

METHODS AND COMPOSITIONS FOR DETERMINING RESISTANCE TO ANDROGEN RECEPTOR THERAPY

INCORPORATION BY REFERENCE OF SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0001] The instant application contains a Sequence Listing, which has been submitted as a computer readable text file in ASCII format via EFS-Web and is hereby incorporated in its entirety by reference herein. The text file, created date of July 16, 2013, is named 38937-725-601_SEQ.txt and is 132 KB in size.

BACKGROUND OF THE INVENTION

[0002] The androgen receptor ("AR") is a ligand-activated transcriptional regulatory protein that mediates induction of a variety of biological effects through its interaction with endogenous androgens. Endogenous androgens include steroids such as testosterone and dihydrotestosterone. Testosterone is converted to dihydrotestosterone by the enzyme 5 alpha-reductase in many tissues.

[0003] The actions of androgens with androgen receptors have been implicated in a number of diseases or conditions, such as androgen dependent cancers, virilization in women, and acne, among others. Compounds that diminish the effects of androgen signaling via the androgen receptor and/or lower the concentrations of androgen receptors find use in the treatment of diseases or conditions in which androgen receptors play a role.

SUMMARY OF THE INVENTION

[0004] Described herein is the identification of modifications in the androgen receptor (AR) that confer resistance of patients to treatment with a first- or second-generation androgen receptor antagonist. In some embodiments, the modification is a substitution of phenylalanine at position 876 of an AR polypeptide. In some embodiments, the modification is F876L. In some embodiments, the modified AR polypeptide is resistant to inhibition by a second-generation androgen receptor antagonist selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the modified AR polypeptide is resistant to inhibition by a first-generation androgen receptor antagonist selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide. In some embodiments, the patient has a cancer. In some embodiments, the cancer is a prostate cancer, breast cancer, liver (i.e. hepatocellular) cancer, or bladder cancer. In some embodiments, the cancer is a castration-resistant prostate cancer. In some embodiments, the modified AR polypeptide exhibits one or more activities of a wildtype AR receptor, including but not limited to, co-activator binding, DNA binding, ligand binding, or

nuclear translocation. In some embodiments, the first- or second generation-antagonist exhibits decreased antagonistic activity against a modified AR polypeptide compared to a wild type AR polypeptide.

[0005] Described herein, in certain embodiments, are methods for determining whether a subject is or will become resistant to therapy with a first- or second-generation androgen receptor antagonist, comprising, consisting of, and/or consisting essentially of: (a) testing a sample that contains a nucleic acid molecule encoding an androgen receptor polypeptide from the subject to determine whether the encoded androgen receptor polypeptide is modified at amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or will become resistant to therapy with a first- or second-generation androgen receptor antagonist if the subject has the modification. In some embodiments, the methods further comprise a step of obtaining the sample from the subject. In some embodiments, the subject has been administered a first- or second-generation AR antagonist for the treatment of a cancer. In some embodiments, the cancer is a prostate cancer, breast cancer, liver (i.e. hepatocellular) cancer, or bladder cancer. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is a castration-resistant prostate cancer. In some embodiments, the methods further comprise, consists of, and/or consists essentially of discontinuing treatment with the first- or second-generation androgen receptor antagonist if the sample from the subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of continuing treatment with a first- or second-generation androgen receptor antagonist if the sample from the subject does not have the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of administering a third-generation androgen receptor antagonist that inhibits the modified AR if the sample from subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the first-generation androgen receptor antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide.

[0006] Described herein, in certain embodiments, are methods for selecting a subject for therapy with a third-generation androgen receptor antagonist, comprising, consisting of, and/or consisting essentially of: (a) testing a sample that contains a nucleic acid molecule encoding an androgen receptor polypeptide from the subject to determine whether the encoded androgen receptor polypeptide is modified at amino acid position 876 of the amino acid sequence set forth

in SEQ ID NO: 1; and (b) characterizing the subject as a candidate for therapy with a third-generation androgen receptor antagonist if the subject has the modification. In some embodiments, the methods further comprise a step of obtaining the sample from the subject. In some embodiments, the methods further comprise, consists of, and/or consists essentially of discontinuing treatment with the first- or second-generation androgen receptor antagonist if the sample from the subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of continuing treatment with a first- or second-generation androgen receptor antagonist if the sample from the subject does not have the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of administering a third-generation androgen receptor antagonist that inhibits the modified AR if the sample from subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the first-generation androgen receptor antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide.

[0007] Described herein, in certain embodiments, are methods for characterizing a subject's androgen receptor to determine whether such subject may be resistant to inhibition with a first- or second-generation androgen receptor antagonist, comprising, consisting of, and/or consisting essentially of: (a) testing a sample that contains a nucleic acid molecule encoding an androgen receptor polypeptide from the subject to determine whether the encoded androgen receptor polypeptide is modified at amino acid position 876 of SEQ ID NO: 1; and (b) if the subject has the modification at position 876 of SEQ ID NO: 1, characterizing the androgen receptor of the subject as being resistant to inhibition with a first- or second-generation androgen receptor antagonist. In some embodiments, the methods further comprise a step of obtaining the sample from the subject. In some embodiments, the first- or second generation-antagonist exhibits decreased antagonistic activity against a modified AR polypeptide compared to a wild type AR polypeptide. In some embodiments, the methods further comprise, consists of, and/or consists essentially of discontinuing treatment with the first- or second-generation androgen receptor antagonist if the sample from the subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of continuing treatment with a first- or second-generation androgen receptor antagonist if the sample from the subject does not have the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of

administering a third-generation androgen receptor antagonist that inhibits the modified AR if the sample from subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the first-generation androgen receptor antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide.

[0008] Described herein, in certain embodiments, are methods for monitoring whether a subject receiving a first- or second-generation androgen receptor antagonist for treatment of a cancer has developed or will develop resistance to the therapy, comprising: (a) testing a sample that contains a nucleic acid molecule encoding an androgen receptor polypeptide from the subject to determine whether the encoded androgen receptor polypeptide is modified at amino acid position 876 of SEQ ID NO: 1; and (b) characterizing the subject as resistant or will become resistant to therapy with a first- or second-generation androgen receptor antagonist if the subject has the modification. In some embodiments, the methods further comprise a step of obtaining the sample from the subject. In some embodiments, the methods further comprise, consists of, and/or consists essentially of discontinuing treatment with the first- or second-generation androgen receptor antagonist if the sample from the subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of continuing treatment with a first- or second-generation androgen receptor antagonist if the sample from the subject does not have the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of administering a third-generation androgen receptor antagonist that inhibits the modified AR if the sample from subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the first-generation androgen receptor antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide.

[0009] Described herein, in certain embodiments, are methods for optimizing the therapy of a subject receiving a first- or second-generation androgen receptor antagonist for treatment of a cancer, comprising, consisting of, and/or consisting essentially of: (a) testing a sample that contains a nucleic acid molecule encoding an androgen receptor polypeptide from the subject to determine whether the encoded androgen receptor polypeptide is modified at amino acid position 876 of SEQ ID NO: 1; and (c)(i) if the sample has the modification, discontinuing treatment of the subject with the first- or second-generation androgen receptor antagonist or (ii)

if the sample does not have the modification, continuing treatment of the subject with the first- or second-generation androgen receptor antagonist if the subject does not have the modification. In some embodiments, the methods further comprise a step of obtaining the sample from the subject. In some embodiments, the methods further comprise, consists of, and/or consists essentially of discontinuing treatment with the first- or second-generation androgen receptor antagonist if the sample from the subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of continuing treatment with a first- or second-generation androgen receptor antagonist if the sample from the subject does not have the modification at position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises, consists of, and/or consists essentially of administering a third-generation androgen receptor antagonist that inhibits the modified AR if the sample from subject has the modification at position 876 of SEQ ID NO: 1. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the first-generation androgen receptor antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide or nilutamide.

[0010] In some embodiments, the modified AR polypeptide comprises, consists of, and or consists essentially of a substitution or a deletion of the amino acid at position 876 of SEQ ID NO: 1. In some embodiments, the modified AR polypeptide comprises, consists, and/or consists essentially of a substitution of phenylalanine to an amino acid selected from the group consisting of leucine, isoleucine, valine, alanine, glycine, methionine, serine, threonine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid at position 876 of SEQ ID NO: 1. In some embodiments, the modified AR polypeptide comprises, consists of, and/or consists essentially of a substitution of phenylalanine to an amino acid selected from among glycine, alanine, valine, leucine, and isoleucine at position 876 of SEQ ID NO: 1. In some embodiments, the modified AR polypeptide comprises, consists of, and/or consists essentially of a substitution of phenylalanine to leucine at position 876 of SEQ ID NO: 1. In some embodiments, the modified AR polypeptide comprises, consists of, and/or consists essentially of a deletion of nucleic acid encoding amino acid position 876 of SEQ ID NO: 1.

[0011] In some embodiments, the nucleic acid encoding the modified AR polypeptide has (i) a mutation of thymine (t) to cytosine (c) at a nucleic acid position corresponding to nucleic acid position 2626 in the sequence of nucleotides set forth in SEQ ID NO: 18; (ii) a mutation of cytosine (c) to adenine (a) at nucleic acid position corresponding to nucleic acid position 2628 in

the sequence of nucleotides set forth in SEQ ID NO: 18; or (ii) a mutation of cytosine (c) to guanine (g) at nucleic acid position corresponding to nucleic acid position 2628 in the sequence of nucleotides set forth in SEQ ID NO: 18.

[0012] In some embodiments of the method, the nucleic acid sample is RNA or DNA. In some embodiments of the method, the nucleic acid sample is genomic DNA. In some embodiments, the method further comprises, consists of, and/or consists essentially of isolating mRNA from the RNA sample. In some embodiments, the nucleic acid is isolated from a tumor cell sample obtained from the subject. In some embodiments, the sample is a tumor biopsy sample, a blood sample, a serum sample, a lymph sample, or a bone marrow aspirate obtained from the subject. In some embodiments, the sample contains circulating tumor cells. In some embodiments, the sample contains disseminated tumor cells. In some embodiments, the nucleic acid is purified from the sample prior to testing. In some embodiments, the nucleic acid is amplified prior to testing.

[0013] In some embodiments of the method, testing comprises, consists of, and/or consists essentially performing polymerase chain reaction (PCR) amplification of a nucleic acid sample encoding position 876 of SEQ ID NO: 1. In some embodiments, PCR amplification comprises, consists of, and/or consists essentially of using a pair of oligonucleotide primers that flank the region encoding amino acid position 876 of SEQ ID NO: 1. In some embodiments, the method further comprises sequencing the PCR amplified nucleic acid using techniques known to those skilled in the art.

[0014] In some embodiments, testing comprises, consists of, and/or consists essentially of contacting the nucleic acid with a sequence specific nucleic acid probe, wherein the sequence specific nucleic acid probe: (a) binds to nucleic acid encoding a modified receptor that is modified at amino acid position 876; and (b) does not bind to nucleic acid encoding the wild-type receptor having phenylalanine at position 876 of SEQ ID NO: 1.

[0015] In some embodiments, the first- or second-generation androgen receptor antagonist inhibits a wild-type androgen receptor polypeptide by competitive antagonism. In some embodiments, the second-generation androgen receptor antagonist is selected from among ARN-509, enzalutamide (MDV3100), or RD162.

[0016] In some embodiments, the subject has a disease or disorder selected from among cancer, an inflammatory disorder or a proliferative disorder. In some embodiments, the subject has cancer. In some embodiments, the subject has an AR-mediated cancer. In some embodiments, the cancer is selected from a prostate cancer, a breast cancer, liver (i.e., hepatocellular) cancer, or a bladder cancer. In some embodiments, the cancer is prostate cancer.

In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the subject has a solid tumor,

[0017] In some embodiments, the subject is treated with the first- or second-generation androgen receptor antagonist prior to obtaining the sample from the subject. In some embodiments, the subject is responsive the treatment with the first- or second-generation androgen receptor antagonist when it is first administered. In some embodiments, the sample for use in the methods is a sample obtained at 1 week, 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, 12 months, 14 months, 16 months, 18 months, 20 months, 22 months, or 24 months following the first administration of with the first- or second-generation AR antagonist. In some embodiments, the sample is obtained 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 times over the course of treatment with the first- or second-generation AR antagonist. In some embodiments, the subject is responsive the treatment with the first- or second-generation AR antagonist when it is first administered.

[0018] Described herein, in certain embodiments, are methods for screening compounds that antagonize a modified androgen receptor, comprising, consisting of and/or consisting essentially of: (a) expressing a modified androgen receptor in a cell, wherein the modified androgen receptor is modified at an amino acid position corresponding to position 876 of SEQ ID NO: 1; (b) contacting the cell with a test compound; and (c) detecting the level of androgen receptor activity in the cell, wherein a decrease in activity expressed indicates that the compound antagonizes the modified AR. In some embodiments, the test compound exhibits full antagonist activity toward the modified AR. In some embodiments, the test compound does not exhibit agonist activity toward the modified AR. Described herein, in certain embodiments, is a third-generation androgen receptor inhibitor identified by the methods described herein. In some embodiments, the modification of the AR polypeptide used in the method is a substitution or deletion of the amino acid at position 876 of the androgen receptor polypeptide. In some embodiments, the modification of the AR polypeptide used in the method is a substitution of phenylalanine to an amino acid selected from among leucine, isoleucine, valine, alanine, glycine, methionine, serine, threonine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid at amino acid position 876 of the androgen receptor polypeptide. In some embodiments, the modification of the AR polypeptide used in the method is a substitution of phenylalanine to an amino acid selected from among glycine, alanine, valine, leucine, and isoleucine at amino acid position 876 of the androgen receptor polypeptide. In some embodiments, the modification of the AR polypeptide used in the

method is a substitution of phenylalanine to leucine at amino acid position 876 of the androgen receptor polypeptide.

[0019] In some embodiments, the cell employed in the method is deficient for the expression of wild-type androgen receptor, expresses a low level of wild-type androgen receptor, or expresses a modified AR receptor. In some embodiments, the cell is selected from among HeLa, CV1, COS7, HepG2, HEK-293, DU145, PC3, TSY-PR1, LNCaP, CWR, VCaP and LAPC4. In some embodiments, the cell comprises a reporter gene operably linked to an androgen responsive promoter. In some embodiments, the activity of the AR polypeptide is determined by analyzing the expression of the reporter gene. In some embodiments, the promoter comprises androgen response element. In some embodiments, the androgen response element is 4XARE or a probasin element. In some embodiments, the promoter is a probasin, a prostate specific antigen, MMTV LTR, FASN, STEAP4, TMPRSS2, ORM1, or NKX3.1 promoter. In some embodiments, the reporter gene encodes a protein selected from among a luciferase, a fluorescent protein, a bioluminescent protein, or an enzyme.

[0020] Described herein, in certain embodiments, is an isolated androgen receptor polypeptide or a variant thereof having androgen receptor activity comprising a modification at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1, wherein the modification confers resistance to an androgen receptor antagonist on the modified androgen receptor polypeptide or variant. In some embodiments, the modification comprises substitution of the amino acid at position 876 compared to a wild-type androgen receptor set forth in SEQ ID NO: 1. In some embodiments, the substitution is F876L. In some embodiments, the modification comprises a deletion of amino acid position 876. In some embodiments, the modified AR polypeptide has the sequence of amino acids set forth in SEQ ID NO: 5. In some embodiments, the modified AR polypeptide is a recombinant polypeptide. In some embodiments, the modified AR polypeptide comprises a substitution of the amino acid at position 876 compared to a wild-type androgen receptor set forth in SEQ ID NO: 1 and one or more additional amino acid substitutions. In some embodiments, the modified AR polypeptide comprises a substitution at amino acid at position 876 that is F876L. In some embodiments, the modified AR polypeptide comprises one or more additional amino acid substitutions selected from among one or more amino acid substitutions associated with castration resistant prostate cancer. In some embodiments, the one or more additional amino acid substitutions is selected from among one or more substitutions at amino acid positions 701, 741, 874 and 877 compared to a wild-type androgen receptor set forth in SEQ ID NO: 1. In some embodiments, the one or more additional amino acid substitutions is selected from among

T877A, W741C, W741L, W741R, L701H and H874Y. In some embodiments, the modified AR polypeptide is a recombinant polypeptide. In some embodiments, the modified AR polypeptide comprises a sequence of amino acids set forth in SEQ ID NO: 5 or a variant that has at least or at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the polypeptide having the sequence set forth in SEQ ID NO: 5, wherein the amino acid at position 876 is not phenylalanine.

[0021] Described herein, in certain embodiments, is an isolated nucleic acid molecule encoding the modified androgen receptor polypeptide provided herein. In some embodiments, the nucleic acid is a DNA or an RNA molecule. In some embodiments, the nucleic acid is a cDNA molecule. In some embodiments, the nucleic acid is a PCR amplification product. In some embodiments, the nucleic acid is a recombinant molecule. In some embodiments, the nucleic acid is a synthetic molecule. In some embodiments, the nucleic acid comprises the sequence of nucleic acids set forth in SEQ ID NO: 19 or a variant that has at least or at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity with the polypeptide having the sequence set forth in SEQ ID NO: 19, wherein the nucleic acid codon encoding amino acid at position 876 does not encode phenylalanine.

[0022] Described herein, in certain embodiments, is a vector, comprising a nucleic acid molecule encoding the modified androgen receptor polypeptide provided herein. In some embodiments, the vector is a viral or plasmid vector. In some embodiments, the nucleic acid is operably linked to a promoter. In some embodiments, the promoter is a constitutive or an inducible promoter. Described herein, in certain embodiments, is a host cell, comprising the vector. In some embodiments, the cell is a prokaryotic cell or a eukaryotic cell. Described herein, in certain embodiments, is a mutant AR polypeptide expressed by the host cell.

[0023] Described herein, in certain embodiments, is a pharmaceutical composition comprising a third-generation androgen receptor inhibitor identified by the methods provided herein and a pharmaceutically acceptable excipient. Described herein, in certain embodiments, are uses of a third-generation androgen receptor inhibitor identified by the methods provided herein for the manufacture of a medicament. Described herein, in certain embodiments, are methods of treatment comprising administering to a subject in need thereof a therapeutically effective amount of the pharmaceutical composition, wherein the composition comprises a suitable pharmaceutical carrier. In some embodiments, the subject has cancer. In some embodiments, the subject has an AR-mediated cancer. In some embodiments, the cancer is a prostate cancer, breast cancer, liver (i.e. hepatocellular) cancer or bladder cancer. In some embodiments, the cancer is a castration resistant prostate cancer. In some embodiments, the subject expresses a mutant AR. In

some embodiments, the mutant AR comprises a substitution or a deletion of the amino acid at amino acid position 876 in the androgen receptor polypeptide. In some embodiments, the substitution is F876L. In some embodiments, the third-generation AR antagonist is administered with an additional therapeutic agent. In some embodiments, the third-generation AR antagonist and the additional therapeutic agent are administered sequentially, simultaneously or intermittently. In some embodiments, the additional therapeutic agent is selected from among hormones, hormone receptor agonists or antagonists, corticosteroids, anti-emetic agents, analgesics, anti-cancer agents, anti-inflammatory agents, kinase inhibitors, HSP90 inhibitors, histone deacetylase (HDAC) inhibitors. In some embodiments, the additional therapeutic agent is a gonadotropin-releasing hormone (GnRH) agonist or antagonist. In some embodiments, the GnRH agonist is leuprolide, bruserelin or goserelin.

[0024] Described herein, in certain embodiments, is a microchip comprising the mutant AR polypeptide provided herein or a nucleic acid encoding modified AR polypeptide provided herein. In some embodiments, the modified AR polypeptide comprises a modification at amino acid 876. In some embodiments, the modification is an amino acid substitution that is F876L.

[0025] Described herein, in certain embodiments, are kits comprising the mutant AR polypeptide provided herein. Described herein, in certain embodiments, is a kit comprising the isolated nucleic acid encoding a mutant AR polypeptide provided herein. Described herein, in certain embodiments, are kits comprising one or more reagents for the detection of a mutant AR polypeptide comprising a modification at amino acid position 876. Described herein, in certain embodiments, is a kit comprising one or more reagents for the detection of nucleic acid encoding a mutant AR polypeptide comprising a modification at amino acid position 876. In some embodiments, the modification is an amino acid substitution that is F876L. In some embodiments, the kits comprises a pair oligonucleotide primers that flank the nucleic acid region encoding amino acid 876 of a AR polypeptide. In some embodiments, the kits comprises an oligonucleotide primer that (a) binds to nucleic acid encoding a modified AR that is modified at amino acid position 876; and (b) does not bind to nucleic acid encoding the wild-type AR having phenylalanine at amino acid position 876. In some embodiments, the kits comprises a microchip comprising (a) a modified AR polypeptide having a modification that is F876S, or a portion thereof comprising a modification that is F876S; or (b) a nucleic acid molecule encoding a mutant AR polypeptide having a modification that is F876S, or a portion thereof comprising a modification that is F876S.

[0026] Described herein, in certain embodiments, are systems for detecting a modified AR that is resistant to inhibition with an a first- or second-generation AR antagonist in a subject,

comprising: (a) a sample containing a nucleic acid molecule encoding a AR polypeptide from the subject; and (b) a microarray comprising nucleic acid encoding a mutant AR polypeptide or a portion thereof that is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the microarray is contained on a microchip. Described herein, in certain embodiments, are systems for detecting a modified AR that is resistant to inhibition with a first- or second- generation AR antagonist in a subject, comprising: (a) a sample containing a nucleic acid molecule encoding a AR polypeptide from the subject; and (b) a sequence specific nucleic acid probe, wherein the sequence specific nucleic acid probe: (i) binds to nucleic acid encoding a modified AR that is modified at amino acid position 876; and (ii) does not bind to nucleic acid encoding the wild-type AR having phenylalanine at amino acid position 876. Described herein, in certain embodiments, are systems for detecting a modified AR that is resistant to inhibition with a first- or second- generation AR antagonist in a subject, comprising: (a) a sample containing a nucleic acid molecule encoding a AR polypeptide from the subject; and (b) a pair oligonucleotide primers that flank the nucleic acid region encoding amino acid 876 of a AR polypeptide.

[0027] Described herein, in certain embodiments, are isolated antibodies that bind to a modified androgen receptor polypeptide provided herein, wherein the antibody does not bind to or binds with lower affinity to a wild-type AR polypeptide having the sequence of amino acids set forth in SEQ ID NO: 1. In some embodiments, the modified AR polypeptide comprises a modification at amino acid 876. In some embodiments, the modification is an amino acid substitution that is F876L.

[0028] Described herein, in certain embodiments, are methods for maintenance therapy in a patient having a cancer, comprising: (a) administering to the patient a maintenance therapy regimen comprising administering a therapeutically effective dose of first- or second- generation AR antagonist; and (b) monitoring the patient at predetermined intervals of time over the course of the maintenance therapy regimen to determine whether the subject has mutation in an endogenous gene encoding AR that results in a modification at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, monitoring comprises: testing a sample containing a nucleic acid molecule encoding a AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the methods further comprise discontinuing maintenance therapy regimen if the subject has the mutation or continuing maintenance therapy regimen if the subject does not have the modification. In some

embodiments, the methods further comprise administering third-generation AR antagonist that inhibits the modified AR if the subject has the modification. In some embodiments, the modification in the AR polypeptide is F876L. In some embodiments, the first- or second-generation AR antagonist inhibits a wild-type AR polypeptide by competitive antagonism. In some embodiments, the second-generation AR antagonist is selected from among ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the cancer is a prostate cancer, a breast cancer, a bladder cancer, or a hepatocellular cancer. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is a castration resistant prostate cancer. In some embodiments, the predetermined interval of time is every week, every month, every 2 months, every 3 months, every 4 months, every 5 months, every 6 months, every 7 months, every 8 months, every 9 months, every 10 months, every 11 months, or every year.

[0029] Other objects, features and advantages of the polypeptides, nucleic acids, compounds, methods and compositions described herein will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments, are given by way of illustration only, since various changes and modifications within the spirit and scope of the instant disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **Figure 1** illustrates ARN-509 and enzalutamide resistance. (A) LNCaP and LNCaP ARN-509r1 cell proliferation. LNCaP and LNCaP ARN-509r1 cells were cultured in the presence of hormone depleted medium for 2 days followed by ligand addition. Proliferation is quantified by CellTiter-Glo® (Promega Corp.) luminescence based viability assay after 7 day compound treatment. (B) Agonist proliferation assay of LNCaP/AR-Luc, LNCaP/AR-Luc ENZr2 and LNCaP ARN-509r2 cell lines. Cells were cultured in the presence of hormone depleted medium for 2 days followed by ligand treatment for 7 days. Proliferation is quantified by CellTiter-Glo® luminescence based viability assay. (C) Antagonist proliferation assay of parental and 2nd generation anti-androgen resistant cell lines. Cells were cultured in the presence of hormone depleted medium for 2 days followed by ligand treatment in the presence of R1881 (final concentration = 100 pM). Proliferation is quantified by CellTiter-Glo luminescence based viability assay. (D) Schematic representation of AR domain structure showing amino acids that when mutated display altered ligand activity in CRPC.

[0031] **Figure 2** illustrates AR levels in parental and 2nd generation anti-androgen resistant cell lines. Protein extracts were generated from cells cultured in hormone depleted medium for 3

days. AR protein levels were analyzed and by western blot. AR levels were quantified and normalized to α -tubulin and expressed relative to LNCaP cells.

[0032] **Figure 3** illustrates ARN-509 and enzalutamide are partial agonists of AR F876L. (A) Transcriptional agonist and antagonist activity of ARN-509 and enzalutamide on wild-type or F876L AR. Transcriptional activation of a 4X ARE-luciferase reporter was measured in the presence of increasing compound concentration in the absence or presence of 1 nM R1881 (for wild-type AR) or 5 nM R1881 (for F876L AR). (B) Transcriptional agonist activity of 1st and 2nd generation anti-androgens and prednisone on wild-type, F876L, T877A, F876L/T877A, L701H, H874Y and W741C AR dependent activation of 4X ARE-Luciferase reporter was measured in transiently transfected HepG2 cells.

[0033] **Figure 4** illustrates VP16-AR (A) and F876L VP16-AR (B) agonist and antagonist activity of ARN-509 and enzalutamide. 4X ARE-luciferase reporter activity was monitored in the presence of increasing compound concentration in the absence or presence of 90 pM R1881 (for wild-type VP16-AR) or 1 nM R1881 (for F876L VP16-AR).

[0034] **Figure 5** illustrates a competitive binding assay of wild-type AR (A) vs. F876L AR (B). ³H-R1881 binding performed in PC3 cell extracts expressing wild-type or F876L AR. Data is representative of 3 independent experiments. Error bars, SEM; n=2.

[0035] **Figure 6** illustrates AR levels in AR overexpressing cell lines. Protein extracts were generated from LNCaP, LNCaP/AR(cs), LNCaP/SR α F876L and LNCaP/pCDNAF876L cells cultured in hormone depleted medium for 3 days. AR protein levels were analyzed and by Western blot. AR levels were quantified and normalized to actin and expressed relative to LNCaP cells.

[0036] **Figure 7** illustrates F876L AR mutation confers partial agonist activity to ARN-509 and enzalutamide. (A) LNCaP/AR(cs) and LNCaP/SR α F876L cell proliferation. Cells were cultured in the presence of hormone depleted medium for 2 days followed by ligand treatment for 7 days. Proliferation is quantified by CellTiter-Glo luminescence based viability assay. (B) LNCaP/AR(cs), LNCaP/SR α F876L and LNCaP/pCDNAF876L cell proliferation. Cells were cultured in hormone depleted medium for 2 days followed by ligand treatment for 7 days. For antagonist assays, compounds were added in the presence of 200 pM R1881 (100 pM final concentration). Proliferation was quantified using the luminescence based CellTiter-Glo® assay.

[0037] **Figure 8** illustrates AR N/C interaction assay. Ligand induced N/C interaction was monitored via mammalian two hybrid assay in HepG2 cells. Antagonists were assayed at 8 μ M, R1881 at 1nM.

[0038] **Figure 9** illustrates AR ChIP analysis of AR target genes. ChIP assays were performed on LNCaP/AR(cs) and LNCaP/SRαF876L cells incubated for 3 days in hormone depleted medium followed by 4 hour ligand treatment. Cells were treated in with 10 μM antagonist in the presence or absence of 1 nM R1881. Data for AR and non-specific IgG control is presented as percent input.

[0039] **Figure 10** illustrates AR F876L mutation confers ARN-509 and enzalutamide resistance in vivo. LNCaP/AR(cs) and LNCaP/SRαF876L tumor xenografts. Castrate male mice bearing tumors were treated daily with vehicle or 30 mg/kg/day compound. Tumor growth for each group is presented as the average tumor volume ± SEM.

[0040] **Figure 11** illustrates the dosing schedule for the open-label phase 1/2 safety, pharmacokinetic, and proof-of-concept study of ARN-509 in patients with progressive advanced, metastatic Castration-Resistant Prostate Cancer.

[0041] **Figure 12** illustrates Identification of AR-F876L in ARN-509 treated patients. (A) PSA response of 29 patients analyzed for F876L mutation. Terminal end of PSA response line is time at which the patient plasma was screened for F876L mutation using BEAMing analysis. The plasma used in this study was initially collected to determine pharmacokinetics of ARN-509 and as such the samples were not prepared using methodology to maximize ratio of ctDNA to lymphocyte DNA. (B) PSA response of patient positive for F876L. PSA response of patient 7 at indicated treatment cycle. Circulating plasma was analyzed for the F876L at times indicated with arrows. The plasma samples with no detectable mutant are notated as “w.t.”, the presence of the F876L mutation is represented by “m”. A plasma sample was called positive for the mutant if the percent of mutant beads was above the cut-off (0.02%) and the number of mutant copies estimated to be ≥ 0.5 (number of genome equivalents in the plasma sample x mutant bead fraction = ≥ 0.5).

[0042] **Figure 13** illustrates the relative amount of prostate specific antigen (PSA) detected over the course of the Phase 1/2 ARN-509 clinical study in each of the three patients (7, 10, and 13) bearing detectable somatic F876L mutations in AR. Arrow indicates sample in which the mutation(s) were detected.

DETAILED DESCRIPTION OF THE INVENTION

Certain Terminology

[0043] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. All patents, patent applications, published applications and publications, GENBANK sequences, websites and other published materials referred to throughout the entire

disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information. Generally, the procedures for cell culture, cell infection, antibody production and molecular biology methods are methods commonly used in the art. Such standard techniques can be found, for example, in reference manual, such as, for example, Sambrook et al. (2000) and Ausubel et al. (1994).

[0044] As used herein, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. In this application, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of “or” means “and/or” unless stated otherwise. Furthermore, use of the term “including” as well as other forms (e.g., “include”, “includes”, and “included”) is not limiting.

[0045] As used herein, ranges and amounts can be expressed as “about” a particular value or range. About also includes the exact amount. Hence “about 5 μ g” means “about 5 μ g” and also “5 μ g.” Generally, the term “about” includes an amount that would be expected to be within experimental error.

[0046] As used herein, an androgen receptor (AR) polypeptide refers to any androgen receptor protein or polypeptide, including, but not limited to, a recombinantly produced protein, a synthetically produced protein, a native androgen receptor protein, and an androgen receptor protein extracted from cells or tissues. An AR polypeptide includes related polypeptides from different species including, but not limited to animals of human and non-human origin. AR polypeptides of non-human origin include, but are not limited to, non-human primate (e.g. chimpanzee and ape), murine (e.g., mouse and rat), canine (dog), feline (cat), leporine (rabbit), avian (bird), bovine (cow), ovine (sheep), porcine (pig), equine (horse), piscine (fish), ranine (frog) and other mammalian or non-mammalian AR polypeptides. Exemplary AR polypeptides include, for example, SEQ ID NOS: 1-17. An androgen receptor polypeptide includes wild-type androgen receptor, allelic variant isoforms, somatic mutations including those found in tumors, synthetic molecules from nucleic acids, protein isolated from human tissue and cells, and modified forms thereof. The androgen receptor polypeptides provided herein can be further modified by modification of the primary amino acid sequence, by deletion, addition, or substitution of one or more amino acids. An androgen receptor polypeptide includes any AR

polypeptide or a portion thereof having AR activity, including for example, fusion polypeptides of an AR ligand binding domain to a heterologous DNA binding domain.

[0047] As used herein, a mutant androgen receptor (AR) polypeptide, a mutant AR protein, a modified AR polypeptide, or a modified AR protein or are used interchangeably herein and refer to an androgen receptor polypeptide that is modified at one or more amino acid positions. Exemplary modifications include, but are not limited to, substitutions, deletions or additions of amino acids.

[0048] As used herein, the term “anti-androgen” refers to a group of hormone receptor antagonist compounds that are capable of preventing or inhibiting the biologic effects of androgens on normally responsive tissues in the body.

[0049] As used herein, the term “AR inhibitor” or “AR antagonist” are used interchangeably herein and refer to an agent that inhibits or reduces at least one activity of an AR polypeptide. Exemplary AR activities include, but are not limited to, co-activator binding, DNA binding, ligand binding, or nuclear translocation.

[0050] As used herein, a “full antagonist” refers to an antagonist which, at an effective concentration, essentially completely inhibits an activity of an AR polypeptide. As used herein, a “partial antagonist” refers an antagonist that is capable of partially inhibiting an activity of an AR polypeptide, but that, even at a highest concentration is not a full antagonist. By ‘essentially completely’ is meant at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% at least about 99%, or greater inhibition of the activity of an AR polypeptide.

[0051] As used herein, the term “third-generation AR inhibitor” or “third-generation AR antagonist” are used interchangeably herein and refer to an agent that inhibits at least one activity of an AR polypeptide containing one or more amino acid modifications that confers resistance to inhibition by a second-generation AR antagonist, such as, for example, ARN-509 (CAS No. 956104-40-8), enzalutamide (also known as MDV3100; CAS No: 915087-33-1), or RD162 (CAS No. 915087-27-3). In some embodiments, a third-generation AR inhibitor inhibits at least one activity of a wild-type AR polypeptide, such as, but not limited to, co-activator binding, DNA binding, ligand binding, or nuclear translocation. In some embodiments, a third-generation AR inhibitor inhibits an activity of a mutant AR polypeptide that is also inhibited by a first- or second-generation AR inhibitor.

[0052] As used herein, the phrase “resistant to inhibition” refers to a decrease in ability of an AR antagonist to inhibit (i.e. antagonize) one or more activities of a modified AR polypeptide compared to a wild type AR polypeptide. For example, the AR antagonist is less effective at

inhibiting a modified AR polypeptide compared to a wild type AR polypeptide. In some embodiments, the decreased antagonistic activity against a modified AR polypeptide is about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98% or 100% compared to the antagonistic activity against a wildtype AR polypeptide. Exemplary AR activities include, but are not limited to, co-activator binding, DNA binding, ligand binding, or nuclear translocation. In some embodiments, the AR antagonist is unable to bind to or binds with decreased affinity to a modified AR polypeptide.

[0053] As used herein, the term “second-generation AR inhibitor” or “second-generation AR antagonist” are used interchangeably herein and refer to an agent that exhibits full antagonist activity of a wild-type AR polypeptide, but does not exhibit full antagonist activity of an AR polypeptide containing one or more amino acid modifications that confers resistance to inhibition, such as a modification at amino acid position 876 in the AR polypeptide. In some embodiments, the second-generation AR inhibitor does not exhibit full antagonist activity on an AR polypeptide having an amino acid substitution that is F876L. In some embodiments, the second-generation AR inhibitor induces activity of AR (i.e. is an AR agonist) at concentrations that are equivalent or higher than the concentration required to inhibit a wild-type AR. Second-generation AR inhibitors differ from first-generation AR inhibitors, such as bicalutamide and flutamide, in that second-generation AR inhibitors act as full antagonists in cells expressing elevated levels of AR, such as for example, in castration resistant prostate cancers (CRPC). First-generation AR inhibitors, such as bicalutamide and flutamide, act as agonists in CRPC. Exemplary second-generation AR inhibitors include ARN-509, enzalutamide and RD162. In some embodiments, a second-generation AR inhibitor is an agonist of a mutant AR polypeptide provided herein. In some embodiments, a second-generation AR inhibitor binds to an AR polypeptide at or near the ligand binding site of the AR polypeptide.

[0054] The term “nucleic acid” refers to deoxyribonucleotides, deoxyribonucleosides, ribonucleosides, or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogs of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless specifically limited otherwise, the term also refers to oligonucleotide analogs including PNA (peptidonucleic acid), analogs of DNA used in antisense technology (e.g., phosphorothioates, phosphoroamidates). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (including but not limited to, degenerate codon substitutions) and complementary sequences as well as the sequence explicitly

indicated. Specifically, degenerate codon substitutions are achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al. (1991)*Nucleic Acid Res.* 19:5081; Ohtsuka et al. (1985)*J. Biol. Chem.* 260:2605-2608; and Cassol et al. (1992)*Mol. Cell. Probes* 6, 327-331; and Rossolini et al. (1994) *Mol. Cell. Probes* 8:91-98).

[0055] The term “amino acid” refers to naturally occurring and non-naturally occurring amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refers to agents that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (such as, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid.

[0056] Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, are referred to by their commonly accepted single-letter codes.

[0057] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers in which one or more amino acid residues is a non-naturally occurring amino acid, e.g., an amino acid analog. The terms encompass amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

[0058] As used herein, modification refers to modification of a sequence of amino acids of a polypeptide or a sequence of nucleotides in a nucleic acid molecule and includes deletions, insertions, and replacements of amino acids and nucleotides, respectively.

[0059] To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences can be aligned for optimal comparison purposes (e.g., gaps are introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions can then be compared. When a

position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). In some embodiments the two sequences are the same length.

[0060] To determine percent homology between two sequences, the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877 is used. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described or disclose herein. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See the website of the National Center for Biotechnology Information for further details (on the World Wide Web at ncbi.nlm.nih.gov). Proteins suitable for use in the methods described herein also includes proteins having between 1 to 15 amino acid changes, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions, deletions, or additions, compared to the amino acid sequence of any protein described herein. In other embodiments, the altered amino acid sequence is at least 75% identical, e.g., 77%, 80%, 82%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any protein described herein. Such sequence-variant proteins are suitable for the methods described herein as long as the altered amino acid sequence retains sufficient biological activity to be functional in the compositions and methods described herein. Where amino acid substitutions are made, the substitutions should be conservative amino acid substitutions. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* Molecular

Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224). The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff et al (1992) *Proc. Natl. Acad. Sci. USA*, 89:10915-10919). Accordingly, the BLOSUM62 substitution frequencies are used to define conservative amino acid substitutions that, in some embodiments, are introduced into the amino acid sequences described or disclosed herein. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the language “conservative amino acid substitution” preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

[0061] As used herein, corresponding residues refers to residues that occur at aligned loci. Related or variant polypeptides are aligned by any method known to those of skill in the art. Such methods typically maximize matches, and include methods such as using manual alignments and by using the numerous alignment programs available (for example, BLASTP) and others known to those of skill in the art. By aligning the sequences of polypeptides, one skilled in the art can identify corresponding residues, using conserved and identical amino acid residues as guides. Corresponding positions also can be based on structural alignments, for example by using computer simulated alignments of protein structure. In other instances, corresponding regions can be identified.

[0062] As used herein, an allelic variant or allelic variation references to a polypeptide encoded by a gene that differs from a reference form of a gene (i.e. is encoded by an allele). Typically the reference form of the gene encodes a wild-type form and/or predominant form of a polypeptide from a population or single reference member of a species. Typically, allelic variants, which include variants between and among species, have at least 80%, 90% or greater amino acid identity with a wild-type and/or predominant form from the same species; the degree of identity depends upon the gene and whether comparison is interspecies or intraspecies. Generally, intraspecies allelic variants have at least about 80%, 85%, 90% or 95% identity or greater with a wild-type and/or predominant form, including 96%, 97%, 98%, 99% or greater identity with a wild-type and/or predominant form of a polypeptide.

[0063] As used herein, species variants refer to variants of the same polypeptide between and among species. Generally, interspecies variants have at least about 60%, 70%, 80%, 85%, 90%, or 95% identity or greater with a wild-type and/or predominant form from another species, including 96%, 97%, 98%, 99% or greater identity with a wild-type and/or predominant form of a polypeptide.

[0064] As used herein, the terms "treat," "treating" or "treatment," and other grammatical equivalents, include alleviating, abating or ameliorating one or more symptoms of a disease or condition, ameliorating, preventing or reducing the appearance, severity or frequency of one or more additional symptoms of a disease or condition, ameliorating or preventing the underlying metabolic causes of one or more symptoms of a disease or condition, inhibiting the disease or condition, such as, for example, arresting the development of the disease or condition, relieving the disease or condition, causing regression of the disease or condition, relieving a condition caused by the disease or condition, or inhibiting the symptoms of the disease or condition either prophylactically and/or therapeutically. In a non-limiting example, for prophylactic benefit, a third-generation AR inhibitor compound disclosed herein is administered to an individual at risk of developing a particular disorder, predisposed to developing a particular disorder, or to an individual reporting one or more of the physiological symptoms of a disorder. In some embodiments, a third-generation AR inhibitor compound disclosed herein is administered to a subject following treatment with one or more therapeutic agents. In some embodiments, a third-generation AR inhibitor compound disclosed herein is administered to a subject in combination with treatment with one or more therapeutic agents.

[0065] As used herein, prevention or prophylaxis refers to the reduction in the risk of developing a disease or condition.

[0066] The terms "effective amount", "therapeutically effective amount" or "pharmaceutically effective amount" as used herein, refer to an amount of an AR inhibitor compound that is sufficient to treat a disorder. In some embodiments, the result is a reduction in and/or alleviation of the signs, symptoms, or causes of a disorder, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses is the amount of the composition comprising an AR inhibitor compound disclosed herein required to provide a clinically significant decrease in a disorder. An appropriate "effective" amount in any individual case is determined using any suitable technique, (e.g., a dose escalation study).

[0067] The term "pharmaceutically acceptable" as used herein, refers to a material, (e.g., a carrier or diluent), which does not abrogate the biological activity or properties of an AR inhibitor compound described herein, and is relatively nontoxic (i.e., the material is administered

to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained).

[0068] As used herein, a control refers to a sample that is substantially identical to the test sample, except that it is not treated with a test parameter, or, if it is a plasma sample, it can be from a normal volunteer not affected with the condition of interest. A control also can be an internal control.

[0069] As used herein, the terms "subject", "individual" and "patient" are used interchangeably. None of the terms are to be interpreted as requiring the supervision of a medical professional (e.g., a doctor, nurse, physician's assistant, orderly, hospice worker). As used herein, the subject can be any animal, including mammals (e.g., a human or non-human animal) and non-mammals. In one embodiment of the methods and compositions provided herein, the mammal is a human.

[0070] As used herein, "contacting" refers to the act of touching, making contact, or of bringing substances into immediate proximity. "Contacting" can be achieved by mixing the components in a fluid or semi-fluid mixture.

Overview: AR Function and Drug Resistance in Cancer

[0071] Androgen receptor (AR) is a member of the steroid and nuclear receptor superfamily. Among this large family of proteins, only five vertebrate steroid receptors are known and include the androgen receptor, estrogen receptor, progesterone receptor, glucocorticoid receptor, and mineralocorticoid receptor. AR is a soluble protein that functions as an intracellular transcription factor. AR function is regulated by the binding of androgens, which initiates sequential conformational changes of the receptor that affect receptor-protein interactions and receptor-DNA interactions.

[0072] AR is mainly expressed in androgen target tissues, such as the prostate, skeletal muscle, liver, and central nervous system (CNS), with the highest expression level observed in the prostate, adrenal gland, and epididymis. AR in certain instance is activated by the binding of endogenous androgens, including testosterone and 5 α -dihydrotestosterone (5 α -DHT).

[0073] AR is a 110 kD nuclear receptor that, upon activation by androgens, mediates transcription of target genes that modulate growth and differentiation of prostate epithelial cells. AR is encoded by the *AR* gene located on the X chromosome at Xq11-12. The AR gene comprises 8 exons encoding the full-length androgen receptor. Similar to the other steroid receptors, unbound AR is mainly located in the cytoplasm and associated with a complex of heat shock proteins (HSPs) through interactions with the ligand-binding domain. Upon agonist binding, AR goes through a series of conformational changes: the heat shock proteins dissociate from AR, and the transformed AR undergoes dimerization, phosphorylation, and translocation to

the nucleus, which is mediated by the nuclear localization signal. The translocated receptor then binds to the androgen response element (ARE), which is characterized by the two hexameric six-nucleotide half-site consensus sequence 5'-TGTTCT-3' arranged as inverted repeats spaced by three random nucleotides and is located in the promoter or enhancer region of AR gene targets. Recruitment of other transcription co-regulators (including co-activators and co-repressors) and transcriptional machinery further ensures the appropriate modulation of AR-regulated gene expression. These processes are initiated by the ligand-induced conformational changes in the ligand-binding domain.

[0074] AR signaling is crucial for the development and maintenance of male reproductive organs including the prostate gland, as genetic males harboring loss of function AR mutations and mice engineered with AR defects do not develop prostates. This dependence of prostate cells on AR signaling continues even upon neoplastic transformation. Androgen depletion (e.g. using gonadotropin-releasing hormone (GnRH) agonists) continues to be the mainstay of prostate cancer treatment. However, androgen depletion is usually effective for a limited duration and prostate cancer evolves to regain the ability to grow despite low levels of circulating androgens.

[0075] Prostate cancer is the most prevalent cancer in men. Prostate cancer represents approximately 29 percent of all new cancer cases diagnosed and 10 percent of cancer deaths in males. In addition, American men over their lifetime have a roughly 17 percent chance of developing invasive prostate cancer. At initial diagnosis, a large percentage of prostate cancers have low to medium risk, meaning that the 10 year mortality risk is relatively low (up to 24%) with little intervention. However, advanced and metastatic prostate cancers have mean survival of 2.5-3 years and are subject to aggressive treatment including surgery and chemical castration therapy.

[0076] Given that most prostate cancer cells depend on AR for their proliferation and survival, treatment generally consists of administration of agents that block production of testosterone (e.g. GnRH agonists), alone or in combination with anti-androgens (e.g. bicalutamide), which antagonize the effect of any residual testosterone. Unfortunately, while often initially successful as evidenced by a drop in prostate specific antigen (PSA) and regression of visible tumor if present, metastatic tumors inevitably become resistant to hormonal therapy at which stage no curative treatment exists.

[0077] Prostate cancers resistant to hormonal therapies are currently referred to as 'castration resistant', implying that they have progressed beyond the point at which drugs targeting any point on the androgen axis would have any clinical utility. Pre-clinical and clinical evidence

indicate that the AR is a viable therapeutic target even in castration resistant cancers. AR mutations have been reported to occur in up to 33 percent of prostate cancer cases and are most commonly observed following treatment in the AR dependent castration resistant state. Mutations have been found that alter ligand specificity and potency and that result in ligand independent receptor activity. The specific mutations vary widely but appear to be dependent upon treatment regimen. Additionally, upregulation of the AR itself has been associated with progression to the castration resistant state in both patients and animal models.

[0078] Two agents, abiraterone acetate (Zytiga; CAS No. 154229-19-3) and MDV3100 (enzalutamide; CAS No. 915087-33-7), have recently been employed in late stage clinical testing for the treatment of men with castration-resistant prostate cancer (CRPC). Abiraterone acetate targets 7- α -hydroxylase/17,20-lyase (CYP17A), thereby inhibiting residual androgen biosynthesis. MDV3100 is an anti-androgen discovered in a screen for potent anti-androgens lacking agonist activity in context of AR overexpression. The clinical efficacies of MDV3100 and abiraterone acetate support the hypothesis that AR continues to promote growth and survival of castration resistant prostate cancer. Unfortunately, similar to first-generation androgen ablation therapies, prolonged treatment with abiraterone acetate or second-generation AR antagonists ultimately results in resistance.

[0079] Resistance to abiraterone acetate and MDV3100 has been observed in both models of prostate cancer and in patients. Preliminary data suggests that, similar to castration resistant prostate cancer, second-generation anti-androgen resistance develops through multiple mechanisms and AR is thought to remain a therapeutic target in this setting. In both xenograft models and in patients, CYP17A upregulation and the presence of picogram levels of androgens have been noted in resistant populations. CYP17A upregulation presumably promotes resistance via AR activation by intratumoral androgen synthesis. In other cases, resistance correlates with increased AR levels as well as nuclear localization. Additionally, numerous cellular signaling pathways known to activate AR in the absence of endogenous ligands may promote second-generation therapy resistance. The observation that tumors with high levels of activated Src respond poorly to MDV3100 and abiraterone acetate treatment supports this hypothesis. To date, no AR mutations have been described that confer resistance to MDV3100, ARN-509 or abiraterone.

[0080] ARN-509 is a synthetic thiohydantoin compound discovered using structure-activity relationship (SAR)-guided medicinal chemistry to identify nonsteroidal anti-androgens that retain full antagonist activity in the setting of increased AR expression. ARN-509 exhibits anti-

tumor activity in castration-sensitive and resistant xenograft models of prostate cancer and anti-androgenic effects in dogs that phenocopy castration.

Identification of AR Inhibitor-Resistant Cell Lines and a Mutant AR polypeptide

[0081] Described herein are working examples demonstrating the production of drug resistant cell lines. In some embodiments, the methods provided are employed for the generation of a cell line that is resistant to inhibition by a second-generation-AR antagonist, such as, but not limited to, ARN-509, enzalutamide (MDV3100) or RD162. In some embodiments, the methods provided are employed for the generation of a cell line that is resistant to inhibition by an AR antagonist that inhibits androgen production and exhibits binding to an AR receptor. In some embodiments, the AR antagonist that inhibits androgen production is a CYP17A inhibitor and exhibits binding to AR. In some embodiments, the AR antagonist that inhibits androgen production and exhibits AR binding is galeterone (TOK001) or abiraterone acetate. In some embodiments, the AR antagonist that inhibits androgen production and exhibits AR binding is TAK-700.

[0082] As described herein, resistant cell lines were generated *in vivo* in a castration resistant prostate cancer (CRPC) cell xenograft and *in vitro* in androgen responsive prostate cancer cell lines by exposure to increasing concentrations of the anti-androgen drugs ARN-509 or MDV3100. In cell proliferation and transcriptional assays, the cell lines segregated into two distinct classes.

[0083] Class 1 cell lines expressed a higher level of AR compared to their parental cell lines and proliferated in the absence of added androgens. Ligand-independent growth of the class 1 cells was unaltered in the presence of ARN-509, MDV3100 or bicalutamide. The synthetic androgen, R1881, inhibited proliferation in the class 1 cells and is antagonized by either MDV3100 or ARN-509, indicating that AR in these cell lines still binds to MDV3100 and ARN-509.

[0084] Class 2 resistant cell lines remained androgen dependent for growth similar to their parental cell lines. While ARN-509 and MDV3100 inhibited proliferation of the parental cell line, both compounds exhibited agonist activity and stimulated proliferation of the class 2 cell lines. Analysis of the nucleic acid encoding AR in the class 2 cell lines revealed that the cell lines expressed a mutant AR with a mutation in the ligand binding domain. The mutation was a thymidine (T) to cytosine (C) missense mutation that resulted in an amino acid substitution of phenylalanine at position 876 of the wild-type AR for leucine (F876L).

[0085] As described herein, in the examples, mutations in the AR gene have been identified in plasma samples from prostate cancer patients undergoing ARN-509 Phase 1/2 clinical studies.

The mutations identified include a thymidine (T) to cytosine (C) missense mutation at position 2988 (first position of the codon encoding phenylalanine, which is encoded in exon 8 of the AR gene), and a cytosine (C) to alanine (A) missense mutation at position 2990 (third position of the codon encoding phenylalanine) in an amino acid substitution of phenylalanine at position 876 of the wild-type AR for leucine (F876L). The patients identified as having these mutations also exhibited increasing levels of prostate specific antigen (PSA) over the course of the study indicating increasing resistance to treatment.

[0086] Described herein are mutant AR polypeptides that contain an amino acid substitution of phenylalanine at position 876 of the wild-type AR for leucine (F876L) and nucleic acids encoding the polypeptides. Also described herein are methods of producing the mutant AR nucleic acids and polypeptides described herein. Also described herein are compositions, combinations and kits containing the mutant AR nucleic acids and polypeptides described herein. Also provided are methods of using the mutant AR polypeptides for identifying mutant AR interacting molecules, including androgen inhibitors, including third-generation AR inhibitor compounds. Also provided are compositions containing the mutant AR interacting molecules, including pharmaceutical compositions thereof. Also provided are methods of treatment using the identified mutant AR interacting molecules. Also described herein are mutant AR nucleic acids that are synthetic nucleic acids. Also described herein are mutant AR nucleic acids that are cDNA molecules. Also described herein are mutant AR polypeptides produced by mutant AR nucleic acids that are synthetic nucleic acids. Also described herein are mutant AR polypeptides produced by mutant AR nucleic acids that are cDNA molecules. Also described herein are mutant AR nucleic acids that do not contain AR genomic DNA. Also described herein are mutant AR nucleic acids that are unmethylated. Also described herein are mutant AR nucleic acids that do not contain AR intron sequences. Also described herein are mutant AR nucleic acids that comprises a sequence of nucleotides from two or more exons of the AR genomic sequence. In some embodiments, the mutant AR nucleic acids comprise a sequence of nucleotides that encode phenylalanine at a position corresponding to position 876 of the wild-type AR polypeptide.

[0087] As described herein, identification of a mutation at position 876 in the AR, such as for example F876L, allows for the design and screening of inhibitors effective for inhibition of a mutant AR having one or more resistance mutations. Such inhibitors are useful in clinical and therapeutic applications. In some embodiments, the inhibitors are useful for the treatment of a cancer, such as for example, an AR-mediated cancer, such as, for example, a prostate cancer,

breast cancer, liver (i.e. hepatocellular) cancer, or bladder cancer, such as for example, a drug resistant prostate, breast, liver (i.e. hepatocellular), or bladder cancer.

[0088] As described herein, in some embodiments, subjects are screened for the identification of a mutation at position 876 in AR, such as for example F876L. In some embodiments, identification of such a mutation allows for the prescription of a cancer treatment or modification of a cancer treatment. In some embodiments, identification of such a mutation is used to stratify subjects for a particular therapy, such as for example, therapy with an inhibitor that inhibits the activity of the mutant AR (i.e. a third-generation AR inhibitor). In some embodiments, identification of such a mutation is used to characterize a subject as having a high risk of relapse of a disease or condition, such as, for example, a prostate cancer, breast cancer, liver (i.e. hepatocellular) cancer, or bladder cancer. In some embodiments, identification of such a mutation is used to characterize a subject as lacking responsiveness to particular AR inhibitor, such as for example ARN-509, MDV3100, or RD162. In some embodiments, identification of such a mutation is used to characterize a subject as lacking responsiveness to an AR inhibitor that is a CYP17A inhibitor, such as, for example galeterone (TOK001), TAK-700 or abiraterone acetate.

Mutant AR Polypeptides

[0089] Provided herein are mutant AR polypeptides. In some embodiments, the isolated mutant AR polypeptides are isolated mutant AR polypeptides. In some embodiments, the isolated mutant AR polypeptides are non-native mutant AR polypeptides. In some embodiments, the isolated mutant AR polypeptides are recombinant mutant AR polypeptides. In some embodiments, the mutant AR polypeptides provided herein exhibit androgen receptor activity. For example, the mutant AR polypeptides bind to androgen response elements and promote the expression of AR-responsive genes. In some embodiments, the mutant AR polypeptides contain one or more amino acid substitutions that confers resistance to full antagonism by an anti-androgen. In some embodiments, the mutant AR polypeptides contain one or more amino acid substitutions that confers resistance to full antagonism by a second-generation AR inhibitor, such as, but not limited to, ARN-509, enzalutamide (MDV3100) or RD162. In some embodiments, the mutant AR polypeptides contain one or more amino acid substitutions that confers resistance to inhibition by ARN-509. In some embodiments, the mutant AR polypeptides contain one or more amino acid substitutions that confers resistance to inhibition by enzalutamide (MDV3100). In some embodiments, the mutant AR polypeptides contain one or more amino acid substitutions that confers resistance to inhibition by RD162.

[0090] In some embodiments, treatment of a mutant AR polypeptide provided herein with a second-generation AR inhibitor induces AR activity. For example, the second-generation AR inhibitor acts as an agonist of the mutant AR polypeptide. In some embodiments, treatment of a mutant AR polypeptide provided herein with ARN-509 induces AR activity. In some embodiments, treatment of a mutant AR polypeptide provided herein with enzalutamide (MDV3100) induces AR activity. In some embodiments, treatment of a mutant AR polypeptide provided herein with RD162 induces AR activity.

[0091] In some embodiments, the mutant AR polypeptide comprises a modification at a position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 (Accession No. P10275) or a corresponding position in the wild-type AR polypeptide set forth in SEQ ID NO: 2 (Accession No. NP_000035.2). In some embodiments, the modification is a substitution of the amino acid phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the mutant androgen receptor (AR) polypeptide does not comprise phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1.

[0092] In some embodiments, the modification is a substitution of the amino acid phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1, and the substituted amino acid is selected from among leucine, isoleucine, valine, alanine, glycine, methionine, serine, threonine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid. In some embodiments, the mutant androgen receptor (AR) polypeptide comprises a leucine, isoleucine, valine, alanine, glycine, methionine, serine, threonine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid or glutamic acid at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the modification is a substitution of the amino acid phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1, and the substituted amino acid is selected from among leucine, isoleucine, valine, alanine, glycine, methionine and tryptophan. In some embodiments, the mutant androgen receptor (AR) polypeptide comprises a leucine, isoleucine, valine, alanine, glycine, methionine or tryptophan at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the modification is a substitution of the amino acid phenylalanine to leucine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID

NO: 1. In some embodiments, the mutant AR polypeptide comprises a leucine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1.

[0093] In some embodiments, the modification comprises a deletion of the amino acid phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1.

[0094] In some embodiments, the mutant AR polypeptide comprises a sequence of amino acids set forth in SEQ ID NO: 5. In some embodiments the mutant AR polypeptide comprises a polypeptide having a leucine at the position corresponding to amino acid position 876 and having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to the polypeptide having the sequence set forth in SEQ ID NO: 5. In some embodiments the mutant AR polypeptide comprises a polypeptide not having a phenylalanine at the position corresponding to amino acid position 876 and having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to the polypeptide having the sequence set forth in SEQ ID NO: 5.

[0095] In some embodiments, the mutant AR polypeptide comprises a modification at amino acid position 876 and a modification at one or more additional amino acid positions. In some embodiments, the mutant AR polypeptide comprises a modification at amino acid position 876 and a modification at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid positions. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification at one additional amino acid position. In some embodiments, the mutant AR polypeptide comprises a leucine at amino acid position 876 and a modification at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more additional amino acid positions. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a modification at one additional amino acid position. In some embodiments, the modification at amino acid position 876 is a substitution that is F876L.

[0096] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification at an additional amino acid position that confers resistance to a first- or second- generation AR antagonist or an AR antagonist that inhibits androgen production. In some embodiments, the modification at the amino acid position in addition to the modification at amino acid 876 increases the resistance of the AR polypeptide to a first- or second- generation AR antagonist or an AR antagonist that inhibits androgen production compared to an AR polypeptide comprising the modification at amino acid position 876 alone. In some embodiments, the modification at amino acid position 876 is a substitution that is F876L.

[0097] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification selected from among AR modifications described in, for example, Grasso et al. (2012) *Nature* 487(7406):239-43; Ning et al. (2012) *Urology* 80(1):216-8; Cong et al. (2012) *Gene* 500(2):220-3; Hay et al. (2012) *PLoS One* 2012;7(3):e32514; Koochekpour (2010) *Asian J Androl.* 12(5):639-57 ; Waltering et al. (2012) *Mol Cell Endocrinol.* 360:38-43; Robbins (2012) *Mol Cell Endocrinol.* 352(1-2):26-33; or Gottlieb et al. (2012) *Hum Mutat.* 33(5):887-94. In some embodiments, the modification at amino acid position 876 is a substitution that is F876L.

[0098] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification selected from among AR modifications associated with castration resistant prostate cancer. In some embodiments, the modification associated with castration resistant prostate cancer is an amino acid substitution such as, for example, T877A, W741C, W741L, W741R, L701H or H874Y. In some embodiments, the modification at amino acid position 876 is a substitution that is F876L.

[0099] In some embodiments, the mutant AR polypeptide comprises a modification at amino acid position 876 and a modification at amino acid position 877. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a modification at amino acid position 877. In some embodiments, the modification at amino acid position 877 is a substitution of the amino acid threonine. In some embodiments, the substituted amino acid at amino acid position 877 is selected from among leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid. In some embodiments, the substituted amino acid at amino acid position 877 is alanine. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and an alanine at amino acid position 877. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and an alanine at amino acid position 877.

[00100] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a modification at amino acid position 741. In some embodiments, the modification at amino acid position 741 is a substitution of the amino acid tryptophan. In some embodiments, the substituted amino acid at amino acid position 741 is selected from among leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, threonine, cysteine, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid. In some embodiments, the substituted amino acid at amino acid

position 741 is leucine, cysteine or arginine. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a leucine at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a cysteine at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and an arginine at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a leucine at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a cysteine at amino acid position 741. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and an arginine at amino acid position 741.

[00101] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification at amino acid position 701. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a modification at amino acid position 701. In some embodiments, the modification at amino acid position 701 is a substitution of the amino acid leucine. In some embodiments, the substituted amino acid at amino acid position 701 is selected from among isoleucine, valine, alanine, phenylalanine, glycine, methionine, histidine, serine, threonine, cysteine, lysine, arginine, tryptophan, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid. In some embodiments, the substituted amino acid at amino acid position 701 is histidine. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a histidine at amino acid position 701. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a histidine at amino acid position 701.

[00102] In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a modification at amino acid position 874. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a modification at amino acid position 874. In some embodiments, the modification at amino acid position 874 is a substitution of the amino acid histidine. In some embodiments, the substituted amino acid at amino acid position 874 is selected from among leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, threonine, cysteine, lysine, arginine, tryptophan, proline, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid. In some embodiments, the substituted amino acid at amino acid position 874 is tyrosine. In some embodiments, the mutant AR polypeptide comprises a modification at position 876 and a tyrosine at amino acid position 874. In some embodiments, the mutant AR polypeptide comprises a leucine at position 876 and a tyrosine at amino acid position 874.

[00103] In some embodiments, the mutant AR polypeptide is an AR polypeptide variant that comprises a modification at position 876 and one or more additional amino acid positions relative to the wild-type AR polypeptide set forth in SEQ ID NO: 1. Exemplary variants include, for example, species variants, allelic variants, RNA splicing variants and variants that contain conservative and non-conservative amino acid mutations. In some embodiments, the AR polypeptide variant comprises a polyglutamine tract of about 6 consecutive glutamine residues to about 39 consecutive glutamine residues. In some embodiments, the AR polypeptide variant comprises a polyglutamine tract of about 16 consecutive glutamine residues to about 29 consecutive glutamine residues. In some embodiments, the AR polypeptide variant comprises a polyglutamine tract of about 21 consecutive glutamine residues. In some embodiments, the AR polypeptide variant comprises a polyglutamine tract of about 22 consecutive glutamine residues. In some embodiments, the AR polypeptide variant comprises a polyglutamine tract of about 23 consecutive glutamine residues. In some embodiments, the AR polypeptide variant comprises a polyglycine tract of about 10 consecutive glycine residues to about 27 consecutive glycine residues. In some embodiments, the AR polypeptide variant comprises a polyglycine tract of about 23 consecutive glycine residues. In some embodiments, the AR polypeptide variant comprises a polyglycine tract of about 24 consecutive glycine residues.

[00104] In some embodiments, the mutant AR polypeptide comprises a portion of the mutant AR polypeptide set forth in SEQ ID NO: 5. In some embodiments, the portion exhibits an activity of an AR polypeptide. In some embodiments, the mutant AR polypeptide comprises the DNA binding domain of an AR polypeptide and the ligand binding domain of the AR polypeptide comprising the modification at amino acid position 876 of the mutant AR polypeptide set forth in SEQ ID NO: 5. In some embodiments, the mutant AR polypeptide consists essentially of the DNA binding domain of an AR polypeptide and the ligand binding domain of the AR polypeptide comprising the modification at amino acid position 876 of the mutant AR polypeptide set forth in SEQ ID NO: 5. In some embodiments, the mutant AR polypeptide comprises the sequence of amino acids from about amino acid position 554 to about amino acid position 919 of the mutant AR polypeptide set forth in SEQ ID NO: 5. In some embodiments, the mutant AR polypeptide comprises the ligand binding domain of the mutant AR polypeptide. In some embodiments, the mutant AR polypeptide consists essentially of the ligand binding domain of the mutant AR polypeptide. In some embodiments, the mutant AR polypeptide comprises the sequence of amino acids from about amino acid position 554 to about amino acid position 919.

[00105] In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of an AR polypeptide comprising the modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a heterologous polypeptide. In some embodiments, the modification is an amino acid substitution that is F876L. Methods for the generation of fusion proteins are known in the art and include standard recombinant DNA techniques. For example, in some embodiments, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In some embodiments, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. In some embodiments, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). In some embodiments, expression vectors are commercially available that encode a fusion moiety (e.g., a GST polypeptide). A nucleic acid encoding a modified AR polypeptide can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the modified AR polypeptide.

[00106] In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a heterologous DNA binding domain. In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 linked to a heterologous DNA binding domain. In some embodiments the heterologous DNA binding domain is GAL4 DNA binding domain. In some embodiments the heterologous DNA binding domain is LexA DNA binding domain. In some embodiments, the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a heterologous DNA binding domain via a peptide linker. In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 linked to a heterologous DNA binding domain via a peptide linker.

[00107] In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of

the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a heterologous peptide for use in an protein interaction assay, such as, but not limited to a yeast two hybrid assay, a mammalian cell based two hybrid (M2H) system, a Förster (Fluorescence) Resonance Energy Transfer (FRET) assay, a Bioluminescence Resonance Energy Transfer (BRET), or a Homogeneous Time Resolved Fluorescence (HTRF®) assay. In some embodiments, an AR polypeptide is a fusion protein comprising the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 linked to a heterologous peptide for use in an protein interaction assay, such as, but not limited to a yeast two hybrid assay, a mammalian cell based two hybrid (M2H) system, a Förster (Fluorescence) Resonance Energy Transfer (FRET) assay, a Bioluminescence Resonance Energy Transfer (BRET), or a Homogeneous Time Resolved Fluorescence (HTRF®) assay.

[00108] In some embodiments, the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a detectable polypeptide. In some embodiments, the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 is linked to a detectable polypeptide. In some embodiments, the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a fluorescent protein, such as, but limited to, a green (GFP), red (RFP), cyan (CFP), yellow (YFP), or blue (BFP) fluorescent protein. In some embodiments, the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 is linked to a fluorescent protein, such as, but limited to, a green (GFP), red (RFP), cyan (CFP), yellow (YFP), or blue (BFP) fluorescent protein. In some embodiments, the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a bioluminescent protein. In some embodiments, the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 is linked to a bioluminescent protein. In some embodiments, the ligand binding domain of an AR polypeptide comprising a modification at amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1 linked to a peptide tag. In some embodiments, the ligand binding domain of a mutant AR polypeptide set forth in SEQ ID NO: 5 is linked to a peptide tag. In some embodiments, the peptide tag is an epitope tag recognized by a tag-specific antibody. In some embodiments the tag is an epitope tag, such as, but not limited to a c-myc, V-5, hemagglutinin (HA), FLAG. In some embodiments the tag is an affinity tag, such as, but not limited to, biotin, strep-tag, chitin binding protein (CBP), maltose binding protein (MBP), glutathione-S-transferase (GST), or a poly(His) tag.

[00109] In some embodiments, provided herein is an array comprising a mutant AR polypeptide provided herein. In some embodiments, the mutant AR polypeptide is bound to a microchip. In some embodiments, the mutant AR polypeptide is bound directly to the microchip. In some embodiments, the mutant AR polypeptide is bound indirectly to the microchip via a linker. In some embodiments, provided herein is a microchip array comprising a mutant AR polypeptide provided herein.

Nucleic Acids

[00110] Provided herein are nucleic acids encoding mutant AR polypeptides. Provided herein are nucleic acids encoding any of the mutant AR polypeptides described herein. Methods for deducing nucleic acids that encode particular polypeptides are known in the art and involve standard molecular biology techniques. Exemplary nucleic acids encoding mutant AR polypeptides provided herein are provided. It is understood that due to the degeneracy of the genetic code multiple variants nucleic acids exist that encode the same polypeptide. Nucleic acids that encode the mutant AR polypeptides provided herein encompass such variants. In some embodiments, the mutant AR nucleic acids are synthetic nucleic acids. In some embodiments, the mutant AR nucleic acids are cDNA molecules. In some embodiments, the mutant AR nucleic acids do not contain genomic DNA. In some embodiments, the mutant AR nucleic acids are unmethylated. In some embodiments, the mutant AR nucleic acids do not contain AR genomic intron sequences. In some embodiments, the mutant AR nucleic acids comprise a sequence of nucleotides from two or more exons of the AR genomic sequence, including exon 8 or a portion thereof comprising the nucleic acid sequence encoding position 876 of the AR polypeptide. In some embodiments, the mutant AR nucleic acids comprise a sequence of nucleotides that encode phenylalanine at a position corresponding to position 876 of the wild-type AR polypeptide.

[00111] In some embodiments, the nucleic acid encoding mutant AR polypeptides comprises a modification relative to a nucleic acid encoding a wild-type AR polypeptide. In some embodiments, the nucleic acid encoding a mutant AR polypeptide comprises a modification where the encoded polypeptide comprises a substitution of the amino acid phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the nucleic acid encoding a mutant AR polypeptide comprises a modification where encoded polypeptide does not comprise phenylalanine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1.

[00112] In some embodiments the nucleic acid modification is a missense mutation or a deletion of one or more codons that encode the polypeptide. In some embodiments, the modification is a missense mutation that changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide. In some embodiments, the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide is TTC or TTT. In some embodiments, the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide is TTC. In some embodiments, the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide is TTT. In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTC to a nucleic acid codon that encodes leucine. In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTT to a nucleic acid codon that encodes leucine. In some embodiments, the nucleic acid codon that encodes leucine is selected from among TTA, TTG, CTT, CTC, CTA or CTG.

[00113] In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C). In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTC to CTC. In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C) at nucleic acid position 2626 of the AR nucleic acid sequence set forth in SEQ ID NO:18.

[00114] In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Adenine (A). In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTT to TTA. In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Adenine (A) at nucleic acid position 2628 of the AR nucleic acid sequence set forth in SEQ ID NO:18.

[00115] In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Guanine (G). In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTT to TTG. In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Guanine (G) at nucleic acid position 2628 of the AR nucleic acid sequence set forth in SEQ ID NO:18.

[00116] In some embodiments, the modification comprises a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C) at the first position of the codon that encodes

F876 of the AR polypeptide and a second missense mutation that comprises a substitution of Thymine (T) for Adenosine (A) at the third position of the codon that encodes F876 of the AR polypeptide. In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTT to CTA. In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C) at nucleic acid position 2626 and a second missense mutation that comprises a substitution of Thymine (T) for Adenosine (A) at nucleic acid position 2628 of the AR nucleic acid sequence set forth in SEQ ID NO:18.

[00117] In some embodiments, the modification comprises a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C) at the first position of the codon that encodes F876 of the AR polypeptide and a second missense mutation that comprises a substitution of Thymine (T) for Guanine (G) at the third position of the codon that encodes F876 of the AR polypeptide. In some embodiments, the modification changes the nucleic acid codon that encodes phenylalanine at amino position 876 of the AR polypeptide from TTT to CTG. In some embodiments, the modification is a missense mutation that comprises a substitution of Thymine (T) for Cytosine (C) at nucleic acid position 2626 and a second missense mutation that comprises a substitution of Thymine (T) for Guanine (G) at nucleic acid position 2628 of the AR nucleic acid sequence set forth in SEQ ID NO:18.

[00118] In some embodiments the nucleic acid encoding the mutant AR polypeptide comprises a sequence of nucleotides set forth in SEQ ID NO: 19. In some embodiments the nucleic acid encoding the mutant AR polypeptide comprises a nucleic acid having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% nucleotide acid sequence identity to the nucleic acid having the sequence of nucleotides set forth in SEQ ID NO: 19, where the encoded mutant AR comprises a modification relative to the wild-type AR polypeptide at a position corresponding to amino acid position 876. In some embodiments the nucleic acid encoding the mutant AR polypeptide comprises a nucleic acid having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% nucleotide acid sequence identity to the nucleic acid having the sequence of nucleotides set forth in SEQ ID NO: 19, where the encoded mutant AR does not comprise a phenylalanine at the position corresponding to amino acid position 876. In some embodiments the nucleic acid encoding the mutant AR polypeptide comprises a nucleic acid having 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% nucleotide acid sequence identity to the nucleic acid having the sequence of nucleotides set forth in SEQ ID NO: 19, where the encoded mutant AR comprises a leucine at the position corresponding to amino acid position 876.

[00119] In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide is an isolated nucleic acid. In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide is a DNA molecule. In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide is a cDNA molecule. In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide is an RNA molecule. In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide is an inhibitory RNA molecule (i.e. RNAi). In some embodiments, the nucleic acid provided herein is a nucleic acid molecule that is complementary, or binds to, an nucleic acid encoding a mutant AR polypeptide.

[00120] In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide or a portion thereof contains nucleic acid encoding an amino acid at position 876 that is not phenylalanine. In some embodiments, the nucleic acid provided herein encoding a mutant AR polypeptide or a portion thereof contains nucleic acid encoding leucine at amino acid position 876.

[00121] In some embodiments, the nucleic acid provided herein is an oligonucleotide that encodes a portion of the mutant AR polypeptide. In some embodiments the nucleic acid provided herein is an oligonucleotide that encodes a portion of the mutant AR polypeptide that contains a nucleotide codon encoding the amino acid corresponding to amino acid position 876. In some embodiments, the codon encodes an amino acid that is not phenylalanine. In some embodiments, the codon encodes an amino acid that is leucine.

[00122] In some embodiments, the nucleic acid provided herein is a vector that comprises nucleic acid encoding any of the mutant AR polypeptides provided herein. In some embodiments, the nucleic acid provided herein is a vector that comprises nucleic acid encoding any of the mutant AR polypeptides provided herein is an expression vector. In some embodiments, the nucleic acid provided herein is a vector that comprises nucleic acid encoding any of the mutant AR polypeptides provided herein is operably linked to a promoter for the expression of the mutant AR polypeptides.

[00123] In some embodiments, the vector is a plasmid vector. In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is a DNA or RNA viral vector. Exemplary viral vectors include, but are not limited to, a vaccinia, adenovirus, adeno-associated virus (AAV), retrovirus, or herpesvirus vector.

[00124] In some embodiments, provided herein is an array comprising a nucleic acid encoding any of the mutant AR polypeptides provided herein. In some embodiments, the mutant AR nucleic acid is bound to a microchip. In some embodiments, the mutant AR nucleic acid is

bound directly to the microchip. In some embodiments, the mutant AR nucleic acid is bound indirectly to the microchip via a linker. In some embodiments, provided herein is a microchip array comprising a nucleic acid encoding any of the mutant AR polypeptides provided herein.

Production of Nucleic Acids and Polypeptides

[00125] In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is generated by standard recombinant methods. In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is generated by amplification of a mutant AR sequence from genomic DNA. In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is generated by polymerase chain reaction using AR sequence specific primers. In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is generated by reverse transcription of mRNA encoding a mutant AR polypeptide.

[00126] In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is inserted into an expression vector and expressed in a host cell or a non-cell extract. In some embodiments, an isolated nucleic acid molecule encoding a mutant AR polypeptide provided herein is operatively linked to a promoter for expression of the encoding polypeptide in a cell or non-cell extract. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is an inducible promoter.

[00127] In some embodiments, the nucleic acid molecule encoding a mutant AR polypeptide provided herein is "exogenous" to a cell, which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques, which are described in Sambrook et al., 1989 and Ausubel et al., 1996, both incorporated herein by reference.

[00128] Methods for the expression of a protein in a cell are well known in the art and include, for example, expression in cells, such as animal and plant cells. Exemplary animal cells for the expression of mutant AR polypeptides provided herein include but are not limited to bacteria, yeast, insect cells, and mammalian cells, such as for example, human, primate, rodent, bovine, and ovine cells. In some embodiments, the nucleic acid encoding the mutant AR is integrated into the genome of the host cell.

[00129] In some embodiments, a method for the expression of a mutant AR polypeptide provided herein comprises culturing a host cell containing an expression vector encoding a

mutant AR polypeptide such that the mutant AR polypeptide is produced by the cell. In some methods, the nucleic acid encoding the mutant polypeptide is connected to nucleic acid encoding a signal sequence such that the signal sequence is expressed as a fusion peptide with the mutant AR polypeptide. In some embodiments the signal sequence allows for the secretion of the mutant AR polypeptide by the host cell.

[00130] In some embodiments the mutant AR polypeptide is isolated from a host cell expressing the mutant polypeptide. In some embodiments an extract is prepared from the host cell and the mutant AR polypeptide is isolated by purification methods such as but not limited to chromatography or immunoaffinity with an antibody that is specific for AR polypeptides or specific to the mutant AR polypeptide in particular.

Antibodies

[00131] In some embodiments the antibody binds to a mutant AR polypeptide provided herein, and binds with less affinity or does not bind to a wild-type AR polypeptide. In some embodiments the antibody binds to a mutant AR polypeptide in the presence of a second-generation inhibitor, such as, but not limited to ARN-509, enzalutamide (MDV3100) or RD162.

[00132] In some embodiments, mutant AR polypeptide provided herein are detected using antibodies that specifically recognize the mutant AR polypeptides, but do not recognize wild-type AR polypeptides. In some embodiments, mutant AR polypeptide provided herein are detected using antibodies that specifically recognize a mutant AR polypeptide having a leucine at amino acid position 876, but do not recognize wild-type AR polypeptides. In some embodiments, antibodies are raised against one or more allelic forms of the mutant AR polypeptide provided herein. Techniques for using a specific protein or an oligopeptide as an antigen to elicit antibodies that specifically recognize epitopes on the peptide or protein are well known. In one embodiment, the DNA sequence of the desired allelic form of the target gene is cloned by insertion into an appropriate expression vector and translated into protein in a prokaryotic or eukaryotic host cell. The protein can be recovered and used as an antigen to elicit the production of specific antibodies. In another embodiment, the DNA of the desired allelic form of the target gene is amplified by PCR technology and is subsequently translated *in vitro* into protein to be used as the antigen to elicit the production of specific antibodies. In another embodiment, the DNA sequence of the alternative alleles is used as a basis for the generation of synthetic peptides representing the amino acid sequence of the alleles for use as the antigen to elicit the production of specific antibodies.

[00133] In some embodiments, antibodies are generated either by standard monoclonal antibody techniques or generated through recombinant based expression systems. See generally,

Abbas, Lichtman, and Pober, Cellular and Molecular Immunology, W. B. Saunders Co. (1991). The term "antibodies" is meant to include intact antibody molecules as well as antibody fragments or derivatives, such as Fab and F(ab')₂, which are capable of specifically binding to antigen. The antibodies so produced preferentially bind only the mutant protein produced in the allelic form which was used as an antigen to create the antibody. Methods of generating allele-specific antibodies are also described in U.S. Pat. No. 6,200,754 and U.S. Pat. No. 6,054,273, the entire contents of which are incorporated herein by reference.

[00134] In some embodiments, the antibody provided herein is a humanized antibody. A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin(s). In some embodiments, framework support residues are altered to preserve binding affinity (see, e.g., Queen et al. Proc. Natl. Acad. Sci. USA, 86:10029-10032 (1989), Hodgson et al. Bio/Technology, 9:421 (1991)). In some embodiments, a suitable human acceptor antibody is one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. In some embodiments, a human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) is suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. In some embodiments, a suitable acceptor antibody capable of donating light chain constant or variable framework regions is selected in a similar manner. In some embodiments, the acceptor antibody heavy and light chains originate from the same acceptor antibody. In some embodiments, the acceptor antibody heavy and light chains originate from the different acceptor antibodies. The prior art describes several ways of producing such humanized antibodies—see, for example, EP-A-0239400 and EP-A-054951.

[00135] In some embodiments, antibodies specific for mutant AR polypeptide provided herein can be used to detect the presence of a mutant AR polypeptide provided herein in a sample, e.g., an assay sample, a cell sample, a cell extract, a biological sample, or a patient sample, using techniques known in the art. These techniques include, for example, Western blot, immunohistochemistry, indirect immunofluorescence, and antibody microarray. In some embodiments, antibodies which specifically recognize mutant AR polypeptide are third-generation AR inhibitors. In some embodiments, the ability of an antibody which specifically recognizes a mutant AR polypeptide to inhibit the biological activity of the mutant AR

polypeptide can be determined using the methods described herein for identifying third-generation AR inhibitors.

Diagnostic Assays For Detecting Mutant AR Polypeptides and Nucleic Acids Encoding Mutant AR Polypeptides

[00136] Provided herein are diagnostic methods that involve the detection of a mutant AR polypeptide in a subject or a nucleic acid encoding a mutant AR polypeptide in a subject. In some embodiments, the subject has an AR-mediated disease or condition. In some embodiments, the diagnostic methods are employed for the screening subjects having a cancer that is resistant to therapy with an anti-androgen, such as a first- or second-generation AR antagonist, identifying subjects for the treatment with anti-androgen, such as a first- or second-generation AR antagonist, monitoring the therapy of subjects receiving an anti-androgen therapy, such as a first- or second-generation AR antagonist, optimizing the therapy of subjects receiving an anti-androgen therapy, such as a first- or second-generation AR antagonist, and combinations thereof. In some embodiments, the methods comprises selecting a subject for therapy with a third-generation AR antagonist. In some embodiments, the methods further comprise administering to the subject a third-generation AR antagonist as described herein. In some embodiments, the mutant AR polypeptide detected comprises a modification at a position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the mutant AR polypeptide detected comprises a substitution of the amino acid phenylalanine to leucine at the position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, a subject having a mutant AR polypeptide comprising a modification at amino acid position 876 is resistant to inhibition with a first- or a second- generation antagonist.

[00137] In some embodiments, a subject having a mutant AR polypeptide comprising a modification at amino acid position 876 is resistant to inhibition with a first- or a second-generation antagonist. In some embodiments, a subject having a mutant AR polypeptide comprising a modification at amino acid position 876 is resistant to inhibition with a first- and a second- generation antagonist. In some embodiments, a subject having a mutant AR polypeptide comprising a leucine at amino acid position 876 is resistant to inhibition with a first- or a second- generation antagonist. In some embodiments, a subject having a mutant AR polypeptide comprising a leucine at amino acid position 876 is resistant to inhibition with a first- and a second- generation antagonist. In some embodiments, a subject having a mutant AR polypeptide comprising a modification at amino acid position 876 is resistant to inhibition with a CYP17A inhibitor that binds to AR, such as for example, galeterone (TOK001), TAK-700 or abiraterone

acetate. In some embodiments, a subject having a mutant AR polypeptide comprising a modification at amino acid position 876 is resistant to inhibition with a CYP17A inhibitor that binds to AR, such as for example, galeterone (TOK001), TAK-700 or abiraterone acetate.

[00138] In some embodiments, provided are methods for characterizing an AR polypeptide that is resistant to inhibition with a second-generation AR antagonist in a subject, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the AR as resistant to inhibition with a second-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the method further comprises not administering a second generation AR antagonist. In some embodiments, the method further comprises administering a second generation AR antagonist if the subject does not have the modification. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00139] In some embodiments, provided are methods for characterizing an AR that is resistant to inhibition with a first-generation AR antagonist in a subject, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the AR as resistant to inhibition with a first-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the method further comprises not administering a second generation AR antagonist. In some embodiments, the method further comprises administering a second generation AR antagonist if the subject does not have the modification. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises obtaining the nucleic acid sample from the subject.

[00140] In some embodiments, provided are methods for characterizing an AR that is resistant to inhibition with an AR antagonist that is a CYP17A inhibitor in a subject, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the

subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the AR as resistant to inhibition with an AR antagonist that is a CYP17A inhibitor if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the method further comprises not administering a second generation AR antagonist. In some embodiments, the method further comprises administering a second generation AR antagonist if the subject does not have the modification. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments the CYP17A inhibitor is galeterone (TOK001), TAK-700 or abiraterone acetate. In some embodiments, the method further comprises obtaining the nucleic acid sample from the subject.

[00141] In some embodiments, provided are methods for selecting a subject for therapy with a second-generation AR antagonist, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as a candidate for therapy with a second-generation AR antagonist if the subject does not have the modification. In some embodiments, the method further comprises characterizing the subject as not a candidate for therapy with a second-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00142] In some embodiments, provided are methods for selecting a subject for therapy with a first-generation AR antagonist, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as a candidate for therapy with a first-generation AR antagonist if the subject does not have the modification. In some embodiments, the method further comprises characterizing the subject as not a candidate for therapy with a first-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-

generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00143] In some embodiments, provided are methods for selecting a subject for therapy with an AR antagonist that is a CYP17A inhibitor, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as a candidate for therapy with an AR antagonist that is a CYP17A inhibitor if the subject does not have the modification. In some embodiments, the method further comprises characterizing the subject as not a candidate for therapy with a CYP17A inhibitor if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments the CYP17A inhibitor is galeterone (TOK001), TAK-700 or abiraterone acetate. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00144] In some embodiments, provided are methods for selecting a subject for therapy with a third-generation AR antagonist, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as a candidate for therapy with a third-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00145] In some embodiments, provided are methods for determining whether a subject is or is likely to become resistant to therapy with a second-generation AR antagonist, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position

corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or is likely to become resistant to therapy with a second-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR inhibitor provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00146] In some embodiments, provided are methods for determining whether a subject is or is likely to become resistant to therapy with a first-generation AR antagonist, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or is likely to become resistant to therapy with a first-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR inhibitor provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00147] In some embodiments, provided are methods for determining whether a subject is or is likely to become resistant to therapy with an AR antagonist that is a CYP17A inhibitor, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or is likely to become resistant to therapy with an AR antagonist that is a CYP17A inhibitor if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR inhibitor provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments the CYP17A inhibitor is galeterone (TOK001), TAK-700 or abiraterone acetate. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00148] In some embodiments, provided are methods for monitoring whether a subject receiving a second-generation AR antagonist for treatment of a cancer has developed or will develop resistance to the therapy, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or will become resistant to therapy with a second-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00149] In some embodiments, provided are methods for monitoring whether a subject receiving a first-generation AR antagonist for treatment of a cancer has developed or will develop resistance to the therapy, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or will become resistant to therapy with a first-generation AR antagonist if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00150] In some embodiments, provided are methods for monitoring whether a subject receiving an AR antagonist that is a CYP17A inhibitor for treatment of a cancer has developed or will develop resistance to the therapy, comprising (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) characterizing the subject as resistant or will become resistant to therapy with an AR antagonist that is a CYP17A inhibitor if the subject has the modification. In some embodiments, the method further comprises administration of a third-generation AR antagonist provided herein for inhibition of the mutant

AR. In some embodiments, the subject has cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a castration resistant prostate cancer. In some embodiments the CYP17A inhibitor is galeterone (TOK001), TAK-700 or abiraterone acetate. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00151] In some embodiments, provided are methods for optimizing the therapy of a subject receiving a second-generation AR antagonist for treatment of a cancer, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) discontinuing treatment with the second- generation AR antagonist if the subject has the modification or continuing treatment with the second-generation AR antagonist if the subject does not have the modification. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00152] In some embodiments, provided are methods for optimizing the therapy of a subject receiving a first-generation AR antagonist for treatment of a cancer, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) discontinuing treatment with the first-generation AR antagonist if the subject has the modification or continuing treatment with the first-generation AR antagonist if the subject does not have the modification. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00153] In some embodiments, provided are methods for optimizing the therapy of a subject receiving an AR antagonist that is a CYP17A inhibitor for treatment of a cancer, comprising: (a) assaying a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject to determine whether the encoded AR polypeptide is modified at an amino acid position corresponding to amino acid position 876 of the amino acid sequence set forth in SEQ ID NO: 1; and (b) discontinuing treatment with the AR antagonist that is a CYP17A inhibitor if the subject has the modification or continuing treatment with the AR antagonist that is a CYP17A inhibitor if the subject does not have the modification. In some embodiments the CYP17A inhibitor is galeterone (TOK001), TAK-700 or abiraterone acetate. In some embodiments, the method further comprises a step of obtaining the nucleic acid sample from the subject.

[00154] In some embodiments, the modified AR is resistant to full antagonism by a second-generation AR antagonist, such as, for example, ARN-509, enzalutamide (MDV3100) or RD162. In some embodiments, a second-generation AR antagonist, such as, for example, ARN-509, enzalutamide (MDV3100) or RD162 exhibits agonist activity toward the modified AR. In some embodiments, the modified AR is resistant to full antagonism by a first-generation AR antagonist. In some embodiments, the modified AR is resistant to full antagonism by an AR antagonist that is a CYP17A.

[00155] In some embodiments, the subject has an AR dependent or AR mediated disease or condition. In some embodiments, the AR dependent or AR mediated disease or condition is benign prostate hyperplasia, hirsutism, acne, adenomas and neoplasms of the prostate, benign or malignant tumor cells containing the AR, hyperpilosity, seborrhea, endometriosis, polycystic ovary syndrome, androgenic alopecia, hypogonadism, osteoporosis, suppression of spermatogenesis, libido, cachexia, anorexia, androgen supplementation for age related decreased testosterone levels, prostate cancer, breast cancer, bladder cancer, liver cancer, endometrial cancer, uterine cancer, hot flashes, Kennedy's disease, muscle atrophy and weakness, skin atrophy, bone loss, anemia, arteriosclerosis, cardiovascular disease, loss of energy, loss of well-being, type 2 diabetes or abdominal fat accumulation.

[00156] In some embodiments, the subject has cancer. In some embodiments, the subject has a solid tumor. In some embodiments, the cancer is a prostate cancer, a breast cancer, a liver cancer, or a bladder cancer. In some embodiments, the cancer is a prostate cancer.

[00157] In some embodiments, the sample is from any tissue or fluid from an organism. Samples include, but are not limited, to whole blood, dissociated bone marrow, bone marrow aspirate, pleural fluid, peritoneal fluid, central spinal fluid, abdominal fluid, pancreatic fluid, cerebrospinal fluid, brain fluid, ascites, pericardial fluid, urine, saliva, bronchial lavage, sweat, tears, ear flow, sputum, hydrocele fluid, semen, vaginal flow, milk, amniotic fluid, and secretions of respiratory, intestinal or genitourinary tract. In particular embodiments, the sample is a tumor biopsy sample. In particular embodiments, the sample is from a fluid or tissue that is part of, or associated with, the lymphatic system or circulatory system. In some embodiments, the sample is a blood sample that is a venous, arterial, peripheral, tissue, cord blood sample. In particular embodiments, the sample is a serum sample. In some embodiments, the sample contains one or more circulating tumor cells (CTCs). In some embodiments, the sample contains one or more disseminated tumor cells (DTC, e.g. in a bone marrow aspirate sample).

[00158] Methods for the isolation of nucleic acids and proteins from cells contained in tissue and fluid samples are well-known in the art. In particular embodiments, the nucleic acid sample

obtained from the subject is isolated from cells contained in a tumor biopsy from the subject. In particular embodiments, the nucleic acid sample obtained from the subject is isolated from cells in a bone marrow aspirate. In particular embodiments, the nucleic acid sample obtained from the subject is isolated from cells contained serum sample. In particular embodiments, the nucleic acid sample obtained from the subject is isolated from cells contained lymph sample.

[00159] In some embodiments, the samples are obtained from the subject by any suitable means of obtaining the sample using well-known and routine clinical methods. Procedures for obtaining fluid samples from a subject are well known. For example, procedures for drawing and processing whole blood and lymph are well-known and can be employed to obtain a sample for use in the methods provided. Typically, for collection of a blood sample, an anti-coagulation agent (e.g. EDTA, or citrate and heparin or CPD (citrate, phosphate, dextrose) or comparable substances) is added to the sample to prevent coagulation of the blood. In some examples, the blood sample is collected in a collection tube that contains an amount of EDTA to prevent coagulation of the blood sample.

[00160] In some embodiments, the sample is a tissue biopsy and is obtained, for example, by needle biopsy, CT-guided needle biopsy, aspiration biopsy, endoscopic biopsy, bronchoscopic biopsy, bronchial lavage, incisional biopsy, excisional biopsy, punch biopsy, shave biopsy, skin biopsy, bone marrow biopsy, and the Loop Electrosurgical Excision Procedure (LEEP).

Typically, a non-necrotic, sterile biopsy or specimen is obtained that is greater than 100 mg, but which can be smaller, such as less than 100 mg, 50 mg or less, 10 mg or less or 5 mg or less; or larger, such as more than 100 mg, 200 mg or more, or 500 mg or more, 1 gm or more, 2 gm or more, 3 gm or more, 4 gm or more or 5 gm or more. The sample size to be extracted for the assay depends on a number of factors including, but not limited to, the number of assays to be performed, the health of the tissue sample, the type of cancer, and the condition of the patient. In some embodiments, the tissue is placed in a sterile vessel, such as a sterile tube or culture plate, and is optionally immersed in an appropriate media. Typically, the cells are dissociated into cell suspensions by mechanical means and/or enzymatic treatment as is well known in the art.

Typically, the cells are collected and then subjected to standard procedures for the isolation of nucleic acid for the assay.

[00161] In some embodiments, the samples are obtained from the subject at regular intervals, such as, for example, one day, two days, three days, four days, five days, six days, one week, two weeks, weeks, four weeks, one month, two months, three months, four months, five months, six months, one year, daily, weekly, bimonthly, quarterly, biyearly or yearly. In some embodiments, the collection of samples is performed at a predetermined time or at regular

intervals relative to treatment with one or more anti-cancer agents. In some embodiments, the collection of samples is performed at a predetermined time or at regular intervals relative to treatment with an AR antagonist, such as a first- or second- generation AR antagonist. For example, a sample is collected at a predetermined time or at regular intervals prior to, during, or following treatment or between successive treatments. In particular examples, a sample is obtained from the subject prior to administration of an anti-cancer therapy and then again at regular intervals after treatment has been effected. In particular examples, a sample is obtained from the subject prior to administration of an AR antagonist, such as a first- or second-generation AR antagonist, therapy and then again at regular intervals after treatment has been effected. In some embodiments, the AR antagonist is selected from among bicalutamide, flutamide, hydroxyflutamide, nilutamide, ARN-509, enzalutamide (MDV3100), and RD162. In some embodiments, the AR antagonist is a CYP17A inhibitor. In some embodiments, the CYP17A inhibitor is selected from among galeterone (TOK001), TAK-700 or abiraterone acetate.

[00162] The volume of a fluid sample can be any volume that is suitable for the detection of an AR mutant in the methods provided. In some examples, the volume for the fluid sample is dependent on the particular assay method used. For example, particular assay methods can require a larger or smaller fluid sample volumes depending on factors such as, but not limited to, the capacity of the device or method used and level of throughput of the assay method. In some examples a fluid sample is diluted in an appropriate medium prior to application of the assay method. In some examples, a fluid sample is obtained from a subject and a portion or aliquot of the sample is used in the assay method. The portion or aliquot can be diluted in an appropriate medium prior to application of the assay method.

[00163] In some embodiments, the sample is obtained from a subject that is mammal. Exemplary mammalian subjects include, but are not limited to primates, such as humans, apes and monkeys; rodents, such as mice, rats, rabbits, and ferrets; ruminants, such as goats, cows, deer, and sheep; horses, pigs, dogs, cats, and other animals. In some embodiments, the sample is obtained from a patient. In some examples, the patient is a human patient.

[00164] In some embodiments, the nucleic acid sample obtained from the subject is a genomic nucleic acid sample. In some embodiments, the nucleic acid sample obtained from the subject is an RNA sample. In some embodiments, mRNA is isolated from the total RNA in an RNA sample. In some embodiments, the RNA sample is reverse transcribed into cDNA. In some embodiments, the genomic nucleic acid sample is amplified by a nucleic acid amplification method. In some embodiments, the nucleic acid amplification method is polymerase chain

reaction (PCR). In some embodiments, the genomic nucleic acid sample is amplified using a set of nucleotide primers specific for the AR gene. In some embodiments, the set of nucleotide primers flank the nucleic acid sequence encoding amino acid position 876 of the AR polypeptide. In some embodiments, the amplification product is a nucleic acid encoding amino acid position 876 of the AR polypeptide. In some embodiments, a sequence specific primer is conjugated to a detectable molecule, such as a fluorescent label, a bioluminescent label, a chemiluminescent label, a radiolabel, an enzyme label, a detectable substrate, or a peptide or molecule that binds to a second detectable molecule.

[00165] In some embodiments, assaying comprises sequencing the nucleic acid sample. In some embodiments, the nucleic acid encoding AR in a nucleic acid sample is first amplified by a method such as polymerase chain reaction (PCR) using sequence specific primers, and the amplified PCR fragment is then sequenced. Exemplary sequencing methods for use in the methods provide herein are well known in the art and include, but are not limited to, dideoxy or chain termination methods, Maxam-Gilbert sequencing, massively parallel signature sequencing (or MPSS), polony sequencing, pyrosequencing, Illumina dye sequencing, SOLiD (or sequencing by ligation) sequencing, ion semiconductor sequencing, DNA nanoball sequencing, heliscope sequencing, and single molecule real time (SMRT) sequencing.

[00166] In some embodiments, the samples is a plasma or serum sample containing circulating tumor DNA (ctDNA), RNA (ctRNA) or microRNA (see e.g. Chan et al. (2007) *Br J Cancer*. 96(5):681-5). In some embodiments, the DNA encoding the mutant AR is assessed by BEAMing (beads, amplification, emulsion, magnetic) PCR sequencing method (see, e.g. Li et al. (2006) *Nat Methods*. 3(2):95-7; Li et al. (2006) *Nat Methods*. 3(7):551-9; and Diehl et al. (2008) *Nat Med*. 14(9): 985-990). BEAMing is a technique in which individual DNA molecules are attached to magnetic beads in water-in-oil emulsions and then subjected to compartmentalized PCR amplification. The mutational status of DNA bound to beads is then determined by hybridization to fluorescent allele-specific probes for mutant or wild-type AR. Flow cytometry is then used to quantify the level of mutant DNA present in the plasma or serum (see e.g. Higgins et al. (2012) *Clin Cancer Res* 18: 3462-3469).

[00167] In some embodiments, assaying a sample for detecting the presence of DNA encoding the mutant AR comprises detection of the mutation with a sequence specific oligonucleotide probe that is specific for nucleic acid that encodes the mutant AR but not the wild-type AR. In some embodiments, assaying comprises (a) contacting a sample with a mutant AR nucleic acid sequence specific oligonucleotide probe, whereby if the mutant nucleic acid sequence is present in the sample, a probe-DNA complex is formed, and (b) detecting the probe-DNA complex. In

some embodiments, the oligonucleotide probe is specific for nucleic acid encoding leucine at a position corresponding to amino acid 876 of an AR polypeptide. In some embodiments, the sequence specific probe is conjugated to a detectable molecule, such as a fluorescent label, a bioluminescent label, a chemiluminescent label, a radiolabel, an enzyme label, a detectable substrate, or a peptide or molecule that binds to a second detectable molecule.

[00168] In some embodiments, single nucleotide changes are detectable by PCR using PCR-based cleaved amplified polymorphic sequences (CAPS) markers which create restriction sites in the mutant sequences (Michaels et al (1998) *Plant J.* 14(3):381-5) or sequence specific hairpin probes attached to detectable moieties, such as, but not limited to, a fluorophore (Mhlanga and Malmberg (2001) *Methods* 25:463-471). In some embodiments, the sequence specific probe is conjugated to a detectable molecule, such as a fluorescent label, a bioluminescent label, a chemiluminescent label, a radiolabel, an enzyme label, a detectable substrate, or a peptide or molecule that binds to a second detectable molecule. In some embodiments, the oligonucleotide probe is specific for nucleic acid encoding leucine at a position corresponding to amino acid 876 of an AR polypeptide.

[00169] In some embodiments, assaying a sample for detecting the presence of DNA encoding the mutant AR is performed using an oligonucleotide array (see e.g. Hastia et al. (1999) *J Med Genet.* 36(10):730-6). In some embodiments, the sample containing nucleic acid from the subject is hybridized directly to the chip. In some embodiments, the sample containing nucleic acid from the subject is amplified using an amplification method, such as, but not limited to polymerase chain reaction (PCR), and the amplified nucleic acid is hybridized to the chip. In some embodiments, the oligonucleotide array is contained on a microchip. In some embodiments, single nucleotide changes are detectable using microchips.

[00170] In some embodiments, assaying a sample comprises detection of the mutation with an antibody specific for the mutant AR polypeptide. In some embodiments, the method of detecting a mutant AR polypeptide comprises obtaining a sample from a subject, wherein the sample comprises an AR polypeptide and testing the sample for the presence of a mutant AR polypeptide by contacting the sample with an antibody that is specific for binding to the mutant AR polypeptide, and does not bind or bind with decreased affinity for the wild-type AR polypeptide, wherein the presence of the mutant AR polypeptide creates an antibody-mutant AR polypeptide complex. In some embodiments, the method further comprises detecting the antibody-mutant AR polypeptide complex. In some embodiments, the method further comprises detecting the antibody-mutant AR polypeptide complex with a detection reagent. In some embodiments, the mutant AR specific antibody is conjugated to a detectable molecule, such as a

fluorescent label, a bioluminescent label, a chemiluminescent label, a radiolabel, an enzyme label, a detectable substrate, or a peptide or molecule that binds to a second detectable protein (e.g. a secondary antibody). In some embodiments, binding of the mutant AR specific antibody is detected by assaying for the detectable molecule. In some embodiments, binding of the mutant AR specific antibody is detected by using a secondary (e.g. anti-IgG) antibody. In some embodiments, the sample is a tumor biopsy sample, a bone marrow aspirate, a blood sample, a serum sample, or a lymph sample.

Identification of Molecules that Interact with Mutant Androgen Receptor

[00171] Provided herein are methods of using the mutant AR polypeptides for screening of compounds that inhibit the mutant receptor (i.e. third-generation AR inhibitor compounds). In some embodiments, the methods are employed for the identification of third-generation AR inhibitor compounds for the treatment of cancer. In some embodiments, the methods are employed for the identification of third-generation AR inhibitor compounds for the treatment of resistant cancers, such as prostate cancer resistant to treatment with second-generation AR antagonists, such as ARN-509, enzalutamide (MDV3100) or RD162.

[00172] In some embodiments, a method for identifying third-generation AR inhibitor compounds comprises (a) expressing a mutant AR polypeptide provided herein in a cell, (b) contacting the cell with a test compound, and (c) detecting the level of AR activity in the cell. In some embodiments, the cell is contacted with an AR agonist prior to or at the same time as contacting the cell with the test compound. In some embodiments the cell is contacted with an AR agonist about 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours or longer prior to contacting the cell with the test compound. In some embodiments the cell is contacted with an AR agonist at the same time as the cell is contacted with test compound. In some embodiments, the AR agonist is selected from among methyltrienolone (R1881), DHT, mibolerone (Mb) and testosterone. In some embodiments, the mutant AR polypeptide comprises an amino acid substitution at a position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the mutant AR polypeptide does not contain a phenylalanine at amino acid position 876 in the polypeptide. In some embodiments, the mutant AR polypeptide contains a leucine at amino acid position 876 in the polypeptide.

[00173] In some embodiments, a cell line that can be transfected with nucleic acid encoding the mutant AR polypeptide and in which AR activity can be monitored is used. In some embodiments, the cell does not express the wild-type AR. In some embodiments, the cell

expresses a low level of wild-type AR. In some embodiments, the cell expresses an endogenous mutant AR polypeptide. In some embodiments, the endogenous mutant AR polypeptide comprises a modification at an amino acid corresponding to amino acid 877 of a wild-type AR polypeptide. In some embodiments, the endogenous mutant AR polypeptide comprises a modification that is a substitution of Threonine to Alanine at amino acid position 877 (T877A). In some embodiments, the mutant AR polypeptide comprises a modification at an amino acid corresponding to amino acid 874 of a wild-type AR polypeptide. In some embodiments, the mutant AR polypeptide comprises a modification that is a substitution of Histidine to Tyrosine at amino acid position 874 (H874Y). In some embodiments, the cell is selected from among HeLa, CV1, COS7, HepG2, HEK-293, DU145, PC3, and TSY-PR1. In some embodiments, the cell line is a prostate cancer cell line. In some embodiments, the cell line is selected from among CWR, LNCaP, VCaP and LAPC4.

[00174] In some embodiments, the cell stably expresses the mutant AR polypeptide. In some embodiments, the nucleic acid encoding the mutant AR is integrated into the genome of the cell.

[00175] In some embodiments, the level of AR activity is detected using a reporter gene operably linked to an AR-responsive promoter. In some embodiments, the AR-responsive promoter comprises one or more androgen response elements (AREs) to which the mutant AR polypeptide binds. In some embodiments, the promoter is selected from among a probasin (Pb), a prostate specific antigen (PSA), mouse mammary tumor virus long terminal repeat (MMTV LTR), fatty acid synthase (FASN), six transmembrane epithelial antigen of the prostate 4 (STEAP4), transmembrane protease, serine 2 (TMPRSS2), alpha-1-acid glycoprotein 1 (ORM1), or human homeobox gene NKX3.1 promoter. In some embodiments, the promoter is a synthetic promoter containing one or more AREs.

[00176] In some embodiments, the AR-responsive promoter is operably linked to a suitable reporter gene that encodes a detectable protein. Exemplary detectable proteins include, but are not limited to, luciferase, fluorescent proteins, bioluminescent proteins, β -galactosidase, alkaline phosphatase, and chloramphenicol acetyltransferase. In some embodiments, a decrease in the expression of the reporter gene following exposure to the test compound compared to a suitable control indicates that the test compound is effective for inhibition of the mutant AR polypeptide. In some embodiments, the control is basal expression of the reporter gene prior to exposure of the cell to the test compound. In some embodiments the expression of the reporter gene is assayed using a cell extract prepared from the test cells.

[00177] In some embodiments, the level of AR activity is detected by measuring the expression of one or more endogenous androgen responsive genes in a cell. In some embodiments, the

androgen responsive gene is upregulated (i.e. induced) in response to androgen treatment. In some embodiments, the androgen responsive gene is downregulated (i.e. repressed) in response to androgen treatment. Exemplary androgen responsive genes include, but are not limited to, prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), prostasin, snail homolog 2 (SLUG), transmembrane protease, serine 2 (TMPRSS2), six transmembrane epithelial antigen of prostate family member 4 (STEAP4), FK506 binding protein 5 (FKBP5), orosomucoid 1/alpha-1-acid glycoprotein 1 (ORM1), solute carrier family 35 (SLC35F2/NOV), insulin-like growth factor I (IGF-1) IGF binding protein-3 and -5, CCAAT-enhancer binding protein- δ , phosphatase and tensin homolog deleted on chromosome 10 (PTEN), FASN, NKX3.1, AMIGO2, BDNF, CAMK2N1, HPGD, NCAPD3, PLD1, IL-15, IL-18, and ERBB2/HER2.

[00178] In some embodiments, the level of AR activity is detected by measuring the expression of one or more endogenous genes that are induced by exposure to an androgen or an AR agonist. In some embodiments, a decrease in the expression of one or more androgen inducible genes following exposure to the test compound compared to a suitable control indicates that the test compound is effective for inhibition of the mutant AR polypeptide. Exemplary androgen inducible genes include but are not limited to, prostate specific antigen (PSA), prostasin, snail homolog 2 (SLUG), transmembrane protease, serine 2 (TMPRSS2), six transmembrane epithelial antigen of prostate family member 4 (STEAP4), FK506 binding protein 5 (FKBP5), and orosomucoid 1/alpha-1-acid glycoprotein 1 (ORM1). In some embodiments, expression of the inducible gene is assessed in the presence of an AR agonist. In some embodiments, the cells are contacted with an androgen agonist prior to or simultaneously with the test compound.

[00179] In some embodiments, the level of AR activity is detected by measuring the expression of one or more endogenous genes that are repressed by exposure to an androgen or an AR agonist. In some embodiments, an increase in or failure to repress the expression of one or more androgen repressed genes following exposure to the test compound compared to a suitable control indicates that the test compound is effective for inhibition of the mutant AR polypeptide. In some embodiments, the control is basal expression of the gene prior to exposure of the cell to the test compound. Exemplary androgen repressed genes include, but are not limited to, prostate specific membrane antigen (PSMA), solute carrier family 35 (SLC35F2/NOV), IGF binding protein-3 and -5, CCAAT-enhancer binding protein- δ , phosphatase and tensin homolog deleted on chromosome 10 (PTEN), IL-15, IL-18, and ERBB2/HER2. In some embodiments, expression of the repressible gene is assessed in the presence of an AR agonist. In some

embodiments, the cells are contacted with an androgen agonist prior to or simultaneously with the test compound.

[00180] Methods to measure the expression of endogenous genes is well-known in the art. Exemplary methods for the measurement of gene expression include, but are not limited to protein analytical methods such as, for example, immunohistochemistry, immunoblotting (e.g. Western analysis), chromatography, and nucleic acids analytical methods, such as, for example, polymerase chain reaction (PCR), quantitative PCR (qPCR), real time PCR (RT-PCR), Northern analysis.

[00181] In some embodiments, the activity of a mutant AR polypeptide is measured using an assay such as, but not limited to, a AR coactivator binding assay (e.g. immunoprecipitation assays, two-hybrid assays, Förster (fluorescence) resonance energy transfer assays, for example, LanthaScreen™ TR-FRET androgen receptor coactivator assay), a AR conformational profiling assay (see, e.g., Joseph et al. (2009) *PNAS* 106(29):12178-12183), an AR DNA binding assay (see, e.g., Roche et al. (1992) *Mol. Endocrinol.* 6(12):2229-35), chromatin immunoprecipitation, N/C terminal interaction assay (see, e.g., Hsu et al. (2005) *Mol. Endocrinology* 19(2) 350-361 and Ghali et al. (2003) *J Clin Endocrinol Metab.* 88(5):2185-93).

[00182] In some embodiments, a method for identifying third-generation AR inhibitor compounds comprises selecting a potential third-generation AR inhibitor compound using computer-assisted modeling with a three-dimensional crystal or solution structure of a mutant AR polypeptide provided herein. In some embodiments, the mutant AR polypeptide comprises an amino acid substitution at a position corresponding to amino acid position 876 of the wild-type AR polypeptide set forth in SEQ ID NO: 1. In some embodiments, the mutant AR polypeptide does not contain a phenylalanine at amino acid position 876 in the polypeptide. In some embodiments, the mutant AR polypeptide contains a leucine at amino acid position 876 in the polypeptide. In some embodiments, the method comprises contacting the mutant AR polypeptide with the test compound and detecting the interaction of the test compound with the mutant AR polypeptide. In some embodiments, a test compound that interacts with the mutant AR polypeptide is identified as a candidate third-generation AR inhibitor compound.

[00183] In some embodiments, a test compound for use in the methods provided is a member of a library of compounds. In some embodiments, generation of a library of test compounds is by any suitable method for the production of chemical compounds. A "library of test compounds" refers to a panel comprising a multiplicity of test compounds. An exemplary approach for the synthesis of molecular libraries of small organic molecules has been described (Carell et al. (1994). *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.*

33:2061). In some embodiments the test compounds provided herein are obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the 'one-bead one-compound' library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) *Anticancer Drug Des.* 12:145). Other exemplary methods for the synthesis of molecular libraries are found in the art, for example in: Erb et al. (1994). *Proc. Natl. Acad. Sci. USA* 91:11422; Horwell et al. (1996) *Immunopharmacology* 33:68-; and in Gallop et al. (1994); *J. Med. Chem.* 37:1233-. In some embodiments, libraries of compounds are presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull et al. (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla et al. (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310). In some embodiments, combinatorial polypeptides are produced from a cDNA library. Exemplary compounds that can be screened for activity include, but are not limited to, peptides, nucleic acids, carbohydrates, small organic molecules, and natural product extract libraries.

AR Inhibitors Identified by the Screening Methods

[00184] The third-generation AR inhibitors identified using the screening methods provided herein are AR modulators. In some embodiments, the third-generation AR inhibitor inhibits or reduces at least one activity of an AR polypeptide. Exemplary AR activities include, but are not limited to, co-activator binding, DNA binding, ligand binding, or nuclear translocation. In some embodiments, the third-generation AR inhibitor inhibits an activity of an AR polypeptide by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98% or 100% compared to the activity of the AR polypeptide in the absence of the inhibitor. In some embodiments, the third-generation AR inhibitor compounds identified using the methods provided herein are AR inverse agonists, AR antagonists, AR degraders, AR trafficking modulators and/or AR DNA-binding inhibitors. In some embodiments, a third-generation AR inhibitor compounds identified using the methods provided herein is an AR inverse agonist. In some embodiments, the third-generation AR inhibitor compounds identified using the methods provided herein are AR antagonists. In some embodiments, the third-generation AR inhibitor compounds identified using the methods provided herein are AR degraders. In some embodiments, the third-

generation AR inhibitor compounds identified using the methods provided herein are AR trafficking modulators. In some embodiments, the third-generation AR inhibitor compounds identified using the methods provided herein are AR DNA-binding inhibitors. In some embodiments, the AR inhibitor inhibits at least one activity of a wild-type AR polypeptide. In some embodiments, the AR inhibitor inhibits at least one activity of a mutant AR polypeptide.

[00185] In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein has minimal pro-convulsant activity and/or minimal impact on seizure threshold. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein displays minimal modulation of the GABA-gated chloride channel. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein displays minimal binding to the GABA-gated chloride channel. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein has minimal antagonism of the GABA-gated chloride channel. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein is an AR modulator with minimal interaction with a GABA-gated chloride channel. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein is an AR modulator with minimal interaction with the GABA_A-gated chloride channel. In some embodiments, the third-generation AR inhibitor compound identified using the methods provided herein is an AR modulator with minimal interaction with the GABA_A-gated chloride channel and or minimal blood-brain barrier penetration. GABA assays are known and include, but are not limited to, those described in Ashok K. Mehta and Maharaj K. Ticku "Characterization of the Picrotoxin Site of GABA_A Receptors" *Current Protocols in Pharmacology* (2000) 1.18.1-1.18.17; Copyright © 2000 by John Wiley & Sons, Inc., which is herein incorporated by reference.

[00186] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits AR nuclear translocation. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits AR DNA binding to androgen response elements. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits coactivator recruitment at an AR responsive promoter. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein exhibits no agonist activity in AR-overexpressing prostate cancer cells.

[00187] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate

cancer xenograft models. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing wild-type AR. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing a mutant AR having a F876L mutation. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing the wild-type AR and a mutant AR having a F876L mutation. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing a mutant AR having a T877A mutation (e.g. xenograft tumors formed from LNCaP cells). In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing the wild-type AR and a mutant AR having a T877A mutation (e.g. xenograft tumors formed from LNCaP/AR cells). In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein inhibits growth of castration sensitive and castration resistant prostate cancer xenograft models expressing a mutant AR having a T877A mutation and a mutant AR having a F876L mutation.

Pharmaceutical compositions

[00188] In some embodiments, provided is a pharmaceutical composition comprising a therapeutically effective amount of a third-generation AR inhibitor identified using the screening methods provided herein. In some embodiments, provided is a use of a third-generation AR inhibitor identified using the screening methods provided herein for the preparation of a medicament.

[00189] In some embodiments, the pharmaceutical composition comprising a third-generation AR inhibitor also contains at least one pharmaceutically acceptable inactive ingredient. In some embodiments, the pharmaceutical composition comprising a third-generation AR inhibitor is formulated for intravenous injection, subcutaneous injection, oral administration, or topical administration. In some embodiments, the pharmaceutical composition comprising a third-generation AR inhibitor is a tablet, a pill, a capsule, a liquid, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, or a lotion.

[00190] Pharmaceutical compositions are formulated in a conventional manner using one or more pharmaceutically acceptable inactive ingredients that facilitate processing of the active

compounds into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions described herein can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins 1999), herein incorporated by reference for such disclosure.

[00191] Provided herein are pharmaceutical compositions a third-generation AR inhibitor, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient. In some embodiments, the third-generation AR inhibitor described herein is administered as pharmaceutical compositions in which the third-generation AR inhibitor is mixed with other active ingredients, as in combination therapy. In other embodiments, the pharmaceutical compositions include other medicinal or pharmaceutical agents, carriers, adjuvants, preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure, and/or buffers. In yet other embodiments, the pharmaceutical compositions include other therapeutically valuable substances.

[00192] A pharmaceutical composition, as used herein, refers to a mixture of a the third-generation AR inhibitor or a pharmaceutically acceptable salt thereof, with other chemical components (i.e. pharmaceutically acceptable inactive ingredients), such as carriers, excipients, binders, filling agents, suspending agents, flavoring agents, sweetening agents, disintegrating agents, dispersing agents, surfactants, lubricants, colorants, diluents, solubilizers, moistening agents, plasticizers, stabilizers, penetration enhancers, wetting agents, anti-foaming agents, antioxidants, preservatives, or one or more combination thereof. The pharmaceutical composition facilitates administration of the compound to a subject, such as a mammal.

[00193] A therapeutically effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. The compounds can be used singly or in combination with one or more therapeutic agents as components of mixtures.

[00194] The pharmaceutical formulations described herein are administered to a subject by appropriate administration routes, including but not limited to, oral, parenteral (e.g., intravenous, subcutaneous, intramuscular), intranasal, buccal, topical, rectal, or transdermal administration routes. The pharmaceutical formulations described herein include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions,

aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate and controlled release formulations.

[00195] In some embodiments, the pharmaceutical composition further comprises one or more additional therapeutically active agents, including, but not limited to, corticosteroids, anti-emetic agents, analgesics, anti-cancer agents, anti-inflammatory agents, kinase inhibitors, HSP90 inhibitors, and histone deacetylase (HDAC) inhibitors.

[00196] In some embodiments, provided is a pharmaceutical composition comprising a therapeutically effective amount of a mutant AR polypeptide provided herein. In some embodiments, provided is a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a mutant AR polypeptide provided herein.

Therapeutic Methods

[00197] In some embodiments, the third-generation AR inhibitor compounds identified using the methods provided herein are administered for the treatment of a disease or condition. In some embodiments, described herein are methods of treating an AR dependent or AR mediated disease or condition in mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof.

[00198] In some embodiments, provided are methods comprising administering a third-generation AR inhibitor compound identified using the methods provided herein to a subject (e.g. a human) having a disease or condition that is AR mediated or AR dependent. In some embodiments, provided is a use of a third-generation AR inhibitor compound identified using the methods provided herein for the preparation of medicament for the treatment of a disease or condition that is AR mediated or AR dependent. In some embodiments, provided is a third-generation AR inhibitor compound identified using the methods provided herein for the treatment of a disease or condition that is AR mediated or AR dependent. In some embodiments, the subject (e.g. a human) is currently receiving one or more additional therapeutically active agents other than the third-generation AR inhibitor compound.

[00199] In some embodiments, the method further comprises administering one or more additional therapeutically active agents other than a third-generation AR inhibitor compound identified using the methods provided herein. In some embodiments, the one or more additional therapeutically active agents other than a third-generation AR inhibitor compound identified using the methods provided herein are selected from: hormones, hormone receptor agonists or

antagonists, corticosteroids, anti-emetic agents, analgesics, anti-cancer agents, anti-inflammatory agents, kinase inhibitors, HSP90 inhibitors, histone deacetylase (HDAC) inhibitors. In some embodiments, third-generation AR inhibitor compound is administered prior to, simultaneously, following, or intermittently with the one or more additional therapeutically active agents other than the third-generation AR inhibitor compound.

[00200] In some embodiments, the subject is administered a gonadotropin-releasing hormone (GnRH) agonist or antagonist in combination with third-generation AR inhibitor compound provided herein. In some embodiments, a GnRH receptor agonist such as leuprolide, bruserelin and goserelin is administered to a subject in combination with third-generation AR inhibitor compound provided herein. GnRH receptor agonists cause an initial surge in hormone production (i.e. "clinical flare") followed by the inhibition of lutenizing hormone production, which in turn causes a suppression of testosterone and dihydrotestosterone, on which continued growth of prostate cancer cells depend. In some embodiments, the subject is administered a gonadotropin-releasing hormone (GnRH) agonist or antagonist in combination with third-generation AR inhibitor compound provided herein for the treatment of an AR dependent or AR mediated disease or condition such as a prostate, breast, bladder or hepatocellular cancer. In some embodiments, the subject is administered a gonadotropin-releasing hormone (GnRH) agonist or antagonist in combination with third-generation AR inhibitor compound provided herein for the treatment of a castration resistant prostate cancer (CRPC). In some embodiments, the subject is administered a third-generation AR inhibitor compound provided herein to reduce or inhibit the initial surge in hormone production caused by treatment with a GnRH receptor agonist. In some embodiments, third-generation AR inhibitor compound is administered prior to, simultaneously, following, or intermittently with a GnRH receptor agonist or antagonist.

[00201] In some embodiments, the AR dependent or AR mediated disease or condition is benign prostate hyperplasia, hirsutism, acne, adenomas and neoplasms of the prostate, benign or malignant tumor cells containing the androgen receptor, hyperpilosity, seborrhea, endometriosis, polycystic ovary syndrome, androgenic alopecia, hypogonadism, osteoporosis, suppression of spermatogenesis, libido, cachexia, anorexia, androgen supplementation for age related decreased testosterone levels, prostate cancer, breast cancer, endometrial cancer, uterine cancer, bladder cancer, hepatocellular cancer, hot flashes, and Kennedy's disease, muscle atrophy and weakness, skin atrophy, bone loss, anemia, arteriosclerosis, cardiovascular disease, loss of energy, loss of well-being, type 2 diabetes or abdominal fat accumulation. In some embodiments, the AR dependent or AR mediated disease or condition is an AR dependent or AR mediated cancer, such as, for example, a prostate, breast, bladder or liver (i.e. hepatocellular) cancer.

[00202] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof. In some embodiments, the cancer is a hormone dependent cancer. In some embodiments, the hormone dependent cancer is an AR dependent cancer. In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is castration resistant prostate cancer. In some embodiments, the method of treating cancer further comprises administering to the mammal at least one additional anti-cancer agent.

[00203] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has not received treatment with an anti-cancer agent. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has not received treatment with a chemotherapeutic compound.

[00204] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been administered one or more anti-cancer agents. Exemplary anti-cancer agents include, but are not limited to, hormonal therapeutic agents, including, but not limited to first- and second-generation AR antagonists (e.g. bicalutamide, flutamide, hydroxyflutamide, nilutamide, ARN-509, enzalutamide (MDV3100) and RD162) and compounds that inhibit hormone (e.g. androgen) production, such as, for example, galeterone (TOK001), TAK-700 or abiraterone acetate, chemotherapeutic compounds, anti-metabolites, anti-cancer antibodies, surgery, radiation, and hyperthermal therapy. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been administered one or more chemotherapeutic compounds. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been treated by surgery. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been treated with radiation therapy. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been treated with a hyperthermal therapy.

[00205] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is used to treat prostate cancer in a mammal, wherein the mammal has been administered one or more cycles of treatment with an anti-cancer agent.

[00206] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR inverse agonist, AR antagonist, an AR degrader, an AR trafficking modulator, an AR DNA-binding inhibitor, or combinations thereof.

[00207] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR inverse agonist.

[00208] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR antagonist.

[00209] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR degrader.

[00210] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR trafficking modulator.

[00211] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a pharmaceutically acceptable salt thereof, wherein the compound is an AR DNA-binding inhibitor.

[00212] In some embodiments, described herein are methods of treating cancer in a mammal comprising administering to the mammal a therapeutically effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, or a

pharmaceutically acceptable salt thereof, wherein the compound is an AR protein synthesis inhibitor.

[00213] In some embodiments, the cancer is prostate cancer. In some embodiments, the cancer is castration resistant prostate cancer. In some embodiments, the prostate cancer is an ARN-509-resistant prostate cancer. In some embodiments, the prostate cancer is an enzalutamide (MDV3100)-resistant prostate cancer. In some embodiments, the prostate cancer is an RD162-resistant prostate cancer. In some embodiments, the prostate cancer is an abiraterone acetate-resistant prostate cancer. In some embodiments, the prostate cancer is a galeterone (TOK001)-resistant prostate cancer. In some embodiments, the prostate cancer is a TAK-700-resistant prostate cancer.

[00214] Pharmaceutical formulations described herein are administerable to a subject in a variety of ways by multiple administration routes, including but not limited to, oral, parenteral (e.g., intravenous, subcutaneous, intramuscular), buccal, topical or transdermal administration routes. The pharmaceutical formulations described herein include, but are not limited to, aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate and controlled release formulations. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is administered orally. In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is administered intravenously.

[00215] In some embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is administered topically. In such embodiments, a third-generation AR inhibitor compound identified using the methods provided herein is formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, shampoos, scrubs, rubs, smears, medicated sticks, medicated bandages, balms, creams or ointments. Such pharmaceutical compounds can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives. In one aspect, the anti-androgen compound identified using the methods provided herein is administered topically to the skin.

[00216] In another aspect is the use of a third-generation AR inhibitor compound identified using the methods provided herein in the manufacture of a medicament for treating a disease, disorder or conditions in which the activity of AR contributes to the pathology and/or symptoms

of the disease or condition. In one aspect, the disease or condition is any of the diseases or conditions specified herein.

[00217] In any of the aforementioned aspects are further embodiments in which: (a) the effective amount of the third-generation AR inhibitor compound identified using the methods provided herein is systemically administered to the mammal; and/or (b) the effective amount of the third-generation AR inhibitor compound is administered orally to the mammal; and/or (c) the effective amount of the third-generation AR inhibitor compound is intravenously administered to the mammal; and/or (d) the effective amount of the third-generation AR inhibitor compound is administered by injection to the mammal; and/or (e) the effective amount of the third-generation AR inhibitor compound is administered topically to the mammal; and/or (f) the effective amount of the third-generation AR inhibitor compound is administered non-systemically or locally to the mammal.

[00218] In any of the aforementioned aspects are further embodiments comprising single administrations of the effective amount of the third-generation AR inhibitor compound identified using the methods provided herein, including further embodiments in which (i) the third-generation AR inhibitor compound is administered once; (ii) the third-generation AR inhibitor compound is administered to the mammal multiple times over the span of one day; (iii) continually; or (iv) continuously.

[00219] In any of the aforementioned aspects are further embodiments comprising multiple administrations of the effective amount of a third-generation AR inhibitor compound identified using the methods provided herein, including further embodiments in which (i) the third-generation AR inhibitor compound is administered continuously or intermittently: as in a single dose; (ii) the time between multiple administrations is every 6 hours; (iii) the third-generation AR inhibitor compound is administered to the mammal every 8 hours; (iv) the third-generation AR inhibitor compound is administered to the mammal every 12 hours; (v) the third-generation AR inhibitor compound is administered to the mammal every 24 hours. In further or alternative embodiments, the method comprises a drug holiday, wherein the administration of the third-generation AR inhibitor compound is temporarily suspended or the dose of the compound being administered is temporarily reduced; at the end of the drug holiday, dosing of the compound is resumed. In some embodiments, the length of the drug holiday varies from 2 days to 1 year.

[00220] Also provided are methods of reducing AR activation in a mammal comprising administering to the mammal a third-generation AR inhibitor compound identified using the methods provided herein. In some embodiments, the method comprises reducing AR activation in prostate cells in the mammal. In some embodiments, the method comprises reducing AR

activation in non-prostate cells. In some embodiments, the method of reducing AR activation comprises reducing the binding of androgens to the androgen receptor. In some embodiments, the method of reducing AR activation comprises reducing AR concentration in a cell.

[00221] In some cases disclosed herein is the use of a third-generation AR inhibitor compound identified using the methods provided herein in the manufacture of a medicament for the treatment of diseases or conditions that are AR dependent or AR mediated. In some embodiments, the disease or condition is prostate cancer. In some embodiments, the AR dependent or AR mediated disease or condition is described herein.

[00222] In some cases disclosed herein is the use of a third-generation AR inhibitor compound identified using the methods provided herein in the treatment or prevention of diseases or conditions that are AR dependent or AR mediated. In some embodiments, the AR dependent or AR mediated disease or condition is described herein.

[00223] In any of the embodiments disclosed herein, the mammal is a human. In some embodiments, the third-generation AR inhibitor compound provided herein is administered to a human.

[00224] In some embodiments, the third-generation AR inhibitor compound provided herein is used to diminish, reduce, or eliminate the activity of AR.

[00225] In some embodiments, the third-generation AR inhibitor compounds identified by the methods disclosed herein are selective AR modulators. In some embodiments, the third-generation AR inhibitor compounds identified by the methods disclosed herein have high specificity for the AR and have desirable, tissue-selective pharmacological activities. Desirable, tissue-selective pharmacological activities include, but are not limited to, AR antagonist activity in prostate cells and no AR antagonist activity in non-prostate cells. In some embodiments, the third-generation AR inhibitor compounds disclosed herein are anti-androgens that display negligible or no AR agonist activity.

[00226] In some embodiments, presented herein are third-generation AR inhibitor compounds identified by the methods disclosed herein selected from active metabolites, tautomers, pharmaceutically acceptable solvates, pharmaceutically acceptable salts or prodrugs of an AR inhibitor compound identified using the methods provided herein.

[00227] In some embodiments, the pharmaceutical composition comprising third-generation AR inhibitor compounds is administered in combination with one or more additional therapeutic agents, including, but not limited to, corticosteroids, anti-emetic agents, analgesics, anti-cancer agents, anti-inflammatory agents, kinase inhibitors, HSP90 inhibitors, and histone deacetylase (HDAC) inhibitors. In some embodiments, the pharmaceutical composition comprising third-

generation AR inhibitor compounds is administered in combination with an anti-cancer agent including, but not limited to, a hormonal therapeutic agent, including, but not limited to a first- and second- generation AR antagonists (e.g. bicalutamide, flutamide, hydroxyflutamide, nilutamide, ARN-509, enzalutamide (MDV3100) and RD162), a compound that inhibits hormone (e.g. androgen) production, such as a CYP17A inhibitor, including, for example, galeterone (TOK001), TAK-700 or abiraterone acetate, chemotherapeutic compounds, anti-metabolites, anti-cancer antibodies, surgery, radiation, and hyperthermal therapy. In some embodiments, the third-generation AR inhibitor compound and the additional therapeutic agent is administered in the same composition. In some embodiments, the third-generation AR inhibitor compound and the additional therapeutic agent are administered as separate compositions. In some embodiments, the third-generation AR inhibitor compound and the additional therapeutic agent are administered simultaneously, sequentially, or intermittently. In some embodiments, the third-generation AR inhibitor compound and the additional therapeutic agent are administered by the same route of administration. In some embodiments, the third-generation AR inhibitor compound and the additional therapeutic agent are administered by different routes of administration.

Kits/Articles of Manufacture

[00228] For use in the diagnostic and therapeutic applications described herein, kits and articles of manufacture are also described herein. Such kits can comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers are formed from any acceptable material including, e.g., glass or plastic.

[00229] In some embodiments, the kits provided herein are for use in detecting nucleic acid encoding a mutant AR polypeptide in a subject or for detecting a mutant AR polypeptide in a subject (i.e. a diagnostic kit). In some embodiments the kits are employed for selecting patients for treatment with a third-generation AR antagonist, for identifying subjects as resistant or likely to become resistant to a first- or second-generation AR antagonist, for monitoring the development of resistance to a first- or second-generation AR antagonist therapy, or combinations thereof. The kits provided herein contain one or more reagents for the detection of the nucleic acid encoding a mutant AR polypeptide, for the detection of mutant AR polypeptides, for detection of AR activity in cells from the subject, or combinations thereof. Exemplary reagents include but are not limited to, buffers, PCR reagents, antibodies, substrates for enzymatic staining, chromagens or other materials, such as slides, containers, microtiter

plates, and optionally, instructions for performing the methods. Those of skill in the art will recognize many other possible containers and plates and reagents that can be used for contacting the various materials. Kits also can contain control samples, such as for example, nucleic acids or proteins, such as for example a mutant AR polypeptide provided herein or nucleic acids encoding a mutant AR polypeptide provided herein. In some embodiments, kits contain one or more set of oligonucleotide primers for detection of endogenous androgen gene expression.

[00230] In some embodiments, the container(s) can comprise one or more first- or second-generation AR antagonists or third-generation AR inhibitor compounds identified by the methods described herein, optionally in a composition or in combination with another agent as disclosed herein. The container(s) optionally have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). Such kits optionally comprising a compound with an identifying description or label or instructions relating to its use in the methods described herein.

[00231] A kit will typically comprise one or more additional containers, each with one or more of various materials (such as reagents, optionally in concentrated form, and/or devices) desirable from a commercial and user standpoint for use of a compound described herein. Non-limiting examples of such materials include, but not limited to, buffers, diluents, filters, needles, syringes; carrier, package, container, vial and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[00232] A label can be on or associated with the container. A label can be on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label can be associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. A label can be used to indicate that the contents are to be used for a specific therapeutic application. The label can also indicate directions for use of the contents, such as in the methods described herein.

[00233] Articles of manufacture, which include packaging material, a third-generation AR inhibitor compound identified using the methods provided herein within the packaging material, and a label that indicates that the compound or composition, or pharmaceutically acceptable salt, tautomers, pharmaceutically acceptable N-oxide, pharmaceutically active metabolite, pharmaceutically acceptable prodrug, or pharmaceutically acceptable solvate thereof, is used for reducing, diminishing or eliminating the effects of androgen receptors, or for the treatment, prevention or amelioration of one or more symptoms of a disease or condition that would benefit from a reduction or elimination of androgen receptor activity, are provided.

Production of Anti-Androgen Resistant cell lines

[00234] Provided herein are methods for producing prostate cancer cell lines resistant to treatment with an AR antagonist. In some embodiments, the prostate cancer cell lines are resistant to treatment with ARN-509. In some embodiments, the prostate cancer cell lines are resistant to treatment with enzalutamide (MDV3100). In some embodiments, the resistant cell lines generated by the method provided express a higher level of AR compared to the parental cell line used to generate the resistant cell line. In some embodiments, the resistant cell lines generated by the method express about 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10 times or greater the amount of AR compared to the parental cell line. In some embodiments, the resistant cell lines express a mutant AR protein.

[00235] In some embodiments, the method comprises contacting a prostate cancer cell line (i.e. parental cell line) with an AR antagonist and culturing the cells for a predetermined period of time. In some embodiments, the method comprises culturing the cells in increasing concentrations of the AR antagonist for a predetermined period of time. In some embodiments, the concentration of the AR antagonist ranges from about 0.1 μM to about 100 μM , such as, for example, 1 μM to about 10 μM . In some embodiments, the cells are cultured at 0.8 μM , 1.5 μM , 3 μM and 6 μM of the AR antagonist. In some embodiments, the concentration of the AR antagonist is increased 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times. In some embodiments, the concentration of the AR antagonist is increased 3 times. In some embodiments, the cells are cultured in the presence of the AR antagonist 1 day, 2 days, 3, days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 1 month, 1.5 months, 2 months, 2.5 months, 3 months or more. In some embodiments, the cells are divided and re-plated every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every week or longer. In some embodiments, the culture media containing the AR antagonist is refreshed every day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every week or longer. In some embodiments, the AR antagonist is a second-generation antagonist. In some embodiments, the AR antagonist is ARN-509. In some embodiments, the AR antagonist is enzalutamide (MDV3100).

[00236] In some embodiments, the prostate cancer cell line is a human prostate adenocarcinoma cell line. In some embodiments, the prostate cancer cell line is an LNCaP cell line. In some embodiments, the prostate cancer cell line overexpresses the androgen receptor. In some embodiments, the prostate cancer cell line is LNCaP/AR(cs) or LNCaP/AR(cs)-Luc.

EXAMPLES

[00237] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Example 1: Generation of Drug Resistant Cell Line *In vivo*

[00238] Cell lines resistant to treatment with the anti-AR compound ARN-509 were generated *in vivo* in mice bearing castration resistant human prostate adenocarcinoma (LNCaP/AR(cs)) xenograft tumors. The LNCaP/AR(cs) cell line over-expresses the androgen receptor (AR) 3-5 fold over the parental LNCaP cell line mimicking castration resistant prostate cancer (Chen et al. (2004) *Nature Medicine* 10:33-39).

[00239] LNCaP-AR(cs) xenograft tumors were grown in castrated six week old male SCID Hairless Outbred mice (SHO, Charles Rivers Laboratories). 1×10^6 LNCaP-ARcs cells in 50% serum-free RPMI and 50% MatrigelTM were subcutaneously injected (100 μ l/animal) on the right flank of 10 mice 3-5 days post castration. Tumor size was monitored daily. When tumors reached an average volume of ~ 200 mm³ (approximately 60 days post-injection), the animals began treatment with vehicle alone (n=1) or 30mg/kg ARN-509 (n=9) by QD dosing regimen (i.e. orally in 15% Vitamin E-TPGS and 65% of a 0.5% w/v carboxymethyl cellulose (CMC) solution in 20 mM citrate buffer (pH 4.0)). Initially, treatment with ARN-509 induced tumor regression. Following approximately 75 days of dosing, a single tumor resumed growth and progressed to a size greater than at treatment initiation. Once the resistant tumor reached ~ 800 mm³, the mouse was euthanized and the tumor was harvested. Tumor cells were manually dispersed by homogenization with a 3 mL syringe. Tumor cells were cultured in RPMI plus 10% FBS and 10 μ M ARN-509. One resistant cell line was generated by this method.

Example 2: Generation of Drug Resistant Cell Lines *In vitro*

[00240] Cell lines resistant to treatment with the anti-AR compounds ARN-509 and MDV3100 were generated *in vitro* using LNCaP human prostate adenocarcinoma cell lines.

[00241] LNCaP (ATCC), LNCaP/AR(cs) (Guo et al. (2006) *Cancer Cell* 0:309-19) and LNCaP/AR-Luc (Tran et al. *Science* (2009) 324(5928):787-90; Ellwood-Yen et al. (2006) *Cancer Res.* 66:10513-6) were maintained in RPMI 1640 supplemented with 10% FBS (Hyclone). LNCaP (ATCC), LNCaP/AR(cs) or LNCaP/AR(cs)-Luc cells were cultured in increasing concentrations of either ARN-509 or MDV3100 over a course of 6 months. Initially, 50 mL of cells were seeded into a 225 cm² cell culture flask at a concentration of approximately 80,000 cells/mL and grown in RPMI plus 10% FBS in the presence of 800 nM ARN-509 or MDV3100. Medium and drug were changed semiweekly and the cells were passaged in 75 cm² cell culture flasks as needed. The concentration of each compound was increased several times

from about 1.5 μM to about 6 μM as the growth rate of the drug-treated cells increased to that of the untreated control cells. After approximately 6 months of drug selection, the cells were maintained in RPMI plus 10% FBS and 6 μM ARN-509 or MDV3100. Following selection, 10 independent ARN-509 and MDV3100 resistant cell lines were obtained. Two lines were derived from LNCaP (ATCC) cells selected in the presence of ARN-509. Four cell lines were derived from LNCaP/AR(cs), two following treatment with ARN-509 and two following treatment with MDV3100. LNCaP/AR(cs)-Luc cells were used to derive 4 resistant cell lines, two from ARN-509 treatment and two from MDV3100 treatment.

Example 3: Proliferation Assays to Test Drug Resistance

[00242] Cell proliferation assays were performed to test resistance of the cell lines to ARN-509 and MDV3100 treatment.

[00243] Proliferation assays were performed on all ARN-509 and MDV3100 resistant cell lines by seeding 16 μL /well of cells at a density of 50,000 cells per mL in phenol-red-free RPMI 1640 (with 5% CSS) into 384-well cell culture plate (Flat Clear Bottom Black Polystyrene TC-Treated 384 Well plates (Corning)) and incubated for 2 days at 37 °C. For agonist assays, 11 point semi-log dilutions of each compound were made in culture medium and 16 μL of each dilution was added to the cells. ARN-509, MDV3100 and bicalutamide were run at a final concentration ranging from 3.16×10^{-5} M to 3.18×10^{-10} M, while the synthetic androgen methyltrienolone (R1881) was run at a final concentration range of 3.16×10^{-8} M to 3.18×10^{-13} M. For the antagonist mode assay, the compounds were diluted in culture medium also containing 200 pM R1881 (PerkinElmer, Waltham, MA) (final [R1881] = 100 pM) and then added to the cells (16 μL). After 7 days, 16 μL of CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI) was added directly to the cell cultures and Relative Luminescence Units (RLUs) measured according to the manufacturer's instructions.

[00244] In the agonist mode assay, percent viability of the samples was calculated as: % viability = $100 \times ([\text{RLU sample} - \text{RLU medium without cells}] / [\text{RLU R1881 treated cells} - \text{RLU medium without cells}])$ (Table 1A). In the antagonist mode assay, the percent viability of the samples was calculated as: % viability = $100 \times ([\text{RLU sample} - \text{RLU Day 0}] / [\text{RLU R1881 treated cells} - \text{RLU Day 0}])$ (Table 1B).

Table 1A. Agonist Proliferation Assay (% Viability)

	R1881	Bicalutamide	MDV3100	ARN-509
LNCaP	100.0	+	+	+
LNCaP/AR(cs)	100.0	+	+	+
Class 1	100.0	++	++	++
Class 2	100.0	+	++	++

'+' = < 30, '++' = > 30

[R1881] = 0.1 nM, [Antagonists] = 10 μ M

Table 1B. Antagonist Proliferation Assay (% Viability)

	R1881	Bicalutamide	MDV3100	ARN-509
LNCaP	100.0	+	+	+
LNCaP/AR(cs)	100.0	+	+	+
Class 1	100.0	++	++	++
Class 2	100.0	++	++	++

'+' = < 30, '++' = > 30

[R1881] = 0.1 nM, [Antagonists] = 10 μ M

[00245] In the proliferation assays, both ARN-509 and enzalutamide were full proliferative antagonists in all three LNCaP parental cell lines (Table 1A, Figure 1A). The resistant cell lines segregated into two distinct classes. Unlike their parental cell lines, the first class of ARN-509- and MDV3100-resistant cells (Class 1) proliferate in the absence of added androgens. The ligand independent growth of the cells is unaltered in the presence of ARN-509, MDV3100 or bicalutamide. The synthetic androgen, R1881, inhibits proliferation in the class 1 cells at high concentrations. This growth inhibitory activity of R1881 is antagonized by either MDV3100 or ARN-509, indicating that AR is still capable of binding MDV3100 and ARN-509 in these cell lines.

[00246] Class 1 resistant cell lines included the one cell line derived from the LNCaP-AR(cs) xenograft tumor, the 2 cell lines derived from LNCaP/AR(cs) cell selected in the presence of ARN-509, the 2 cell lines derived from LNCaP/AR(cs) cells selected in the presence of MDV3100, the 2 cell lines derived from LNCaP/AR(cs)-Luc selected in the presence of ARN-509, and one of the cell lines derived from LNCaP/AR(cs)-Luc selected in the presence of MDV3100.

[00247] The second class of MDV3100- and ARN-509- resistant cell lines (Class 2) remains androgen dependent for growth, similar to their parental cell lines. However, unlike their activity on the parental cell lines, ARN-509 and MDV3100 can stimulate proliferation in the class 2 cell lines. Bicalutamide, however, did not stimulate proliferation in the class 2 cell lines. Class 2

resistant cell lines included the two cell lines derived from LNCaP cells selected in the presence of ARN-509 (LNCaP ARN-509r1 and LNCaP ARN-509r2) and one of the cell lines derived from LNCaP/AR(cs)-Luc selected in the presence of MDV3100 (LNCaP ENZr2).

[00248] The partial agonist activity was independent of the compound utilized for selection; ARN-509 and enzalutamide displayed partial agonist activity in all three cell lines regardless of the compound used to derive the resistance variants. Consistent with proliferative activity, ARN-509 or enzalutamide only partially antagonized androgen dependent growth of these resistant lines (Table 1B, Figure 1B,C).

Example 4: Transcriptional Reporter Assays to Test Drug Resistance

[00249] Transcriptional reporter assays using an ARE response element operatively linked to a reporter gene were performed to test resistance of the cells to ARN-509, MDV3100, and bicalutamide treatment.

[00250] Transcriptional reporter assays were performed on all resistant cell lines by seeding 100 μ L of cells at a density of 250,000 cells/mL into 96-well cell culture plates in RPMI 1640 supplemented with 5% charcoal stripped serum and allowed to attach overnight at 37 °C.

[00251] With the exception of the LNCaP/AR(cs)-Luc cells that contain an integrated androgen responsive reporter, cells were transiently transfected using Lipofectin® (Life Technologies) according to the manufacturer's protocol. For LNCaP and LNCaP/AR(cs) cells, triplicate transfections were performed using 428 ng reporter vector (pGL4 Pb-Luciferase (the rat probasin promoter in pGL4 (Promega, Madison, WI))), 50 ng pRL-CMV (normalization vector, Promega, Madison, WI) and 0.7 μ L Lipofectin®. Following transfection, the cells were incubated for 4 hours.

[00252] Cells were then treated with the test compounds ARN-509, MDV3100 and bicalutamide. For agonist assays, the compounds were serially diluted and 50 μ L of compound plus RPMI 1640 plus 5% charcoal stripped FBS was added to the cells. For antagonist assays, the compounds were serially diluted and 50 μ L of compound with RPMI plus 3 nM R1881 supplemented with 5% charcoal stripped serum was added to the cells. Following 48 hour incubation the medium was removed and the cells were lysed in 40 μ L of lysis buffer (25 mM Tris Phosphate, 2 mM CDTA, 10% Glycerol, 0.5% Triton X-100, 2 mM DTT). Firefly luciferase activity was measured immediately following the addition of 40 μ L luciferase buffer (20 mM tricine, 0.1 mM EDTA, 1.07 mM (MgCo₃)₄ Mg(OH)₂•5H₂O, 2.67 mM MgSO₄, 33.3 mM DTT, 270 μ M Coenzyme A, 470 μ M luciferin, 530 μ M ATP). *Renilla* luciferase was measured following the addition of 40 μ L coelenterazine buffer (1.1 M NaCl, 2.2 mM

Na₂EDTA, 0.22 M K_xPO₄ (pH 5.1), 0.44 mg/mL BSA, 1.3 mM NaN₃, 1.43 μ M coelenterazine, final pH adjusted to 5.0).

[00253] The two classes of ARN-509- and MDV3100-resistant cell lines identified in the proliferation assays also exhibited distinct properties in the transcriptional assays. In transient transfection assays using the pGL4 Pb-Luciferase reporters or in assays using the integrated probasin-luciferase reporter (i.e. LNCaP/AR(cs)-Luc-derived cells), ARN-509 and MDV3100 were effective antagonists in the androgen-independent class 1 resistant cells as evidenced by a decrease in luciferase activity relative to no treatment controls (Table 2). Bicalutamide, however, exhibited an increased agonist activity in class 1 resistant cells compared to the parental cells. In class 2 resistant cells, MDV3100 was a weak partial agonist in the probasin-luciferase reporter assay, while bicalutamide and ARN-509 displayed no agonist activity.

Table 2A. Agonist Transcriptional Reporter Assay (% Max Activity)

	R1881	Bicalutamide	MDV3100	ARN-509
LNCaP	>90	+	+	+
LNCaP/AR(cs)	>90	+	+	+
Class 1	>90	++	+	+
Class 2	>90	+	++	+

'+' = < 5, '++' = > 5

[R1881] = 10 nM, [Antagonists] = 50 μ M

Table 2B. Antagonist Transcriptional Reporter Assay (% Max Activity)

	R1881	Bicalutamide	MDV3100	ARN-509
LNCaP	>90	+	+	+
LNCaP/AR(cs)	>90	+	+	+
Class 1	>90	++	+	++
Class 2	>90	+	++	+

'+' = < 5, '++' = > 5

[R1881] = 10 nM, [Antagonists] = 50 μ M

Example 5: Endogenous Gene Transcriptional Assays

[00254] The effects of R1881, MDV3100 and bicalutamide treatment on endogenous gene transcription was examined.

[00255] Endogenous gene transcriptional assays were performed on all resistant lines by plating 0.5 mL of cells at a density of 500,000 cells/mL in 24-well plates in RPMI containing 5% charcoal stripped FBS (hormone depleted) and growing the cells for 3 days at 37 °C. Cells were then treated for 24 h with 10 nM R1881, 30 μ M MDV3100 or 30 μ M bicalutamide.

[00256] Total RNA was isolated using the Aurum[™] total RNA isolation kit (BIO-RAD, Hercules, CA). RNA (1 μ g) was reverse transcribed using the iScript cDNA synthesis kit (BIO-

RAD, Hercules, CA) to produce cDNA. Real-time PCR was performed using the Applied Biosystems 7900HT instrument and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). PCR reactions were performed in 6 μ L according to the manufacturer's protocol and a thermocycle protocol of 95 °C for 10 minutes followed by 40 cycles of 95 °C for 15 seconds and 58 °C for 1 minute. Androgen responsive gene (PSA, SLUG, TMPRSS2, STEAP4, FKBP5, ORM1, NOV, FASN, NKX3.1, AMIGO2, BDNF, CAMK2N1, HPGD, NCAPD3, PLD1) expression was normalized to GAPDH expression and expressed relative to vehicle treatment of the parental cell line. Relative expression results are provided in Table 3A. Primers employed for PCR are listed in Table 3B.

[00257] Similar compound and class selective agonist activities were observed on endogenous androgen-responsive genes, but in the context of the endogenous genes the transcriptional activity of MDV3100 is more evident (Table 3). In class 1 resistant cells, bicalutamide displays robust transcriptional activity and MDV3100 is a weak transcriptional agonist. In contrast, in class 2 resistant cell lines, bicalutamide is a weak transcriptional agonist while MDV3100 displays robust transcriptional agonist activity on the genes tested.

Table 3A. Endogenous Gene Transcriptional Activity (Fold Vehicle)

		PSA	SLUG	TMPRSS2	STEAP4	FKBP5	ORM1	NOV
LNCaP	DMSO	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	R1881	++	+++	++	++++	++	+++	--
	Bicalutamide	+	+	+	+	+	-	+
	MDV3100	+	+	+	-	+	+	+
LNCaP/AR(cs)	DMSO	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	R1881	+++	+++	++	++++	++	++++	--
	Bicalutamide	++	+	+	++	+	+++	+
	MDV3100	+	+	+	+	+	+	+
Class 1	DMSO	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	R1881	++	+++	+	++++	+++	++++	--
	Bicalutamide	+	+++	+	+++	++	++	+
	MDV3100	+	+	+	+	+	+	+
Class 2	DMSO	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	R1881	+++	+++	++	++++	+++	++++	--
	Bicalutamide	+	+	+	+	+	+	+
	MDV3100	+++	+++	++	+++	++	+++	-

[R1881] = 10 nM, [Antagonists] = 30

μ M

-- < 0.1, - = 0.1 - 1, + = 1 - 10, ++ = 10 - 50, +++ 50 - 500, ++++ > 500

Table 3B: Transcriptional Real-time PCR Oligonucleotide Sequence		
Gene	Forward Primer Sequence	Reverse Primer Sequence
AMIGO2	AGAGACTCAGAGGCGACCAT (SEQ ID NO: 20)	ATCAGCAAACACAGCAGCTC (SEQ ID NO: 21)
BDNF	AGAGCTGTTGGATGAGGACCAGAA (SEQ ID NO: 22)	AGGCTCCAAAGGCACTTGACTACT (SEQ ID NO: 23)
CAMK2N1	GACCAAGCGGGTTGTTATTGA (SEQ ID NO: 24)	TGCCTTGTCGGTCATATTTTCA (SEQ ID NO: 25)
FKBP5	CGGAGAACCAAACGGAAAGG (SEQ ID NO: 26)	CTTCGCCCACAGTGAATGC (SEQ ID NO: 27)
HPGD	ACAGCAGCCGGTTTATTGTGCTTC (SEQ ID NO: 28)	TGGCATTTCAGTCTCACACCACTGT (SEQ ID NO: 29)
NCAPD3	ACCACTCACCATCATCTCAAGGCA (SEQ ID NO: 30)	TGCTCTTCTTTGCCAGATCCTCGT (SEQ ID NO: 31)
NOV	GCCTTACCCTTGACAGCTTAC (SEQ ID NO: 32)	GAGCATGCTGTCCACTCTGT (SEQ ID NO: 33)
ORM1	CTTGCGCATTCCCAAGTCAGATGT (SEQ ID NO: 34)	TTTCCTCTCCTTCTCGTGCTGCTT (SEQ ID NO: 35)
PLD1	GAGCCTGCTACAGATGGTCA (SEQ ID NO: 36)	TGTCTACCAGCAGGACGAAG (SEQ ID NO: 37)
PSA	CCTCCTGAAGAATCGATTCC (SEQ ID NO: 38)	GAGGTCCACACACTGAAGTT (SEQ ID NO: 39)
SLUG	TTTCTGGGCTGGCCAAACATAAGC (SEQ ID NO: 40)	ACACAAGGTAATGTGTGGGTCCGA (SEQ ID NO: 41)
STEAP4	CGGCAGGTGTTTGTGTGTGGAAAT (SEQ ID NO: 42)	AGAAGACACACAGCACAGCAGACA (SEQ ID NO: 43)
TMPRSS2	TAGTGAAACCAGTGTGTCTGCCCA (SEQ ID NO: 44)	AGCGTTCAGCACTTCTGAGGTCTT (SEQ ID NO: 45)
FASN	CGCTCTGGTTCATCTGCTCTG (SEQ ID NO: 46)	TCATCAAAGGTGCTCTCGTCTG (SEQ ID NO: 47)
NKX3.1	TGGAGAGGAAGTTCAGCCATCAGA (SEQ ID NO: 48)	AGGAGAGCTGCTTTCGCTTAGTCT (SEQ ID NO: 49)

[00258] In a separate experiment, gene expression of the following genes were analyzed in LNCaP, LNCaP/AR(cs), LNCaP/AR-Luc, LNCaP ARN-509r1, LNCaP ARN-509r2 and LNCaP/AR-Luc ENZr2 cells: PLD, CAM2KN, NOV, BDNF, AMIGO2, FASN, TMPRSS2, NKX3.1, PSA, FKBP5, HPGD, NCAPD3, SLUG, STEAP4, and ORM. Cells were cultured for 3 days in hormone depleted medium followed by treatment with vehicle, 1 nM R1881 or 30 μ M compound. Gene expression was normalized to GAPDH as described above. Results are presented in Tables 4A and 4B.

Table 4A. LNCaP, LNCaP/AR(cs) and resistant line transcription

Gene	LNCaP				LNCaP/AR(cs)				LNCaP ARN-509r1			
	Vehicle	ARN-509	ENZ	R1881	Vehicle	ARN-509	ENZ	R1881	Vehicle	ARN-509	ENZ	R1881
AMIGO2	1.00	1.12	1.20	0.68	1.00	0.72	1.13	0.54	1.00	0.62	0.99	0.21
BDNF	1.00	1.09	0.85	0.40	1.00	0.85	1.13	0.48	1.00	0.90	1.58	0.31
CAM2K N1	1.00	1.17	1.39	0.13	1.00	0.80	1.11	0.05	1.00	0.37	0.59	0.01
FASN	1.00	1.34	1.24	5.58	1.00	0.75	1.19	7.11	1.00	1.04	1.53	3.12
FKBP5	1.00	0.99	1.04	54.95	1.00	0.99	1.71	97.01	1.00	2.58	9.45	53.45
HPGD	1.00	1.48	1.78	100.43	1.00	1.18	2.01	183.55	1.00	2.36	10.20	61.39
NCAPD3	1.00	1.16	1.20	104.69	1.00	0.91	1.22	93.05	1.00	1.29	3.12	90.51
NKX3.1	1.00	0.90	1.66	30.06	1.00	1.13	2.51	8.82	1.00	6.54	11.55	8.11
NOV	1.00	1.73	2.57	0.15	1.00	1.04	1.27	0.04	1.00	1.40	1.39	0.03
ORM1	1.00	0.71	1.13	873.10	1.00	0.84	3.58	3444.31	1.00	15.89	205.07	1296.13
PLD1	1.00	1.09	0.70	0.02	1.00	1.04	0.65	0.02	1.00	0.33	0.24	0.03
PSA	1.00	0.66	1.69	47.84	1.00	1.43	2.69	19.16	1.00	32.67	57.68	85.63
SLUG	1.00	1.27	2.35	129.79	1.00	1.03	2.06	89.88	1.00	4.66	42.52	164.28
STEAP4	1.00	0.78	1.54	639.15	1.00	0.90	1.95	1314.23	1.00	3.03	32.22	1184.45
TMPRSS2	1.00	0.77	1.68	22.16	1.00	1.06	2.36	17.51	1.00	10.20	23.75	37.27
Gene	LNCaP				LNCaP ARN-509r2							
	Vehicle	ARN-509	ENZ	R1881	Vehicle	ARN-509	ENZ	R1881				
AMIGO2	1.00	1.12	1.20	0.68	1.00	0.67	0.63	0.55				
BDNF	1.00	1.09	0.85	0.40	1.00	1.04	0.77	0.47				
CAM2K N1	1.00	1.17	1.39	0.13	1.00	0.46	0.38	0.02				
FASN	1.00	1.34	1.24	5.58	1.00	0.98	0.93	4.82				
FKBP5	1.00	0.99	1.04	54.95	1.00	8.00	3.41	48.50				
HPGD	1.00	1.48	1.78	100.43	1.00	1.49	4.56	59.30				
NCAPD3	1.00	1.16	1.20	104.69	1.00	1.13	1.35	54.95				
NKX3.1	1.00	0.90	1.66	30.06	1.00	3.73	6.28	10.20				
NOV	1.00	1.73	2.57	0.15	1.00	1.39	0.71	0.07				
ORM1	1.00	0.71	1.13	873.10	1.00	3.94	23.75	625.99				
PLD1	1.00	1.09	0.70	0.02	1.00	0.81	0.29	0.02				
PSA	1.00	0.66	1.69	47.84	1.00	1.91	2.48	7.89				
SLUG	1.00	1.27	2.35	129.79	1.00	1.31	9.58	77.17				
STEAP4	1.00	0.78	1.54	639.1	1.00	1.45	4.69	377.41				

				5				
TMPRSS 2	1.00	0.77	1.68	22.16	1.00	3.07	4.86	15.14

Table 4B. LNCaP/AR-Luc and LNCaP/AR-Luc ENZr2 transcription

Gene	LNCaP/AR-Luc				LNCaP/AR-Luc ENZr2			
	Vehicle	ARN-509	ENZ	R1881	Vehicle	ARN-509	ENZ	R1881
AMIGO2	1.00	1.13	1.44	0.54	1.00	0.99	0.78	0.49
BDNF	1.00	1.17	1.08	0.18	1.00	0.56	0.44	0.12
CAM2KN1	1.00	1.13	1.51	0.11	1.00	0.65	0.57	0.11
FASN	1.00	1.08	1.40	3.48	1.00	1.72	1.20	4.44
FKBP5	1.00	1.16	1.46	20.82	1.00	2.46	3.03	23.43
HPGD	1.00	1.88	2.51	2.41	1.00	1.22	1.61	1.39
NCAPD3	1.00	1.09	1.33	17.27	1.00	1.38	1.39	31.12
NKX3.1	1.00	1.14	1.68	11.24	1.00	4.82	5.21	10.13
NOV	1.00	2.55	1.95	0.47	1.00	1.21	1.20	0.27
ORM1	1.00	1.27	1.39	7.89	1.00	15.24	17.88	347.29
PLD1	1.00	1.45	1.33	0.15	1.00	0.46	0.74	0.21
PSA	1.00	0.60	0.44	2.10	1.00	4.59	3.48	16.91
SLUG	1.00	1.13	2.73	48.84	1.00	5.98	12.30	160.90
STEAP4	1.00	0.88	1.23	20.25	1.00	1.66	4.38	79.89
TMPRSS2	1.00	0.74	1.35	3.46	1.00	4.44	4.23	7.73

Example 6: Assays for Mechanisms of Resistance

[00253] Array CGH, mRNA expression profiling and sequence analysis of patient derived prostate cancer tumors as well as many animal and *in vitro* models have implicated multiple pathways in the progression to the castration resistant state. Three of the most commonly activated pathways indentified in castration resistant prostate cancer are the PI3K, Raf and AR pathways. In this example, Akt and Erk phosphorylation was evaluated by Western blot to assess the activation states of the PI3K and Raf pathway respectively.

[00254] To evaluate the mode of drug resistance in the class 1 and class 2 cell lines, Western analysis was performed to assess AR protein levels and the activation state of several cellular signaling pathways known to modulate AR activity and commonly activated in castration resistant prostate cancer. Relative expression of AR, Akt, phosphorylated Akt (Ser473), p44/42 MAPK (Erk1/2), phosphorylated p44/42 MAPK (Erk1/2) (Thr202/Tyr204), tubulin and actin were determined.

[00255] For Western analysis, cells were grown in RPMI 1640 supplemented with 5% charcoal stripped serum for 3 days. Cells were lysed in modified radioimmunoprecipitation buffer (mRIPA; 10 mM Tris, 150 mM NaCl, 1% (v/v) NP-40, 0.5% deoxycholate, 0.1% SDS, 5 mM EDTA, pH 7.4) containing Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo

Scientific). Total protein of the clarified lysates was quantitated by Lowry Assay (Biorad DC protein assay). NuPAGE® LDS Sample Buffer and Sample Reducing Agent were added to the lysates and heated to 70°C for 10 minutes. 20 µg of total cell protein was separated on a NuPAGE 4-12% Bis Tris Acrylamide Gel and transferred to a nitrocellulose membrane using an Xcell II™ blot module (Invitrogen). Membranes were incubated in Blocking Buffer (LI-COR, Lincoln, NE) for 30 minutes at room temperature, followed by 60 minute incubations with primary antibodies against Androgen Receptor (Santa Cruz Biotechnology cat. No. SC-816), Akt and Phospho-Akt (Ser473) (Cell Signaling cat. Nos. 9272 and 4058 respectively), p44/42 MAPK (Erk1/2) and Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling cat. Nos. 4695 and 4376s respectively) and tubulin or actin (Sigma cat. No T6199 and A4700 respectively). Following incubation with an IRDye® Conjugated Goat Anti Mouse or Rabbit IgG (LI-COR), protein bands were quantified using an Odyssey® Infrared Imaging System.

[00256] By Western blot, the AR levels in the class 1 resistant cell lines were approximately 2 to 4-fold higher than observed in the LNCaP/AR(cs) cell line (Figure 2). Analogous to an approximate 3-fold increase in AR levels being sufficient to support growth of LNCaP cells in a castrate setting *in vivo*, the further 2 to 4-fold elevation in AR levels may be sufficient to promote proliferation in the absence of androgens *in vitro*. In contrast, the AR levels of the class 2 resistant lines were similar to that observed in the parental cell lines. Thus, the conversion of enzalutamide and ARN-509 to partial agonists in the class 2 cell lines was not due to AR overexpression. Minor differences in total and phosphorylated Akt and Erk were observed with no consistent changes seen in either class of resistant cell lines.

Example 7: Determination of Mutant AR sequence

[00257] MDV3100 and ARN-509 are transcriptional and proliferative agonists in the class 2 resistant cell lines, while bicalutamide remains an antagonist. AR expression in class 2 resistant cells was similar to the parental cell line (Example 6). Thus, the sequence of AR in class 2 resistant cells was determined to ascertain whether a mutation of the AR ligand binding domain promotes the gain of function activity.

[00258] cDNA generated by reverse transcription of RNA isolated from class 1 and class 2 cells (see Example 5) was used as a template to sequence AR. Using a series of oligonucleotides, overlapping segments encompassing the AR ligand binding domain (c.2013-2757) were generated by PCR using Phusion® polymerase (New England Biolabs) using the manufacturer's protocol. The PCR products were gel purified to remove non-specific bands as well as unincorporated oligonucleotides. The purified PCR products were sequenced using internal oligonucleotides.

[00259] A single nucleic acid mutation was identified within the AR ligand binding domain at nucleotide position 2626 in three independently derived cell lines. The missense mutation identified in all lines was Thymine (T) to Cytosine (C) that converts Phenylalanine (F) at amino acid position 876 of the encoded polypeptide to Leucine (L) (SEQ ID NO: 19). Additionally, sequencing of individual subcloned PCR products from the LNCaP/AR-Luc ENZr2 cell line indicated that in all cell lines the F876L mutation arose in the endogenous AR allele.

[00260] F876 lies in helix 11 in a region of AR ligand binding pocket that is a hotspot for CRPC AR mutations (Figure 1D). However, unlike T877 and L701, which coordinate hydrogen bonding to the 17 α -OH group of dihydrotestosterone, F876 contributes to a small hydrophobic core formed by residues in helix 11 (F786, L880), the loop between helices 11 and 12 (F891) and helix 3 (F697, L701). While similar residues and hydrophobic ligand interactions are conserved in the estrogen and progesterone receptor, F876 has not been implicated in high affinity binding or steroid selectivity. Consistent with the relatively minor role F876 is predicted to play in steroid binding, the AR-F876L mutation has not been reported in prostate cancer or androgen insensitive populations.

Example 8: Expression of the Mutant AR in AR-deficient Cells

[00261] To confirm that the F876L mutation confers agonist activities to ARN-509 and MDV3100, the point mutation was generated in the context of the full-length wild-type AR and the LNCaP T877A mutant receptor. The F876L and F876L/T877A mutants were generated in the plasmid pcDNA3-AR using the QuickChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol.

[00262] Transcriptional reporter assays using an ARE response element operatively linked to a reporter gene were performed to test the transcriptional activity of the mutant AR in response to ARN-509, MDV3100 and bicalutamide treatment.

[00263] Transcriptional reporter assays were performed by seeding 100 μ L of HEPG2 cells at a density of 250,000 cells/mL into 96-well cell culture plates in MEM supplemented with 10% charcoal stripped serum and allowed to attach overnight at 37 $^{\circ}$ C.

[00264] Cells were transiently transfected using Lipofectin $^{\circledR}$ (Life Technologies) according to the manufacturer's protocol. Triplicate transfections were performed using 378 ng reporter vector (4X ARE-Luciferase or pGL4 Pb-Luciferase (the rat probasin promoter in pGL4 (Promega, Madison, WI)), 50 ng pcDNA-AR (wild-type or mutant) 50 ng pRL-CMV (normalization vector) and 0.7 μ L Lipofectin $^{\circledR}$. Following transfection, the cells were incubated for 4 hours.

[00265] Following the incubation, the cells were treated with the test compounds ARN-509, MDV3100 and bicalutamide. For agonist assays, the compounds were diluted 1:6 and 50 μ L of compound in MEM plus 5% charcoal stripped FBS was added to the cells for a final concentration range of 30 μ M to 0.64 nM. For antagonist assays, the compounds were serially diluted with 1 nM R1881 (for wild-type AR) or 5 nM R1881 (for F876L AR).

[00266] Following 48 hour incubation the medium was removed and the cells were lysed in 40 μ L of lysis buffer (25 mM Tris Phosphate, 2 mM CDTA, 10% Glycerol, 0.5% Triton X-100, 2 mM DTT). Firefly luciferase activity was measured immediately following the addition of 40 μ L luciferase buffer (20 mM tricine, 0.1 mM EDTA, 1.07 mM $(\text{MgCo}_3)_4$ $\text{Mg}(\text{OH})_2 \cdot 5\text{H}_2\text{O}$, 2.67 mM MgSO_4 , 33.3 mM DTT, 270 μ M Coenzyme A, 470 μ M luciferin, 530 μ M ATP). *Renilla* luciferase was measured following the addition of 40 μ L coelenterazine buffer (1.1 M NaCl, 2.2 mM Na_2EDTA , 0.22 M K_xPO_4 (pH 5.1), 0.44 mg/mL BSA, 1.3 mM NaN_3 , 1.43 μ M coelenterazine, final pH adjusted to 5.0).

[00267] In transient transfection assays, second-generation AR antagonists ARN-509 and MDV3100 induced AR dependent transcriptional activity in the context of the F876L or F876L/T877A mutant AR, while induction from bicalutamide was minimal (Table 5). For example, in HepG2 cells using either a 4XARE-luciferase or Pb-luciferase reporter, ARN-509 and MDV3100, but not bicalutamide, induced AR dependent transcriptional activity in the context of the F876L or F876L/T877A mutant AR. This indicates that mechanism of resistance in the class 2 cell lines is the AR F876L mutation.

Table 5. AR Transcriptional Activity (Fold DMSO)

	R1881	Bicalutamide	MDV3100 (enzalutamide)	ARN509
AR WT	+++	+	+	+
AR F876L	+++	+	++	++

+ = < 20, ++ = 10 - 200, +++ > 200

[R1881] = 100 nM, [Antagonists] = 6.3 μ M

[00268] A second experiment was performed 4X-ARE-Luciferase report to confirm the above results. In this experiment, first generation anti-androgens nilutamide and hydroxyflutamide were also compared along with bicalutamide, ARN-509 and enzalutamide. The AR transcriptional reporter assays were performed essentially as described above with minor modifications. Triplicate transfections were performed using 50 ng pCDNA3-AR or pCDNA3-AR mutant, 65 ng 4X ARE-Luciferase, 20 ng pRL (Promega), and 25 ng pCMX. For agonist assays, compounds were serially diluted, and 50 μ L of compound plus RPMI 1640 supplemented with charcoal stripped serum was added to the cells. For antagonist assays, the

compounds were serially diluted, and 50 μ L of compound with RPMI supplemented with charcoal stripped serum plus methyltrienolone (R1881) were added to the cells. The final R1881 concentration used in the antagonist assays was 1 nM with the exception of F876L for which 5nM R1881 was used.

[00269] As shown in Figure 4, in the context of wild-type AR, ARN-509 and enzalutamide were full antagonists and with minimal agonist activity in 4X-ARE androgen responsive transcriptional reporter assays. However, in cells expressing AR-F876L or AR F876L/T877A, enzalutamide and ARN-509 were partial transcriptional agonists (Figure 4A). Conversely, the first generation anti-androgens bicalutamide, nilutamide and hydroxyflutamide displayed minimal agonist activity on the F876L mutant (Table 6, Figure 4) (E_{max} = percent maximal R1881 response). Enzalutamide and ARN-509 were full antagonists on AR mutants T877A, L701H, H874Y and W741C that either confer resistance to first generation AR antagonists or broaden steroidal ligand specificities in CRPC patients.

Table 6.

Compound	4X ARE Reporter Assay			
	WT IC ₅₀ (μ M)	WT Emax	F876L IC ₅₀ (μ M)	F876L Emax
ARN-509	0.79 $\pm$ 0.15	1.3 $\pm$ 0.3	0.09 $\pm$ 0.06	49.7 $\pm$ 11.1
Enzalutamide	1.12 $\pm$ 0.17	0.4 $\pm$ 0.1	0.13 $\pm$ 0.04	20.2 $\pm$ 5.2
Bicalutamide	1.65 $\pm$ 0.93	10.0 $\pm$ 2.9	3.63 $\pm$ 0.04	0.7 $\pm$ 0.3
Hydroxyflutamide	0.36 $\pm$ 0.04	28.9 $\pm$ 5.0	2.60 $\pm$ 6.61	0.8 $\pm$ 0.2
Nilutamide	1.11 $\pm$ 0.17	5.1 $\pm$ 2.1	7.59 $\pm$ 1.18	0.6 $\pm$ 0.1

[00270] To test DNA binding competency of the F876L mutants VP16-AR fusion constructs were generated. pVP16-AR was generated by subcloning full-length human AR into pVP16 (Clontech). AR point mutations were generated in VP16-AR using the QuickChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol.

[00271] Transcription assays were performed essentially as described above. Triplicate transfections were performed using 35 ng pVP16-AR or pVP16-F876L, 70 ng 4X ARE-Luciferase, 20 ng pRL (Promega), and 35 ng pCMX. 4X ARE-luciferase reporter activity was monitored in the presence of increasing compound concentrations in the absence or presence of 90 pM R1881 (for wild-type VP16-AR) or 1 nM R1881 (for F876L VP16-AR). Luciferase activity was measured as described above.

[00272] Reflective of the transcriptional reporter assay, in the wild-type VP16-AR assay, which monitors the DNA binding competency of the receptor, enzalutamide and ARN-509 were full antagonists (Table 7, Figure 4) (E_{max} = percent maximal R1881 response). However, in the

context of the VP16-AR-F876L, ARN-509 and enzalutamide stimulated AR DNA binding. Thus, the mutation of AR F876 to L was sufficient to convert the 2nd generation anti-androgens, enzalutamide and ARN-509, to partial agonists.

Table 7.

Compound	AR-VP16			
	WT IC ₅₀ (□M)	WT Emax	F876L IC ₅₀ (□M)	F876L Emax
ARN-509	0.16 ± 0.06	3.98 ± 0.27	0.03 ± 0.02	53.98 ± 1.45
Enzalutamide	0.21 ± 0.07	2.65 ± 0.73	0.05 ± 0.01	34.32 ± 5.38
Bicalutamide	0.18 ± 0.10	32.77 ± 5.76	2.51 ± 1.11	2.20 ± 1.35
Hydroxyflutamide	0.03 ± 0.01	42.28 ± 4.44	0.97 ± 0.27	5.36 ± 5.35
Nilutamide	0.13 ± 0.08	33.53 ± 9.75	2.12 ± 0.68	2.90 ± 1.80

Example 9: Stable Cell Line Generation

[00273] In this example, cell lines were generated with stable expression of AR F876L mutant. pSRαF876L, pQCXIN-AR and pQCXIN-F876L retroviruses were first generated by co-transfecting GP2-293 cells with pVSV-G (Clontech) according to the manufacturer's protocol.

[00274] PC3 cells stably expressing wild-type or AR-F876L were generated by transducing PC3 cells with either pQXIN-AR or pQXIN-F876L retrovirus and selection in RPMI 1640 medium containing 500 µg/mL gentamycin.

[00275] LNCaP cells stably expressing AR-F876L were generated by either transfecting LNCaP cells with pCDNA-F876L or transducing LNCaP cells with SRαF876L retrovirus. Individual clones of LNCaP/pCDNA-F876L were isolated following selection in 400 µg/mL gentamycin. LNCaP/SRαF876L cell pools were selected 400 mg/mL gentamycin.

[00276] AR protein expression of all cell lines was validated by western analysis using AR N-20 antibody (Santa Cruz Biotechnology).

Example 10: Equilibrium AR binding assays

[00277] Competitive binding assays of wild-type AR vs. F876L AR were performed as described in Clegg et al. (2012) *Cancer Research* 72:1494-503 using PC3 cells stably expressing wild-type human AR or AR-F876L as described above. Ki was calculated as $K_i = IC_{50} / (1 + ([^3H-R1881]/K_d))$, $[^3H-R1881] = 0.6$ nM.

[00278] In equilibrium AR binding assays, ARN-509 and enzalutamide bound the mutant with 30 and 48-fold higher affinity, respectively (Table 8, Figure 5) (Kd for R1881: AR = 0.5 nM; AR-F877L = 0.64 nM). Thus, increased agonist activity on AR is associated with increased binding affinity to both wild-type and F876L receptor, suggesting that the agonist confirmation enables higher affinity through decreased dissociation constant.

Table 8.

Compound	AR binding, WT K _i (nM)	AR binding, F876L K _i (nM)
ARN-509	18.07 ± 7.46	0.68 ± 0.15
Enzalutamide	26.30 ± 12.77	0.60 ± 0.17
Bicalutamide	26.56 ± 12.51	360.36 ± 283.85
Hydroxyflutamide	14.56 ± 8.25	150.57 ± 55.10
Nilutamide	17.74 ± 5.65	197.42 ± 9.26

Example 11: Expression of AR target genes in and Proliferation of F876L stable prostate cancer cell lines

[00279] As shown above, expression of AR-F876L was sufficient to confer enzalutamide and ARN-509 resistance in transient reporter based assays. In this example, the effects of F876L on endogenous AR target genes and proliferation in prostate cancer cells stably expressing the mutant was examined. Two LNCaP cell lines (LNCaP/SRαF876L and LNCaP/pCDNAF876L) were engineered as described in Example 9 to overexpress AR-F876L at levels comparable to the LNCaP/AR(cs) model.

[00280] To measure AR levels in the cells, protein extracts were generated from LNCaP, LNCaP/AR(cs), LNCaP/SRαF876L and LNCaP/pCDNAF876L cells cultured in hormone depleted medium for 3 days. AR protein levels were analyzed by western blot. AR levels were quantified and normalized to actin and expressed relative to AR expression in LNCaP cells (Fig. 6)

[00281] For endogenous target gene analysis, LNCaP/AR(cs), LNCaP SRαF876L, and LNCaP/pCDNAF876L cells were cultured for 3 days in hormone depleted medium followed by treatment with vehicle, 1 nM R1881 or 1, 3, 10 and 30 μM ARN-509 or enzalutamide in the presence or absence on 1 nM R1881.

[00282] For the proliferation assays, LNCaP/AR(cs), LNCaP SRαF876L, and LNCaP/pCDNAF876L cells were cultured in the presence of hormone depleted medium for 2 days followed by ligand treatment for 7 days as described above. For antagonist assays, ARN-509 or enzalutamide were added in the presence of 200 pM R1881 (100 pM final concentration). Proliferation was quantified by CellTiter-Glo luminescence based viability assay as described above.

[00283] In LNCaP/AR(cs) cells, ARN-509 and enzalutamide had little effect on the induction of AR target genes or proliferative activity (Figure 7A, Table 9A). Both antagonists blocked R1881 induced transcription and proliferation to levels consistent with their agonist activity at the highest concentration (Figure 7B, Table 9B). In contrast, in F876L-AR expressing cells, both

enzalutamide and ARN-509 demonstrated robust transcriptional and proliferative agonist activity (Figs. 7A and 7B, Tables 9C-F).

Table 9A. LNCaP/AR(cs) Agonist Transcription

Gene	Vehicle	1nM R1881	- R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.98	0.68	0.61	0.62	0.66	1.22	1.14	0.69	0.89	0.65	2.41
BDNF	1.00	0.86	1.04	0.92	0.88	0.95	0.98	1.15	0.93	1.03	1.03	1.80
CAM2KN1	1.00	0.04	0.80	0.70	0.71	0.64	0.64	0.90	0.76	0.99	0.78	1.56
FASN	1.00	4.12	0.47	0.32	0.29	0.31	0.58	0.46	0.40	0.42	0.41	1.30
FKBP5	1.00	100.51	0.91	0.62	0.75	0.61	0.85	0.99	0.71	0.91	0.75	1.87
HPGD	1.00	324.60	0.74	0.57	0.61	0.63	1.29	0.81	0.56	0.78	0.61	1.86
NCAPD3	1.00	95.54	0.66	0.57	0.47	0.56	0.79	0.72	0.74	0.73	0.74	1.34
NKX3.1	1.00	12.72	0.71	0.52	0.51	0.86	1.63	1.11	0.68	0.73	0.85	2.24
NOV	1.00	0.05	1.12	0.91	0.80	1.02	1.01	1.12	1.00	1.11	0.98	2.07
ORM1	1.00	6987.01	0.92	0.90	1.06	0.71	2.02	1.37	1.79	1.20	0.97	1.35
PLD1	1.00	0.03	0.77	0.62	0.63	0.56	0.56	1.28	0.66	0.88	0.65	1.33
PSA	1.00	22.14	0.42	0.32	0.38	0.74	1.60	0.44	0.49	0.58	0.61	1.13
SLUG	1.00	91.84	0.53	0.55	0.31	0.51	0.91	0.38	0.42	0.52	0.56	1.36
STEAP4	1.00	1498.46	0.75	0.73	0.52	1.16	1.28	0.94	0.77	1.05	0.56	1.01
TMPRSS2	1.00	35.42	0.75	0.53	0.63	0.89	1.45	0.99	0.64	1.04	0.62	2.78

Table 9B. LNCaP/AR(cs) Antagonist Transcription

Gene	Vehicle	1nM R1881	+ 1nM R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.98	0.49	0.19	0.48	0.78	0.92	0.30	0.31	0.52	0.60	0.76
BDNF	1.00	0.86	0.61	0.28	0.63	0.99	1.12	0.43	0.35	0.67	0.74	0.88
CAM2KN1	1.00	0.04	0.04	0.06	0.27	0.50	0.56	0.04	0.06	0.19	0.43	0.42
FASN	1.00	4.12	1.30	0.42	0.71	0.46	0.59	1.92	1.52	1.08	0.60	0.64
FKBP5	1.00	100.51	23.51	5.64	3.32	1.41	1.50	33.14	22.49	12.56	2.74	1.08
HPGD	1.00	324.60	99.16	16.30	13.19	3.85	4.25	105.97	76.25	32.19	8.36	2.78
NCAPD3	1.00	95.54	23.74	4.35	3.14	1.29	1.27	43.93	32.00	15.57	2.39	0.96
NKX3.1	1.00	12.72	7.70	3.77	6.72	4.70	5.03	8.32	9.92	12.01	7.22	5.49
NOV	1.00	0.05	0.11	0.14	0.61	1.00	0.92	0.03	0.09	0.31	0.86	0.82
ORM1	1.00	6987.01	3521.95	503.02	257.85	43.91	22.70	5100.90	2679.73	1031.40	156.90	16.42
PLD1	1.00	0.03	0.02	0.02	0.13	0.42	0.54	0.02	0.02	0.04	0.11	0.32
PSA	1.00	22.14	22.76	8.82	11.75	6.59	5.65	19.09	21.75	23.57	12.90	7.20
SLUG	1.00	91.84	50.08	10.20	10.56	5.26	5.22	71.33	62.00	50.70	11.15	5.49
STEAP4	1.00	1498.46	585.81	109.37	74.44	13.61	7.79	1019.98	742.61	256.89	32.98	6.86
TMPRSS2	1.00	35.42	19.88	7.72	10.51	3.58	4.62	19.07	24.11	23.38	11.45	5.94

Table 9C. LNCaP/SRαF876L Agonist Transcription

Gene	Vehicle	1nM R1881	+ R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.29	0.58	0.32	0.47	0.40	0.31	0.27	0.47	0.23	0.34	0.27
BDNF	1.00	1.65	1.04	1.30	1.01	0.99	1.55	0.79	1.07	0.75	0.84	0.94
CAM2KN1	1.00	0.03	0.52	0.48	0.38	0.23	0.37	0.30	0.24	0.17	0.21	0.18
FASN	1.00	3.76	0.50	0.64	0.58	1.09	1.34	0.64	1.01	0.93	1.50	2.77
FKBP5	1.00	66.89	1.38	1.14	2.54	9.61	23.67	2.66	5.95	5.56	13.02	27.89
HPGD	1.00	182.19	0.68	0.96	2.79	18.98	55.76	4.18	7.90	10.12	25.79	69.22
NCAPD3	1.00	31.69	0.77	1.01	1.09	3.56	8.83	1.39	1.58	1.69	3.53	9.35
NKX3.1	1.00	10.80	4.26	5.54	7.05	11.94	14.20	7.12	9.47	7.96	9.85	12.67
NOV	1.00	0.06	0.55	0.28	0.55	0.42	0.21	0.27	0.51	0.31	0.29	0.17
ORM1	1.00	6535.38	2.17	4.85	18.77	242.08	2114.41	44.44	58.96	101.45	459.30	1357.12
PLD1	1.00	0.02	0.67	0.76	0.60	0.42	0.41	0.49	0.44	0.30	0.45	0.36
PSA	1.00	3.43	1.95	2.25	3.02	4.05	5.02	3.71	3.26	3.00	5.07	5.16
SLUG	1.00	99.20	1.21	1.55	3.28	16.87	107.64	5.56	5.91	8.15	26.35	56.48
STEAP4	1.00	1706.02	1.13	2.15	9.98	96.53	343.81	20.36	27.49	46.06	187.24	479.64
TMPRSS2	1.00	25.55	3.11	3.85	5.39	9.20	18.61	6.36	6.29	5.84	10.90	14.20

Table 9D. LNCaP/SRαF876L Antagonist Transcription

Gene	Vehicle	1nM R1881	+ 1nM R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.29	0.23	0.29	0.33	0.39	0.40	0.17	0.22	0.14	0.18	0.18
BDNF	1.00	1.65	0.53	0.64	0.62	0.51	0.71	0.74	0.76	0.57	0.50	0.63
CAM2KN1	1.00	0.03	0.08	0.12	0.19	0.24	0.32	0.03	0.03	0.06	0.11	0.17
FASN	1.00	3.76	1.29	1.22	0.85	0.94	1.58	2.23	2.03	1.39	1.30	2.66
FKBP5	1.00	66.89	17.90	14.89	10.21	17.33	40.52	36.57	39.48	26.46	29.94	22.71
HPGD	1.00	182.19	49.66	30.03	21.62	34.77	93.52	149.34	134.70	79.93	70.80	131.79
NCAPD3	1.00	31.69	6.34	4.28	2.21	3.44	13.64	19.83	15.47	8.71	6.50	11.82
NKX3.1	1.00	10.80	21.66	20.78	16.19	11.84	15.26	12.63	14.12	11.34	10.15	13.64
NOV	1.00	0.06	0.12	0.28	0.25	0.33	0.26	0.05	0.06	0.09	0.11	0.09
ORM1	1.00	6535.38	386.40	216.67	137.73	661.51	3496.87	3335.14	2343.29	1469.18	1608.16	3440.86
PLD1	1.00	0.02	0.04	0.08	0.14	0.37	0.48	0.01	0.01	0.02	0.08	0.20
PSA	1.00	3.43	5.84	5.63	4.47	4.54	4.64	4.93	4.38	4.59	5.28	4.17
SLUG	1.00	99.20	85.97	64.63	35.50	48.80	161.58	85.97	64.63	35.50	48.80	161.58
STEAP4	1.00	1706.02	259.94	147.25	83.20	275.88	645.04	259.94	147.25	83.20	275.88	645.04
TMPRSS2	1.00	25.55	14.05	13.81	11.78	12.92	20.19	14.05	13.81	11.78	12.92	20.19

Table 9E. LNCaP/pCDNAF876L Agonist Transcription

Gene	Vehicle	1nM R1881	- R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.92	1.28	0.82	0.87	0.72	0.91	1.07	0.86	1.25	0.60	1.55
BDNF	1.00	1.01	1.54	1.04	1.11	0.82	0.64	1.01	0.76	0.87	0.85	1.16
CAM2KN1	1.00	0.02	0.55	0.96	0.51	0.37	0.23	0.47	0.55	0.34	0.41	0.27
FASN	1.00	22.20	0.91	0.72	0.48	1.27	3.66	1.16	1.42	1.59	1.79	7.86
FKBP5	1.00	145.63	2.18	2.91	4.28	10.54	42.25	7.24	7.82	9.52	17.39	55.55
HPGD	1.00	854.42	4.70	6.32	13.26	26.78	105.81	15.36	19.27	29.25	47.33	173.82
NCAPD3	1.00	169.67	1.95	1.94	3.68	9.41	35.36	5.83	6.52	8.28	20.42	51.13
NKX3.1	1.00	9.67	3.76	3.71	3.57	4.69	8.12	6.07	5.72	5.93	7.05	11.69
NOV	1.00	0.08	1.73	1.62	2.19	0.88	0.49	1.24	1.01	0.82	0.86	0.72
ORM1	1.00	12816.69	116.91	195.36	659.73	2530.13	4760.77	932.41	1371.33	2307.29	3322.63	5541.08
PLD1	1.00	0.75	1.37	0.80	0.69	0.29	0.45	0.78	0.69	1.01	0.68	0.48
PSA	1.00	12.17	3.56	4.04	5.29	9.53	11.70	6.74	7.19	8.25	10.06	12.99
SLUG	1.00	268.77	1.99	3.53	4.74	14.91	55.93	7.84	8.70	10.91	21.21	71.08
STEAP4	1.00	3084.81	10.38	15.58	46.50	240.68	520.15	113.39	113.29	173.77	317.80	597.09
TMPRSS2	1.00	26.85	4.98	6.46	6.09	8.51	21.78	9.49	8.51	9.70	11.09	23.01

Table 9F. LNCaP/pCDNAF876L Antagonist Transcription

Gene	Vehicle	1nM R1881	+ 1nM R1881									
			Enzalutamide					ARN-509				
			0.3μM	1μM	3μM	10μM	30μM	0.3μM	1μM	3μM	10μM	30μM
AMIGO2	1.00	0.92	0.51	0.43	0.39	0.49	0.52	0.24	0.31	0.43	0.41	0.40
BDNF	1.00	1.01	0.59	0.71	0.53	0.50	0.41	0.28	0.41	0.42	0.45	0.34
CAM2KN1	1.00	0.02	0.05	0.16	0.17	0.21	0.14	0.01	0.02	0.06	0.11	0.06
FASN	1.00	22.20	5.29	2.44	1.55	2.08	4.22	3.89	5.52	3.22	2.10	3.98
FKBP5	1.00	145.63	53.18	26.29	10.03	14.22	32.97	45.69	48.95	38.89	24.82	30.26
HPGD	1.00	854.42	149.44	59.67	21.66	35.90	96.55	192.05	170.69	107.79	73.28	87.03
NCAPD3	1.00	169.67	101.65	31.49	14.34	16.69	42.06	68.41	90.52	62.27	22.96	44.10
NKX3.1	1.00	9.67	8.53	7.05	5.69	5.54	7.61	3.08	5.95	5.42	4.81	5.24
NOV	1.00	0.08	0.08	0.17	0.32	0.49	0.39	0.02	0.10	0.12	0.26	0.18
ORM1	1.00	12816.69	4375.20	1581.76	587.00	1377.92	3765.54	4802.10	4943.10	3366.19	2576.38	2470.99
PLD1	1.00	0.75	0.08	0.12	0.09	0.11	0.12	0.04	0.12	0.05	0.06	0.04
PSA	1.00	12.17	16.08	12.73	7.92	8.12	14.27	10.36	14.01	12.21	10.27	13.14
SLUG	1.00	268.77	166.04	86.01	30.23	36.56	98.34	112.06	134.56	113.34	69.55	85.38
STEAP4	1.00	3084.81	524.97	156.24	47.11	92.80	214.69	633.57	587.91	376.66	195.78	232.63
TMPRSS2	1.00	26.85	19.03	16.05	8.14	8.92	15.32	12.13	15.75	14.19	12.99	12.67

Example 12: Interaction of N and C Expression of AR target genes in and Proliferation of F876L stable prostate cancer cell lines

[00284] Interaction of the AR amino-terminus with the AR carboxy-terminus is important for the full AR transactivation capacity. Many AR full and partial agonists stimulate the AF2 dependent N-C interaction. A N-C two hybrid interaction assay was performed to assess the interaction of the AR N- and C-termini in the F87L mutant in the presence of ARN-509 and enzalutamide.

[00285] pM-AR1-660, pVP16-AR507-919 and pVP16-F876L507-919 were generated by subcloning appropriate PCR products from pCDNA3-AR and pCDNA3-F876L into pM and

pVP16 (Clontech). For N-C terminal interaction assays, triplicate transfections were performed using 50 ng pM-AR1-660, 75 ng pVP16-AR507-919 or pVP16-F876L507-919, 25 ng pMH100-Luc and 10 ng pRL (Promega). Transfected cells were incubated 4 hours then treated with ligand. ARN-509 and enzalutamide were assayed at 8 μ M and R1881 at 1nM.

[00286] Consistent with their transcriptional activities, enzalutamide and ARN-509 promoted the N-C interaction of AR-F876L but not wild-type AR (Figure 8). Thus, the agonist activity of ARN-509 and enzalutamide on AF-F876L is associated with an agonist-like AF-2 conformation.

Example 13: Chromatin immunoprecipitation assay of AR

[00287] Transcriptional activation of androgen regulated AR target genes requires agonist-induced DNA binding and subsequent recruitment of transcriptional coregulators. To confirm the VP16-AR reporter results indicating ARN-509 and enzalutamide stimulate AR-F876L DNA binding, we performed chromatin immunoprecipitation (ChIP) analysis of 6 AR target genes from cells treated with R1881 and/or each antagonist was performed.

[00288] ChIP assays were performed as described in Joseph et al. (2009) PNAS USA 106:12178-83]. Briefly, LNCaP/AR(cs) and LNCaP SR α F876L cells were plated in 150 mm dishes (7×10^6 cells in 20 mL) in RPMI 1640 supplemented with 10% CSS for 3 days. Cells were treated with 10 μ M antagonist in the presence or absence of 1 nM R1881 for 4 hours. Following ligand treatment, formaldehyde was added to the media to a final concentration of 1%, incubated for 10 min and quenched with glycine (125 mM final concentration) for 5 minutes. Cells were washed 3X with PBS containing 1X Halt™ Protease & Phosphatase Single-Use Inhibitor Cocktail (1X PI, Thermo Scientific), pelleted, lysed in 1 mL RIPA buffer (50 mM Tris pH7.5, 0.15 M NaCl, 1% NP-40, 0.5% Na-deoxycholate, 0.05% SDS, 1 mM EDTA, 1X PI) and sonicated until the average DNA size fragment was $\approx$ 500 bp. The sonicated cross-linked chromatin was diluted into 3.3 mL RIPA and precleared with 100 mL 50% protein A/G agarose slurry (SC-2003, Santa Cruz Biotechnology) containing 200 mg per mL sonicated salmon sperm DNA and 500 mg per mL of BSA. One mL of precleared chromatin was then immunoprecipitated with 15 μ g anti-AR (SC-816, Santa Cruz Biotechnology) or normal rabbit IgG (SC-2027, Santa Cruz Biotechnology), for 2 hours at 4°C and 100 mL of a 50% slurry of protein A/G agarose beads were added and incubated overnight at 4°C. Beads were washed 2 times sequentially in low-salt buffer (50 mM HEPES pH 7.8, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS), high-salt buffer (same as low-salt with 500 mM NaCl), LiCl buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate), and TE buffer (50 mM Tris pH 8.0, 1 mM EDTA). All washing steps were performed in the presence of 1X PI. Protein-DNA complexes were eluted in 225 mL Elution

buffer (50 mM Tris pH 8.0, 1 mM EDTA, 1% SDS) at 65°C twice for 15 minutes. Eluted protein-DNA complexes were reverse cross-linked in the presence of NaCl overnight at 65°C and further treated with EDTA and proteinase K at 42°C for 1 hour. The DNA fragments were purified in 10 mM Tris pH 8.5 using the QIAquick PCR purification kit (Qiagen), diluted and analyzed by real-time PCR using iTaq SYBR Green Supermix with ROX (Bio-Rad). The samples were amplified on the ABI 7900HT instrument. Oligonucleotide primer sequences are listed in Table 8.

Table 8. ChIP Real-time PCR Oligonucleotide Sequence		
Gene	Forward Primer Sequence	Reverse Primer Sequence
PSA E2	ACCTGCTCAGCCTTTGTCTCTGAT (SEQ ID NO: 50)	AGATCCAGGCTTGCTTACTGTCCT (SEQ ID NO: 51)
PSA D1	ATTCTGGGTGGGAGTGCAAGGA A (SEQ ID NO: 52)	AGGAGACATGCCCAGGATGAAAC A (SEQ ID NO: 53)
STEAP4	ACTAGGCAGGACATTGACATCCC A (SEQ ID NO: 54)	ACAGTAAACCTCTCCACACATGG C (SEQ ID NO: 55)
FASN	TATGACACCCAGGGCTTTCGTTC A (SEQ ID NO: 56)	TAACGTTCCCTGCGCGTTTACAGA (SEQ ID NO: 57)
TMPRSS 2	TCCCAAATCCTGACCCCA (SEQ ID NO: 58)	ACCACACAGCCCCTAGGAGA (SEQ ID NO: 59)
NKX3.1	ACAGGGTGGCCCAAATAGAAC (SEQ ID NO: 60)	CCTGTCTTGGACAAGCGGAGA (SEQ ID NO: 61)
ORM1	GGGTCATTTCACACCTCAAAC A (SEQ ID NO: 62)	GGAGAAAGGCCTTACAGTAGTCT C (SEQ ID NO: 63)

[00289] In the LNCaP/AR(cs) cells, R1881 promoted AR DNA (Fig. 9). Consistent with the VP16-AR reporter data, both ARN-509 and enzalutamide promoted AR DNA binding LNCaP/SR α F876L cells. In the presence of R1881, all antagonists inhibited R1881-stimulated AR DNA binding to levels consistent with their partial agonist or antagonist activity in both cell lines (Supplementary Figure S9).

Example 14: *In vivo* Effects of AR F876L

[00290] To determine whether the F876L alteration conveys resistance to enzalutamide and ARN-509 *in vivo*, LNCaP cell lines stably expressing F876L AR were injected (s.c.) into castrated immune-deficient mice and tumors established.

[00291] All animal studies were carried out under protocols approved by the Institutional Animal Care and Use Committees and institutional guidelines for the proper, humane use of animals in research were followed. *In vivo* xenograft experiments were carried out in SCID

Hairless Outbred (SHO) male mice (Charles River Laboratories). Mice were orchietomized under isoflurane anesthesia and were given 7-10 days to recover. LNCaP/AR(cs) or LNCaP/SR α F876L cells (as described above) were suspended in 50% RPMI, 50% Matrigel (BD Biosciences), and 3×10^6 cells/xenograft were injected in a volume of 100 μ L. Animals were observed weekly until tumor growth was apparent. After 40-60 days post-injection, animals were randomized into cohorts of equivalent tumor burden mean (150-250 mm³) and range. All compounds were administered daily by oral gavage at a dose of 30 mg/kg/day compound. For all LNCaP/AR(cs) xenograft studies ARN-509 and enzalutamide drug stocks were prepared in 18% PEG-400, 1% Tween-80 and 1% povidone, and were formulated for dosing in 15% Vitamin E-TPGS and 65% of a 0.5% w/v CMC solution in 20 mM citrate buffer (pH 4.0). ARN-509 and enzalutamide pharmacokinetics were assessed at the end of study as described previously (Clegg et al. (2012) *Cancer Research* 72:1494-503).

[00292] Consistent with the *in vitro* data, neither ARN-509 nor enzalutamide 30 mg/kg/day impacted the growth of LNCaP/AR/SR α F876L tumors (Figure 10). This lack of activity was not a function of unexpectedly low compound exposure as day 28 plasma drug levels were quantified and were consistent with previous LNCaP/AR xenograft studies (Table 9). In addition, in a parallel experiment, ARN-509 30 mg/kg/day exhibited robust anti-tumor activity in the LNCaP/AR(cs) model, consistent with previous results (Figure 10).

Table 9. LNCaP/SR α F876L Xenograft Pharmacokinetics

Compound	Dose	C ₂₄ (μ g/mL)	T _{1/2} (hr)	AUC ₀₋₂₄ (μ g·hr/mL)	C _{max} (μ g/mL)	T _{max} (hr)
Enzalutamide	30 mg/kg	9.14	9.9	527.3	33.5	1.0
ARN-509	30 mg/kg	1.02	7.1	98.9	9.11	1.0

Example 15: An Open-Label, Phase 1/2 Safety, Pharmacokinetic, and Proof-of-Concept Study of ARN-509 in Patients with Progressive Advanced Castration-Resistant Prostate Cancer (CRPC)

[00293] In this study, DNA was isolated from 29 patient plasma samples patients participating in a Phase 1/2 clinical study ARN-509 treatment for prostate cancer. These were analyzed using the emulsion PCR-based BEAMing Technology method (Dressman et al. (2003) PNAS USA100:8817-22). BEAMing has been successfully used to detect a variety of tumor derived mutations in driver oncogenes such as PIK3a and K-ras in ctDNA derived from human plasma (Diehl et al. (2008) *Nature Medicine* 14:985-90). The AR F876L mutation was identified in 3 of the 29 patient samples tested.

[00294] The clinical study performed was multi-institution, first in man, Phase 1/2, dose-escalation and proof-of-concept study across 9 dose levels in which eligible patients with progressive advanced CRPC received oral doses of ARN-509 on an outpatient basis to determine the safety, pharmacokinetics (PK) and preliminary evidence of the anti-tumor effects of ARN-509.

[00295] Patients with mCRPC were assigned sequentially to dose levels in cohorts of 3 to 6 patients per dose level using standard 3x3 dose-escalation criteria.

[00296] The objective was to determine the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D) of ARN-509 that leads to a dose-limiting toxicity (DLT) in a maximum of 30% of patients. A DLT is generally defined as any Grade 1 or higher seizure, any Grade 3-4 non-hematologic toxicity (GI toxicities must persist at Grade 3-4 despite maximal medical therapy) and/or grade 4 hematologic toxicity of more than 5 days duration, defined by CTCAE V4.0, and that is assessed as related to ARN-509 treatment. A schematic of the study design is provided in Fig. 11.

[00297] Eligible patients who signed informed consent documents were initially enrolled into a dose escalation cohort where they will receive a single oral dose of ARN-509 followed by a one-week observation period (PK Week). Continuous dosing began on Cycle 1 Day 1 assuming no unacceptable toxicities were observed.

[00298] A minimum of 3 subjects at each dose level were monitored for a DLT through day 28 of Cycle 1. If no DLTs were observed in the first 3 patients at each dose level, subsequent enrollment proceeded at the next dose level. If 2 or more patients experienced a DLT at a given dose level or a single episode of seizure of any grade was observed at a given dose level, dose escalation was stopped and the MTD was defined as the previous dose level. If no DLTs were observed, RP2D was based on the overall pharmacokinetic and safety profile of ARN-509 and the optimal biological dose determined from preclinical data, and not necessarily the MTD.

[00299] The starting dose was 30 mg/day, with escalations to 60 mg, 90 mg, 120 mg, 180 mg, 240 mg, 300 mg, 390 mg, and 480 mg daily. It was anticipated that these dose levels span the anticipated pharmacologically active dose range and be within the safety margin indicated by the preclinical toxicology results.

[00300] Following the selection of 240 mg as the RP2D, additional eligible patients were enrolled in the Phase 2 portion of the study, consisting of 3 concurrent expansion cohorts at the MTD and/or RP2D to gather additional safety information and provide an initial signal of anti-tumor activity. The three expansion cohorts included:

[00301] 1. Non-metastatic CRPC treatment naïve (50 patients with non-metastatic CRPC who are chemotherapy and abiraterone treatment naïve);

[00302] 2. Metastatic CRPC treatment-naïve (20 patients with mCRPC who are chemotherapy and abiraterone naïve); and

[00303] 3. Metastatic CRPC abiraterone treated (10-20 patients).

[00304] It was expected that each patient with mCRPC would receive at least 3 cycles (12 weeks) of continuous treatment and each patient with non-metastatic CRPC will receive at least 4 cycles (16 weeks) of continuous treatment. Treatment was discontinued at any time for protocol-defined disease progression or unacceptable toxicity. Tumor evaluations were performed every 3 cycles (12 weeks) for patients with mCRPC and every 4 cycles (16 weeks) for patients with non-metastatic CRPC. Safety was assessed from the first dose through at least four weeks after the last dose or until resolution of drug-related toxicity, or when toxicity is deemed irreversible, whichever was longer. The effect of food on the absorption of ARN-509 and the effect of ARN-509 on ventricular repolarization was evaluated in the Phase 2 expansion phase at selected clinical sites.

[00305] Analysis of the samples using BEAMing technology in the current ARN-509 clinical study was carried out by Inostics GMBH. Blood was collected in K2-EDTA evacuated tubes and mix thoroughly by slowly inverting several times. Within 30 minutes of collection tubes were spun at 2000 x g for 15 minutes. Plasma was decanted, transferred to cryo storage tubes. Within 90 minutes of decantation, plasma was stored at -70°C or lower until analysis. DNA was purified from 300-500 µl plasma aliquots and extracted as described in Diehl et al. (2008) *Nature medicine* 14:985-90. Mutation detection was performed according to BEAMing technology as described in Diehl et al. Briefly, in the initial PCR step, the target region (~100 bp) was amplified using gene-specific primers with tag sequences and subjected to an emulsion PCR containing primer coated magnetic beads. After emulsion PCR discrimination of wildtype and mutant beads was performed by allele-specific hybridization followed flow cytometry. Flow cytometry results were analyzed using FCS Express (De Novo Software, Los Angeles, CA) resulting in the quantification of the ratio of the mutant allele over the wild type alleles.

[00306] Samples were analyzed for the presence of wild type AR or 3 single point mutant alleles that could result in F876L (t2988c, c2990a, and c2990g (or t2626c, c2628a, and c2928g, respectively, relative to the AR nucleic acid coding sequence set forth in SEQ ID NO: 18). Analyzed mutations and the technical sensitivity of the BEAMing Method are shown in Table 10. For detection, the frequency has to be above the total amount of genome equivalent used per assay. For example, if in a sample, 1,000 genomic equivalent are present, yet the

calculated fraction of mutant DNA molecules is 0.02% (1 mutant allele in 5,000 wild-type alleles), the sample is scored as wild type.

Table 10. AR nucleotide changes monitored by BEAMing assay

Nucleotide Position	Nucleotide Change	Amino Acid Position	Amino Acid Change
c.2626	t > c	876	F>L
c.2628	c > a	876	F>L
c.2628	c > g	876	F>L

Results

[00307] A subset of patients across all dose groups exhibited PSA response with 14/30 patients exhibiting a 12 week $\geq 50\%$ decline in PSA compared to baseline. The PSA response for the 29 patients screened is depicted in Fig. 12. Pre-treatment and during treatment plasma samples were analyzed. Time of BEAMing analysis is indicated by the terminal end of the PSA response line. Eighteen out of the 29 patients had PSA above of baseline at time of analysis indicating either intrinsic or acquired resistance to ARN-509.

[00308] Three probes were designed to monitor the 3 nucleotide changes that can encode for the F876L amino acid substitution. Dilution mixing experiments with the mutant sequence and wild-type DNA indicated a technical sensitivity of 0.02% (potential to detect 1 mutant sequence among 5000 wild-type). In an initial screen of plasma samples of 29 ARN-509 treated patients, evidence of the mutation was detected in 3 patients (Tables 11 and 12). At time of BEAMing analysis, Patient 7 and 10 had PSA levels above baseline, whereas Patient 13 has evidence of rising PSA above the treatment nadir (Fig. 13). In all 3 patients, the nucleotide change c2628a was detected. In one of these 3 patients (Patient 10) the t2626c mutant was also detected, indicative of polyclonal disease. F876L encoding mutations were not detected in any of pre-treatment samples (0/29) suggesting that if present prior to ARN-509 treatment they were below the limit of detection or that the mutations arose de novo during ARN-509 treatment. In either scenario, the data support the hypothesis that the selective outgrowth of lesions bearing the mutant allele to levels sufficient to detect in ctDNA is dependent on chronic exposure to ARN-509 and is associated with rising PSA. To further establish the association of F876L with progressive disease, we analyzed plasma samples taken at additional timepoints from the 3 patients scored positive during the initial screen (Table 12) In patient 10, the mutation was not detected at the one other timepoint analyzed (Cycle 4; PSA 102% of T0). In Patient 13, the mutation was not detected at Cycle 4 (PSA 16.2 % of baseline) or at Cycle 12 (patient was scored positive at Cycle 11). The mutant sequence at Cycle 11 was at the limit of detection and is estimated to arise via amplification of a single mutant molecule. Although PSA of Patient 13

was slowly rising from the treatment nadir at Cycle 11 and 12, at both time points PSA was still >60% below study start, and thus frank resistance had not yet emerged. Identification of the mutant sequence at the limit of detection likely reflects presence of a relatively rare, mutant clone that has potential to expand under continued selective pressure and eventually drive progressive disease.

[00309] Given the relatively long duration of treatment of Patient 7, plasma from additional time points during evident PSA reduction; (> 90% decline from baseline; Cycle 4, 8 and 10) and at initial PSA rise from its nadir (Cycle 15 and 19) (Fig. 13) was analyzed. Interestingly, mutations were not detected in the 3 samples from treatment cycle 4, 8 and 10 whereas the c2628a mutation was detected in the 2 samples analyzed from initial PSA rise (Cycle 15 and 19). These clinical data are consistent with the preclinical data indicating that the F876L amino acid change is sufficient to convey resistance to ARN-509.

Table 11. BEAMing Results from F876L Positive Patients

Patient	Treatment Cycle*	PSA [Percent of Day 0]	Genotypic Call	Mutant Frequency [mutant/w.t. beads x 100]		
				c2990a	c2990g	t2988c
7	0	100	Wild-type	-	-	-
7	4	1.4	Wild-type	-	-	-
7	8	3.3	Wild-type	-	-	-
7	10	5.6	Wild-type	-	-	-
7	15	41.2	Mutant	0.162	-	-
7	19	97.2	Mutant	5.005	-	-
7	22	281.0	Mutant	1.002	-	-
10	0	100	Wild-type	-	-	-
10	4	102	Wild-type	-	-	-
10	7	245	Mutant	0.051	-	0.12
13	0	100	Wild-type	-	-	-
13	4	16.2	Wild-type	-	-	-
13	11	31.7	Mutant	0.065	-	-
13	12	39.5	Wild-type	-	-	-

*Treatment cycle was 4 weeks; Cycle 0 is pre-treatment timepoint.

Table 12. Primary F876L BEAMing of ARN-509-001 Patients

Patient	12 week PSA Response	BEAMing Analysis Treatment Cycle*	PSA at Time of BEAMing Analysis [Percent of Baseline]	Baseline Genotypic Call [#]	During Treatment Genotypic Call
1	30.49	19	195.4	w.t.	w.t.
2	-61.8	10	337.08	w.t.	w.t.
3	22.7	4	122	w.t.	w.t.
4	12.4	5	134	w.t.	w.t.
5	-70.65	8	16	w.t.	w.t.
6	-90.02	10	84.6	w.t.	w.t.
7	-98.6	22	281	w.t.	Mutant
8	-62.2	16	10.2	w.t.	w.t.
9	26.38	7	185	w.t.	w.t.
10	2.33	7	245	w.t.	Mutant
11	-49.76	8	143.88	w.t.	w.t.
12	-56.65	7	99.47	w.t.	w.t.
13	-83.79	11	31.7	w.t.	Mutant
14	-43.21	11	150.5	w.t.	w.t.
15	164.14	3	220	w.t.	w.t.
16	89.09	4	189	w.t.	w.t.
17	-45.86	4	54.14	w.t.	w.t.
18	-95.65	6	7.34	w.t.	w.t.
19	-71.19	11	196.86	w.t.	w.t.
20	-30.88	21	89.3	w.t.	w.t.
21	-74.43	10	46.91	w.t.	w.t.
22	-82.7	25	0.19	w.t.	w.t.
23	97.78	8	222.46	w.t.	w.t.
24	-74.86	25	4.89	w.t.	w.t.
25	-30.07	12	161	w.t.	w.t.
26	96.51	5	214.85	w.t.	w.t.
27	-0.89	13	20.69	w.t.	w.t.
28	-33.59	10	126	w.t.	w.t.
29	-58.44	13	105	w.t.	w.t.

Example 16: Method for Generation of Cell Lines for Drug Screening

[00310] To identify compounds that retain AR antagonist activity in the context of the F876L mutation of the androgen receptor, a number of *in vitro* and *in vivo* assays as described above are adapted.

[00311] In an *in vitro* setting, a transient transfection transcription assays similar to that described in Example 8 is used to identify compounds that are devoid of agonist activity and fully antagonize both the wild-type as well as F876L mutant AR transcriptional activity. HEPG2 cells or any eukaryotic cell in which AR transcriptional activity can be monitored is employed for this screen.

[00312] As an alternative to transient transfection, the transcriptional reporter and F876L AR is stably integrated in a number of cell lines and used for screening compounds. The stable integration of the mutant F876L AR into an androgen sensitive prostate cancer cell line such as LNCaP, LaPC4 or VCaP through the use of plasmid (i.e. pCDNA3.1) or viral based integration allows the screening and evaluation of compounds in transcriptional, proliferation and xenograft setting. Briefly, the F876L AR is cloned into a retroviral expression vector such as pQCXIP (Clontech, Mountain View, CA). The resultant plasmid is then used to generate high titer viral stocks for use in the generation of stably transduced cell lines according to the manufacturer's protocol. The resulting cell lines are used in transient transfection transcriptional assays as described in Example 4, endogenous gene transcriptional assays as described in Example 5, proliferation assays as described in Example 3 or xenograft studies as described in Example 1.

[00313] Alternatively the cells are further modified by the stable integration of an AR responsive reporter such as Signal Lenti AR Reporter (Qiagen, Valencia, CA) allowing reporter based compound screening without the need for transient transfection.

[00314] The examples and embodiments described herein are for illustrative purposes only and various modifications or changes suggested to persons skilled in the art are to be included within the spirit and purview of this application and scope of the appended claims.

Claims:

1. A fusion protein comprising an androgen receptor (AR) polypeptide-comprising at least 95% sequence identity to a ligand binding domain having the amino acid sequence of amino acid positions 554 to 919 of SEQ ID NO: 1 and comprising an F876L amino acid substitution in SEQ ID NO: 1, wherein said AR polypeptide is fused to a heterologous polypeptide, a secretion signal sequence, an epitope tag, or an affinity tag.
2. The fusion protein of claim 1, wherein the AR polypeptide comprises the sequence of amino acids set forth in SEQ ID NO: 5.
3. The fusion protein of claim 1, wherein the AR polypeptide further comprises one or more amino acid substitutions in addition to the F876L amino acid substitution.
4. The fusion protein of claim 3, wherein the one or more additional amino acid substitutions is T877A, W741C, W741L, W741R, L701H or H874Y.
5. The fusion protein of claim 1, wherein the AR polypeptide further comprises at least one of:
 - (a) a substitution of the threonine at amino acid position 877 in SEQ ID NO: 1 by leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, cysteine, tryptophan, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid or glutamic acid;
 - (b) a substitution of the tryptophan at amino acid position 741 in SEQ ID NO: 1 by leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, threonine, cysteine, lysine, arginine, histidine, proline, tyrosine, asparagine, glutamine, aspartic acid or glutamic acid;
 - (c) a substitution of the leucine at amino acid position 701 in SEQ ID NO: 1 by isoleucine, valine, alanine, phenylalanine, glycine, methionine, histidine, serine, threonine, cysteine, lysine, arginine, tryptophan, proline, tyrosine, asparagine, glutamine, aspartic acid, or glutamic acid; and
 - (d) a substitution of the histidine at amino acid position 874 in SEQ ID NO: 1 by leucine, isoleucine, valine, alanine, phenylalanine, glycine, methionine, serine, threonine,

cysteine, lysine, arginine, tryptophan, proline, tyrosine, asparagine, glutamine, aspartic acid or glutamic acid.

6. A cDNA molecule encoding an androgen receptor (AR) polypeptide comprising at least 95% sequence identity to a ligand binding domain having the amino acid sequence of amino acid positions 554 to 919 of SEQ ID NO:1 and comprising an F876L amino acid substitution in SEQ ID NO:1.

7. The cDNA molecule of claim 6, wherein the cDNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 19.

8. A vector, comprising the cDNA molecule of claim 6.

9. The vector of claim 8, wherein the vector is a viral or plasmid vector.

10. The vector of claim 8, wherein the cDNA molecule is operably linked to a promoter.

11. The vector of claim 10, wherein the promoter is a constitutive or an inducible promoter.

12. An isolated host cell, comprising the vector of claim 8.

13. The isolated host cell of claim 12, wherein the isolated host cell is a prokaryotic cell.

14. The isolated host cell of claim 13, wherein the isolated host cell is a bacterial cell.

15. A microchip comprising the fusion protein of claim 1 or the cDNA molecule of claim 6.

16. A system for detecting a modified AR that is resistant to inhibition with a first- or second-generation AR antagonist in a subject, comprising:

(a) a sample containing a nucleic acid molecule encoding an AR polypeptide from the subject; and

(b) a microarray comprising the cDNA molecule of claim 6.

17. The system of claim 16, wherein the microarray is contained on a microchip.

18. The fusion protein of claim 1, wherein the heterologous polypeptide is a heterologous DNA binding domain, a fluorescent protein, or a bioluminescent protein.

19. The fusion protein of claim 18, wherein the heterologous DNA binding domain is selected from the group consisting of GAL4 and LexA.
20. The fusion protein of claim 18, wherein the AR polypeptide is linked to the heterologous DNA binding domain via a peptide linker.
21. The fusion protein of claim 18, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein (GFP), red fluorescent protein (RFP), cyan fluorescent protein (CFP), yellow fluorescence protein (YFP), and blue fluorescent protein (BFP).
22. The fusion protein of claim 1, wherein the epitope tag is selected from the group consisting of c-myc, V-5, hemagglutinin (HA), and FLAG.
23. The fusion protein of claim 1, wherein the affinity tag is selected from the group consisting of biotin, strep-tag, chitin binding protein (CBP), maltose binding protein (MBP), glutathione-S-transferase (GST), and a poly(His) tag.
24. The isolated host cell of claim 12, wherein the isolated host cell is a eukaryotic cell.
25. The isolated host cell of claim 24, wherein the isolated host cell is a mammalian cell, a yeast cell, an insect cell, a plant cell, or an amphibian cell.
26. The fusion protein of claim 1, wherein the AR polypeptide has at least 96% sequence identity to the ligand binding domain.
27. The fusion protein of claim 1, wherein the AR polypeptide has at least 97% sequence identity to the ligand binding domain.
28. The fusion protein of claim 1, wherein the AR polypeptide has at least 98% sequence identity to the ligand binding domain.
29. The cDNA molecule of claim 6, wherein the AR polypeptide has at least 96% sequence identity to the ligand binding domain.
30. The cDNA molecule of claim 6, wherein the AR polypeptide has at least 97% sequence identity to the ligand binding domain.

31. The cDNA molecule of claim 6, wherein the AR polypeptide has at least 98% sequence identity to the ligand binding domain.