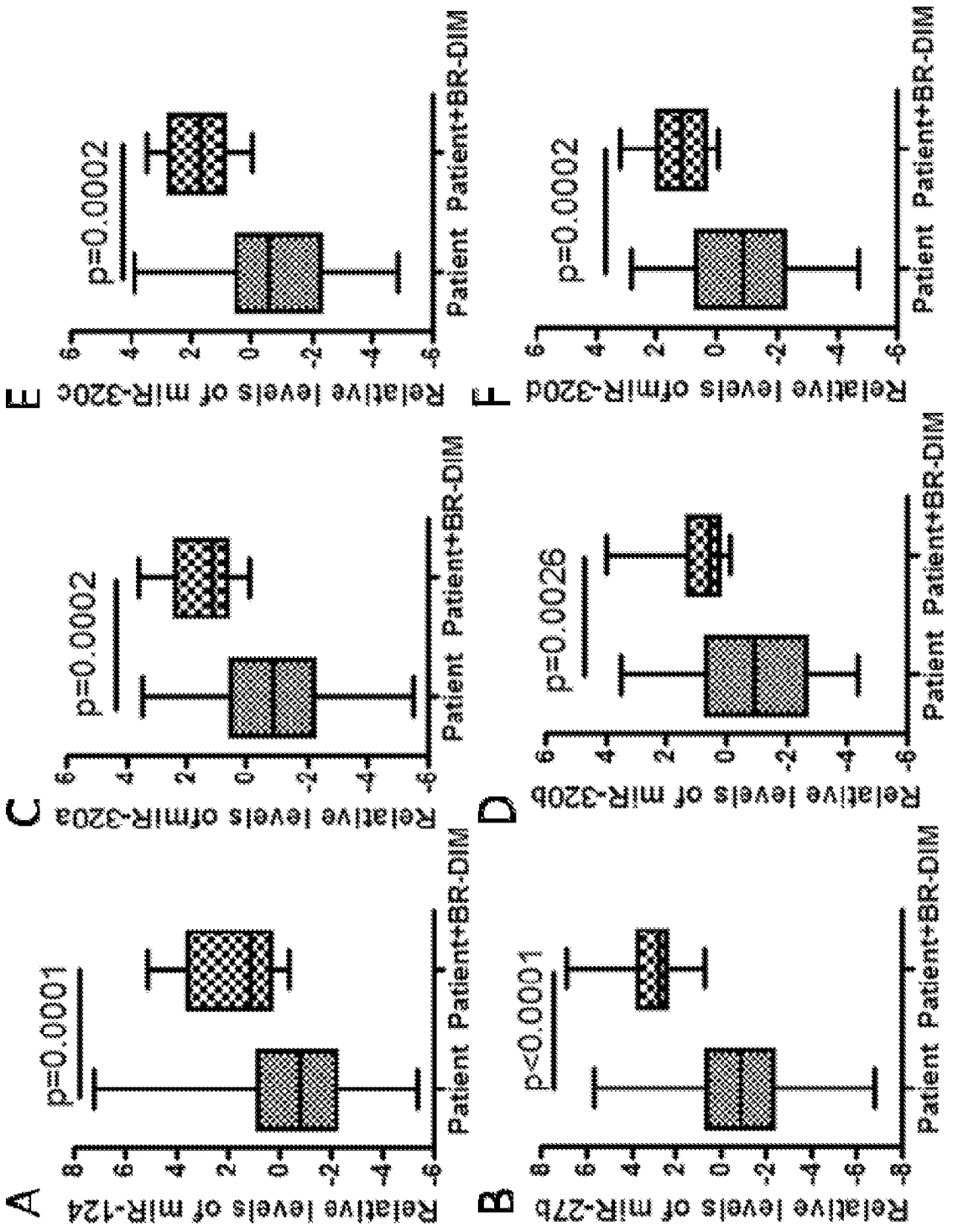

A
Relative levels of miR-124
p=0.0001
Patient
Patient+BR-DIM
B
Relative levels of miR-27b
p<0.0001
Patient
Patient+BR-DIM
C
Relative levels ofmiR-320a
p=0.0002
Patient
Patient+BR-DIM
D
Relative levels of miR-320b
p=0.0026
Patient
Patient+BR-DIM
E
Relative levels of miR-320c
p=0.0002
Patient
Patient+BR-DIM
F
Relative levels ofmiR-320d
p=0.0002
Patient
Patient+BR-DIM

Fig. 8

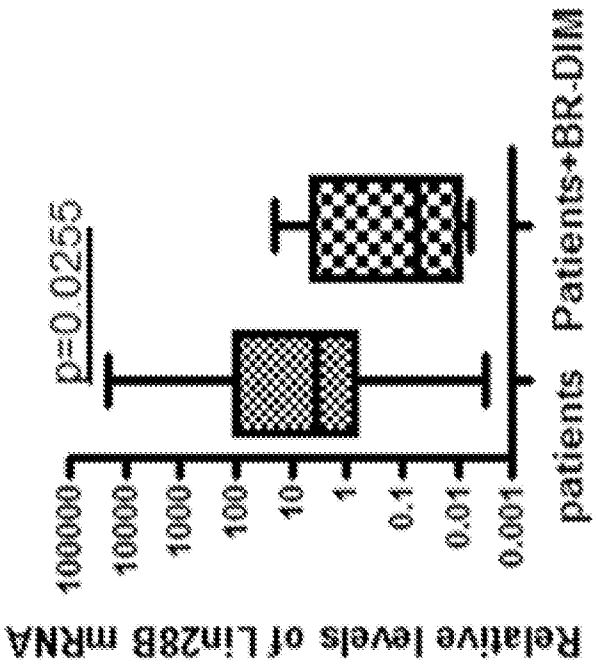
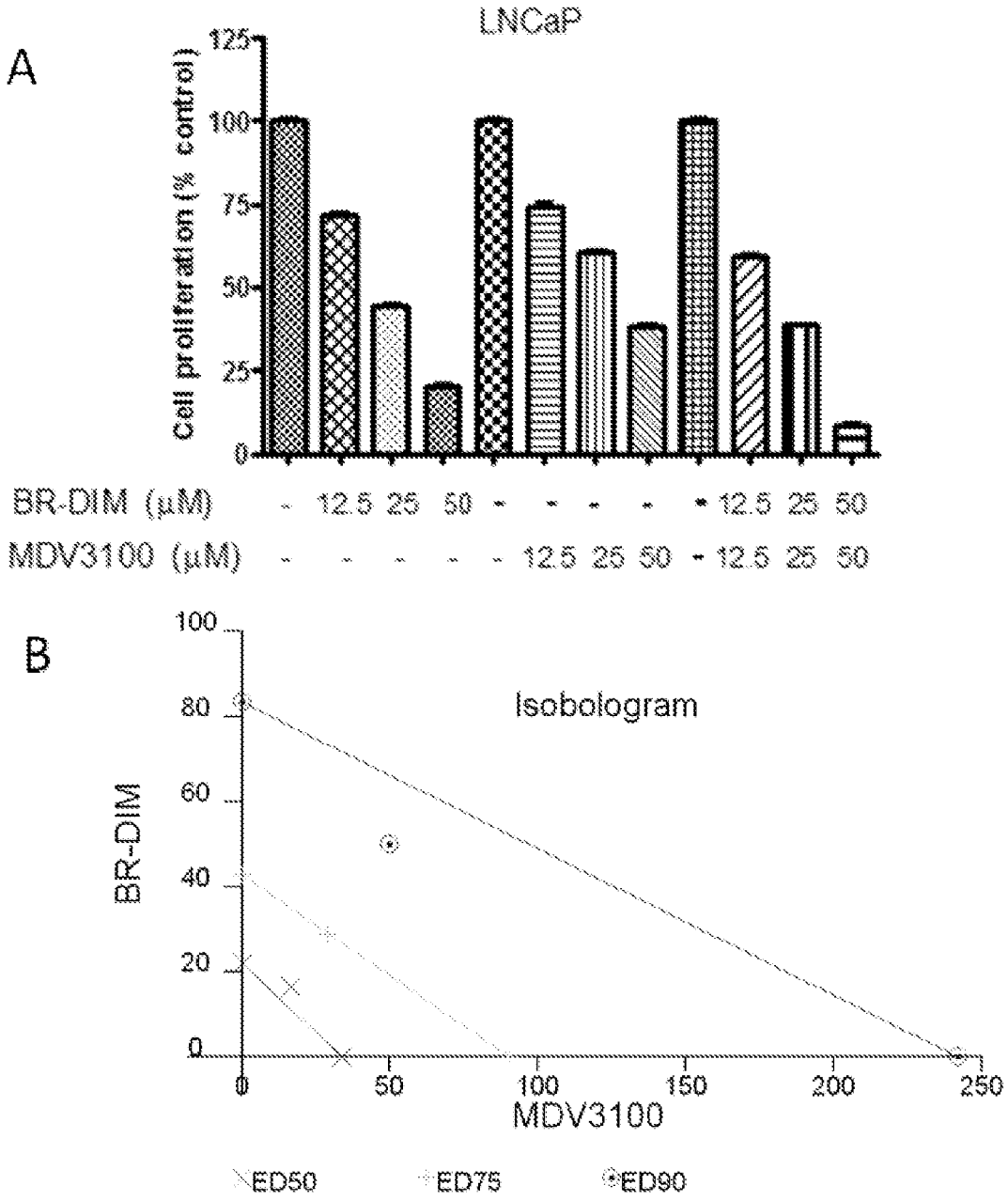

Relative levels of Lin28B mRNA
100000
10000
1000
100
10
1
0.1
0.01
0.001
p=0.0255
patients
Patients+BR-DIM

Fig. 9

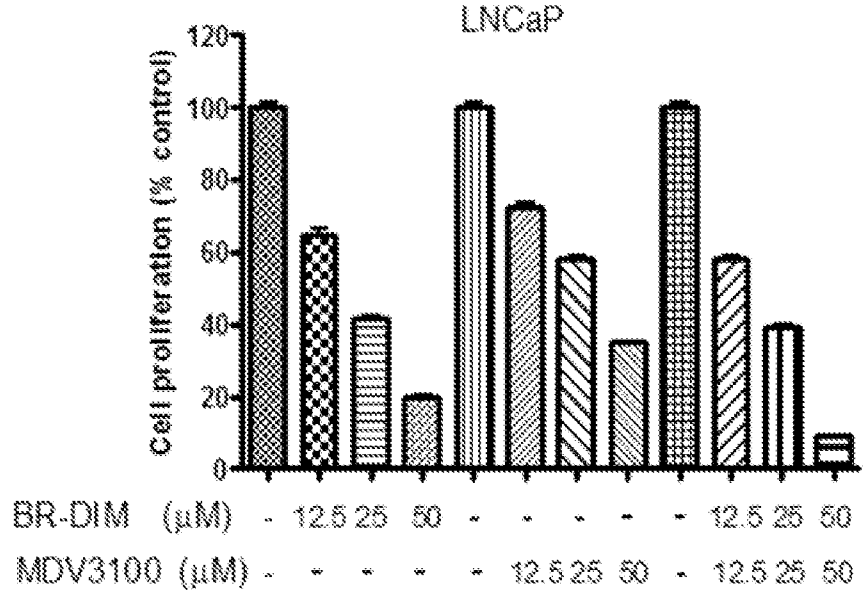


C

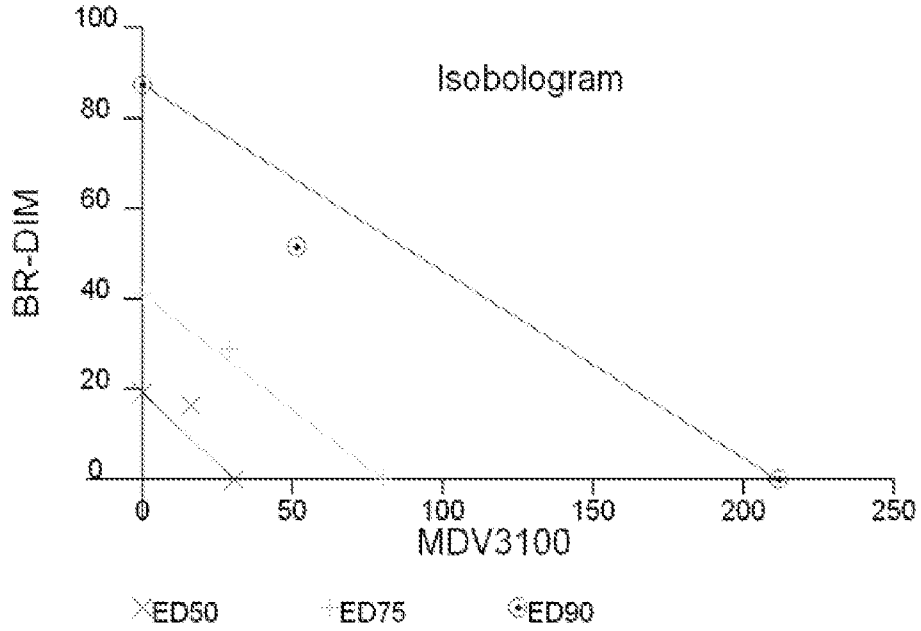
Drug	CI Values at		
	ED50	ED75	ED90
BR-DIM+MDV3100	1.24447	0.99005	0.80511

Fig. 10

A



B

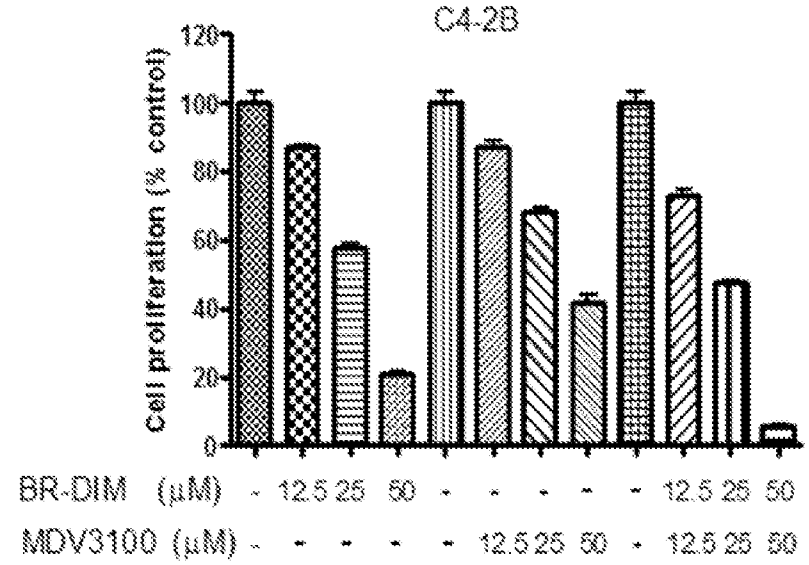


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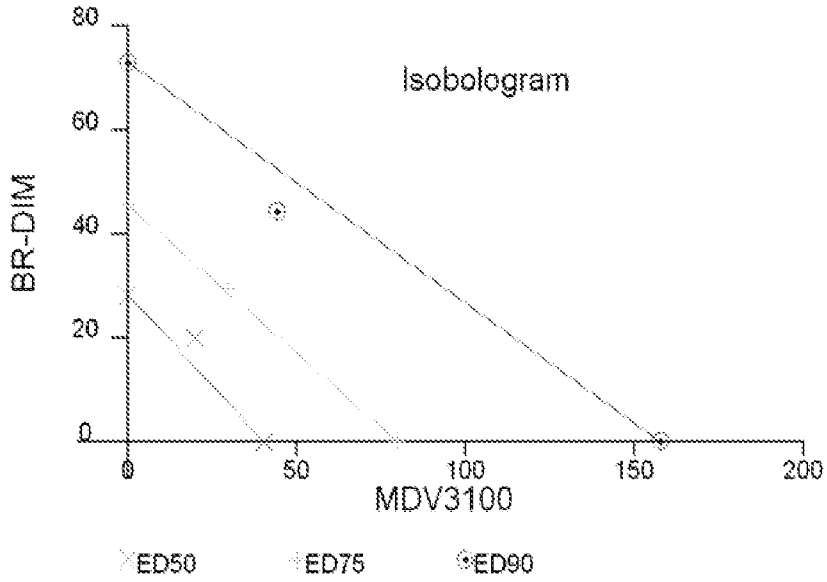
Drug	CI Values at ED50	ED75	ED90
BR-DIM+MDV3100	1.3825	1.06817	0.83397

Fig. 11

A



B

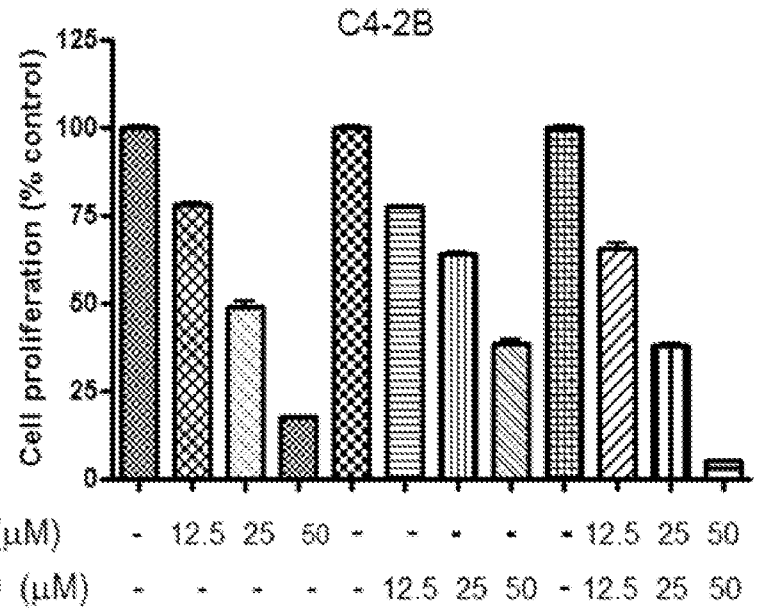


C

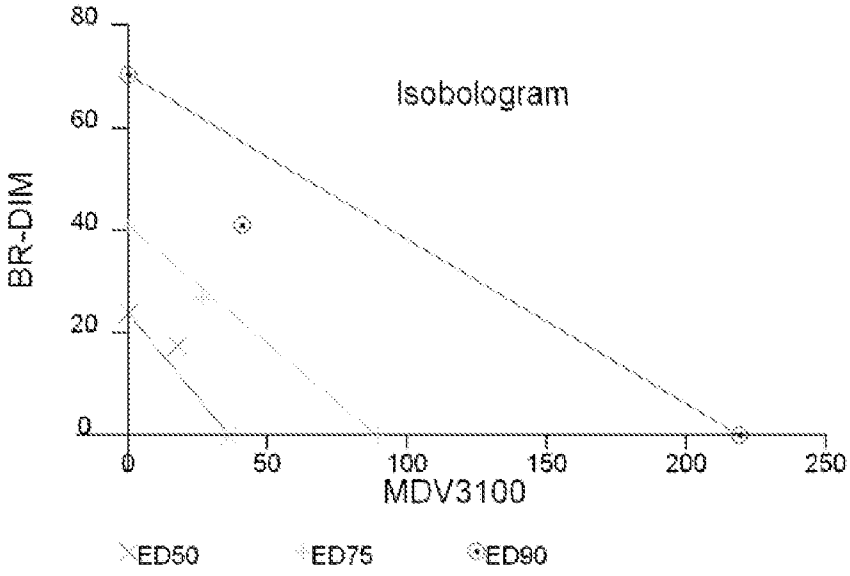
Drug	CI Values at		
	ED50	ED75	ED90
BR-DIM+MDV3100	1.18861	1.02173	0.88716

Fig. 12

A



B

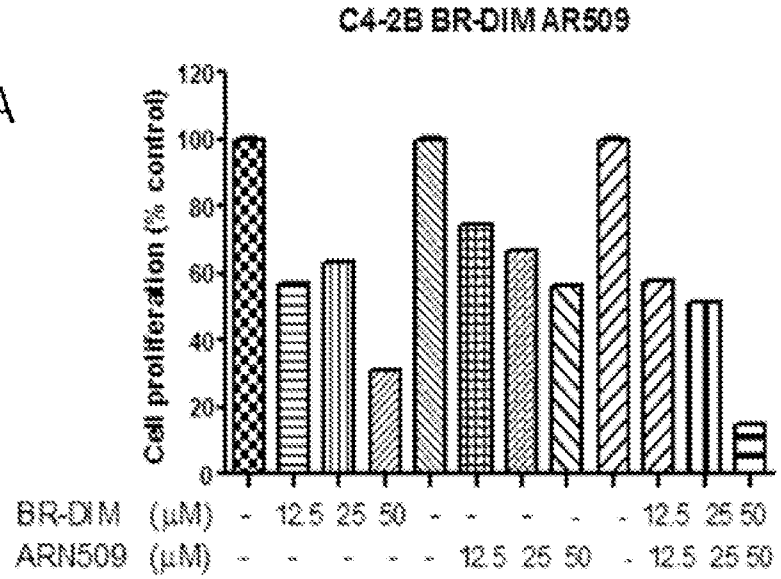


C

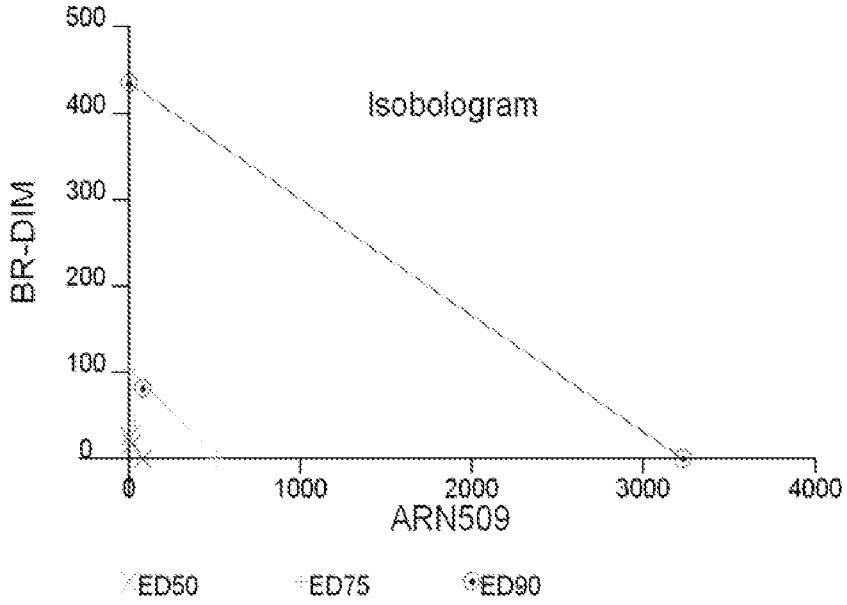
Drug	CI Values at ED50	ED75	ED90
BR-DIM+MDV3100	1.22061	0.95654	0.77066

Fig. 13

A



B

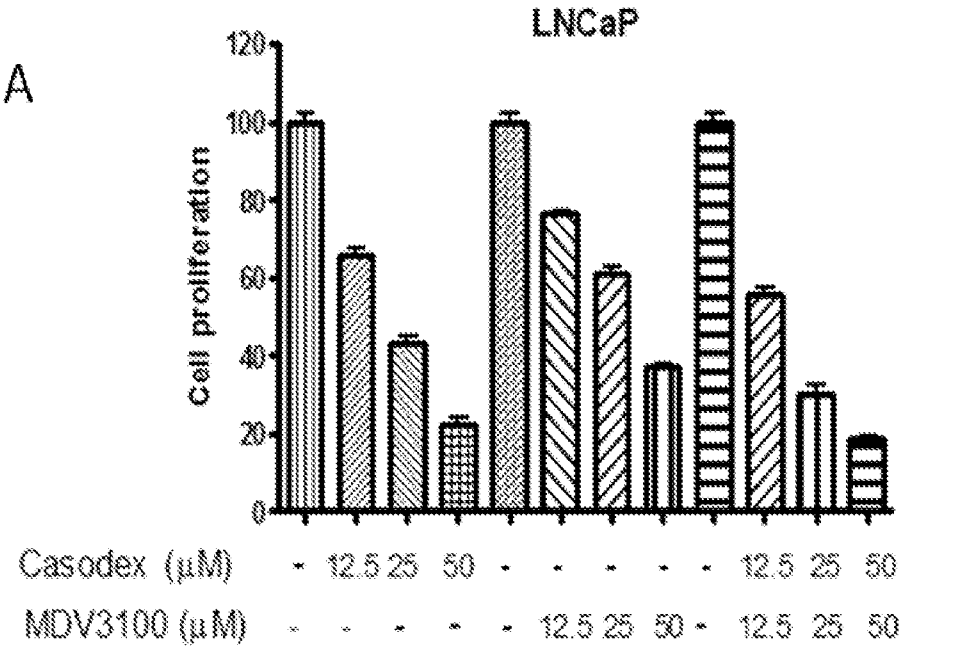


C

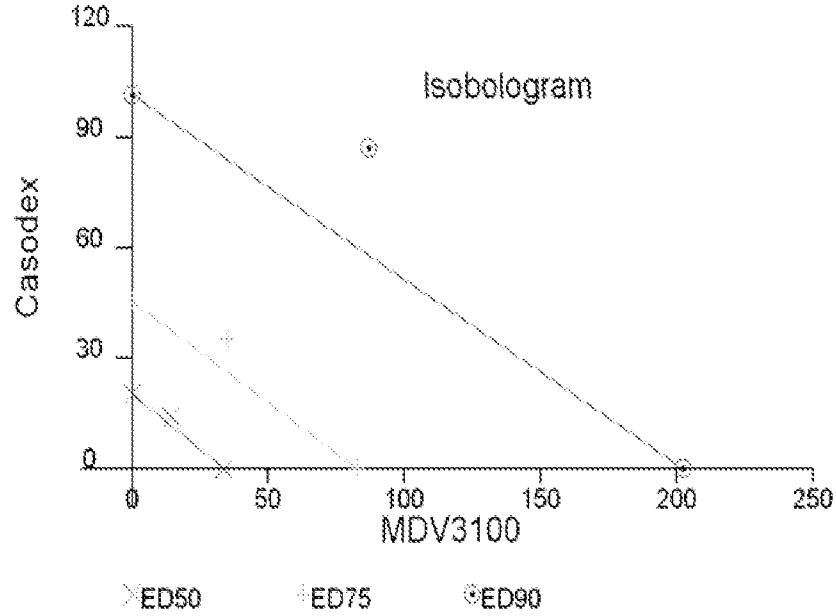
Drug	CI Values at ED50	ED75	ED90
BR-DIM+ARN509	0.95722	0.44619	0.21361

Fig. 14

A



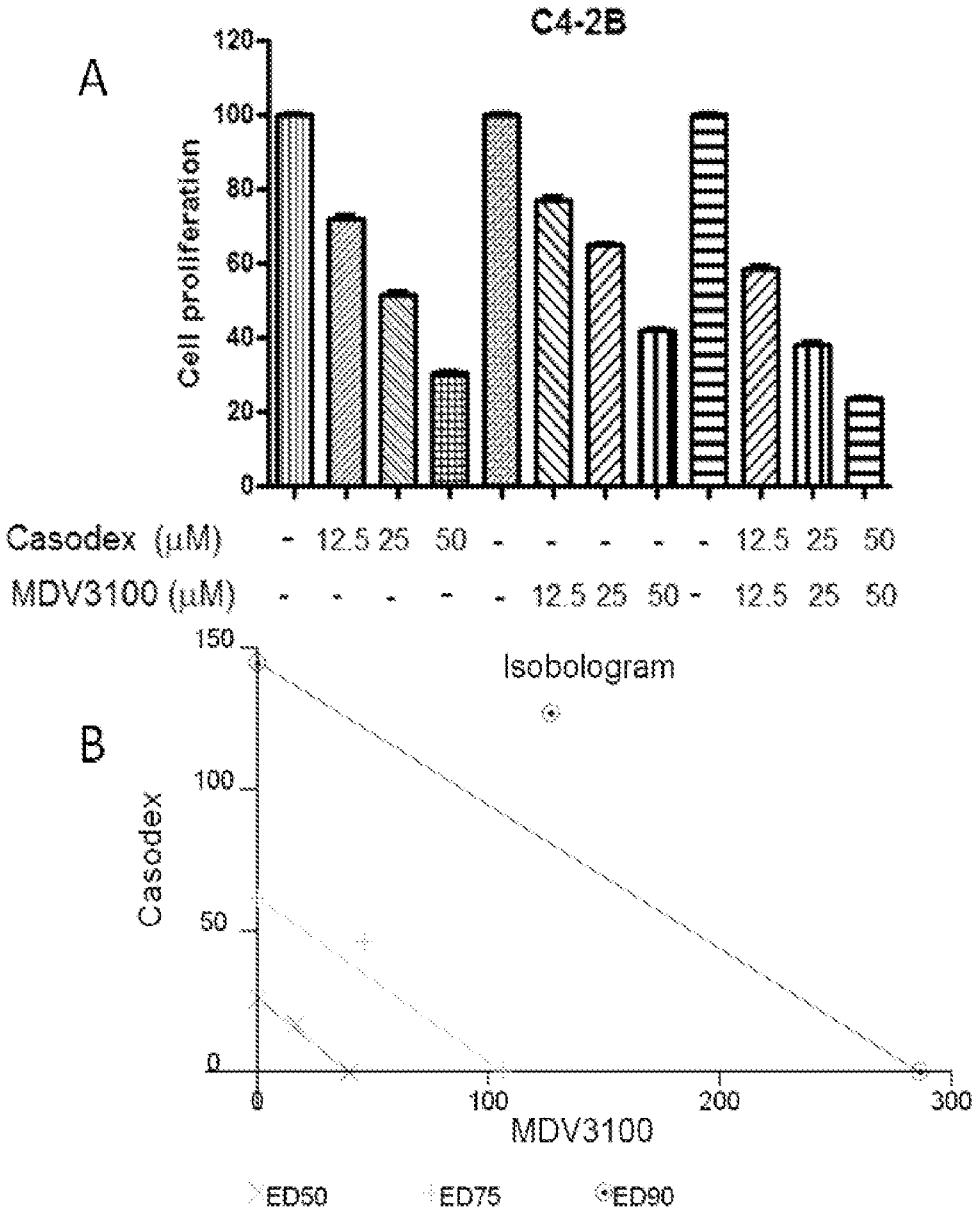
B



C

Drug	CI Values at		
	ED50	ED75	ED90
casodex+MDV3100	1.12256	1.20109	1.2877

Fig. 15

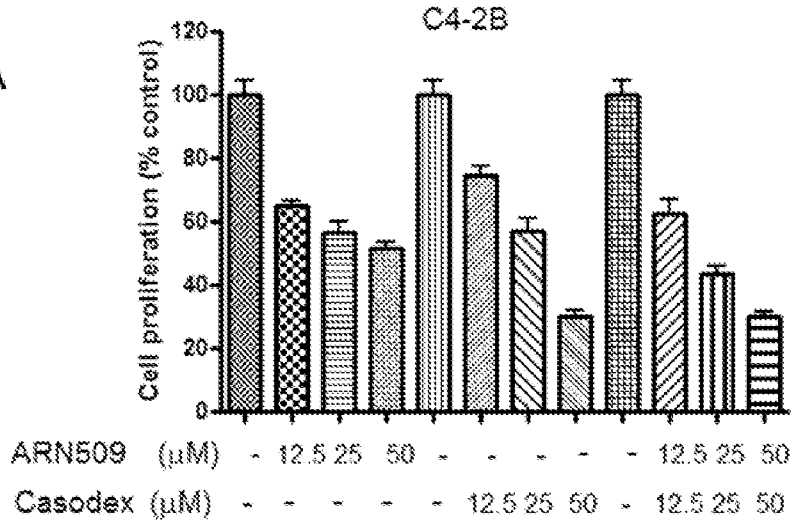


C

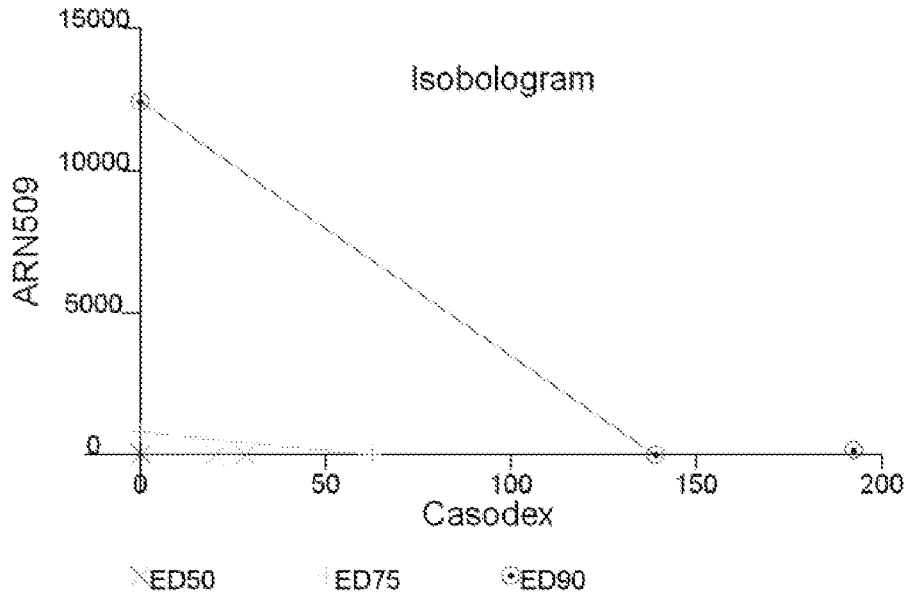
Drug	CI Values at		
	ED50	ED75	ED90
Casodex+MDV3100	1.06368	1.18137	1.31796

Fig. 16

A



B



C

Drug	CI Values at ED50	ED75	ED90
ARN509+Casodex	1.09464	1.07361	1.39959

Fig. 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/056128

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/404 A61K31/407 A61K31/4166 A61K31/4178 A61K31/5025
A61P35/00
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

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RU 2 377 984 C2 (SHCHELKANOVA LARISA ANATOL EVN [RU]) 10 January 2010 (2010-01-10) pages 7,8; examples 1,2	1,33, 67-69, 102
A	US 2004/152672 A1 (CARSON DENNIS A [US] ET AL) 5 August 2004 (2004-08-05) paragraph [0117]	1-128

☐ 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
15 December 2014	05/01/2015

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	Trifilieff-Riolo, S

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/056128

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
RU 2377984 C2	10-01-2010	NONE	
US 2004152672 A1	05-08-2004	US 2004152672 A1	05-08-2004
		WO 2005034944 A1	21-04-2005