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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING PROSTATE CANCER WITH ENZALUTAMIDE AND AN INHIBITOR OF MONOAMIN OXIDASE A

(57) **Abstract:** The invention provides novel therapeutically methods and pharmaceutical compositions for treating prostate cancer with increased survival rate and improved treatment outcome. Methods and compositions of the invention can be used to prevent, delay, and/or reduce drug resistance and to increase, restore and/or prolong the effective treatment of prostate cancer with anti -androgen compounds.

COMPOSITIONS AND METHODS FOR TREATING PROSTATE CANCER WITH ENZALUTAMIDE AND AN INHIBITOR OF MONOAMIN OXIDASE A

Priority Claims and Related Applications

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/791,474, filed January 11, 2019, the entire content of which is incorporated herein by reference for all purposes.

Technical Field of the Invention

[0002] The invention generally relates to novel therapeutic methods and pharmaceutical compositions for treating cancer. More particularly, the invention relates to a novel approach to addressing drug resistance in prostate cancer treatment.

Background of the Invention

[0003] Prostate cancer (PCa) is the most common form of cancer and the second leading cause of cancer death among men in the United States. (Centers for Disease Control and Prevention. http://www.cdc.gov/cancer/dcpc/data/men.htm.) Approximately one in seven men will be diagnosed with prostate cancer during his lifetime, and about one in 38 men will die of the disease with an estimated 307,000 deaths worldwide in 2012. (Merseburger, *et al.* **2015** *Ther Adv Urol* 7, 9-21.)

[0004] The current standard treatment for advanced prostate cancer is androgen deprivation therapy (ADT), which may suppress prostate cancer progression by reducing androgen biosynthesis or by preventing androgen from binding to the androgen receptor. (Chang, et al. **1988** *Science* 240, 324-326; Heinlein, *et al.* **2004** *Endocr Rev* 25, 276-308; Chang, *et al.* **2014** *Oncogene* 33, 3225-3234; Niu, *et al.* **2010** *Oncogene* 29, 3593-3604.)

[0005] A newly developed anti-androgen compound, Enzalutamide (Enz), was shown to suppress castration resistant prostate cancer (CRPC) and could further extend patients overall survival by an average of 4.8 months. (Scher, *et al.*, **2012** *N Engl J Med* 367, 1187-1197.) Unfortunately, most ADT, including Enz, may fail after 1-2 years treatment due to development of castration resistance (EnzR). (Miyamoto, *et al.* **2005** *Nature clinical practice. Oncology* 2,

236-237; Miyamoto, *et al.* **2004** *The Prostate* 61, 332-353.) The detail mechanism, however, remains unclear.

[0006] Recent human clinical survey indicated that Enz might induce the expression of ARv7, an AR splicing variant that lacks androgen-binding-domain, to constitutively activate its downstream genes in the castration concentration of androgen (near 1-3 nM), which can then lead to the development of castration resistance. (Kaiser, J. **2009** *Science* 324, 165; Ryan, *et al.* **2013** *The New England journal of medicine* **368**, 1458-1459.) The detailed mechanism why Enz-induced ARv7 may lead to the development of castration resistance, however, remains unclear.

[0007] Currently, there is not an effective way to delay, reduce or reverse such drug resistance, which has seriously limited the overall effectiveness and treatment outcome of anti-androgen therapy. There remains an urgent need for novel and improved approaches that effectively address these issues.

Summary of the Invention

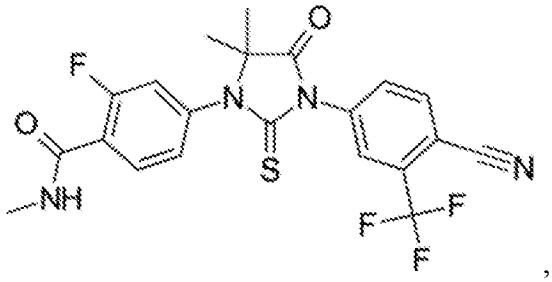
[0008] The invention is based in part on the unexpected discovery that inhibitors of MAOA can be used to prevent, delay, reduce and/or reverse EnzR resistance and to increase, restore and/or prolong the effective treatment of prostate cancer with anti-androgen compounds, such as Enz. As disclosed herein, MOAO inhibitors can delay the onset and development and/or reduce the magnitude of EnzR and suppress EnzR tumors.

[0009] As is disclosed herein for the first time, MAOA is highly expressed in various Enz resistant PCa cells. Targeting MAOA with its specific inhibitors, for example clorgyline or phenelzine, both FDA-proved for treating Parkinson and depression, can lead to re-sensitized Enz-R cells in response to Enz treatment to further suppress Enz-R cell growth. This result indicates that certain currently FDA-proved drugs may be immediately developed and used as new therapy to overcome the Enz-induced castration resistance.

[0010] Studies have shown that MAOA increase hypoxia signaling to promote the Enz resistance. Together, this newly identified mechanism showing Enz can function *via* altering the ARv7 to increase MAOA expression lays the foundation for the quick development of a new therapy for CRPC using current FDA-proved drugs (*e.g.*, clorgyline or phenelzine) to further extend CRPC patients survival that already developed Enz-resistance.

[0011] The present invention thus may fundamentally alter the treatment protocol for castration resistant prostate cancer patients. Therapeutic methods and pharmaceutical compositions of the invention may be used to treat castration resistant prostate cancer, or a related disease or condition, in ways that delay, reduce or reverse drug resistance while maximizing treatment outcome.

[0012] In one aspect, the invention generally relates to a pharmaceutical composition, which includes: a first compound of Formula (I)



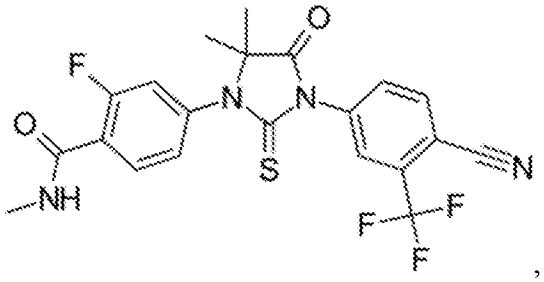
(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of monoamine oxidase A, wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in the treatment of prostate cancer, or a related disease or condition thereof, in a mammal, including a human, and a pharmaceutically acceptable carrier.

[0013] In another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition disclosed herein.

[0014] In yet another aspect, the invention generally relates to a method for treating castration resistant prostate cancer, or a related disease or condition thereof. The method includes administering to a subject in need thereof a pharmaceutical composition disclosed herein.

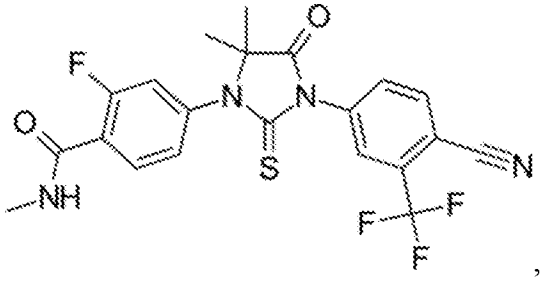
[0015] In yet another aspect, the invention generally relates to a method for treating castration resistant prostate cancer, or a related disease or condition thereof. The method includes administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of monoamine oxidase A, in amounts effective in the treatment of castration resistant prostate cancer, or a related disease or condition thereof, in a mammal, including a human, wherein the subject has been detected of the development of drug resistance to the first compound.

[0016] In yet another aspect, the invention generally relates to a method for treating castration resistant prostate cancer, or a related disease or condition thereof. The method includes: administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, in an amount effective in the treatment of castration resistant prostate cancer or a related disease or condition thereof in a mammal, including a human; monitoring the subject to detect a development of drug resistance to the first compound; upon the subject being detected of the development of drug resistance to the first compound, administering to the subject a second compound which is an inhibitor of monoamine oxidase A, in an amount effect to reduce or eliminate drug resistance to the first compound; and monitoring the subject to detect a level of drug resistance to the first compound.

[0017] In yet another aspect, the invention generally relates to a method for treating a drug resistance in connection with a cancer treatment. The method includes: administering to a subject in need thereof a second compound which is an inhibitor of monoamine oxidase A, in an amount

effective to prevent, delay, reduce or reverse resistance to a treatment of prostate cancer, or a related disease or condition thereof in a mammal, including a human.

Brief Description of the Drawings

[0018] FIG. 1. MAOA expression is associated with the development of Enz-resistance. (A) Heat map of most significantly changed genes in EnzR cells. (B) Statistical analysis of MAOA expression in published datasets. The expressions of MAOA in normal prostate tissues and PCa tissues were analyzed based on Singh (normal, n=50; cancer, n=52) and Vanaja (normal, n=8; cancer, n=32) datasets. The MAOA expressions in different Gleason score PCa samples were analyzed based on the Taylor dataset (Gleason score 6, n=79; Gleason score 7, n=50 and Gleason score 8, n=10; Gleason score 9, n=9).The MAOA expression in PCa samples with recurrence and no recurrence were analyzed based on Lapointe (no recurrence, n=14; recurrence, n=3) dataset. (C) The qPCR and Western blot analysis of MAOA levels in EnzR1-C4-2 and EnzS1-C4-2 cells. (D) MAOA activity analysis in EnzS1-C4-2 and EnzR1-C4-2 cells by using MAO-Glo assay. (E) MAOA and ARv7 mRNA levels by qPCR following treatment with DMSO or 10 μM Enz in EnzS1-C4-2 cells for 6 days. (F) MAOA activity analyzed in EnzS1-C4-2 cells treated with 10 μM Enz for 6 days. (G) Absolute copy numbers of MAOA mRNA normalized to copy number of RPL13A in CTCs isolations from 288 patients. Those samples were grouped by CTC and ARv7 status: CTC-, CTC+/ARv7-, and CTC+/ARv7+. (H) MAOA copy numbers normalized by RPL13A in patients whose ARv7 status remained negative after Enz treatment (N=30). (I) MAOA copy numbers normalized by RPL13A in patients whose ARv7 status changed from negative to positive after Enz treatment (N=13). (J-K) Relative MAOA copy number values at baseline (before Enz) and after Enz treatment (after Enz) in each patient whose ARv7 status changed from negative to positive after Enz treatment (N=13) (J) and in patients whose ARv7 status was positive at baseline (before Enz) and remained positive after Enz treatment (N=12) (K). Quantitation is mean ± SEM, P-value was determined by two-tailed paired t test. P-values are, **<0.005, ***<0.001.

[0019] FIG. 2. Targeting MAOA re-sensitizes EnzR cells to Enz and suppresses EnzR cell growth. (A) EnzS1-C4-2 cells were treated with/without (w/o) 10 μM Enz and 5 μM clorgyline and cell viability analyzed by MTT assay. (B-D) EnzR1-C4-2, EnzR2-C4-2, EnzR3-

22Rv1 cells, respectively, were treated (w/o) 10 μM Enz and 5 μM clorgyline, and cell viability was analyzed by MTT assay. (E-G) EnzR1-C4-2, EnzR2-C4-2, EnzR3-22Rv1 cells, respectively, were treated w/o 10 μM Enz and 5 μM phenelzine, and cell viability was analyzed. (H-J) The EnzR1-C4-2, EnzR2-C4-2, EnzR3-22Rv1 cells with pLKO or shMAOA cells were treated w/o Enz and cell viability was analyzed. (K) MAOA was overexpressed (oeMAOA) in EnzS1-C4-2 cells and then the cells treated w/o Enz and cell viability was analyzed. (L) EnzS1-C4-2 cells were treated w/o 10 μM Enz and cell viability was analyzed. (M) EnzS1-C4-2 cells were first treated (w/o) 10 μM Enz for 1.5 month. And then the cells were seeding and the cell viability under 10 μM Enz treatment was analyzed by MTT assay. (N-O) EnzS1-C4-2 cells were treated w/o 10 μM Enz and 1 μM,2.5μM clorgyline (clg) for 1.5 month. And then the cell viability under 10 μM Enz treatment was analyzed. Quantitation is mean ± SEM, P-value was determined by two-tailed paired t test. P-values are, *<0.05, **<0.005.

[0020] FIG. 3. Mechanism dissection of how Enz increases the MAOA expression: *via* increasing ARv7. (A-B) PC3 cells were treated with/without (w/o) 10 μM Enz and 5 μM clorgyline or 10μM clorgyline. (The cell viability was analyzed by MTT assay. (C) EnzS1-C4-2 cells were treated with 10 μM Enz for different time points. The ARv7 and MAOA mRNA level were analyzed by qPCR (left) and western blot (right). (D) The mRNA levels of ARv7 and MAOA were analyzed in EnzR1-C4-2 PWPI and PWPI-ARv7 cells. (E) The expression of ARv7 and MAOA were analyzed in EnzS1_C4-2pWPI and pWPI-ARv7(oeARv7) cells by q-PCR (left) and western blot (right). (F-H) The mRNA and protein level of ARv7 and MAOA were analyzed in EnzR1_C4-2, EnzR2_C4-2 (no protein shown), EnzR3_CWR22Rv1 pLKO and shARv7 cells. (I) EnzS1-C4-2 cells were infected with pLKO and shARv7 viruses. And then the cells were treated w/o Enz for 2 and 6 days, the ARv7 and MAOA levels were examined by qPCR. (J) EnzS1 cells were infected by pWPI-ARv7, pLKO-shMAOA or both viruses. And then the cells were treated w/o10 μM Enz and the cell viability analyzed by MTT assay. For C and F-I, quantitations are presented as mean±SEM, P-value was determined by two-tailed paired t test. P-values are **<0.005, ***<0.001.

[0021] FIG. 4. Enz up-regulates the MAOA transactivation in transcriptional level and protein level. (A) Schematic depiction of putative ARE on MAOA promoter region. The mutant ARE was marked by italic font. (B) ChIP assay was performed to identify that endogenous AR

and Flag-ARv7 bind to the putative ARE on MAOA promoter in EnzR1-C4-2 and EnzS1-pWPI-flag-ARV7 cells. (C) EnzR1 cells were infected by Flag-ARv7 or Flag-ARfl virus. The ChIP assay were performed to analyze Flag-AR and Flag-ARv7 binding on MAOA promoter region in EnzR1 cells. (D) PC3 pWPI and pWPI-ARV7 cells were transfected by the PGL3-MAOA promoter plasmid, and the promoter activity was determined by luciferase assay. (E) EnzS1-C4-2 cells were treated with Enz at different time points and the MAOA promoter activity was identified by luciferase reporter assay. (F) MAOA promoter with mutated ARE was constructed into PGL3 plasmid, and then the MAOA promoter activity was analyzed in PC3 and PC3-oeARv7 cells. (G) ARv7 and flAR were transfected into EnzS1-C4-2 cells. And then the PGL3-MAOA promoter luciferase activity was assayed. (H) ARv7 and flAR were transfected into EnzS1-C4-2 cells. And then the MAOA expression was analyzed by WB. (I) EnzS1-C4-2 and EnzR1-C4-2 cells were treated with cycloheximide (CHX) for different time points. The MAOA protein level was analyzed by western blot. (J) EnzS1-C4-2 and EnzR1-C4-2 cells were treated with MG-132. The MAOA protein level was analyzed by western blot. (K) MAOA phosphorylation is increased in EnzR1-C4-2 cells and the lysine phosphorylation of MAOA was detected by specific antibody. (L) The phosphorylation of p38 (p-p38) protein levels were detected by western blot in EnzS1-C4-2 and EnzR1-C4-2 cells. (M) EnzS1 and EnzR1-C4-2 cells were treated with p38 inhibitor. The MAOA protein levels were analyzed by western blot. (N) EnzR1 cells were treated with p38 inhibitors for 48hrs, and then the MAOA protein was pulled down, MAOA p-Ser level was examined by western blot. For C and D, Quantitation is mean ± SEM, P-value was determined by two-tailed paired t test. P value is <**0.005.

[0022] FIG. 5. MAOA activates Hypoxia signaling to promote the Enzalutamide-resistance. (A) The mRNA levels of Glut1, N-Cadherin, Timp and VEGF-A in EnzS1-C4-2 and EnzR1-C4-2 cells were analyzed by qPCR. (B) In EnzR1-C4-2 cells, MAOA was knocked down by shRNA. And the mRNA levels of MAOA, Slug, VEGF-A and HIF-1α were analyzed by qPCR (left), as well asHIF-1α and VEGF-A expression by western blot (right). (C) EnzR1-C4-2 cells were treated with/without 5μM clorgyline, and the mRNA and protein level of MAOA, HIF-1α and VEGF-A were analyzed by qPCR (left) and western blot (right).(D) MAOA was overexpressed (oeMAOA) in EnzS1-C4-2 cells, and the mRNA and protein levels of MAOA, HIF-1α and VEGF-A were analyzed by qPCR (left) and western blot (right). (E) HIF-1αwas

knocked down (shHIF-1α) in EnzR1-C4-2 cells, and then the cells were treated with/without Enz. The cell viability was analyzed by MTT assay. Quantitation is presented as mean ± SEM, P-value was determined by two-tailed paired t test. P values are *<0.05, **<0.005, ***<0.001.

[0023] FIG. 6. Clorgyline and phenelzine, inhibitors of MAOA activity can overcome Enzalutamide-resistance. (A) The *in vivo* PDX-PCa mouse model data revealed that injection with Enz (30mg/kg/every other day) increased the MAOA, ARv7 and p-p38 level.(B, C) Mice implanted with EnzR3-22Rv1 xenografts were treated with vehicle control, Enz (30 mg/kg), clorgyline (10 mg/kg), phenelzine (30 mg/kg), Enz+clorgyline (30 mg/kg+10 mg/kg), or Enz+phenelzine (30 mg/kg+30 mg/kg). After sacrifice, tumors of the 6 groups were collected and weighed. (D, E) Mice implanted with EnzR1-C4-2 xenografts received the same treatments as EnzR3-22Rv1 After sacrifice, tumors of the 6 groups were collected and weighed. (F) IHC staining of Ki-67 and VEGFA in EnzR3-22Rv1 tumors were performed. (G) IHC staining of Ki-67 and VEGF-A in EnzR1-C4-2 tumors were performed. For C, E, F and G, quantitation is mean ± SEM, P-value was determined by two-tailed paired t test. P value is ***<0.001.

[0024] FIG. 7. (A) Quantitative IHC analysis of MAO-A protein expression in normal prostate (n = 7), PCa clinical samples (gleason score 3) (n = 5), PCa clinical samples (gleason score 4) (n = 7), and PCa clinical samples (gleason score 5) (n = 8). Arrows indicate tumor areas. (B) Western blot analysis of MAO-A expression in EnzS4_C4-2B parental and Enz-R4_C4-2B EnzR cells. (C) VCaP cells were treated by Enz for 10 days and then the ARv7 and MAO-A protein levels were examined by WB. For A Quantitations are mean±SEM, P-value was determined by two-tailed paired t test. P values are **<0.005, ***<0.001.

[0025] FIG. 8. AR-FL copy numbers at baseline (before) and after Enzalutamide treatment (after) in patients whose ARv7 status (A) remained negative (N=30), (B) changed from negative to positive (N=13), and (C) remained positive (N=12) after Enzalutamide treatment. P-values were determined by two-tailed paired t-test.

[0026] FIG. 9. (A) MGEA11 and HSD3B1 expression EnzR and EnzS cells were analyzed by qPCR. (B) The EnzR1 cells were treated w/o 10uM clorgyline for 24 hours. The MAO-A and ARv7 expression were analyzed by WB.

[0027] **FIG. 10**. The ARfl and ARv7 binding on MAO-A promoter regions in presence of DHT (A) or in the absence of DHT (Etoh) (B) were analyzed based on ChIP-seq data (GEO: GSE106559). (C) The quantification of ARfl and ARv7 binding on the MAO-A promoter region.

[0028] **FIG. 11**. (A) Western blot analysis to identify the knocking efficiency of MAO-A in EnzR1_C4-2 and EnzR2 cells. (B) The qPCR analysis (left) to identify the mRNA level and Western blot analysis (right) of HIF-1α and VEGF-A in EnzR1_C4-2 pLVTHM and shHIF1a cells. For B Quantitations are mean±SEM, P-value was determined by two-tailed paired t test. P values are **<0.005, ***<0.001.

[0029] **FIG. 12**. (A) Mice implanted with EnzR3-22RV1-luc xenografts were treated with vehicle control, Enz (30 mg/kg), clorgyline (10 mg/kg), phenelzine (30 mg/kg), Enz+clorgyline (30 mg/kg+10 mg/kg), or Enz+phenelzine (30 mg/kg+30 mg/kg). The tumor sizes were monitored by In vivo imaging system (IVIS). (B-C) After sacrifice, tumors of the 6 groups were collected (B) and weighed (C). (D) IHC staining of Ki-67 and VEGF-A in EnzR3-22Rv1-luc tumors were performed. For D Quantitations are mean±SEM, P-value was determined by two-tailed paired t test. P values are **<0.005.

[0030] **FIG. 13**. Exemplary primer and plasmid sequences.

Definitions

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. General principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 2006.

[0032] Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

[0033] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

[0034] If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic methods well known in the art, and subsequent recovery of the pure enantiomers.

[0035] Enzalutamide (*a.k.a.*, MDV3100 and Xtandi, CAS No. 915087-33-1) is a synthetic, non-steroidal pure antiandrogen that was developed for the treatment of metastatic castration-resistant prostate cancer. The IUPAC name of the compound is 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide. The structure of Enzalutamide is depicted by formula (I).

[0036] As used herein, the term "effective amount" of an active agent refers to an amount sufficient to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound of the invention may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the disease being treated, the mode of administration, and the patient.

[0037] As used herein, the terms "treatment" or "treating" a disease or disorder refers to a method of reducing, delaying or ameliorating such a condition before or after it has occurred. Treatment may be directed at one or more effects or symptoms of a disease and/or the underlying pathology. The treatment can be any reduction and can be, but is not limited to, the complete ablation of the disease or the symptoms of the disease. As compared with an equivalent untreated control, such reduction or degree of prevention is at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, or 100% as measured by any standard technique.

[0038] As used herein, the terms “prevent”, “preventing”, or “prevention” refer to a method for precluding, delaying, averting, or stopping the onset, incidence, severity, or recurrence of a disease or condition. For example, a method is considered to be a prevention if there is a reduction or delay in onset, incidence, severity, or recurrence of a disease or condition or one or more symptoms thereof in a subject susceptible to the disease or condition as compared to a subject not receiving the method. The disclosed method is also considered to be a prevention if there is a reduction or delay in onset, incidence, severity, or recurrence of osteoporosis or one or more symptoms of a disease or condition in a subject susceptible to the disease or condition after receiving the method as compared to the subject's progression prior to receiving treatment. Thus, the reduction or delay in onset, incidence, severity, or recurrence of osteoporosis can be about a 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between.

[0039] As used herein, the term “pharmaceutically acceptable” excipient, carrier, or diluent refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0040] As used herein, the terms "isolated" or "purified" refer to a material that is substantially or essentially free from components that normally accompany it in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography.

[0041] As used herein, the term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0042] As used herein, the term "low dosage" refers to at least 5% less (*e.g.*, at least 10%, 20%, 50%, 80%, 90%, or even 95%) than the lowest standard recommended dosage of a particular compound formulated for a given route of administration for treatment of any human disease or condition. For example, a low dosage of an agent that is formulated for administration by inhalation will differ from a low dosage of the same agent formulated for oral administration.

[0043] As used herein, the term "high dosage" is meant at least 5% (*e.g.*, at least 10%, 20%, 50%, 100%, 200%, or even 300%) more than the highest standard recommended dosage of a particular compound for treatment of any human disease or condition.

[0044] As used herein, the term "prodrug" (or "pro-drug") refers to a pharmacological derivative of a parent drug molecule that requires biotransformation, either spontaneous or enzymatic, within the organism to release the active drug. Such prodrugs are pharmaceutically active *in vivo,* when they undergo solvolysis under physiological conditions or undergo enzymatic degradation. Prodrug compounds herein may be called single, double, triple, etc., depending on the number of biotransformation steps required to release the active drug within the organism, and the number of functionalities present in a precursor-type form.

[0045] Prodrug forms often offer advantages of solubility, tissue compatibility, or delayed release in the mammalian organism. (See, Bundgard, Design of Prodrugs, pp. 7-9,21-24, Elsevier, Amsterdam 1985 and Silverman, The Organic Chemistry of Drug Design and Drug Action, pp. 352-401, Academic Press, San Diego, Calif., 1992). Prodrugs commonly known in the art include well-known acid derivatives, such as, for example, esters prepared by reaction of the parent acids with a suitable alcohol, amides prepared by reaction of the parent acid compound with an amine, basic groups reacted to form an acylated base derivative, *etc.* Of

course, other prodrug derivatives may be combined with other features disclosed herein to enhance bioavailability. As such, those of skill in the art will appreciate that certain of the presently disclosed compounds having free amino, arnido, hydroxy or carboxylic groups can be converted into prodrugs. Prodrugs include compounds having an amino acid residue, or a polypeptide chain of two or more (*e.g.*, two, three or four) amino acid residues which are covalently joined through peptide bonds to free amino, hydroxy or carboxylic acid groups of the presently disclosed compounds. The amino acid residues include the 20 naturally occurring amino acids commonly designated by three letter symbols and also include 4-hydroxyproline, hydroxylysine, demosine, isodemosine, 3-methylhistidine, norvalin, beta-alanine, gamma-aminobutyric acid, citrulline homocysteine, homoserine, ornithine and methionine sulfone. Prodrugs also include compounds having a carbonate, carbamate, amide or alkyl ester moiety covalently bonded to any of the above substiruents disclosed herein.

[0046] Isotopically-labeled compounds are also within the scope of the present disclosure. As used herein, an "isotopically-labeled compound" refers to a presently disclosed compound including pharmaceutical salts and prodrugs thereof, each as described herein, in which one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds presently disclosed include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively.

[0047] By isotopically-labeling the presently disclosed compounds, the compounds may be useful in drug and/or substrate tissue distribution assays. Tritiated (^{3}H) and carbon-14 (^{14}C) labeled compounds are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (^{2}H) can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds presently disclosed, including pharmaceutical salts, esters, and prodrugs thereof, can be prepared by any means known in the art.

[0048] Further, substitution of normally abundant hydrogen (^{1}H) with heavier isotopes such as deuterium can afford certain therapeutic advantages, *e.g.*, resulting from improved absorption,

distribution, metabolism and/or excretion (ADME) properties, creating drugs with improved efficacy, safety, and/or tolerability. Benefits may also be obtained from replacement of normally abundant ^{12}C with ^{13}C. (See, WO 2007/005643, WO 2007/005644, WO 2007/016361, and WO 2007/016431.)

[0049] Stereoisomers (*e.g.*, cis and trans isomers) and all optical isomers of a presently disclosed compound (*e.g.*, R and S enantiomers), as well as racemic, diastereomeric and other mixtures of such isomers are within the scope of the present disclosure.

[0050] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 95% ("substantially pure"), which is then used or formulated as described herein. In certain embodiments, the compounds of the present invention are more than 99% pure.

[0051] Solvates and polymorphs of the compounds of the invention are also contemplated herein. Solvates of the compounds of the present invention include, for example, hydrates.

[0052] Any appropriate route of administration can be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intraventricular, intracorporeal, intraperitoneal, rectal, or oral administration. Most suitable means of administration for a particular patient will depend on the nature and severity of the disease or condition being treated or the nature of the therapy being used and on the nature of the active compound.

[0053] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds described herein or derivatives thereof are admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (i) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (ii) binders, as for example, carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (iii) humectants, as for example, glycerol, (iv) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (v) solution retarders, as for example, paraffin, (vi) absorption accelerators, as for example, quaternary ammonium compounds, (vii) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (viii) adsorbents, as for example, kaolin and bentonite, and (ix) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or

mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard- filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like. Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others known in the art.

[0054] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers, such as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan, or mixtures of these substances, and the like. Besides such inert diluents, the composition can also include additional agents, such as wetting, emulsifying, suspending, sweetening, flavoring, or perfuming agents.

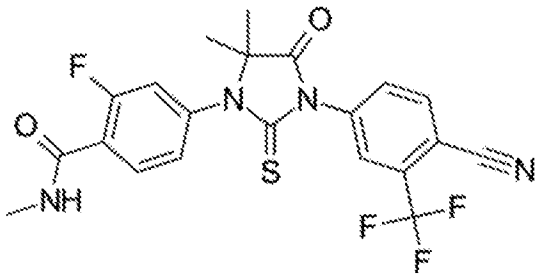
[0055] Materials, compositions, and components disclosed herein can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. It is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a method is disclosed and discussed and a number of modifications that can be made to a number of molecules including in the method are discussed, each and every combination and permutation of the method, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each

such combination or subset of combinations is specifically contemplated and should be considered disclosed.

Detailed Description of the Invention

[0056] The invention provides a unique approach to treatment of prostate cancer, particularly drug resistance prostate cancer. The novel therapeutic methods and compositions provided herein can benefit prostate cancer patients in terms of increased survival rate and improved treatment outcome. Methods and compositions of the invention can be used to prevent, delay, reduce and/or reverse drug resistance and to increase, restore and/or prolong the effective treatment of prostate cancer with anti-androgen compounds.

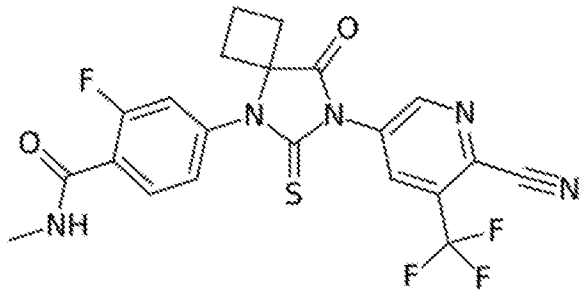
[0057] Enzalutamide, an anti-androgen agent, has been successfully used as the last line therapy to extend lives of CRPC patients. After prostate cancer patients develop CRPC, the current standard therapy to further suppress CRPC involves either docetaxel (Doc)-chemotherapy or ADT with either using Enz to prevent androgens binding to AR or using abiraterone (ABI) to further suppress the androgen synthesis in renal. Unfortunately, many patients still develop Enz resistance after an average of 4.8 months response to Enz. (Scher, *et al.* **2012** *N Engl J Med* 367, 1187-1197; Dhingra, *et al.* **2013** *Mini Rev Med Chem* 13, 1475-1486.)



Enzalutamide (Enz)

[0058] The mechanism by which CRPC patients received Enz develop EnzR remains not fully understood. Certain studies indicated the possible involvement of multiple mechanisms. For instance, Enz or its derivative ARN-509 (*a.k.a.*, JNJ-56021927) may induce an AR point mutation at AR876 (a missense mutation of phenylalanine 876 to leucine in the Ligand-Binding-

Domain (LBD) of AR, named AR-F876L) that is no longer sensitive to Enz treatment. (Korpal, *et al.* 2013 *Cancer Discovery* 3, 1030-1043; Joseph *et al.* **2013** *Cancer Discovery* 3, 1020-1029.)



ARN-509

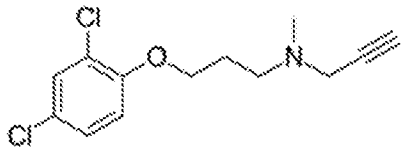
[0059] Other studies showed increased glucocorticoid receptor (GR) signals by Enz in a subset of prostate cancer cells due to relief of AR-mediated feedback repression of GR expression. GR and AR are closely related members of the nuclear receptor superfamily with similar DNA-binding-domains. It is possible that the GR could replace some AR roles during development of EnzR. Studies also found that the GR agonist dexamethasone was sufficient to confer EnzR, whereas a GR antagonist could partially restore sensitivity. (Arora, *et al.* **2013** *Cell* 155, 1309-1322; Yemelyanov, *et al.* **2012** *Cell Cycle* **11**, 395-406; Sharifi **2014** *New England Journal of Medicine* **370**, 970-971.)

[0060] Development of AR splicing variant ARv7 is also linked to the development of EnzR. Recent clinical sample survey reported that CRPC patients received ADT-Enz had increased ARv7, and ARv7 expression was higher in patients developed Enz resistance. (Antonarakis, *et al.* **2014** *The New England journal of medicine* 371, 1028-1038.) For example, clinical studies from CRPC patients demonstrated that 39% of metastatic CRPC patients treated with Enz had detectable ARv7 in their circulating tumor cells. These ARv7 positive patients had lower Prostate-Specific Antigen (PSA) response rates than ARv7 negative patients with shorter PSA progression–free survival (median, 1.4 months vs. 6.0 months), indicating that CRPC patients with ARv7 might have poor response to Enz treatment and that Enz treatment might enhance ARv7 expression. (Antonarakis *et al.* **2014** *New England Journal of Medicine* **371**, 1028-1038.)

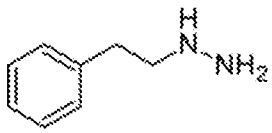
[0061] Instead of directly targeting ARv7, however, compounds identified herein target MAOA, a key gene downstream of ARv7, to mediate the ARv7-indiced Enz resistance. The advantages of using MAOA inhibitors to re-sensitize Enz resistance include improved efficacy to increase the Enz sensitivity in Enz resistant cells (**FIG. 2B-D**) as well as delaying the development of Enz resistance during combination therapy with Enz to treat CRPC cells (**FIG. 2J**).

[0062] MAOA is a key enzyme in catalyzing the deamination of amines, and play key roles in inducing some neurotransmitters including norepinephrine, dopamine and serotonin. (Dorfman, *et al.* **2014** *Current topics in behavioral neurosciences* 17, 297-313.) Interestingly, recent studies also reported that higher MAOA might be linked to the PCa progression *via* inducing the hypoxia and EMT signals. (Flamand, *et al.* **2010** *Journal of cancer research and clinical oncology* **136**, 1761-1771; Wu, *et al.* **2014** *The Journal of clinical investigation* **124**, 2891-2908.)

[0063] It has now been unexpectedly discovered, as disclosed herein, that inhibitors of MOAO, such as clorgyline or phenelzine, can be used to effectively prevent, delay, reduce and/or reverse EnzR resistance and to increase, restore and/or prolong the effective treatment of prostate cancer with anti-androgen compounds, such as Enzalutamide and its derivative ARN-509.



Clorgyline



Phenelzine

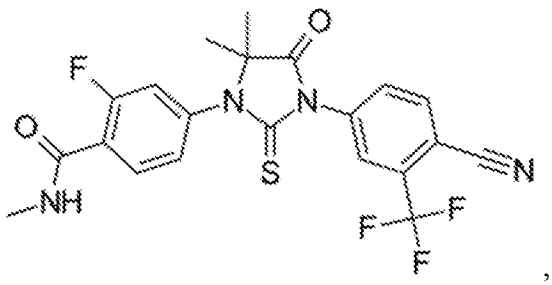
[0064] Clorgyline is a selective and irreversible inhibitor of MAOA. Phenelzine is a non-selective and irreversible MAOA inhibitor of the hydrazine class. Phenelzine is approved and used as an antidepressant and anxiolytic.

[0065] Potential side effects of treatment with MAOA inhibitors such as phenelzine include dizziness, drowsiness, tiredness, weakness, problems sleeping, constipation, and dry mouth. To CRPC patients that have already developed EnzR, the potential benefit of MAOA inhibitors in restoration of Enz sensitivity would in many cases outweigh the risk of such side effects. In addition, where side effects are severe, dosage adjustment may offer patient therapeutic benefit with manageable risk exposure.

[0066] Significantly, certain MAOA inhibitors have been clinically studied and or approved and may be immediately applied to treating Enz resistant CRPC patients. For example, clorgyline or phenelzine has been used to treat Parkinson's disease and depression since 1960s. (Riederer, *et al.* **2011** *Experimental neurobiology* **20**, 1-17.) It's worth noting that the suggested dose of clorgyine for depression is near 30 mg/day, whereas doses used in *in vitro* cell line studies disclosed herein is at 1.5 mg/mL and *in vivo* mice is 1 mg/Kg/day, which is lower than the human dose. This indicates that the dose used to treat the human depression can be also used to effectively re-sensitize the Enz resistance.

[0067] Thus, the present invention enables a novel and ready therapy to suppress the Enz-resistant CRPC progression and to extend survival of CRPC patients. In certain embodiments of the invention, as discussed in details herein, a combination of a MOAO inhibitor and Enz may be used at the beginning of therapy. In certain other embodiments, sequential treatment with Enz therapy at the beginning may be followed by addition of a MOAO inhibitor at or after confirmation of EnzR (*e.g.*, when decreased PSA start to rise again).

[0068] In one aspect, the invention generally relates to a pharmaceutical composition, which includes: a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of monoamine oxidase A, wherein each of the first and second compounds is present

in an amount such that the pharmaceutical composition is effective in the treatment of prostate cancer, or a related disease or condition thereof, in a mammal, including a human, and a pharmaceutically acceptable carrier.

[0069] In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in delaying the onset of or delaying the development of drug resistance. In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in delaying the onset of drug resistance. In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in delaying the development of drug resistance.

[0070] In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reducing or reversing drug resistance. In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reducing drug resistance. In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reversing drug resistance.

[0071] The first and second compounds may be present in the pharmaceutical composition at any suitable ratios. For example, the weight ratio of the first compound to the second compound is from about 10 : 1 to about 1 : 10 (*e.g.*, from about 7 : 1 to about 1 : 7, from about 5 : 1 to about 1 : 5, from about 3 : 1 to about 1 : 3, from about 2 : 1 to about 1 : 2, about 1 : 1).

[0072] The first compound may be present in the pharmaceutical composition in any suitable amount, for example in a unit dosage of, in the range of about 0.1 mg to about 1,000 mg, about 1 mg to about 500 mg, about 1 mg to about 10 mg, about 10 mg to about 250 mg, about 10 mg to about 125 mg, about 10 mg to about 100 mg, about 10 mg to about 75 mg, about 10 mg to about 50 mg, about 10 mg to about 25 mg.

[0073] The second compound may be present in the pharmaceutical composition in any suitable amount, for example in a unit dosage of, in the range of about 0.1 mg to about 1,000 mg, about 1 mg to about 500 mg, about 1 mg to about 10 mg, about 10 mg to about 250 mg, about 10 mg to about 125 mg, about 10 mg to about 100 mg, about 10 mg to about 75 mg, about 10 mg to about 50 mg, about 10 mg to about 25 mg.

[0074] In certain embodiments, the pharmaceutical composition is suitable for oral administration. In certain embodiments, the pharmaceutical composition is suitable for intravenous, intramuscular, or subcutaneous administration.

[0075] Any suitable inhibitor(s) of monoamine oxidase may be used in preparing the pharmaceutical composition of the invention. In certain embodiments, the pharmaceutical composition includes a single inhibitor of monoamine oxidase (*e.g.*, MAOA). In certain embodiments, the pharmaceutical composition includes two or more inhibitors of monoamine oxidase (*e.g.*, MAOA) in a combination.

[0076] Exemplary inhibitors of MAOA include clorgyline and phenelzine.

[0077] In certain embodiments, the second compound is clorgyline. In certain embodiments, the weight ratio of the first compound to clorgyline is from about 7 : 1 to about 1 : 7, from about 5 : 1 to about 1 : 5, from about 3 : 1 to about 1 : 3, from about 2 : 1 to about 1 : 2, about 1 : 1

[0078] In certain embodiments, the second compound is phenelzine. In certain embodiments, the weight ratio of the first compound to phenelzine is from about 7 : 1 to about 1 : 7, from about 5 : 1 to about 1 : 5, from about 3 : 1 to about 1 : 3, from about 2 : 1 to about 1 : 2, about 1 : 1.

[0079] In another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition disclosed herein.

[0080] In certain embodiments, the unit dosage form is in the form of a tablet or capsule suitable for oral administration.

[0081] In certain embodiments, the unit dosage form is in the form of a liquid solution or suspension suitable for intravenous, intramuscular, or subcutaneous administration.

[0082] In yet another aspect, the invention generally relates to a method for treating prostate cancer, or a related disease or condition thereof. The method includes administering to a subject in need thereof a pharmaceutical composition disclosed herein.

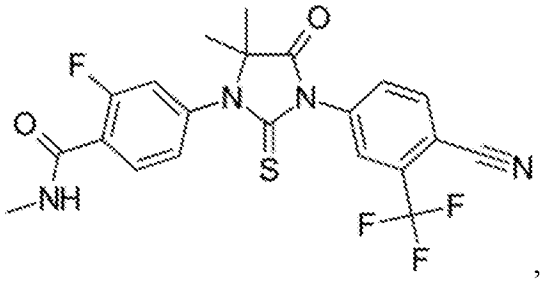
[0083] In certain embodiments, the method further include: administering to the subject one or more other anti-cancer agents. The one or more other anti-cancer agents may be any suitable agent, for example, a chemotherapeutic agent.

[0084] Exemplary chemotherapeutic agents include Erlotinib (TARCEVA®, Genentech/OSI Pharm.), Bortezomib (VELCADE®, Millennium Pharm.), Fulvestrant (FASLODEX®,

AstraZeneca), Sutent (SU11248, Pfizer), Letrozole (FEMARA®, Novartis), Imatinib mesylate (GLEEVEC®, Novartis), PTK787/ZK 222584 (Novartis), Oxaliplatin (Eloxatin®, Sanofi), 5-FU (5-fluorouracil), Leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafarnib (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs), and Gefitinib (IRESSA®, AstraZeneca), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analog topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g. , calicheamicin, especially calicheamicin gammall and calicheamicin omegall (Angew Chem. Intl. Ed. Engl. (1994) 33: 183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6- diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esonibicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as

fludarabine, 6- mercaptopurine, thiamniprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2' ,2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. , TAXOL® (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, 111.), and TAXOTERE® (doxetaxel; Rhone-Poulenc Rorer, Antony, France); chloranmbucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP- 16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11 ; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0085] In yet another aspect, the invention generally relates to a method for treating castration resistant prostate cancer, or a related disease or condition thereof. The method includes administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of monoamine oxidase A, in amounts effective in the treatment of prostate cancer, or a related disease or condition thereof, in a mammal, including a human, wherein the subject has been detected of the development of drug resistance to the first compound.

[0086] In certain embodiments, the second compound is administered simultaneously with the first compound, or subsequently after the administration of the first compound.

[0087] In certain embodiments, the subject has been detected of the development of drug resistance to the first compound and the method is applied.

[0088] In certain embodiments, the subject has not been detected of the development of drug resistance to the first compound and the method is applied.

[0089] In certain embodiments, the drug resistance results from or is related to Arv7 and/or AR mutant.

[0090] In certain embodiments, the first and/or the second compound is administered orally.

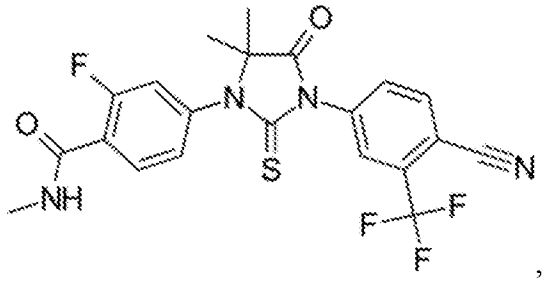
[0091] In certain embodiments, the first and/or the second compound is administered intravenously, intramuscularly, or subcutaneously.

[0092] In certain embodiments, the second compound is clorgyline.

[0093] In certain embodiments, the second compound is phenelzine.

[0094] In certain embodiments, the method further includes administering to the subject one or more other anti-cancer agents (e.g., a chemotherapeutic agent).

[0095] In yet another aspect, the invention generally relates to a method for treating prostate cancer, or a related disease or condition thereof. The method includes: administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, in an amount effective in the treatment of prostate cancer or a related disease or condition thereof in a mammal, including a human; monitoring the subject to detect a development of drug resistance to the first compound; upon the subject being detected of the development of drug resistance to the first compound, administering to the subject a second compound which is an inhibitor of monoamine oxidase A, in an amount effect to reduce or eliminate drug resistance to the first compound; and monitoring the subject to detect a level of drug resistance to the first compound.

[0096] In certain embodiments, the method further includes: upon the subject being detected of the development of drug resistance to the first compound, continuing to administer the subject the first compound.

[0097] In certain embodiments, the method further includes: upon the subject being detected of the development of drug resistance to the first compound, halting the administration of the first compound, and upon the subject being detected of a substantial reduction or disappearance of drug resistance, re-starting the administration of the first compound.

[0098] In certain embodiments, the drug resistance results from or is related to Arv7 and/or AR mutant.

[0099] In certain embodiments, the first and/or the second compound is administered orally.

[00100] In certain embodiments, the first and/or the second compound is administered intravenously, intramuscularly, or subcutaneously.

[00101] In certain embodiments, the second compound is clorgyline.

[00102] In certain embodiments, the second compound is phenelzine.

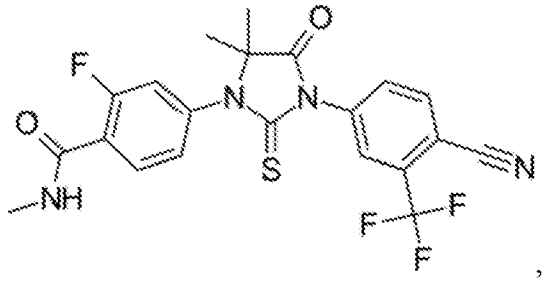
[00103] In certain embodiments, the method further includes administering to the subject one or more other anti-cancer agents (*e.g.*, a chemotherapeutic agent).

[00104] In yet another aspect, the invention generally relates to a method for treating a drug resistance in connection with a cancer treatment. The method includes: administering to a subject in need thereof a second compound which is an inhibitor of monoamine oxidase A, in an amount effective to prevent, delay, reduce or reverse resistance to a treatment of prostate cancer, or a related disease or condition thereof in a mammal, including a human.

[00105] In certain embodiments, the method is effective in delaying the onset of or delaying the development of drug resistance.

[00106] In certain embodiments, the method is effective in reducing or reversing drug resistance.

[00107] In certain embodiments, the resistance is to a compound having Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof.

[00108] The following examples are meant to be illustrative of the practice of the invention, and not limiting in any way.

Examples

[00109] It was unexpectedly found that the expression of MAOA was significantly increased in several EnzR CRPC cells, and higher MAOA expression was associated with positive ARv7 detection in CRPC patients following Enz treatment. Targeting MAOA with specific inhibitors, including clorgyline or phenelzine re-sensitized the EnzR cells to Enz treatment and further suppressed EnzR cell growth in the multiple *in vitro* EnzR CRPC cells and *in vivo* mouse models. Mechanism dissection indicates that Enz *via* increasing ARv7 expression can transcriptionally regulate the MAOA expression *via* binding to ARE on its 5' promoter region, and increased MAOA can then increase Enz-resistance *via* altering the hypoxia HIF-1α signals. Together, results disclosed herein showed that targeting MAOA with anti-depression phenelzine

or clorgyline can restore Enz-sensitivity to further suppress EnzR cell growth *via* altering the Enz/ARv7/MAOA signaling.

Increased MAOA expression after the development of Enz resistance

[00110] The antiandrogen Enz is clinically effective in treating CRPC patients. However, development of Enz-resistance is inevitable, emphasizing the need to further dissect the mechanisms of Enz-resistance in basic preclinical studies using *in vitro* cell lines and *in vivo* mouse models. (Antonarakis, *et al.* **2014** *The New England journal of medicine* 371, 1028-1038.)

[00111] We first searched for potential altered genes after the development of Enz-resistance in EnzR CRPC cells generated after chronic culture of CRPC C4-2 cells in media containing increasing Enz concentrations from 10 to 30 μM for 1 year (named as EnzR1-C4-2), or after continuously culturing the C4-2 cells in media containing fixed 10 μM Enz for 6 months (named as EnzR2-C4-2), We also used the naturally Enz-resistant cell line, CWR22Rv1, and named them as EnzR3-CWR22Rv1. Finally, we also obtained the EnzR C4-2B cells from Dr. Allen Gao and named as EnzR4-C4-2B in these studies.

[00112] We then applied RNAseq assay to compare the expression profiles in EnzR1-C4-2 cells and the C4-2 parental Enz-sensitive (EnzS1-C4-2) cells. Among many genes that were highly differentially expressed in EnzR1-C4-2 cells, MAOA expression was increased significantly in the EnzR1-C4-2 cells by more than 6 folds (**FIG. 1A**). We analyzed MAOA expression in 4 different human PCa datasets. The results revealed that higher expression of MAOA in human PCa tissues compared to normal prostate tissues (**FIG. 1B**), and higher MAOA expression in higher Gleason score tumors and recurrent PCa (**FIG. 1B, FIG. 7A**).

[00113] We further validated the RNAseq data, and found MAOA (mRNA and protein) expression were significantly increased in the EnzR1-C4-2 cells as compared to the EnzS1-C4-2 cells (**FIG. 1C**). In addition, MAOA activity was higher in EnzR1-C4-2 cells than EnzS1-C4-2 cells by MAO-GLO assay (**FIG. 1D**). Similar results were also obtained when we replaced another paired EnzR *vs* EnzS cell line (EnzR4-C4-2B vs EnzS4-C4-2B cells) (**FIG. 7B**).

[00114] Importantly, we found that treating the EnzS1-C4-2 cells with 10 μM Enz for 6 days could increase both MAOA and ARv7 mRNA expressions (**FIG. 1E**), suggesting that Enz may increase MAOA expression by activating key transcription factors, such as ARv7. Similar results

were obtained when we treated EnzS5-VCaP cells with 10uM for 10 days (**FIG. 7C**). Results from MAO-GLO assay also confirmed that adding 10 μM Enz led to increased MAOA activity in EnzS1-C4-2 cells **(FIG. 1F)**.

[00115] Therefore, similar to an increase of ARv7 expression in cells treated with Enz, an increase in MAOA expression in Enz-treated cells may also play a role in mediating the Enz-resistance.

Elevated MAOA expression detected in circulating tumor cells (CTCs) from mCRPC patients treated with Enz

[00116] To further corroborate the *in vitro* cell lines data, we evaluated MAOA expression in a total of 288 blood-CTCssamples collected from men with mCRPC undergoing treatment with standard-of-care systemic therapies. These 288 blood samples were processed for CTCs and then cDNA andfinally were divided into three biomarker groups: 1. CTC negative (CTC-) (n=90); 2. CTC positive (CTC+)/ARV7- (n=127); and 3. CTC+/ARv7+ (n=71). We found significantly increased MAOAmRNAin ARv7+ CTCs compared to ARv7- CTCs (**FIG. 1G**), suggesting a positive correlation between ARv7and MAOA expression.

[00117] Subseqeunt analysis focused on patients treated with Enz with paired samples collected at treatment baseline and at disease progression. Among the 288 samples, there were 30 pairs of samples (n=60) from those that were negative for ARv7 at baseline and remained negative at the time of progression on Enz (ARv7- to ARv7- group), 13 pairs of samples (n=26) from those with a conversion from ARv7- status to ARv7+ status (ARv7- to ARv7+ group), and 12 pairs of samples (n=24) from those who were positive for ARv7 at baseline and remained positive at the time of progression (ARv7+ to ARv7+ group). In those CTCs that remained ARv7- after Enz treatment, MAOA expression showed little difference (**FIG. 1H**). In contrast, in those CRPC patients whose ARv7 expression changed from negative to positive after Enz treatment, the MAOA expression levels increased dramatically, suggesting that MAOA expression may only increase in those ARv7+ patients (**FIG. 1I-J**). In CRPC patients with ARv7+ at baseline and remained positive after Enz treatment, there was no further increase of MAOA expression after Enz treatment, MAOA expression trended higher after treatment with Enz, but the difference was not significant (**FIG. 1K**). This is consistent with lack of further

ARv7 increase after Enz treatment if ARv7 was detected at the baseline. (Antonarakis, *et al.* **2014** *The New England journal of medicine* 371, 1028-1038.) We did not find statistically significant differences for the full-length AR (AR-FL) comparing pre- and post-treatment samples in any of the paired groups (**FIG. 8A-C**), suggesting that elevated MAOA expression following treatment with Enz may be mediated by the ARv7.

[00118] Together, results from human CRPC patients' CTC analysis (**FIG. 1G-K**) demonstrate that MAOA expression is positively associated with ARv7 expression in CRPC patients treated with Enz. Together with our *in vitro* cell lines data (**FIG. 1C-D**), these human clinical findings suggest that Enz can increase both ARv7 and MAOA expression, and Enz-induced MAOA expression may depend on the ARv7 status.

Clorgyline or Phenelzine, the selective inhibitor of MAOA, can restore Enz-sensitivity to further suppress EnzR cell growth

[00119] To further link the increased MAOA expression to the development of Enz-resistance in CRPC cells, we treated the CRPC cells with a selective MAOA inhibitor, Clorgyline, used as anti-depression drug. Results from MTT proliferation assay revealed that while Clorgyline treatment alone failed to suppress EnzS1-C4-2cell proliferation, the combination of Clorgyline and Enz treatment significantly inhibited (85%) EnzS1-C4-2cell growth at day 6 (**FIG. 2A**). Importantly, EnzR1-C4-2 cell proliferation was also suppressed by adding Clorgyline to Enz treatment (49% suppression), suggesting restoration of Enz sensitivity (**FIG. 2B**). Similar results were obtained in EnzR2-C4-2 cells (56% suppression) (**FIG. 2C**) or EnzR3-CWR22RV1(43% suppression) cells (**FIG. 2D**).

[00120] We repeated the same set of experiments with the FDA-approved MAOA inhibitor, Phenelzine (10uM) used to treat depression[19], and results also revealed that suppressing the MAOA activity could re-sensitize EnzR1-C4-2 (51% suppression), EnzR2-C4-2 (46%suppression) and EnzR3-CWR22RV1 cells (48%suppression) to Enz treatment (**FIG. 2E-G**).

[00121] We then applied the gene expression perturbation approach using MAOA-shRNA to suppress MAOA, and results revealed that targeting MAOA with MAOA-shRNA also significantly increased Enz sensitivity and suppressed EnzR cells proliferation (**FIG. 2H-J**), with

suppression rates of 42% in EnzR1-C4-2, 35% in EnzR2-C4-2, and 28% in EnzR3-CWR22Rv1cells. In contrast, overexpressing MAOA in EnzS1-C4-2 cells resulted in Enz-resistance (**FIG. 2K**).

[00122] Together, results from **FIG. 2A-K** using multiple EnzR CRPC cells with various inhibitors/blockers to either suppress MAOA activity or suppress MAOA expression all demonstrate that Enz-induced MAOA expression contributed to the development of Enz-resistance, and suppressing the MAOA activity with anti-depression drugs Clorgyline or Phenelzine (or MAOA-shRNA to suppress its expression) all lead to restore Enz-sensitivity to further suppress EnzR cell proliferation.

Clorgyline delays the development of Enz resistance in CRPC cells

[00123] In addition to suppressing the EnzR cell growth, we are interested to see if Clorgyline can also delay the development of Enz-resistance in CRPC cells. We first treated EnzS1-C4-2 cells with10 μM Enz alone or combined 10 μM Enz with 1 μM or 2.5 μM Clorgyline for 1.5 months. We then treated the cells with Enz and evaluated cell proliferation by MTT assays on days 0, 2, 4, and 6. The results revealed that while1.5 months of Enz treatment alone could induce the development of Enz-resistance by reducing Enz sensitivity from 54% to 24%.(**FIG. 2L *vs* FIG. 2M**), the combination of Enz and Clorgyline delayed the development of Enz-resistance by increasing Enz sensitivity from 54%→24% to 54%→33% (at 1μM Clorgyline, **FIG. 2M *vs* FIG. 2N**) or from 54%→24% to 54%→43% (at 2.5μM Clorgyline, **FIG. 2M *vs* FIG. 2O**).

[00124] Together, these results (**FIG. 2L-O**) suggest that targeting MAOA with the inhibitor Clorgyline can also delay the development of Enz-resistance.

Mechanism dissection of how Enz can induce MAOA expression: via increasing ARv7 expression

[00125] To dissect the molecular mechanism underlying Enz-induced MAOA expression, we focused on the ARv7. We first examined the effect of MAOA inhibition in AR-negative PC-3 cells, and found that adding Clorgyline alone or Clorgyline and Enz resulted in little effects on PC-3 cell proliferation (**FIG. 3A)** at 5 μM Clorgyline (**FIG. 3B)** and 10 μM Clorgyline, suggesting that Enz-increased MAOA expression and effect of MAOA inhibition is rather

specific to AR-positive cells and may depend on AR (or ARv7) signals. As expected, adding Enz to EnzS1-C4-2 cells led to increase the expression of ARv7 and MAOA at both mRNA and protein levels (**FIG. 3C**), In addition, adding ARv7-cDNA (OE-ARv7) also increased MAOA mRNA expression in EnzR1-C4-2 cells **(FIG. 3D,** at mRNA level**)** and EnzS1-C4-2 cells (**FIG. 3E,** at both mRNA and protein levels), while suppressing ARv7 *via* adding ARv7-shRNA led to decrease MAOA mRNA level in multiple EnzR cells, including EnzR1-C4-2, EnzR2-C4-2 and EnzR3-CWR22Rv1 cells (**FIG. 3F-H**, respectively), as well as a decrease in MAOA protein expression in the EnzR1-C4-2 and EnzR3-CWR22Rv1 cells **(FIG. 3F-H)**. To explore whether ARv7 induction by Enz is essential for the MAOA increase, we treated EnzS1-C4-2-pLKO and EnzS1-C4-2-shARv7 cells w/o Enz and then detected the ARv7 and MAOA level. As shown in **FIG. 3I**, only in EnzS1-C4-2-pLKO cells, the ARv7 and MAOA level can be induced by Enz treatment, however, the ARv7 and MAOA expression cannot be induced by Enz in EnzS1-C4-2-shARv7 cells. To identify whether MAOA is the key downstream gene of ARv7 to confer EnzR, we manipulated ARv7 and MAOA expression in the EnzS1-C4-2 cells. As shown in the **FIG. 3J**, OE-ARv7 can decrease the Enz sensitivity, however, knockdown MAOA can reverse the ARv7 effects on Enz sensitivity, suggesting that ARv7 decrease Enz sensitivity dependent on MAOA.

[00126] Finally, to test whether clorgyline can also suppress the expression of MAOA (and ARv7), in addition to suppress the MAOA activity, we found that adding clorgyline failed to impact significantly the expression of MAOA (and ARv7) in the EnzR1 cells during 3 days treatment (**FIG. 9B**), suggesting that clorgyline's effect on the altering Enz sensitivity could be mainly from suppressing the MAOA activity.

[00127] Together, results from **FIG. 3A-J** suggest that Enz may function *via* increasing ARv7 to increase MAOA expression during the development of Enz-resistance.

Mechanism dissection of how Enz-increased ARv7 can increase the MAOA expression: via transcriptional regulation

[00128] Next, to dissect the mechanism of how Enz-increased ARv7 can increase the MAOA expression at the molecular level, we searched for the ARv7 response elements (AREs) on the 3kb MAOA promoter region, and found a putative ARE on the 5' promoter region (**FIG. 4A**).

We first checked ARv7 binding on this ARE and ChIP assay showed strong binding signal of Flag-ARv7, but not endogenous ARfl (**FIG. 4B**). To better compare the binding ability of ARv7 and ARfl, we then overexpressed Flag-flAR and Flag-ARv7 in EnzR1-C4-2 cells (which were maintained in 10uM Enz) and used Flag antibody to pull-down flAR and ARv7, suggesting that ARv7 has much stronger activity to bind *in vivo* to this ARE on the MAOA promoter region as compared to flAR (**FIG. 4C**). Such result is consistent with the previous reports that in EnzR cells, flAR activity is suppressed significantly.

[00129] To confirm the results of ChIP assay, we checked AR and ARv7 binding on 5kb MAOA promoter region through ChIP-seq database (GEO: GSE106559). The results showed that in the presence of DHT, AR and ARv7 can bind to MAOA promoter in different areas (**FIG. 5A**). However, without DHT, AR lost its ability to bind to MAOA promoter, yet ARv7 still showed very strong binding signal (**FIG. 11B**). Interestingly, according to ChIP-seq data, although the strongest signal of ARv7 binding is on 4.5kb upstream of MAOA coding region, the ARv7 can still bind to the predicted ARE area in the absence of DHT. This data is consistent with our **FIG. 4B-C** showing ARv7 has stronger activity to bind to MAOA promoter.

[00130] Last, we also quantitated the enrichment of AR or ARv7 binding on MAOA promoter region. As shown in **FIG. 11C**, ARv7 binding in both conditions (w/o DHT) is stronger than AR.

[00131] Together, results from multiple approaches all confirmed that ARv7 has stronger binding ability than AR.

[00132] Results from luciferase assay *via* constructing the 3Kb MAOA promoter containing this ARE (or mutant ARE) into PGL3 reporter plasmid also confirmed that treating with Enz to increase ARv7 expression or direct adding ARv7-cDNA (OE-ARv7) could increase the MAOA expression at the transcriptional level with wild-type ARE in both EnzS1-C4-2 and PC3 cells (**FIG. 4D-E**), and not with mutant ARE (see sequences in **FIG. 4A**) in the PC3 cells **(FIG. 4F).**

[00133] To further confirm ARv7, and not flAR, has better effects to increase MAOA expression, we overexpressed flAR and ARv7 in the EnzS1-C4-2 cells (cultured in 10%FBS RPMI which has 3nM DHT), and then assayed the MAOA promoter activity. As shown in **FIG. 4G**, only ARv7, and not flAR, could enhance MAOA promoter activity, suggesting that ARv7 had stronger ability to promote the MAOA transcription.

[00134] To further confirm this data, we also assayed the MAOA expression in EnzS1-AR and EnzS1-ARv7 cells. As shown in **FIG. 4H**, both flAR and ARv7 can increase MAOA expression, however, ARv7 showed much better effect compared to flAR.

[00135] Together, results from multiple assays including qPCR, western blot, ChIP-on *in vivo* binding assay, and luciferase reporter assay (**FIG. 4A-H**) all demonstrate that Enz-increased ARv7 expression may lead to increase MAOA expression by transcriptional regulation *via* direct binding to the ARE on the MAOA 5' promoter region.

Mechanism dissection of how Enz-increased ARv7 expression can lead to increase the MAOA expression: via protein stability regulation

[00136] In addition to the transcriptional regulation, we also investigated whether Enz-increased ARv7 can lead to increase the MAOA expression *via* protein stability. By treating Enz-S1-C4-2 and Enz-R1-C4-2 cells with the protein synthesis inhibitor cycloheximide (CHX), we found the MAOA protein degradation rate is much lower in EnzR1-C4-2 cells than that in the EnzS1-C4-2 cells (**FIG. 4I**), suggesting that MAOA protein is more stable in EnzR1-C4-2 cells than in EnzS1-C4-2 cells. In contrast, we found that the MAOA protein expression in EnzS1-C4-2 and EnzR1-C4-2 cells was similar after adding the proteasome inhibitor, MG132, suggesting that the degradation of MAOA protein by the proteasome system is suppressed in EnzR1-C4-2 cells (**FIG. 4J**).

[00137] To further dissect the mechanism why the MAOA protein stability is higher in Enz-R1-C4-2 cells, we compared the phosphorylation of MAOA in EnzS1-C4-2 and EnzR1-C4-2 cells, since early reports suggested that the phosphorylation could influence protein stability[30,31]. The results from immunoprecipitation of MAOA from EnzS1-C4-2 and EnzR1-C4-2 cells revealed that serine phosphorylation (p-Ser) significantly increased in EnzR1-C4-2 cells (**FIG. 4K**).

[00138] We also assayed the p38 effects since it might also phosphorylate MAOA to alter the MAOA protein stability, and results revealed that the phosphorylation of p38 (p-p38) significantly increased in EnzR1-C4-2 cells (**FIG. 4L**), and adding the p38 inhibitor could only reduce MAOA expression and p-MAOA level in EnzR1-C4-2 cells but not in EnzS1-C4-2 cells and (**FIG. 4M&N**), suggesting that p38 activity was enhanced in EnzR1-C4-2 cells and

indicating that MAOA was phosphorylated by higher p38 activity to increase the protein stability.

[00139] Taken together, results from **FIG. 4I-N** suggest that in addition to increase MAOA expression *via* transcriptional regulation, Enz can also increase MAOA expression *via* increasing its protein stability.

Mechanism dissection of how targeting the Enz/ARv7/MAOA signaling can increase the Enz-sensitivity to further suppress the EnzR cell growth

[00140] Next, to dissect the mechanisms of how targeting the Enz/ARv7/MAOA signaling can overcome the Enz-resistance in multiple EnzR cells, we focused on hypoxia signals since recent studies indicated MAOA might exert its biological functions *via* altering the key hypoxia signals[24]. We first examined the MAOA effects on the hypoxia signals with the HIF-1 and its downstream target genes including the Glut1, N-Cadherin, Timp and VEGF-A. The results revealed higher expression of these hypoxia downstream genes in EnzR1-C4-2 cells than those in EnzS1-C4-2 cells **(FIG. 5A).** Suppressing MAOA with MAOA-shRNA (**FIG. 10A**) also led to reduce the expression of HIF-1 and its target genes expression at mRNA (**FIG. 5B, left**) and the protein levels of HIF-1 and VEGF-A (**FIG. 5B, right**) in both EnzS1-C4-2 cells and EnzR1-C4-2 cells**.** Suppressing HIF-1α with HIF-1 -shRNA also reduced its target gene VEGF-A expression at mRNA and protein levels in EnzR1-C4-2cells. **(FIG. 4B).**

[00141] In contrast, treating with MAOA inhibitor Clorgyline decreased these hypoxia-related genes at mRNA and protein levels in EnzR1-C4-2 cells **(FIG. 5C).**

[00142] Using an opposite approach *via* increasing MAOA *via* adding with MAOA-cDNA in EnzS1-C4-2 cells also resulted in increasing the target genes HIF-1 and VEGF-A expression at both mRNA and protein levels **(FIG. 5D)**. More importantly, our results showed that when knocking down HIF-1α led to increase the Enz-sensitivity in EnzR1-C4-2 cells (**FIG. 5E**), which suggested that MAOA can promote EnzR via activating HIF1a signaling.

[00143] Together, results from **FIG. 5A-E** suggest that Enz/ARv7/MAOA axis can overcome the Enz-resistance *via* altering the hypoxia signals in EnzR1-C4-2 and EnzS1-C4-2cells.

Preclinical studies using in vivo mouse models to prove that targeting Enz/ARv7/MAOA signaling with Clorgyline and Phenelzine led to further suppress EnzR tumor growth.

[00144] To establish a preclinical proof-of-principle in the *in vivo* mouse model, we first established the *in vivo* PDX-PCa mouse model in 6 mice, and results revealed that mice received Enz (30 mg/kg/every other day) had an increase in MAOA, ARv7 and p-p38 level in the xenografted PDX tumors (**FIG. 6A**).

[00145] We then established the 2nd *in vivo* mouse model by implanted EnzR3-22Rv1 cells in the nude mice for the purpose of examining the effects of Clorgyline and Phenelzine on EnzR tumors. To make sure all data are repeatable and reach the statistical significance, we performed two sets of experiments, with implanted EnzR3-22RV1-Luc cells (cells which express luciferase plasmid) in the 1st-set and implanted EnzR3-22RV1 cells in the 2nd-set into nude mice. Once the tumor formation was detectable (after 4 weeks), the mice were treated in groups as follows, 1) vehicle, 2) Enz(10mg/kg), 3) clorgyline (10mg/kg), 4) phenelzine(30mg/kg), 5) Enz + clorgyline, and 6) Enz + phenelzine, and i.p. injected every two days for 4 weeks. For EnzR3-22RV1-luc cells, we used *in vivo* imaging system (IVIS) to monitor the tumor sizes weekly. After 4 weeks injections, we sacrificed the mice (4 mice/group in the 1st-set and 10 mice/group in the 2nd-set) 2 days after the final treatment, and then measured the tumor weights.

[00146] The results revealed that adding Enz alone has little effect on the EnzR3-22Rv1 (**FIG. 6B**) or EnzR3-22Rv1-luc (**FIG. 12A-B**) growth. In contrast, combining Enz (10mg/kg every two days for 4 weeks) with Clorgyline (10mg/kg every two days for 4 weeks) or Phenelzine (30mg/kg every two days for 4 weeks) significantly suppressed EnzR3-22Rv1(**FIG. 6B**) or EnzR3-22Rv1-luc (**FIG. 12A-B**) tumors growth. Tumor volumes and weight after sacrifice also confirmed that combining Enz and Clorgyline or Phenelzine can suppress EnzR3-22Rv1 and EnzR3-22Rv1-luc tumors progression in mice compared with Enz alone and control vehicle group (**FIG. 6C** & **FIG. 12C**).

[00147] Finally, to further confirm the above two *in vivo* mouse models (PDX and EnzR3-22Rv1), we also established the 3rd *in vivo* mouse model with implanted another EnzR cells (EnzR1-C4-2) cells into B-NDG mice and repeated the treatments on EnzR3-22Rv1 tumors. As expected, we obtained the similar results showing Clorgyline and Phenelzine can also reverse the EnzR1-C4-2 resistance. (**FIG. 6D-E**)

[00148] We also perform the IHC staining on EnzR3-22Rv1, EnzR3-22Rv1-luc and EnzR1-C4-2 tumors to assay the proliferation markers Ki-67 and VEGF-A, and results showed that in all three EnzR tumors, the expression of these proliferation proteins were significantly inhibited by the combination treatment (**FIG. 6F-G** & **FIG. 12D**).

[00149] Together, results from **FIG. 6A-G** & **FIG. 12A-D** using three preclinical *in vivo* mouse models all conclude that combining Enz with the MAOA inhibitors Clorgyline or Phenelzine can overcome Enz-resistance to further suppress the growth of EnzR tumors in the well-established EnzR *in vivo* models.

Experimental

Cell culture and reagents

[00150] CWR-22RV1, VCaP and PC-3 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in RPMI 1640 with 10% FBS. HEK293T cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM with 10% FBS. The EnzS1-C4-2 cell line was a gift from Dr. Leland W.K Chung from Cedars-Cinai. EnzS4-C4-2B and EnzR4-C4-2B cell lines were gifts from Dr Allen Gao from UC Davis. C4-2 EnzR cell lines were generated *via* chronic culture of CRPC C4-2 cells in media containing increasing Enz (from 10 μM to 30 μM), with the increased concentration added when cells were no longer sensitive (EnzR1_C4-2) or continuous culture with 10 μM Enz for 6 months (EnzR2_C4-2). All cells were maintained in a humidified 5% CO_2 environment at 37°C.All cell lines have been authenticated by ATCC and periodically re-authenticated by PCR and determined to be mycoplasma and bacteria free following ATCC's instructions. The cycloheximide, p38 map kinase inhibitor IV and clorgyine were purchased from Sigma-Aldrich (St. Louis, MO). Enzalutamide was purchased from MedChemExpress(South Brunswick, New Jersey).

Cell Proliferation Assays

[00151] We plated $1x10^4$ C4-2, PC3 and C4-2 EnzR cells into each well of 24-wellplates for MTT assays on days 0, 2, 4, 6 for the PCa cells and on days 0,1,2,3 for the EnzS1_C4-2 and

EnzR1_C4-2. After the various time points, the MTT assay was performed by adding 100 µl of 5 mg/ml MTT to each well. We included one set of wells with MTT, but no cells (control), then incubated for 3 hrs at 37 °C, removed media and added 150 µl DMSO, covered the plates with foil, agitated the cells on an orbital shaker for 15 min, and then read the absorbance at 570 nm.

Lentivirus packaging and cell transfection

[00152] The sh-ARv7 was constructed into the pLKO.1 lentiviral vector as reported previously. The pLKO.1 shMAOA, together with package and envelope plasmids, psPAX2 and pMD2G, were co-transfected into 293T cells for 48hrs to produce the MAOA shRNA lentivirus particle soup, which was then collected and frozen at -80°C for later use in transduction of PCa cells.

RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

[00153] Total RNAs were isolated using Trizol reagent (Invitrogen, Grand Island, NY). One µg of total RNA was subjected to reverse transcription using Superscript III transcriptase (Invitrogen). RT-PCR was conducted using a Bio-Rad CFX96 system with SYBR green to determine the mRNA expression level of a gene of interest. Expression levels were normalized to GAPDH level.

RNAseq

[00154] The EnzS_C4-2 and EnzR1_C4-2 cells were prepared for RNA-seq. For cDNA library construction and sequencing, mRNA was first isolated from total RNA treated with DNase I using Magnetic Oligo (dT) Beads and was fragmented. Then, the double-stranded cDNA was synthesized with random hexamer primers and was further subjected to end-repair and adapter ligation using T4 DNA ligase. The products of ligation reaction were purified on 2% agarose gel and cDNA fragments (about 200 bp) were recovered. PCR was carried out to enrich the purified cDNA template. Finally, the cDNA library was constructed. After validating on Quit and Bioanalyzer, the library was sequenced using Illumina HiSeq 2500 according to the manufacturer's instruction.

Western blot analysis

[00155] Cells were lysed in RIPA buffer and proteins (20–40 μg) were separated on 8–10% SDS/PAGE gel and then transferred onto PVDF membranes (Millipore, Billerica, MA). After blocking membranes, they were incubated with primary antibodies, then HRP-conjugated secondary antibodies, and visualized using ECL system (Thermo Fisher Scientific, Rochester, NY). The MAOA, GAPDH, tubulin, VEGF-A, Ki67and HIF-1 antibodies were from Santa Cruz Biotechnology, Inc (Santa Cruz). The ARv7 antibody was purchased from Precision Antibody (Columbia, MD). P-P38 antibody was purchased from Cell signaling Technology (Danvers, MA).

MAOA activity assay

[00156] MAO-Glo assay systems were purchased from Promega (Madison, WI). The cells were lysed by luciferase lysis buffer, and the cell lysates were applied to analyze the MAOA activity by the MAO-Glo kit.

Chromatin Immunoprecipitation Assay (ChIP)

[00157] Cells were cross-linked with 4% formaldehyde for 10 minutes followed by cell collection and sonication with a predetermined power to yield genomic DNA fragments of 300-1000 bp long. Lysates were precleared sequentially with normal rabbit IgG (sc-2027, Santa Cruz Biotechnology) and protein A-agarose. Anti-ARv7 antibody (2.0 μg) was added to the cell lysates and incubated at 4°C overnight. For the negative control, IgG was used in the reaction. Specific primer sets were designed to amplify a target sequence within human MAOA promoter and PCR products were analyzed by agarose gel electrophoresis.

Luciferase assay

[00158] PC3-Pwpi and PC3-oeARv7 cells were plated in 24-well plates and co-transfected with PGL3-MAOA-promotercontaining the WT-ARE or mutant ARE and pRL-TK, which is used as internal control using Lipofectamine (Invitrogen). After 48hrs transfection, . Luciferase activity was measured by Dual-Luciferase Assay (Promega, Madison, WI) according to the

manufacturer's manual. The EnzS1 cells were transfected with PGL3-MAOA-promoter containing the WT-ARE and pRL-TK using Lipofectamine. After 12 hrs of transfection, the cells were treated with 10uM Enz for 1, 2 and 4 days and then the luciferase activity was measured.

In vivo mouse model PDX implantation and different compound treatments

[00159] The PCa-133 PDX samples are the gifts from Dr. Sankar N. Maity from MD Andersen Cancer center, who generated it from CRPC patients as described before[50]. The PCa-133 samples were implanted into 6 SCID mice subcutaneously as described previously[51]. After average tumor's volumes reached 200mm3, we i.p injected two mice with DMSO and 5 mice with Enz (30mg/kg) every other day. After 10 injections, the mice were sacrificed and the tumors were collected for WB. All the mice were purchased from NCI. All experiments were conducted after approval from University of Rochester medical center and follow the regulations of University Committee on Animal Resources (UCAR).

In vivo tumorigenesis assay

[00160] EnzR3-22Rv1, EnzR3-22Rv1-luc and EnzR1-C4-2 ($1x10^6$) were mixed with Matrigel (1:1) and injected into the prostates of 6- to 7-week old male nude mice (EnzR3-22Rv1-luc and EnzR3-22Rv1) or B-NDG mice (EnzR1-C4-2). Tumor-bearing mice were randomized into four groups and treated by i.p. every other day for 4 weeks as follows: 1) vehicle, 2) Enz (10 mg/kg), 3) clorgyline (10 mg/kg), 4) phenelzine (30 mg/kg), 5) Enz+clorgyline, and 6) Enz+phenelzine. For EnzR3-22Rv1-luc tumors, IVIS was used weekly to monitor tumor growth. We imaged the mice a final time 2 days after the final treatment, sacrificed the mice, and monitored tumor growth with the IVIS (EnzR3-22Rv1-luc) as well as tumor sizes and tumor weights.

Analysis of circulating tumor cells (CTC)

[00161] CTC samples used for MAOA expression analysis were excess "left-over" cDNA samples from an ongoing prospective blood-based CTC ARv7 study in men with metastatic CRPC. Patient enrollment, blood collection, processing, CTC isolation, cDNA prepartion, and ARv7 detection procedures were described previosuly (Antonarakis ES, Lu C, Luber B, et al. Clinical Significance of Androgen Receptor Splice Variant-7 mRNA Detection in Circulating

Tumor Cells of Men With Metastatic Castration-Resistant Prostate Cancer Treated With First- and Second-Line Abiraterone and Enzalutamide. This study was approved by the Johns Hopkins University institutional review board, and patients provided written informed consent. Selection for left-over CTC samples for MAOA expression analysis involved a retrospective review of available cDNA samples prepared from CTCs and stored at -80ºC. A total of 288 frozen cDNA samples were selected on the basis of availability of adequate cDNA for analysis, and processed for quantitative PCR analysis on CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). PCR cycles at 95°C x 5min, 40 cycles of 95°C x 10s, 58°C x 30s, and 72°C x 30s were followed by melting curve analysis. Primer sequences used for MAOA were 5'-AATTCAGCGGCTTCCAATGG-3'(forward) and 5'-CAAGTCGATCAGCTTTCCGG-3'(reverse); Primer sequences used for RPL13A were 5'-CCTGGAGGAGAAGAGGAAAGAGA-3' (forward) and 5'-TTGAGGACCTCTGTGTATTTGTCAA-3' (reverse). resistance. Patient treatment status and sample collection timepoints were unblinded after laboratory data was generated. Among the 288 cDNA samples, 90 were CTC negative (CTC-), 127 were CTC positive (CTC+) but ARv7 negative (ARv7-), and 71 were CTC+ and ARv7 positive (ARv7+). MAOA expression data was normalized to the control gene (RPL13A), and normalized data presnted for each biomaker group according to CTC and ARv7 status, as well as each pre- and post-treatment pairs. Difference of MAOA copy numbers according to CTC and ARv7 status were compared using two-tailed unpaired t test. Changes of MAOA expression before and after Enz treatment were compared by two-tailed paired t test. P-values of 0.05 or less were considered statistically significant. All statistical tests were performed by GraphPad Prism version 7.02 (GraphPad software, San Diego, CA).

[00162] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[00163] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

Incorporation by Reference

[00164] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

Equivalents

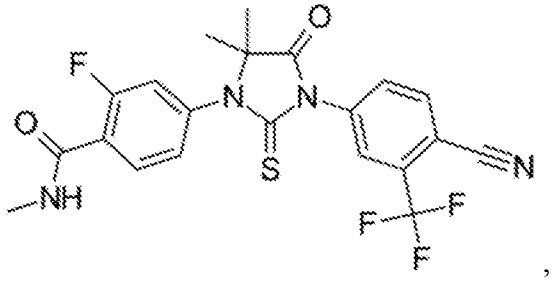
[00165] The representative examples disclosed herein are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. The examples herein contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is claimed is:

CLAIMS

1. A pharmaceutical composition comprising:

a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and

a second compound which is an inhibitor of monoamine oxidase A,

wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in the treatment of prostate cancer, or a related disease or condition thereof, in a mammal, including a human, and a pharmaceutically acceptable carrier.

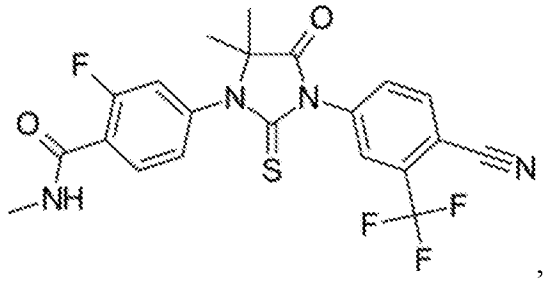
2. The pharmaceutical composition of claim 1, wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in delaying the onset of drug resistance.

3. The pharmaceutical composition of claim 1, wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in delaying the development of drug resistance.

4. The pharmaceutical composition of claim 1, wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reducing drug resistance.

5. The pharmaceutical composition of claim 1, wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reversing drug resistance.

6. The pharmaceutical composition of any of claims 1-5, wherein the weight ratio of the first compound to the second compound is from about 10 : 1 to about 1 : 10.
7. The pharmaceutical composition of any of claims 1-6, being suitable for one or more of oral administration, intravenous, intramuscular, and subcutaneous administration.
8. The pharmaceutical composition of any of claims 1-7, wherein the second compound is clorgyline.
9. The pharmaceutical composition of any of claims 1-7, wherein the second compound is phenelzine.
10. A unit dosage form comprising the pharmaceutical composition of any of claims 1-9.
11. The unit dosage form of claim 10, being in the form of a tablet or capsule suitable for oral administration, or in the form of a liquid solution or suspension suitable for intravenous, intramuscular, or subcutaneous administration.
12. A method for treating prostate cancer, or a related disease or condition thereof, comprising administering to a subject in need thereof the pharmaceutical composition of any of claims 1-11.
13. The method of claim 12, further comprising administering to the subject one or more other anti-cancer agents.
14. The method of claim 13, wherein the one or more other anti-cancer agents comprise a chemotherapeutic agent.
15. The method of any of claims 12-14, wherein the prostate cancer is castration resistant prostate cancer.
16. A method for treating prostate cancer, or a related disease or condition thereof, comprising administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of monoamine oxidase A, in amounts effective in the treatment of

prostate cancer, or a related disease or condition thereof, in a mammal, including a human, wherein the subject has been detected of the development of drug resistance to the first compound.

17. The method of claim 16, wherein the second compound is administered simultaneously with the first compound.

18. The method of claim 16, wherein the second compound is administered after the administration of the first compound.

19. The method of any of claims 16-18, wherein the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.

20. The method of any of claims 16-19, wherein the weight ratio of the first compound to the second compound is from about 10 : 1 to about 1 : 10.

21. The method of any of claims 16-20, wherein the second compound is clorgyline.

22. The method of any of claims 16-20, wherein the second compound is phenelzine.

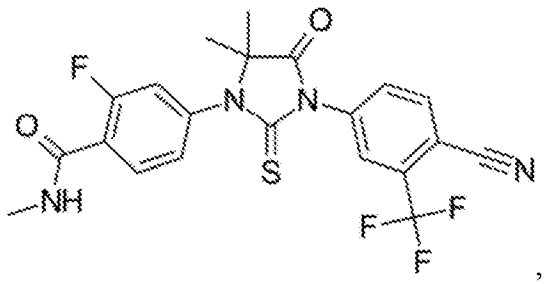
23. The method of any of claims 16-22, further comprising administering to the subject one or more other anti-cancer agents.

24. The method of claim 23, wherein the one or more other anti-cancer agents comprise a chemotherapeutic agent.

25. The method of any of claims 16-24, wherein the prostate cancer is castration resistant prostate cancer.

26. A method for treating prostate cancer, or a related disease or condition thereof, comprising:

administering to a subject in need thereof a first compound of Formula (I)



,

(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, in an amount effective in the treatment of prostate cancer or a related disease or condition thereof in a mammal, including a human;

monitoring the subject to detect a development of drug resistance to the first compound;

upon the subject being detected of the development of drug resistance to the first compound, administering to the subject a second compound which is an inhibitor of monoamine oxidase A, in an amount effect to reduce or eliminate drug resistance to the first compound; and

monitoring the subject to detect a level of drug resistance to the first compound.

27. The method of claim 26, further comprising:

upon the subject being detected of the development of drug resistance to the first compound, continuing to administer the subject the first compound.

28. The method of claim 26, further comprising:

upon the subject being detected of the development of drug resistance to the first compound, halting the administration of the first compound, and

upon the subject being detected of a substantial reduction or disappearance of drug resistance, re-starting the administration of the first compound.

29. The method of any of claims 26-28, wherein the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.

30. The method of any of claims 26-29, wherein the drug resistance is related to Arv7 and/or AR mutant and detecting a development of drug resistance to the first compound is by measuring ARv7.

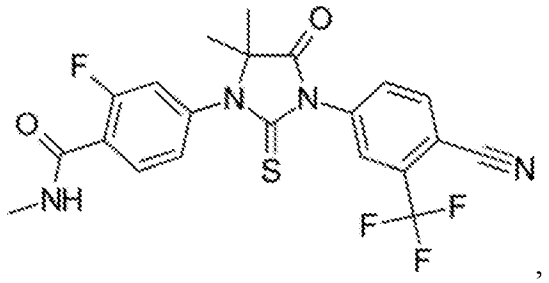
31. The method of any of claims 26-30, wherein the prostate cancer is castration resistant prostate cancer.

32. The method of any of claims 26-31, wherein the second compound is clorgyline or phenelzine.

33. A method for treating a drug resistance in connection with a prostate cancer treatment, comprising administering to a subject in need thereof a compound which is an inhibitor of monoamine oxidase A, in an amount effective to prevent, delay, reduce or reverse resistance to a treatment of prostate cancer, or a related disease or condition thereof in a mammal, including a human.

34. The method of claim 33, wherein the drug resistance is related to Arv7 and/or AR mutant.

35. The method of claim 33 or 34, wherein the resistance is to a compound having Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof.

36. The method of any of claims 33-35, wherein the inhibitor of monoamine oxidase A is clorgyline.

37. The method of any of claims 33-35, wherein the inhibitor of monoamine oxidase A is phenelzine.

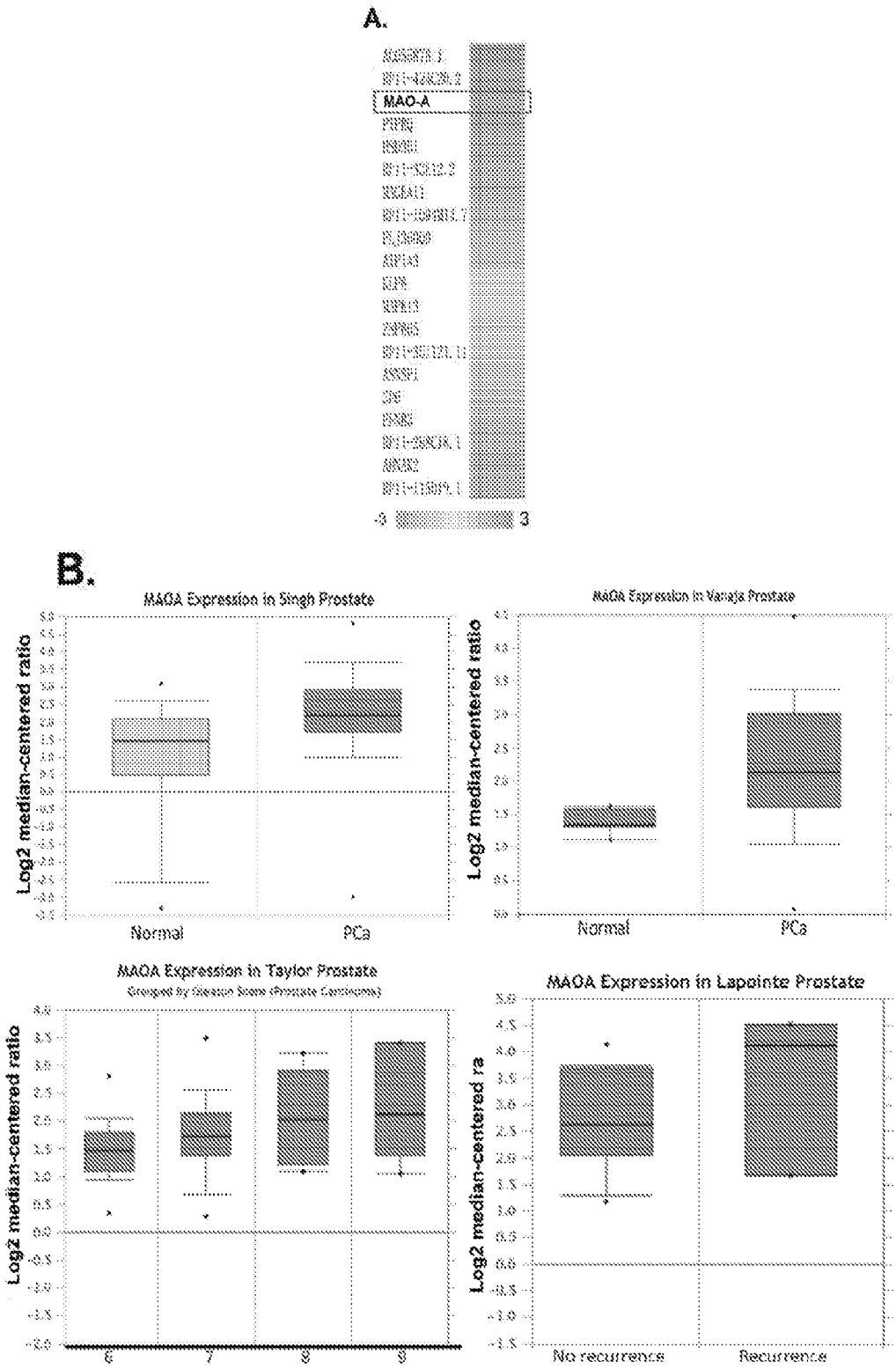

A.
MAO-A
-3
3
B.
MAOA Expression in Singh Prostate
Log2 median-centered ratio
Normal
PCa
MAOA Expression in Varaja Prostate
Log2 median-centered ratio
Normal
PCa
MAOA Expression in Taylor Prostate
Grouped by Gleason Score (Prostate Carcinoma)
Log2 median-centered ratio
6
7
8
9
MAOA Expression in Lapointe Prostate
Log2 median-centered ra
No recurrence
Recurrence

FIG. 1

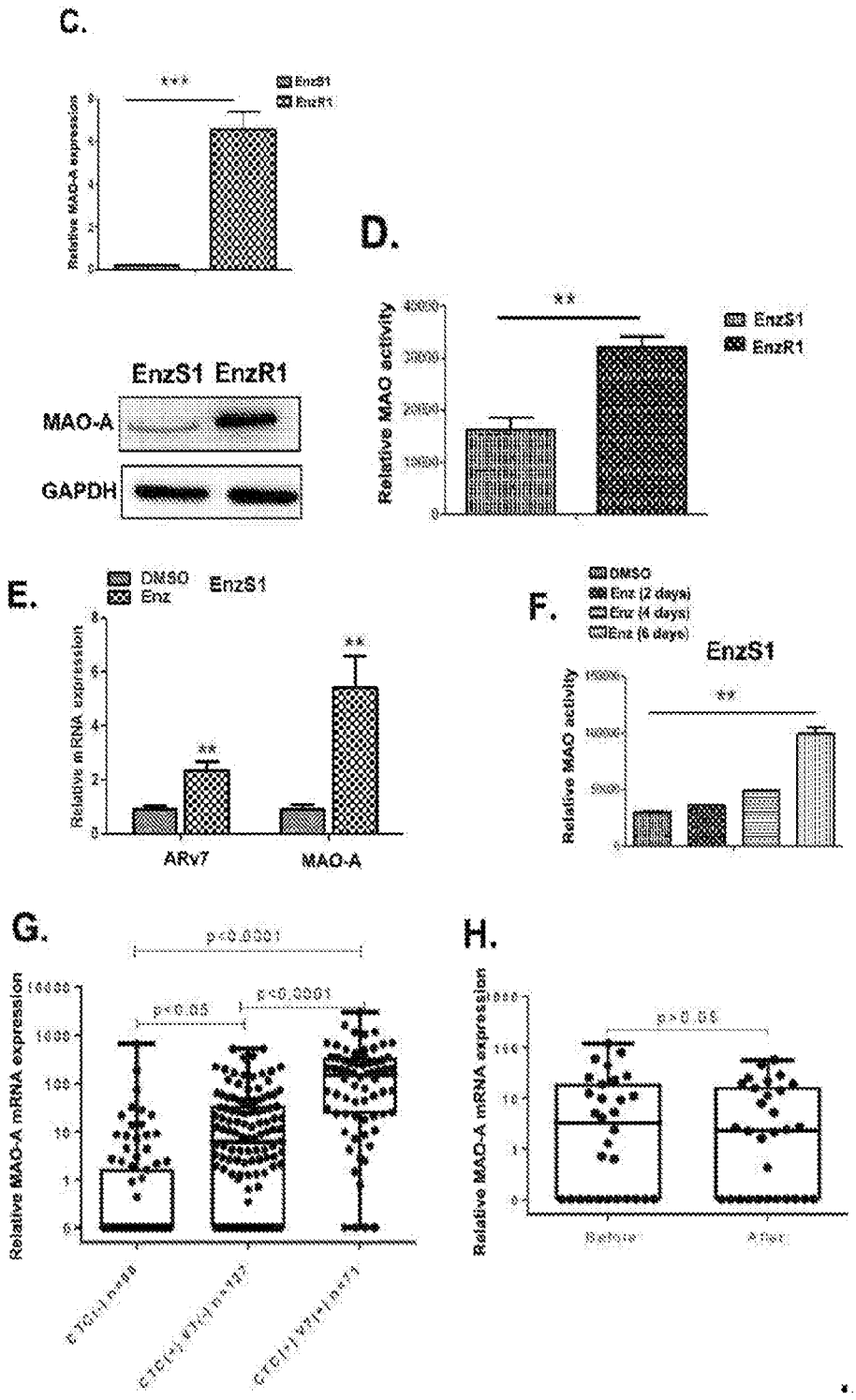

C.
***
EnzS1
EnzR1
Relative MAO-A expression
EnzS1 EnzR1
MAO-A
GAPDH
D.
**
EnzS1
EnzR1
Relative MAO activity
E.
DMSO
Enz
EnzS1
Relative mRNA expression
**
**
ARv7
MAO-A
F.
DMSO
Enz (2 days)
Enz (4 days)
Enz (6 days)
EnzS1
**
Relative MAO activity
G.
p<0.0001
p<0.05
p<0.0001
Relative MAO-A mRNA expression
H.
Relative MAO-A mRNA expression

FIG. 1 (Cont'd)

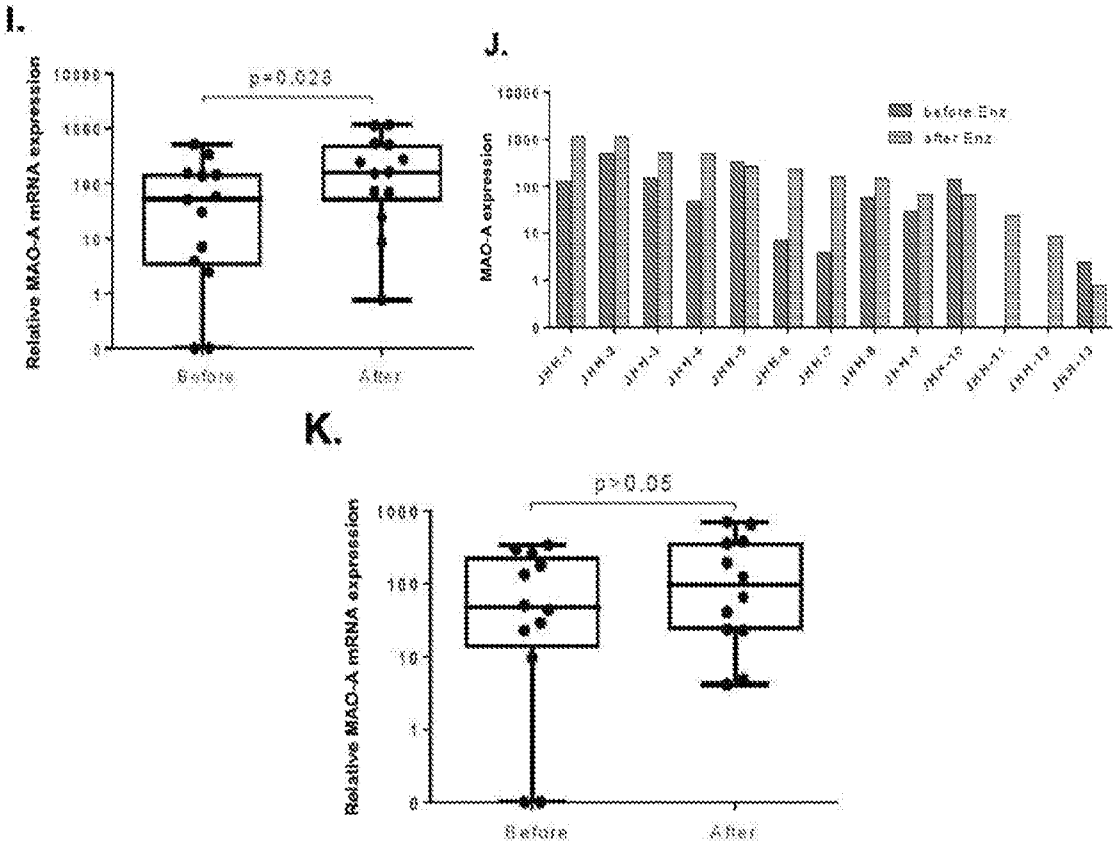


FIG. 1 (Cont'd)

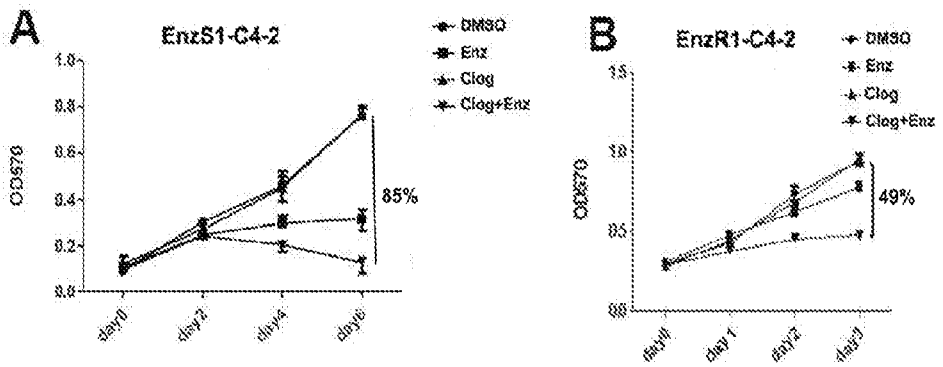


FIG. 2

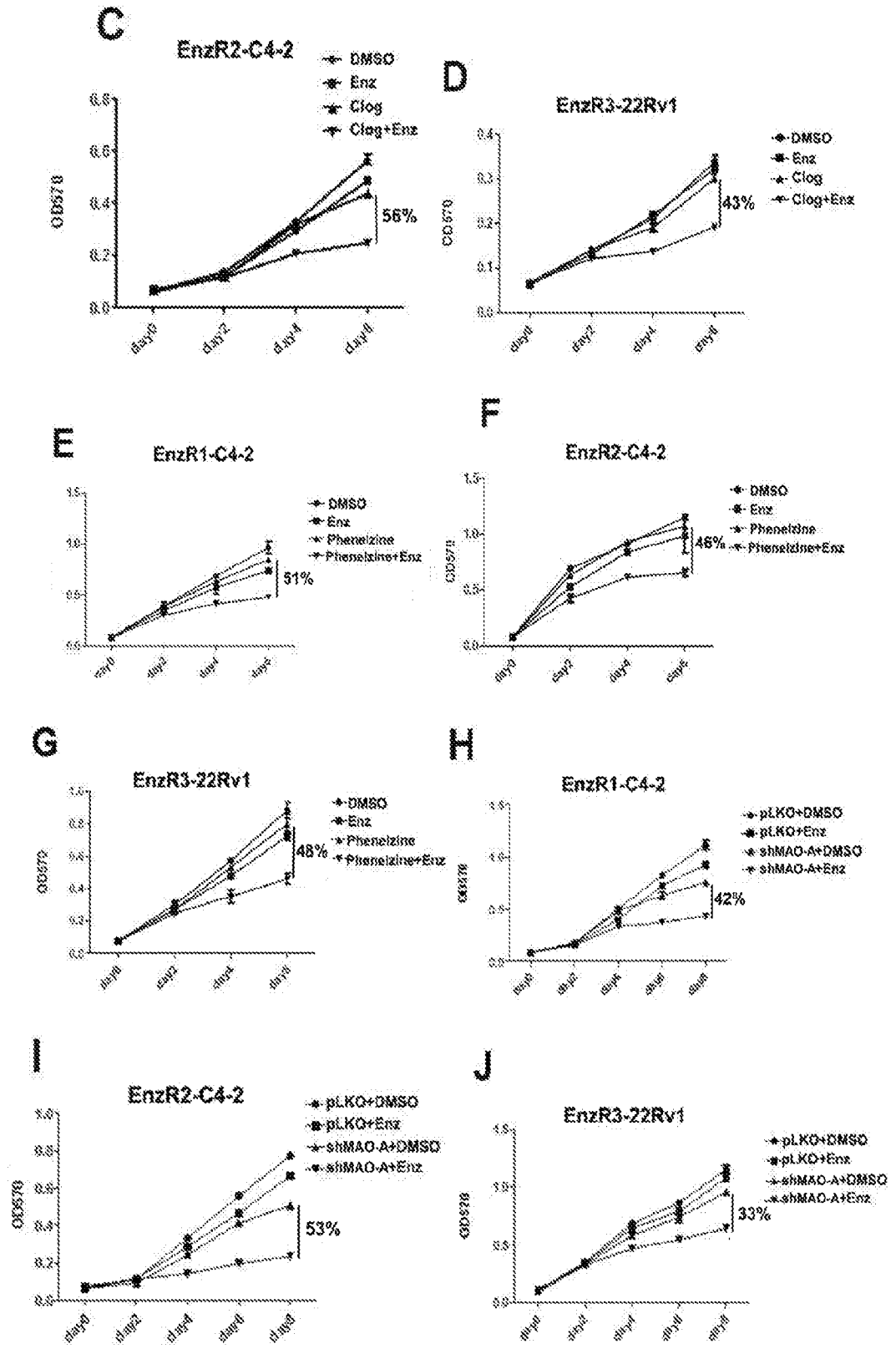

C
EnzR2-C4-2
DMSO
Enz
Clog
Clog+Enz
OD570
56%
D
EnzR3-22Rv1
DMSO
Enz
Clog
Clog+Enz
OD570
43%
E
EnzR1-C4-2
DMSO
Enz
Phenelzine
Phenelzine+Enz
51%
F
EnzR2-C4-2
DMSO
Enz
Phenelzine
Phenelzine+Enz
46%
G
EnzR3-22Rv1
DMSO
Enz
Phenelzine
Phenelzine+Enz
48%
H
EnzR1-C4-2
pLKO+DMSO
pLKO+Enz
shMAO-A+DMSO
shMAO-A+Enz
42%
I
EnzR2-C4-2
pLKO+DMSO
pLKO+Enz
shMAO-A+DMSO
shMAO-A+Enz
53%
J
EnzR3-22Rv1
pLKO+DMSO
pLKO+Enz
shMAO-A+DMSO
shMAO-A+Enz
33%

FIG. 2 (Cont'd)

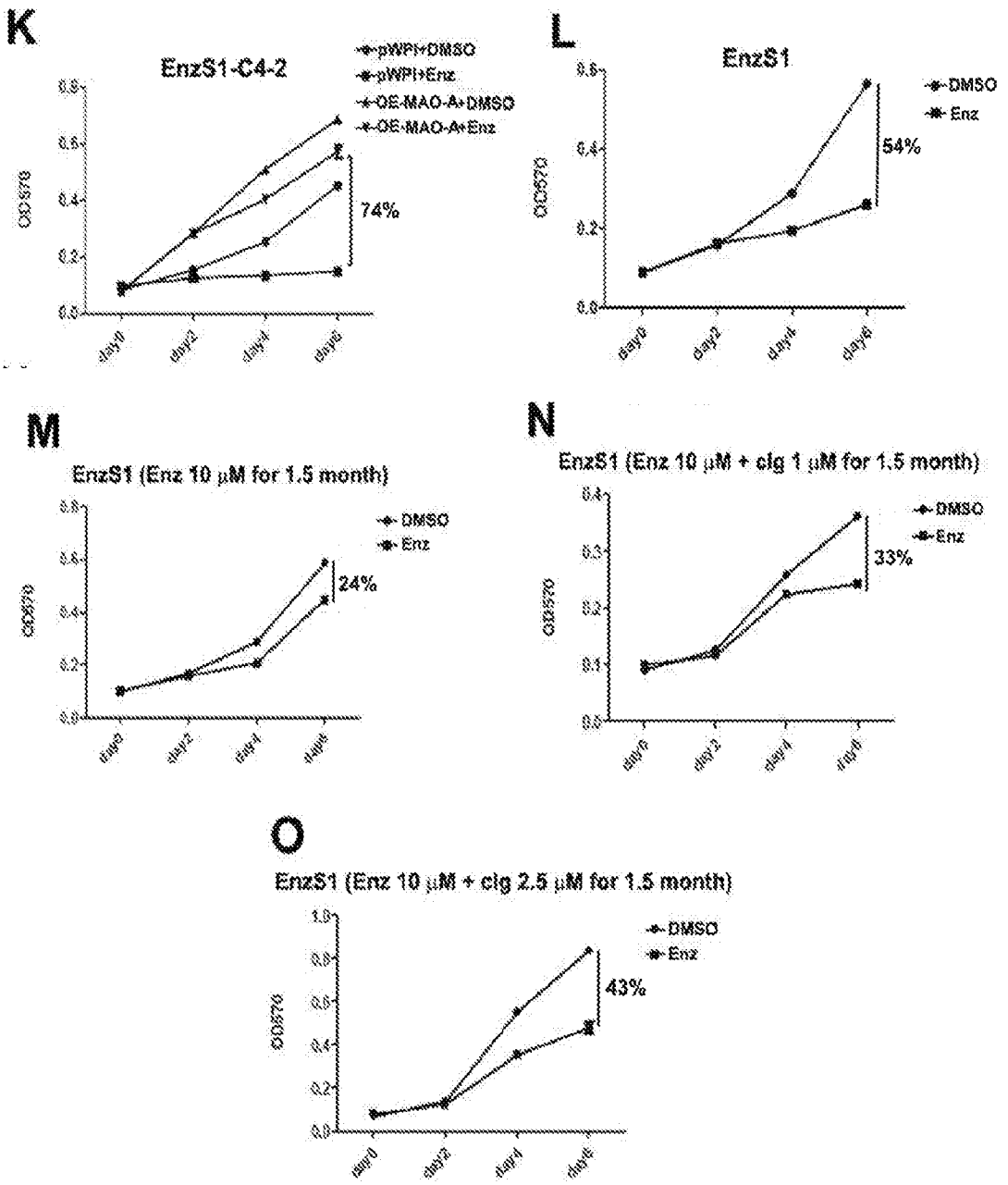

K
EnzS1-C4-2
pWPI+DMSO
pWPI+Enz
OE-MAO-A+DMSO
OE-MAO-A+Enz
74%
OD570
day0
day2
day4
day6
L
EnzS1
DMSO
Enz
54%
M
EnzS1 (Enz 10 μM for 1.5 month)
24%
N
EnzS1 (Enz 10 μM + cig 1 μM for 1.5 month)
33%
O
EnzS1 (Enz 10 μM + cig 2.5 μM for 1.5 month)
43%

FIG. 2 (Cont'd)

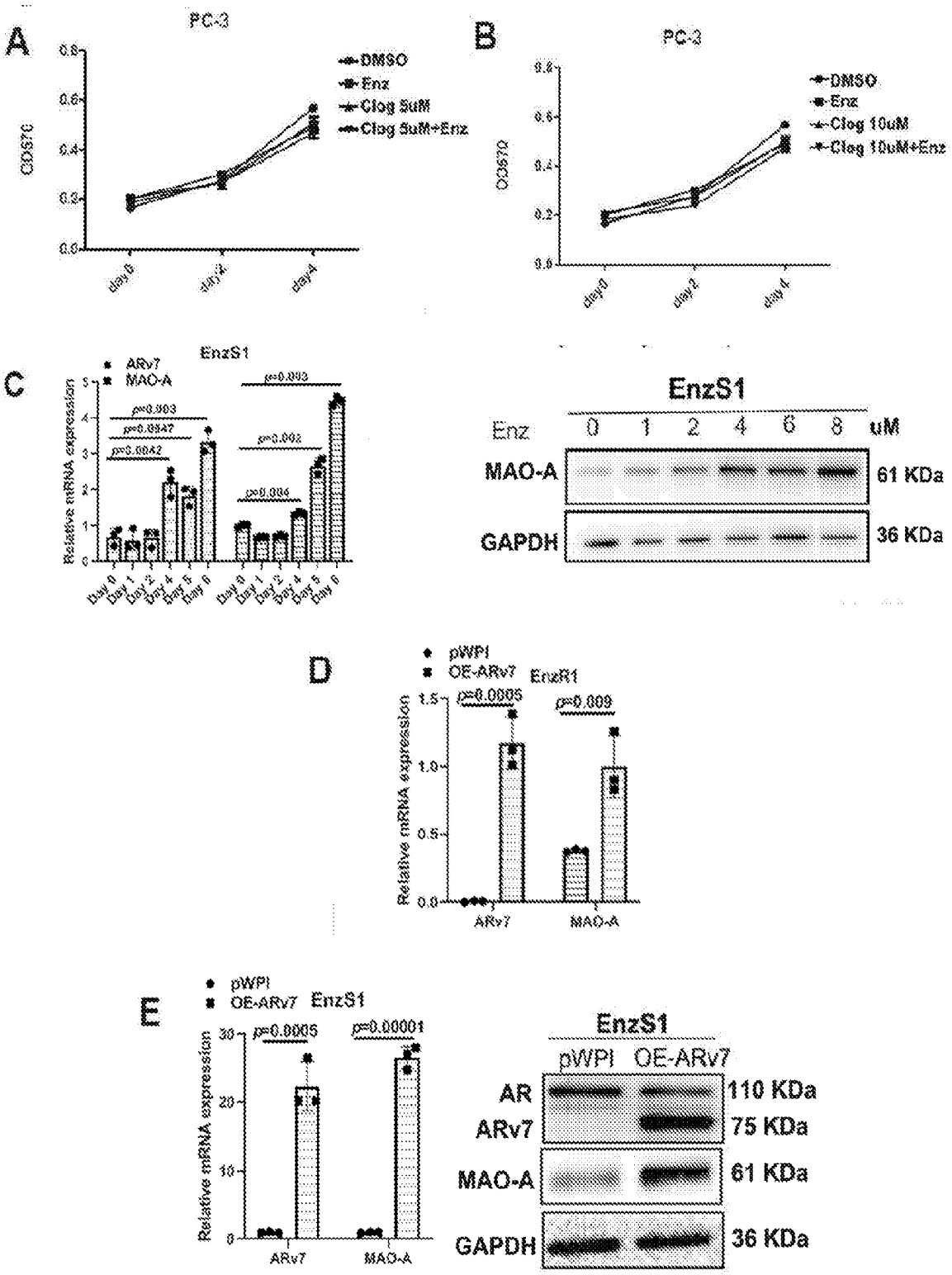

A
PC-3
OD570
DMSO
Enz
Clog 5uM
Clog 5uM+Enz
day0
day2
day4
B
PC-3
OD570
DMSO
Enz
Clog 10uM
Clog 10uM+Enz
day0
day2
day4
C
EnzS1
ARv7
MAO-A
Relative mRNA expression
p=0.003
p=0.0047
p=0.0042
p=0.003
p=0.002
p=0.004
Day 0
Day 1
Day 2
Day 4
Day 5
Day 6
EnzS1
Enz 0 1 2 4 6 8 uM
MAO-A
GAPDH
61 KDa
36 KDa
D
pWPI
OE-ARv7
EnzR1
Relative mRNA expression
p=0.0005
p=0.009
ARv7
MAO-A
E
pWPI
OE-ARv7
EnzS1
Relative mRNA expression
p=0.0005
p=0.00001
ARv7
MAO-A
EnzS1
pWPI OE-ARv7
AR
ARv7
MAO-A
GAPDH
110 KDa
75 KDa
61 KDa
36 KDa

FIG. 3

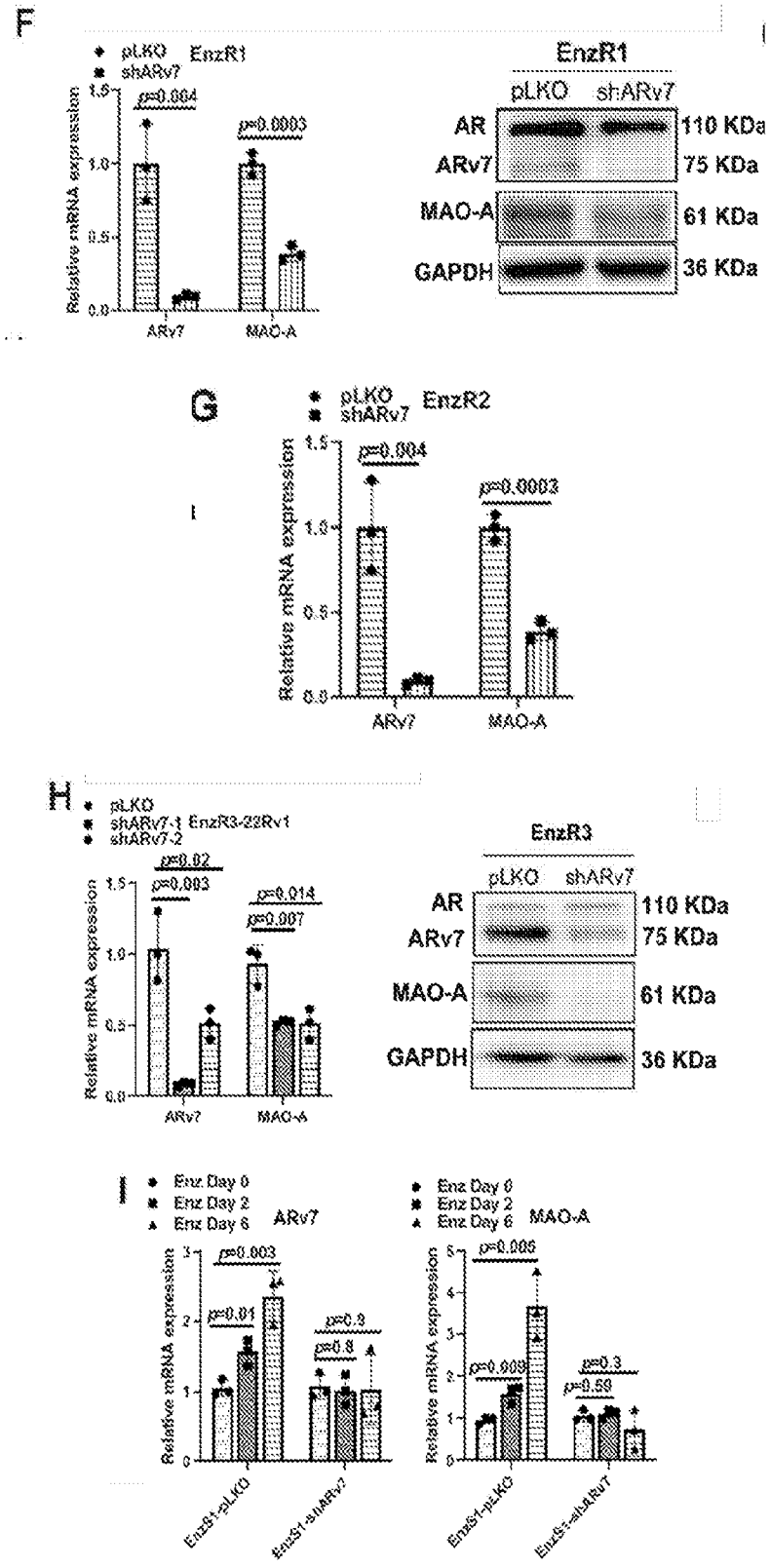

F
pLKO
shARv7
EnzR1
p=0.004
p=0.0003
Relative mRNA expression
ARv7
MAO-A
EnzR1
pLKO
shARv7
AR
ARv7
MAO-A
GAPDH
110 KDa
75 KDa
61 KDa
36 KDa
G
pLKO
shARv7
EnzR2
p=0.004
p=0.0003
Relative mRNA expression
ARv7
MAO-A
H
pLKO
shARv7-1
shARv7-2
EnzR3-22Rv1
p=0.02
p=0.003
p=0.014
p=0.007
Relative mRNA expression
ARv7
MAO-A
EnzR3
pLKO
shARv7
AR
ARv7
MAO-A
GAPDH
110 KDa
75 KDa
61 KDa
36 KDa
I
Enz Day 0
Enz Day 2
Enz Day 6
ARv7
p=0.003
p=0.01
p=0.9
p=0.8
Relative mRNA expression
EnzS1-pLKO
EnzS1-shARv7
Enz Day 0
Enz Day 2
Enz Day 6
MAO-A
p=0.005
p=0.003
p=0.3
p=0.59
Relative mRNA expression
EnzS1-pLKO
EnzS1-shARv7

FIG. 3 (Cont'd)

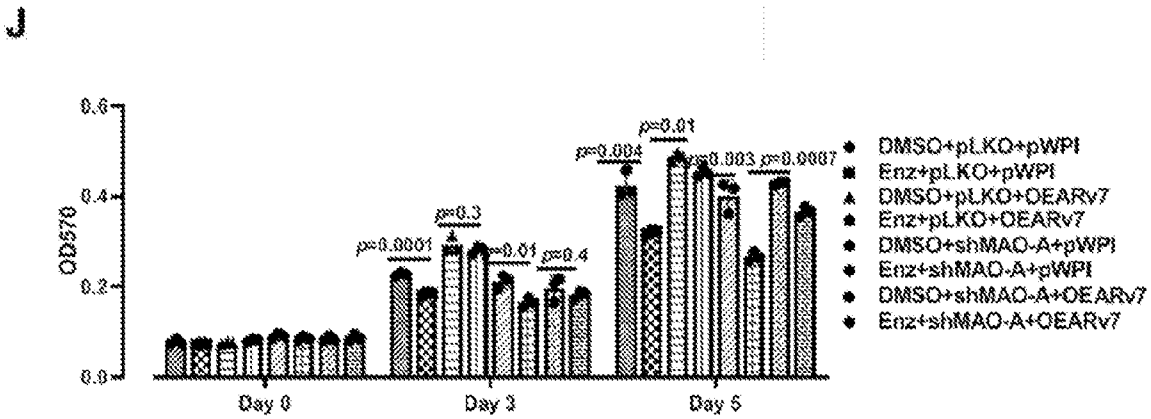


FIG. 3 (Cont'd)

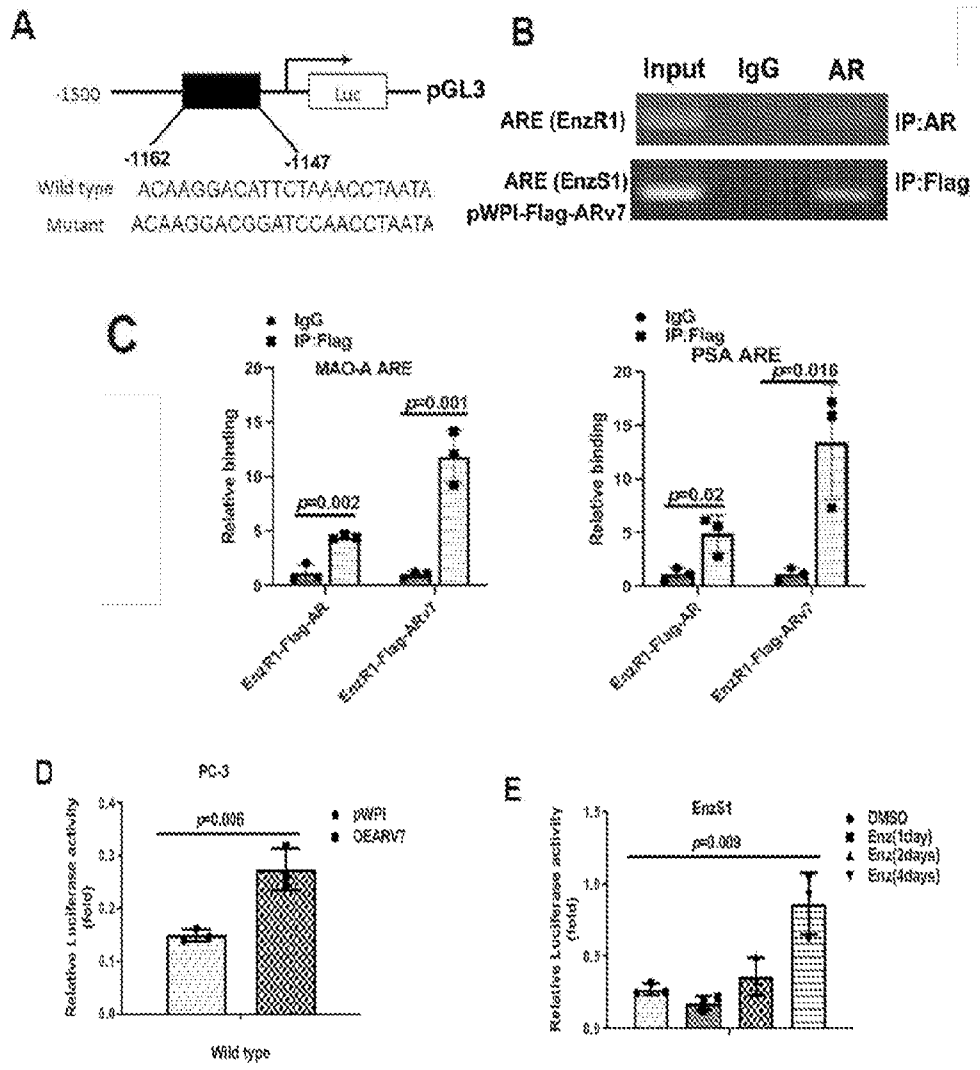


FIG. 4

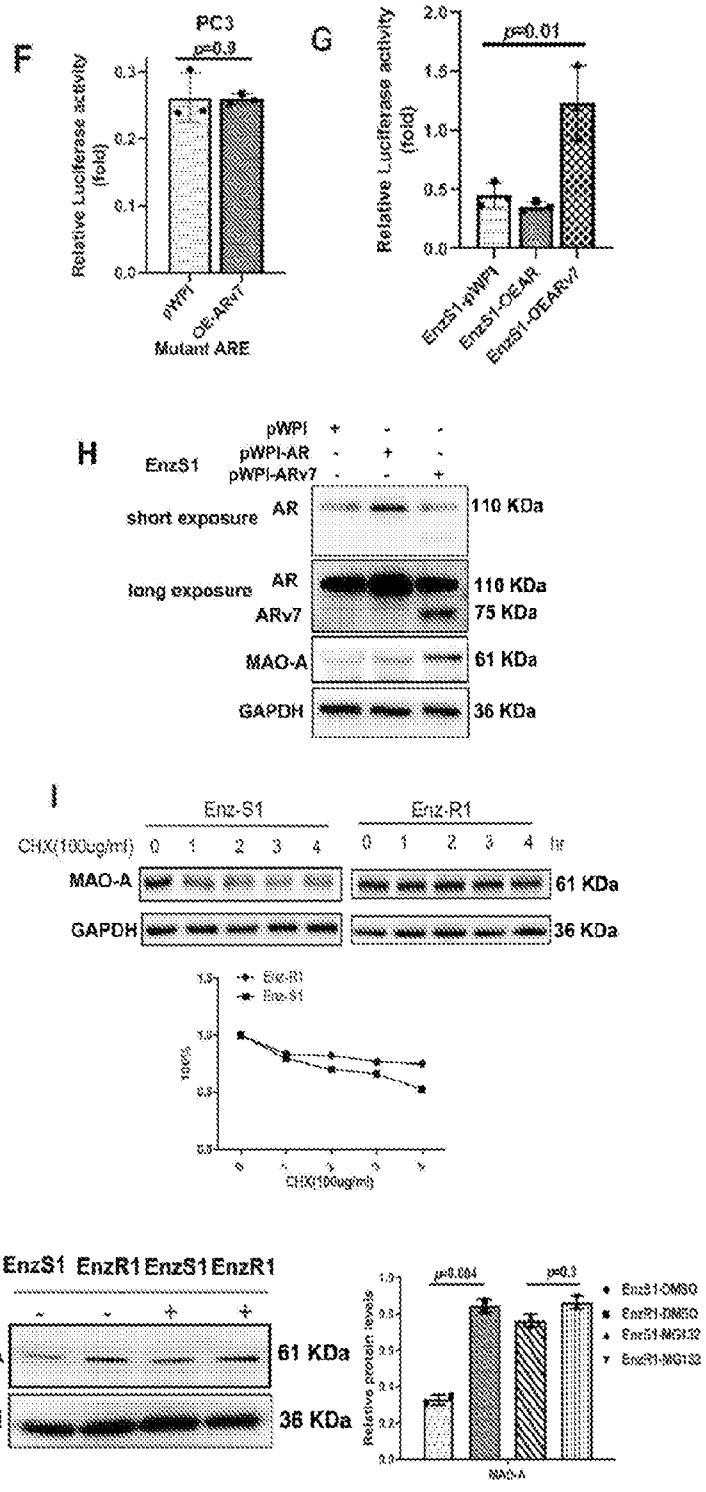

F
PC3
p=0.3
Relative Luciferase activity (fold)
pWPI
OE-ARv7
Mutant ARE
G
p=0.01
Relative Luciferase activity (fold)
EnzS1-pWPI
EnzS1-OE-AR
EnzS1-OE-ARv7
H
EnzS1
pWPI
pWPI-AR
pWPI-ARv7
short exposure
AR
110 KDa
long exposure
AR
ARv7
110 KDa
75 KDa
MAO-A
61 KDa
GAPDH
36 KDa
I
Enz-S1
Enz-R1
CHX(100ug/ml) 0 1 2 3 4 0 1 2 3 4 hr
MAO-A
61 KDa
GAPDH
36 KDa
Enz-R1
Enz-S1
CHX(100ug/ml)
J
EnzS1 EnzR1 EnzS1 EnzR1
MG132
MAO-A
61 KDa
GAPDH
36 KDa
p=0.004
p=0.3
Relative protein levels
MAO-A
EnzS1-DMSO
EnzR1-DMSO
EnzS1-MG132
EnzR1-MG132

FIG. 4 (Cont'd)

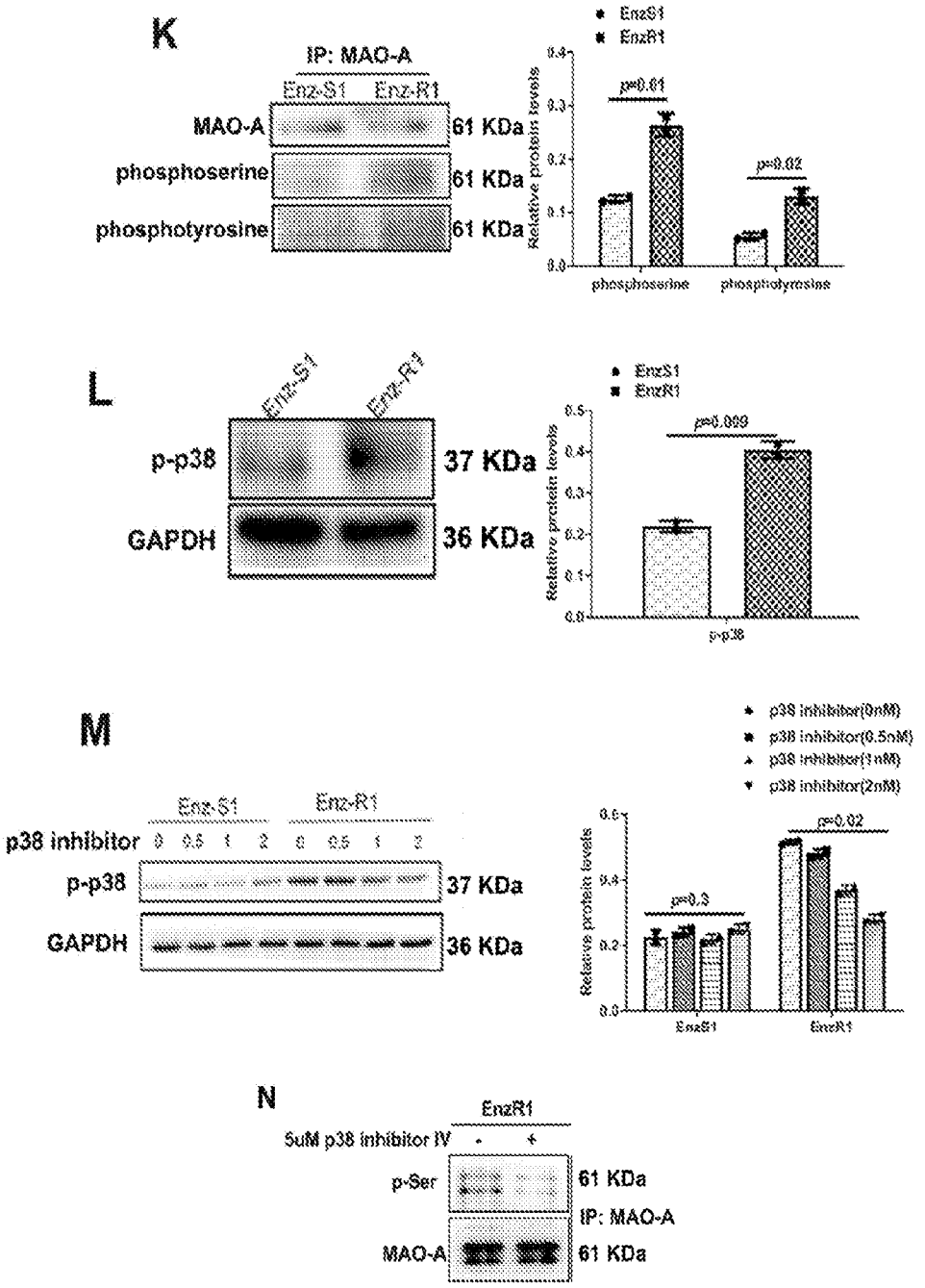

K
IP: MAO-A
Enz-S1
Enz-R1
MAO-A
61 KDa
phosphoserine
61 KDa
phosphotyrosine
61 KDa
EnzS1
EnzR1
p=0.01
p=0.02
Relative protein levels
phosphoserine
phosphotyrosine
L
Enz-S1
Enz-R1
p-p38
37 KDa
GAPDH
36 KDa
EnzS1
EnzR1
p=0.009
Relative protein levels
p-p38
M
Enz-S1
Enz-R1
p38 inhibitor 0 0.5 1 2 0 0.5 1 2
p-p38
37 KDa
GAPDH
36 KDa
p38 inhibitor(0nM)
p38 inhibitor(0.5nM)
p38 inhibitor(1nM)
p38 inhibitor(2nM)
p=0.3
p=0.02
Relative protein levels
EnzS1
EnzR1
N
EnzR1
5uM p38 inhibitor IV - +
p-Ser
61 KDa
IP: MAO-A
MAO-A
61 KDa

FIG. 4 (Cont'd)

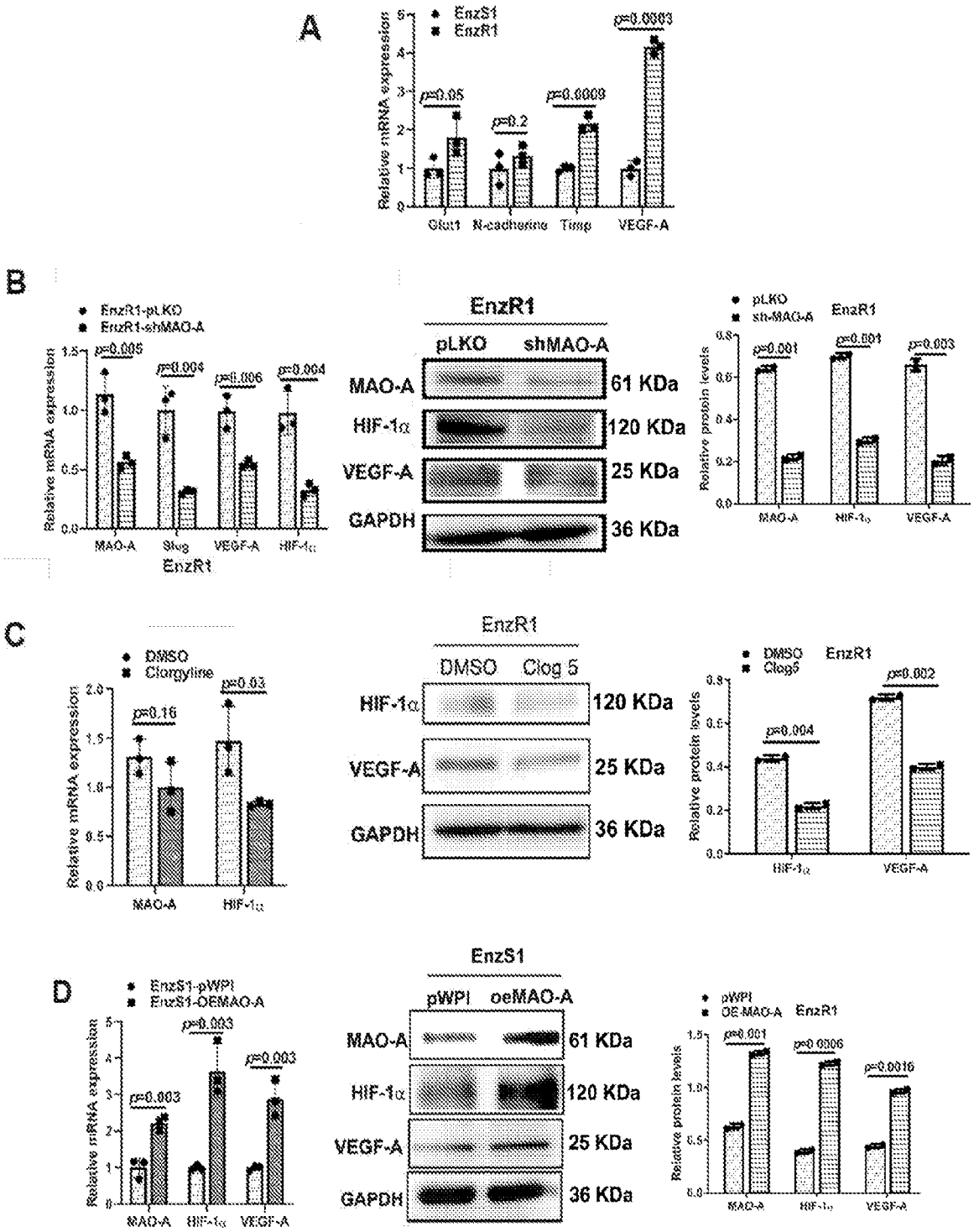

A
EnzS1
EnzR1
Relative mRNA expression
p=0.05
p=0.2
p=0.0009
p=0.0003
Glut1
N-cadherine
Timp
VEGF-A
B
EnzR1-pLKO
EnzR1-shMAO-A
p=0.005
p=0.004
p=0.006
p=0.004
MAO-A
Slug
VEGF-A
HIF-1α
EnzR1
EnzR1
pLKO
shMAO-A
MAO-A
61 KDa
HIF-1α
120 KDa
VEGF-A
25 KDa
GAPDH
36 KDa
pLKO
sh-MAO-A
EnzR1
Relative protein levels
p=0.001
p=0.001
p=0.003
MAO-A
HIF-1α
VEGF-A
C
DMSO
Clorgyline
p=0.16
p=0.03
MAO-A
HIF-1α
EnzR1
DMSO
Clog 5
HIF-1α
120 KDa
VEGF-A
25 KDa
GAPDH
36 KDa
DMSO
Clog5
EnzR1
p=0.004
p=0.002
HIF-1α
VEGF-A
D
EnzS1-pWPI
EnzS1-OEMAO-A
p=0.003
p=0.003
p=0.003
MAO-A
HIF-1α
VEGF-A
EnzS1
pWPI
oeMAO-A
MAO-A
61 KDa
HIF-1α
120 KDa
VEGF-A
25 KDa
GAPDH
36 KDa
pWPI
OE-MAO-A
EnzR1
p=0.001
p=0.0006
p=0.0016
MAO-A
HIF-1α
VEGF-A

FIG. 5

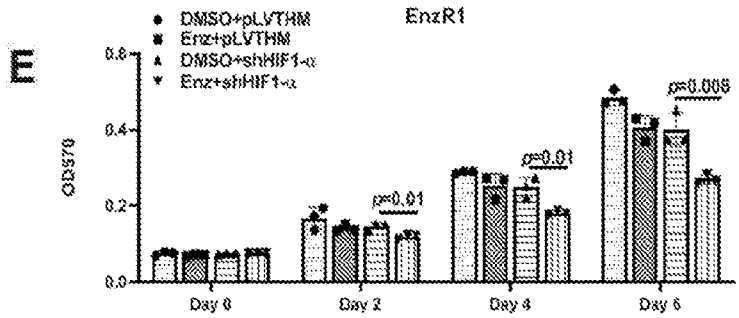


FIG. 5 (Cont'd)

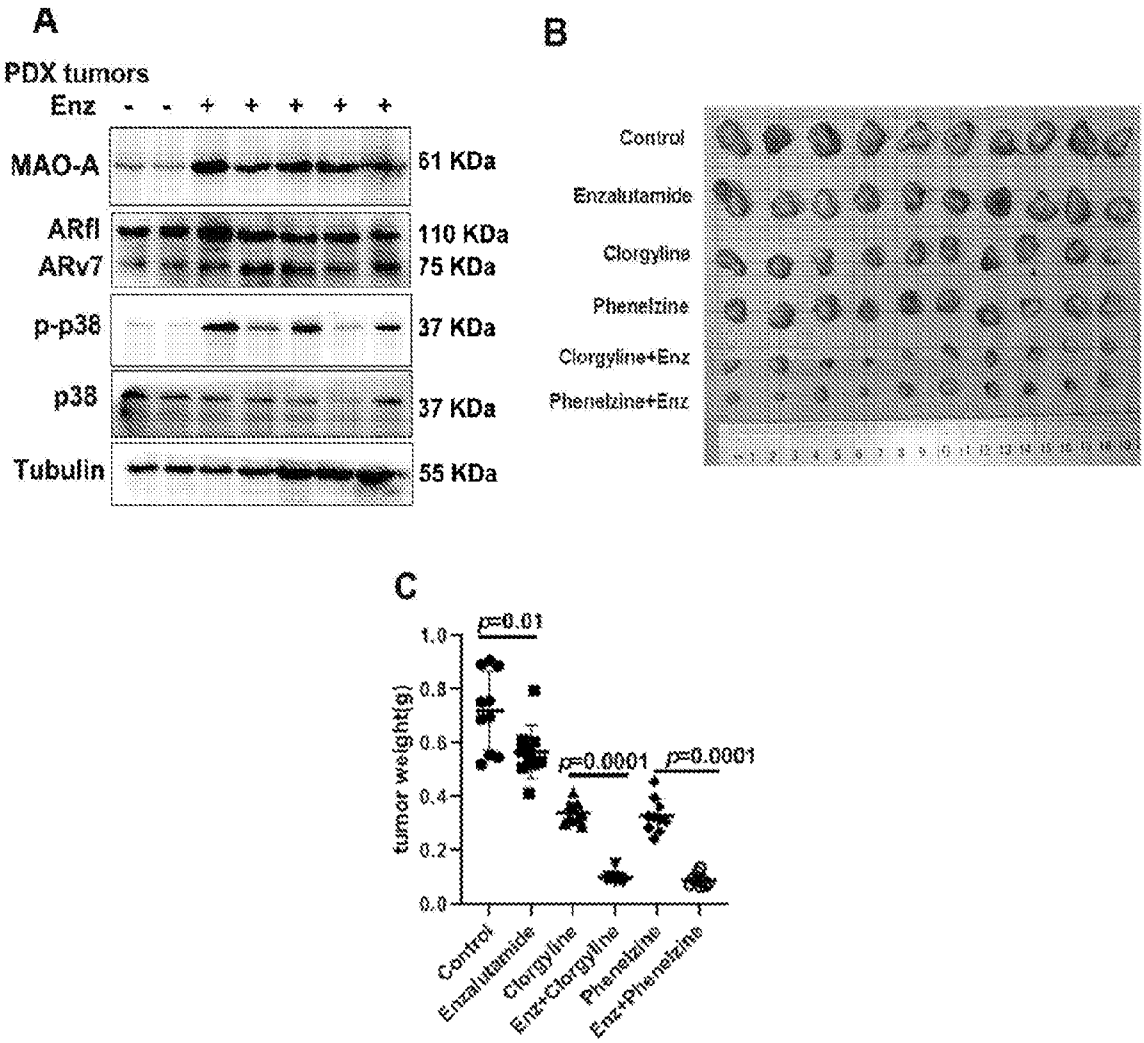


FIG. 6

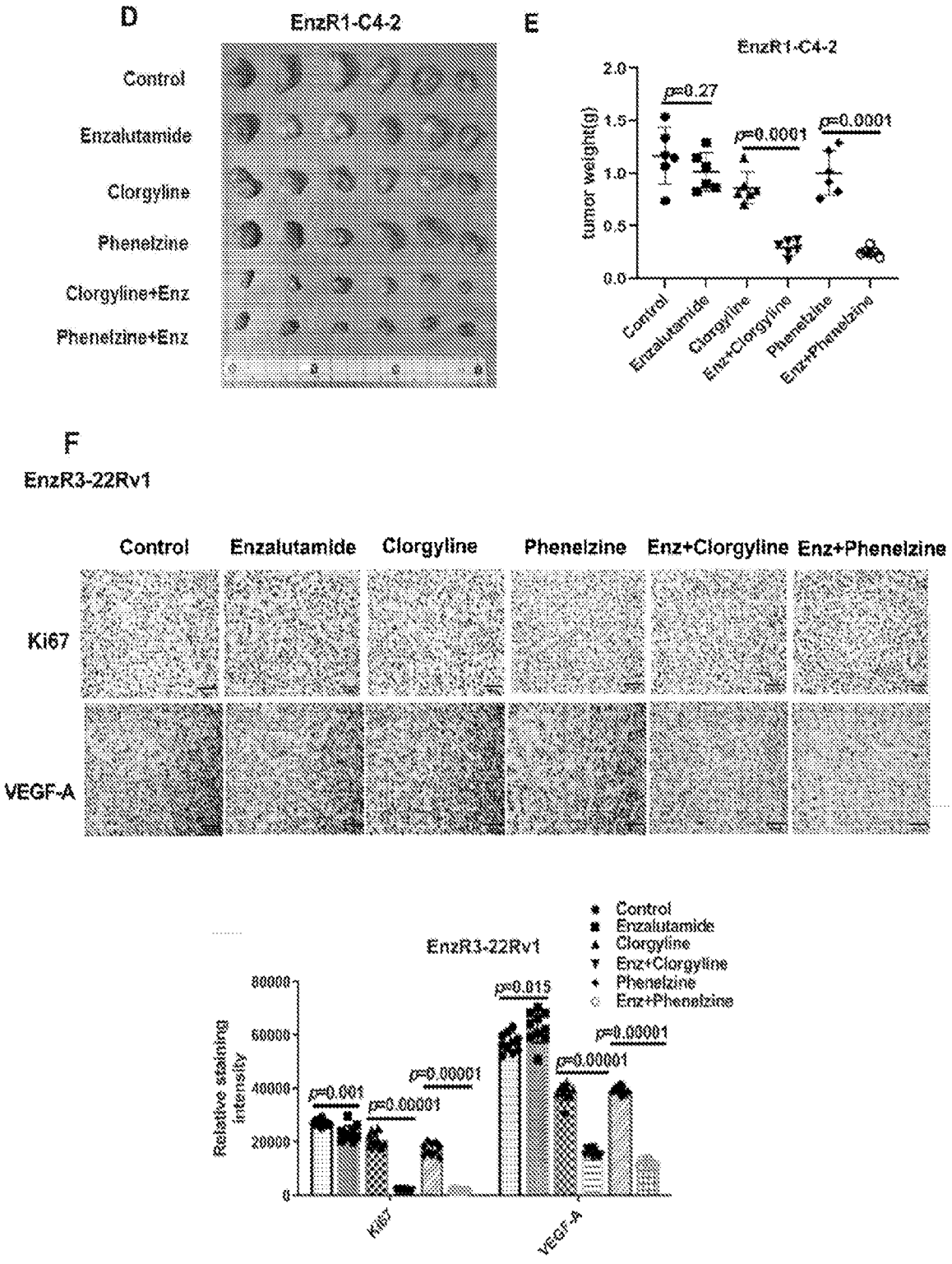

D
EnzR1-C4-2
Control
Enzalutamide
Clorgyline
Phenelzine
Clorgyline+Enz
Phenelzine+Enz
E
EnzR1-C4-2
tumor weight(g)
p=0.27
p=0.0001
p=0.0001
Control
Enzalutamide
Clorgyline
Enz+Clorgyline
Phenelzine
Enz+Phenelzine
F
EnzR3-22Rv1
Control
Enzalutamide
Clorgyline
Phenelzine
Enz+Clorgyline
Enz+Phenelzine
Ki67
VEGF-A
EnzR3-22Rv1
Relative staining intensity
p=0.001
p=0.00001
p=0.00001
p=0.015
p=0.00001
p=0.00001
Ki67
VEGF-A

FIG. 6 (Cont'd)

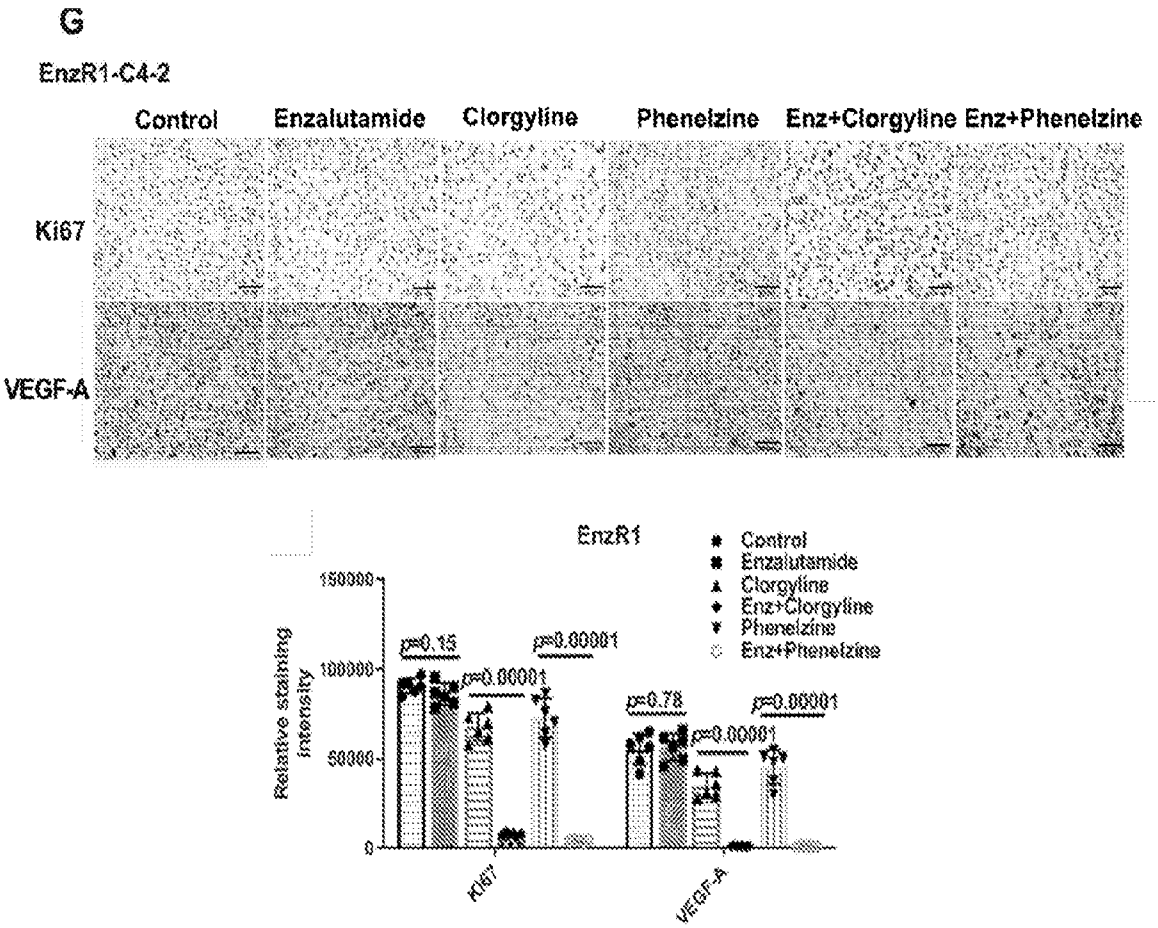

G
EnzR1-C4-2
Control
Enzalutamide
Clorgyline
Phenelzine
Enz+Clorgyline
Enz+Phenelzine
Ki67
VEGF-A
EnzR1
Control
Enzalutamide
Clorgyline
Enz+Clorgyline
Phenelzine
Enz+Phenelzine
Relative staining intensity
150000
100000
50000
0
p=0.15
p=0.00001
p=0.00001
p=0.78
p=0.00001
p=0.00001
Ki67
VEGF-A

FIG. 6 (Cont'd)

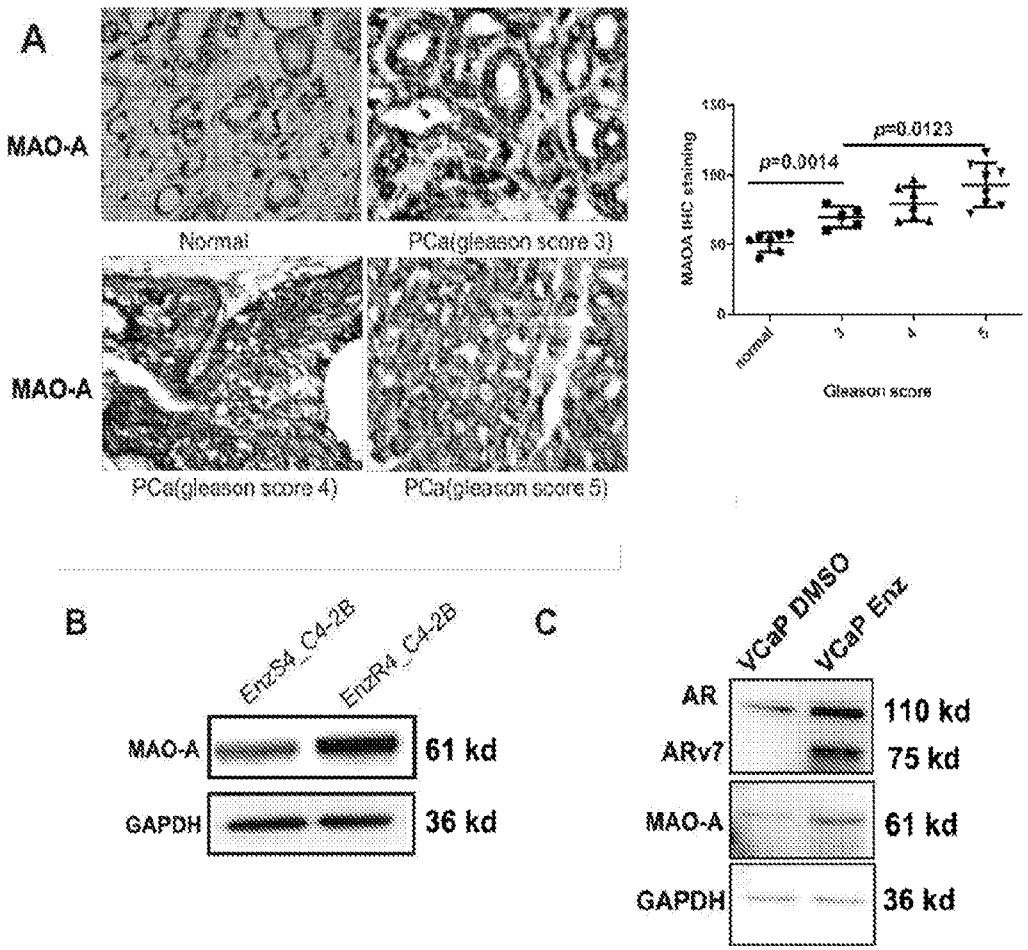

A
MAO-A
Normal
PCa(gleason score 3)
MAO-A
PCa(gleason score 4)
PCa(gleason score 5)
p=0.0014
p=0.0123
MAOA IHC staining
normal
Gleason score
B
EnzSA_C4-2B
EnzRA_C4-2B
MAO-A
61 kd
GAPDH
36 kd
C
VCaP DMSO
VCaP Enz
AR
110 kd
ARv7
75 kd
MAO-A
61 kd
GAPDH
36 kd

FIG. 7

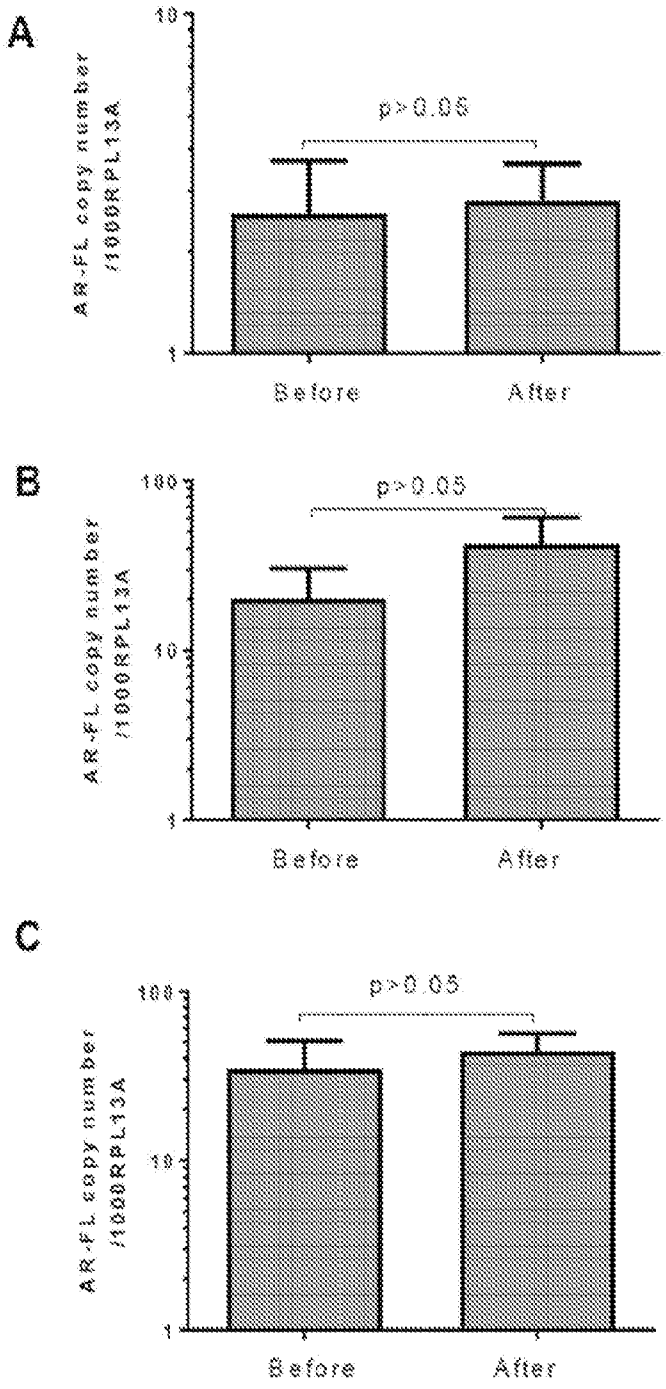

A
AR-FL copy number /1000RPL13A
10
1
p>0.05
Before
After
B
AR-FL copy number /1000RPL13A
100
10
1
p>0.05
Before
After
C
AR-FL copy number /1000RPL13A
100
10
1
p>0.05
Before
After

FIG. 8

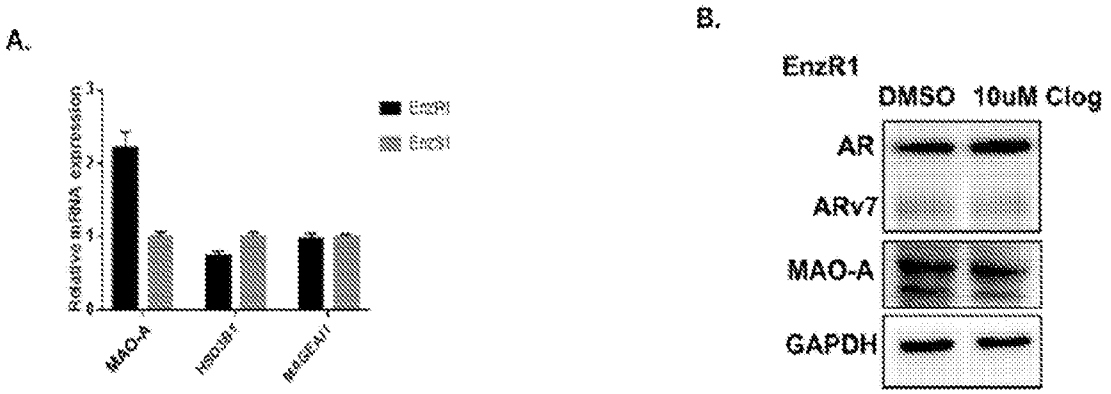


FIG. 9

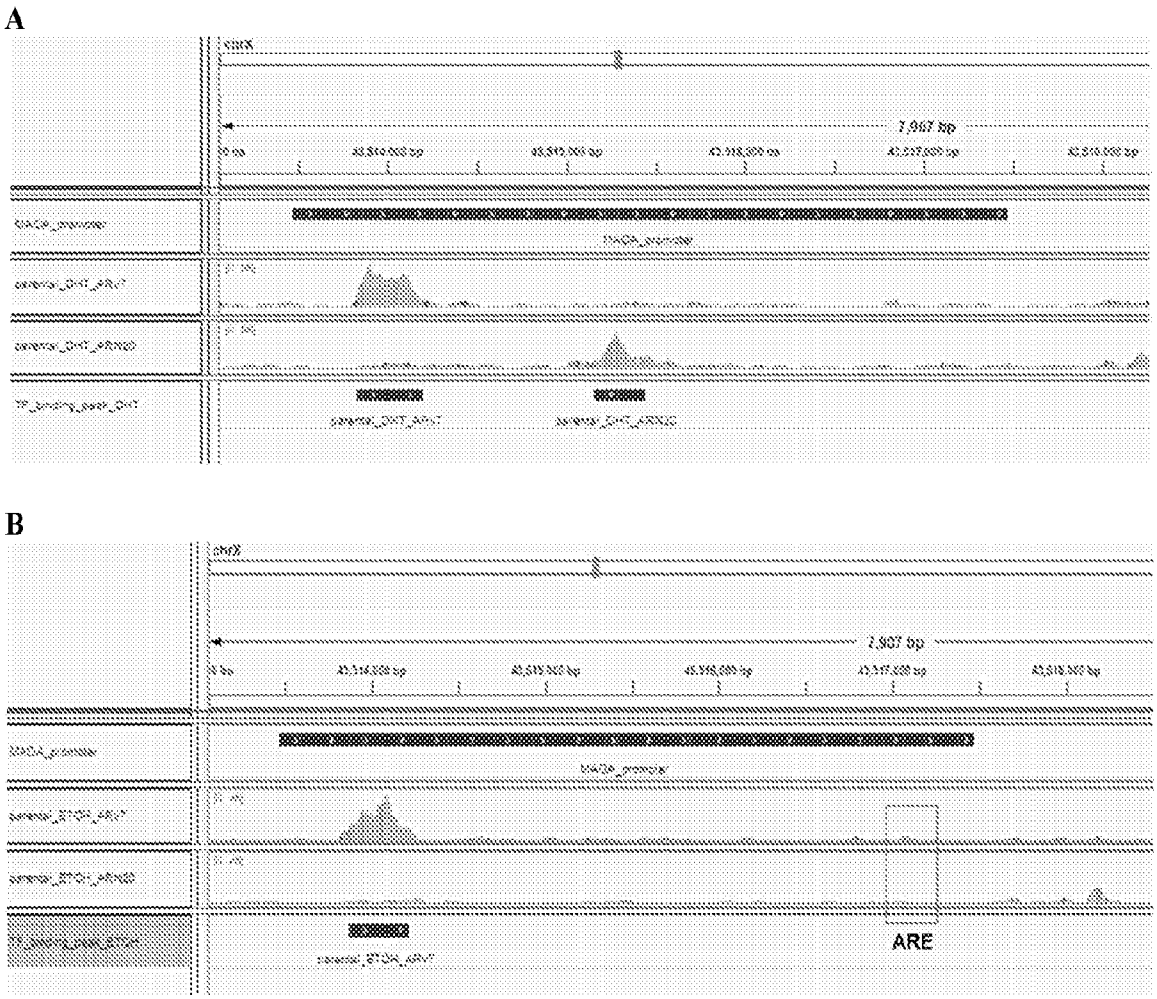


FIG. 10

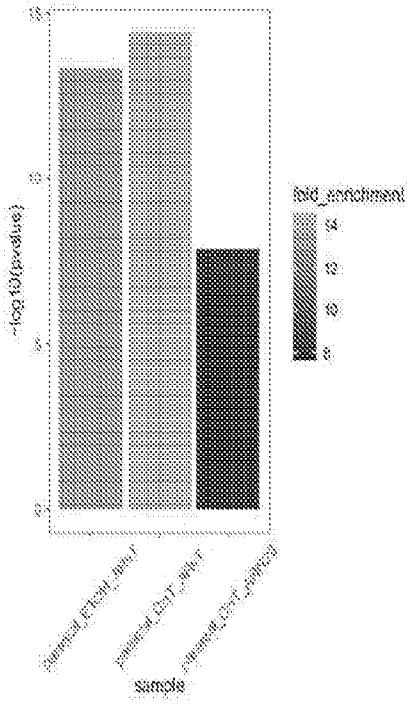


FIG. 10 (Cont'd)

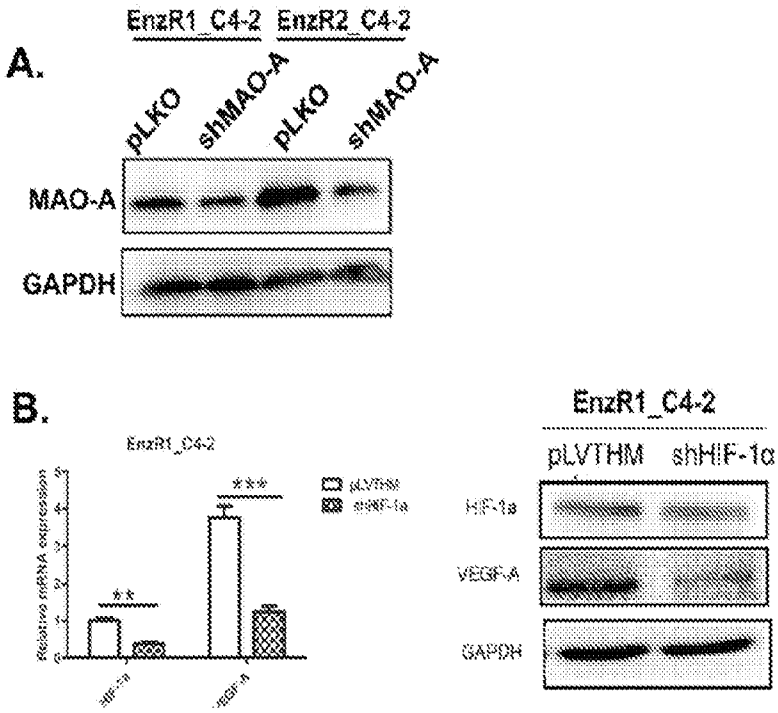
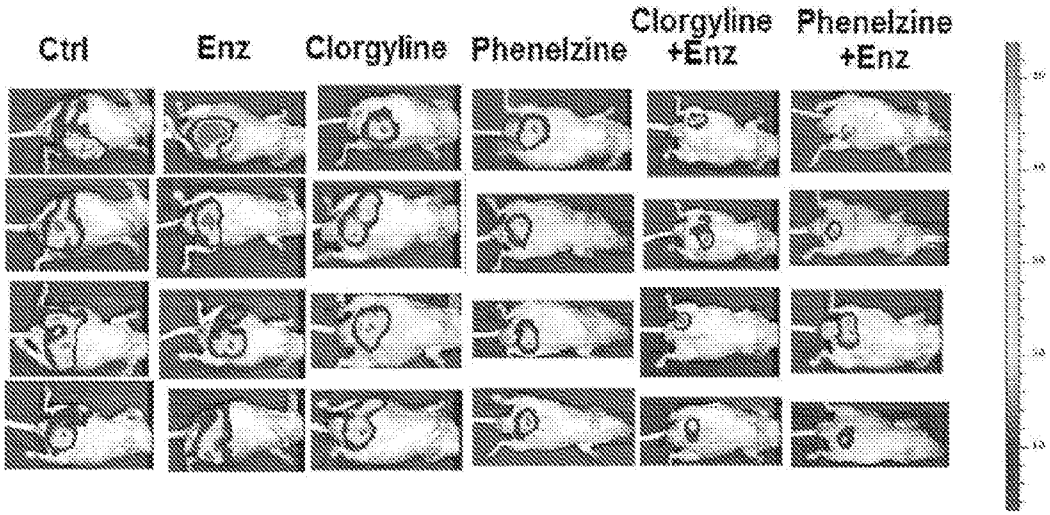
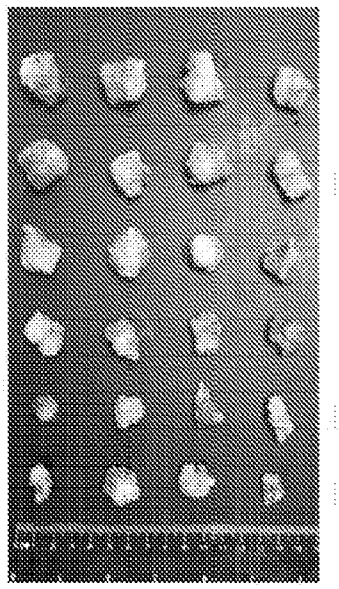


FIG. 11

A.


Ctrl
Enz
Clorgyline
Phenelzine
Clorgyline +Enz
Phenelzine +Enz

B.


CWR-22RV1-luc

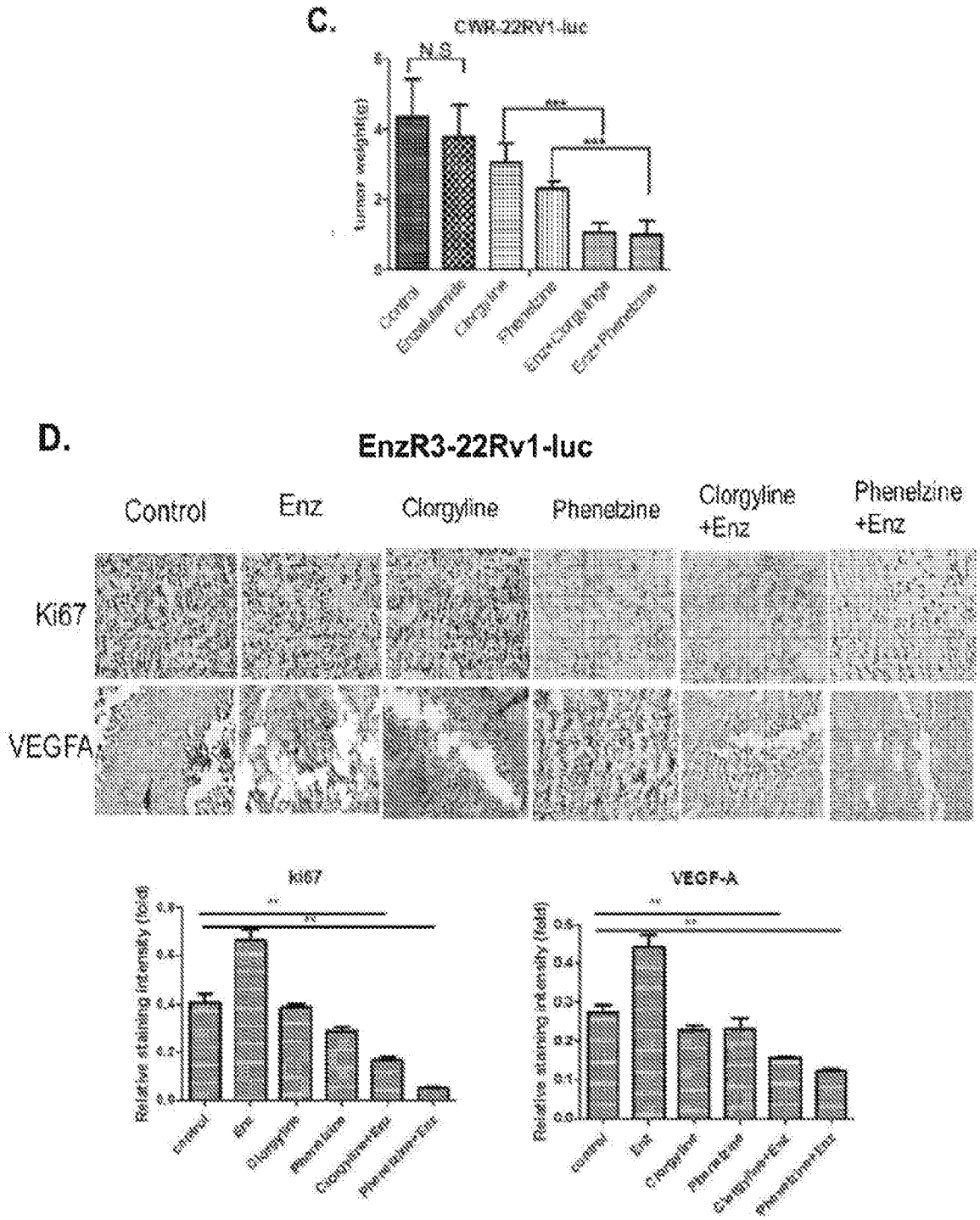

C.
CWR-22RV1-luc
N.S
Control
Enzalutamide
Clorgyline
Phenelzine
Enz+Clorgyline
Enz+Phenelzine
D.
EnzR3-22Rv1-luc
Control
Enz
Clorgyline
Phenelzine
Clorgyline +Enz
Phenelzine +Enz
Ki67
VEGFA
Ki67
VEGF-A

FIG. 12

Table 1

Primer	Sequence
MAOA F	5'-GAATCAAGAGAAGGCGAGTATCG-3'
MAOA R	5'-GGCAGCAGATAGTCCTGAAATG-3'
ARV7 F	5'-CAGGGATGACTCTGGGAGAA-3'
ARV7 R	5'-GCCCTCTAGAGCCCTCATTT-3'
GAPDH F	5'-GCACCGTCAAGGCTGAGAAC-3'
GAPDH R	5'-TGGTGAAGACGCCAGTGGA-3'
GLUT1 F	5'-GGCCAAGAGTGTGCTAAAGAA-3'
GLUT1 R	5'-ACAGCGTTGATGCCAGACAG-3'
N-cadherin F	5'-TCAGGCGTCTGTAGAGGCTT-3'
N-cadherin R	5'-ATGCACATCCTTCGATAAGACTG-3'
Timp F	5'-CATGTGCAGTACATCCATACGG-3'
Timp R	5'-CATCATAGACGCGACCTGTCA-3'
VEGF-A F	5'-AGGGCAGAATCATCACGAAGT-3'
VEGF-A R	5'-AGGGTCTCGATTGGATGGCA-3'
HIF-1a F	5'-GTGTACCCTAACTAGCCGAGG-3'
HIF-1a R	5'-GCAGTGCAATACCTTCCATGTT-3'

Table 2

Plasmid	Sequence
shMAOA-1 top	5'-CCGGAGTGAGCGTCTCGTTCAATATGGATCCATATTGAACGAGACGCTC ACTTTTTTG-3'
shMAOA-1 bottom	5'-AATTCAAAAAAGTGAGCGTCTCGTTCAATATGGATCCATATTGAACGAGA CG CTCACT-3'
shMAOA-2 top	5'CCGGAGCAGAGAGAAACCAGTTAATGGATCCATTAACTGGTTTCTCTCTG TTTTTG 3'
shMAOA-2 bottom	5'-AATTCAAAAAAGCAGAGAGAAACCAGTTAATGGATCCATTAACTGGTTT C TCTGCT-3'
shARv7 top1	5'-CCGG AGGCTAATGAGGTTTATTT-3'
shARv7 bottom1	5'-GGATCC AAATAAACCTCATTAGCCT-3'
shARv7 top2	5'-GGATCC AAATAAACCTCATTAGCCT TTTTTG-3'
shARv7 bottom2	5'-AATTCAAAAA AGGCTAATGAGGTTTATTT-3'
pwpi MAOA gibson F	5'-GTGAGGAATTTCGACATTTAAATTTA ATG GAG AATCAAGAGAAG GC-3'
pwpi MAOA gibson R	5'-TCCTGCAGCCCGTAGTTTTCAAGACCGTGGCAGGAGCT-3'

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/013243

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4166 A61K31/137 A61K45/06 A61P35/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018/055915 A1 (CHANG CHAWNSHANG [US] ET AL) 1 March 2018 (2018-03-01) page 1, paragraph 8 - paragraph 10 page 5, paragraph 53 - page 6, paragraph 56 page 9, paragraph 97 claims 1-31	1-37

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2020	02/06/2020

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

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/013243

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	C. LIU ET AL: "Niclosamide Inhibits Androgen Receptor Variants Expression and Overcomes Enzalutamide Resistance in Castration-Resistant Prostate Cancer", CLINICAL CANCER RESEARCH, vol. 20, no. 12, 16 April 2014 (2014-04-16), pages 1-21, XP055248432, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-3296 the whole document	1-37
Y	EMMANUEL S. ANTONARAKIS ET AL: "AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer", NEW ENGLAND JOURNAL OF MEDICINE, vol. 371, no. 11, 11 September 2014 (2014-09-11), pages 1028-1038, XP055566838, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1315815 abstract	1-37
Y	WO 2013/016580 A2 (UNIV SOUTHERN CALIFORNIA [US]; SHIH JEAN C [US] ET AL.) 31 January 2013 (2013-01-31) page 2, paragraph 8 page 30, paragraph 99 claims 1-25	1-37
Y	US 2018/177786 A1 (SHIH JEAN C [US]) 28 June 2018 (2018-06-28) page 1, paragraph 7 - paragraph 16 claims 1-16	1-37
X,P	SHIKHA GAUR ET AL: "Effect of Monoamine oxidase A (MAOA) inhibitors on androgen-sensitive and castration-resistant prostate cancer cells", PROSTATE., vol. 79, no. 6, 28 January 2019 (2019-01-28), pages 667-677, XP055696637, US ISSN: 0270-4137, DOI: 10.1002/pros.23774 the whole document	1-37

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/013243

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2018055915 A1	01-03-2018	NONE	
WO 2013016580 A2	31-01-2013	CA 2843359 A1	31-01-2013
		CN 103826625 A	28-05-2014
		EP 2736506 A2	04-06-2014
		JP 6502308 B2	17-04-2019
		JP 2014525914 A	02-10-2014
		JP 2017081923 A	18-05-2017
		US 2013039854 A1	14-02-2013
		US 2018185303 A1	05-07-2018
		WO 2013016580 A2	31-01-2013
US 2018177786 A1	28-06-2018	NONE	