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(71) Applicant: UNIVERSITY OF ROCHESTER [US/US];

601 Elmwood Avenue, Box URV, Rochester, NY 14642 (US).

(72) Inventor: CHANG, Chawnshang; c/o University of

Rochester, 601 Elmwood Avenue, Box URV, Rochester, NY 14642 (US).

(74) Agent: ZHANG, Philip, Yin; Milstein Zhang & Wu LLC,

2000 Commonwealth Ave, Suite 400, Newton, MA 02466 (US).

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(54) Title: COMPOSITIONS AND METHODS FOR TREATING PROSTATE CANCER

(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.



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COMPOSITIONS AND METHODS FOR TREATING PROSTATE CANCER

Priority Claims and Related Applications

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/797,210, filed January 26, 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.)

[0006] 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.

[0007] Several mechanisms have been developed to account for the development of Enz-resistance. For example, Korpai *et al.* found that the ARF876L mutation might confer the resistance to Enz, and Liu *et al.* found that AKR1C3 might enhance the Enz-resistance *via* increasing the cellular androgen concentration. Interestingly, Arora *et al.* found that glucocorticoid receptor might also contribute to the Enz-resistance *via* replacing AR to activate some survival related signals. However, among many potential mechanisms to induce the Enz-resistance, the Enz-induced AR splicing mutant ARv7 received the most attention. A recent clinical survey clearly suggested that CRPC patients received ADT with Enz had increased ARv7, and ARv7 expression was higher in patients who developed Enz-resistance. The detailed mechanisms on how Enz-induced ARv7 can lead to Enz resistance and how to overcome Enz-resistance to further suppress CRPC remain to be further elucidated. (Korpai, *et al.* **2015** *Cancer discovery* 3, 1030-1043 (2013); Liu, *et al.* *Cancer research* **75**, 1413-1422; Arora, *et al.* **2013** *Cell* 155, 1309-1322; Antonarakis, *et al.* **2014** *The New England journal of medicine* 371, 1028-1038.)

[0008] 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.

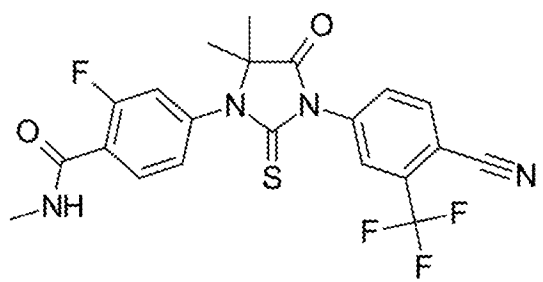
Summary of the Invention

[0009] The invention is based in part of the discovery of a unique approach to treatment of prostate cancer, particularly drug resistance prostate cancer. 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.

[0010] As is disclosed herein, S1P-ceramide axis is altered significantly (with increased S1P yet decreased ceramide) in the Enz-resistant CRPC (EnzR) cells, and targeting this Enz-altered S1P-ceramide axis with SPHK1 inhibitors or tetrahydrocannabinol (THC, the active component of marijuana) can re-sensitize the Enz-R cells to further suppress Enz-R cell growth.

[0011] As disclosed herein, Enz alters the S1P-ceramide rheostat to induce the Enz resistance, and targeting this S1P-ceramide rheostat with THC or Fingolimod/SKI2 all resulted in delaying the Enz resistance development and increasing the Enz sensitivity to further suppress the EnzR cells growth. Mechanism dissection revealed that Enz functions *via* modulating the ARv7-altered miRNAs signaling to decrease the SPHKAP expression, which then leads to increase S1P production *via* increasing SPHK1 activity. The Enz/ARv7/miRNAs/SPHKAP/SPHK1 axis-increased S1P then results in increasing Enz resistance *via* altering the methuosis, a rare cell death in the EnzR cells. Preclinical studies using multiple *in vitro* EnzR cell lines and *in vivo* mouse models demonstrated that targeting this newly identified Enz-altered S1P-ceramide rheostat with THC or Fingolimod/SKI2 all resulted in increasing the Enz sensitivity to further suppress the EnzR cells growth.

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

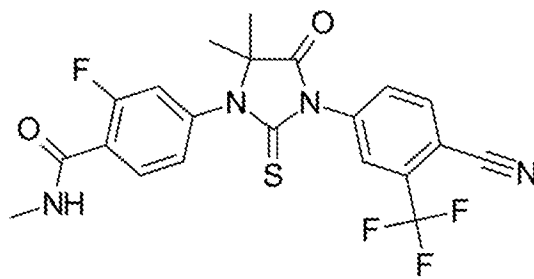


(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound selected from an inhibitor of SPHK and an activator of ceramide, 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 yet another aspect, the invention generally relates to a method for treating prostate cancer, or a related disease or condition thereof, comprising administering to a subject in need thereof a pharmaceutical composition disclosed herein.

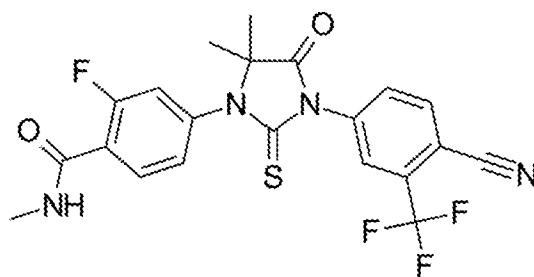
[0014] In yet another aspect, the invention generally relates to a method for treating prostate cancer, or a related disease or condition thereof, comprising administering to a subject in need thereof a first compound of Formula (I)



(I)

or a pharmaceutically acceptable salt, ester or pro-drug thereof, and a second compound which is an inhibitor of SPHK or an activator of ceramide, 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.

[0015] In yet another aspect, the invention generally relates to 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 selected from an inhibitor of an inhibitor SPHK and an activator of ceramide, 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.

[0016] In yet another aspect, the invention generally relates to 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 SPHK or an activator of ceramide, 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

[0017] FIG. 1. S1P signal is activated in EnzR cells. (A) The RNA-seq was performed to compare the different genes expressions in EnzR and EnzS cells. The genes which showed significant difference were picked up and uploaded for the gene ontology analysis. The results showed that the most of the genes were involved in the different metabolism processes. (B) The list of the genes which showed significant change in EnzR cells was analyzed by Pathway Commons pathway analysis. The results showed that the S1P pathway is significantly altered in EnzR cells. (C) The Gene Set Enrichment Analysis (GSEA) was performed based on the RNA-seq results and results showed that Sphingolipid related pathway is highly activated in EnzR cells. (D) The genes expression which were involved in sphingolipid metabolism were increased in EnzR cells. (E) The S1P and Ceramide levels in EnzR1&4 and EnzS1&4 cells were analyzed by Elisa assay (S1P) and HLPC (ceramide). (F) The EnzS1 cells were pre-treated with/without (w/o) 1 uM S1P for 3 days and then the Enz efficacy was analyzed by MTT. (G) The S1P levels in EnzR patients' plasma were analyzed. For E, data are presented as mean $\pm$ SD, * $p < 0.05$, ** $p < 0.005$, N.S, not significant by t-test for two groups or ANOVA for more than two groups.

[0018] FIG. 2. Targeting S1P signal with different compounds can reverse the EnzR. (A) The EnzR1 cells were treated w/o 2 uM Fingolimod and the Enz efficacy was analyzed by MTT. (B) The EnzR1 cells were treated w/o 10uM SKI2 and the Enz efficacy was analyzed by MTT. (C) The EnzR4 cells were treated w/o 2 uM Fingolimod, and Enz efficacy was analyzed by MTT. (E-F) The EnzS1, EnzR1 and EnzR3 cells were treated with different does of SKI2 (E) and Fingolimod (F) for 24hrs, the cell viabilities were analyzed by MTT assay. (G-H) The EnzR1 (G) and EnzR4 (H) cells were treated w/o 20uM ceramide and the Enz efficacy was analyzed by MTT assay. (I) The EnzR1 cells were treated w/o 10uM CBD and the Enz efficacy was analyzed by MTT assay. (J) The EnzR1 cells were treated w/o 6uM THC and the Enz efficacy was analyzed by MTT assay. (K-L) EnzR4 cells were treated w/o 10uM CBD (K) or 9uM THC. (N) The EnzR1 cells were treated w/o different does of THC and CBD, the p-SPHK1

level were analyzed. (P-R) The EnzS1 cells were treated w/o Enz, SKI2 or CBD for 1 month. And then the Enz efficacy on the cells with different kinds of treatments was analyzed by MTT assay.

[0019] FIG. 3. The SPHK1 activity is up-regulated in EnzR cells. (A) The SPHK1, SPHK2, CERK and ACER1 expressions were analyzed in EnzR1 and EnzS1 cells by WB. (B) The SPHKs activity was analyzed in EnzR1 and EnzS1 cells. (C) The EnzS1 cells were treated w/o 10uM Enz for 10 days, and then the SPHKs activity was analyzed. (D) The EnzR1 cells were treated w/o SPHK1 inhibitor PF543 or SPHK2 inhibitor ABC294640, and the Enz efficacy were examined. (E) The SPK1 p-S225 level in EnzR1 and EnzS1 cells were examined by WB. (F) The SPHK1 was knocked down by two shRNAs in EnzR4 cells, and then the Enz efficacy was examined by MTT assay on day 2&4. For B, C and D, data are presented as mean $\pm$ SD, * $p < 0.05$, ** $p < 0.005$, N.S, not significant by t-test for two groups or ANOVA for more than two groups.

[0020] FIG. 4. Low expression of SPHKAP in EnzR cells promote SPHK1 activity and S1P production. (A) Western blot analysis to detect SPHKAP protein level in EnzR1 and EnzS1 cells. (B) The EnzS1 cells were treated with 10uM Enz for different time points and the SPHKAP level was examined. (C) The PDX-133-4C tumors were treated with 30mg/kg Enz for 20 days (I.P injection each other day). The SPHKAP level was examined by IHC. (D) The SPHK activities in EnzS-plKO and EnzS-sh-SPHKAP cells were analyzed. (E) The S1P levels in EnzS-plKO and EnzS-sh-SPHKAP cells were analyzed. (F) The Enz efficacy on EnzR-pWPI and EnzR-OE-SPHKAP cells was analyzed by MTT assay. (G) The Enz efficacy on EnzS-Plko and EnzS-sh-SPHKAP cells was analyzed by MTT assay. (H) The EnzS1-shSPHKAP cells were treated with CBD or Fingolimod, and the Enz efficacy were analyzed by MTT assay. (I) The mRNA levels of SPHKAP in different stages of PCa samples were analyzed. The data is from TCGA database. (J) The SPHKAP protein levels in PCa tissues and normal prostate tissues were analyzed. The data is from The Human Protein Atlas. (K) We applied the tissue micro-array to analyze the SPHKAP protein levels in PCa samples with the different gleason scores. For C and D, data are presented as mean $\pm$ SD, * $p < 0.05$, ** $p < 0.005$, N.S, not significant by t-test for two groups or ANOVA for more than two groups.

[0021] FIG. 5. ARv7 suppresses SPHKAP expression via miRNA. (A) EnzS1 cells were infected with ARv7 and ARF876L cDNA and the SPHKAP levels were examined by WB. (B) The ARv7 was knocked down in EnzR1 cells and SPHKAP level were examined by WB. (C) The S1P levels in EnzS1-pWPI, EnzS1-ARF876L and EnzS1-ARv7 cells were analyzed. (D) The ARv7 was knocked down in EnzR1 cells and S1P levels were examined. (E) The mRNA level of SPHKAP was analyzed in EnzS1 and EnzR1 cells. (F) The enrichment of SPHKAP mRNA in Ago2 complex was analyzed in EnzR1 and EnzS1 cells by RNA-IP assay. (G) The potential miRNAs which can target SPHKAP were screened out from four different miRNA databases. The overlapped miRNAs were picked up for further identification. (H) The candidate miRNAs expressions were examined in EnzS1 and EnzR1 cells by Qpcr.

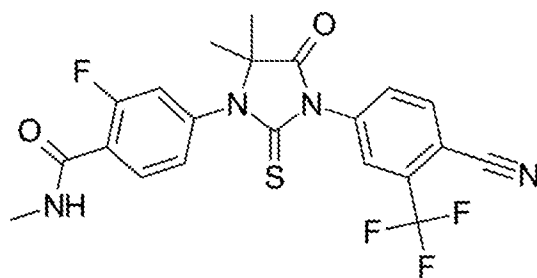
[0022] FIG. 6. Targeting S1P signal in EnzR cells induce methuosis. (A) EnzR1 and EnzS1 cells were treated w/o SKI2 or THC, the cell morphologies were observed. (B) EnzR4 and EnzS4 cells were treated w/o Fingolimod and CBD, the cell morphologies were observed. (C) The EnzR1 cells were treated w/o Z-VAD, chloroquine or EIPA, the SKI2 efficacy was analyzed by MTT assay. (E) The lucifer yellow absorption assay to identify the macropinocytosis in EnzR1 cells treated w/o Fingolimod or SKI2. (F) The EnzR1 cells were treated with SKI2 for 24 hrs. And then the cells were incubated with LY for 30mins. The IF images showed that lucifer yellow locate inside the vacuoles. (G) The EnzR1-GFP-Rab7 cells were treated with 10uM SKI2 for 24hrs. The IF images showed that GFP-Rab7 locate on the membrane of the vacuoles. (H) The S1P downstream genes activities, including Rac1, mTOR and ERK, were analyzed by WB. (I) The Rac1 target genes expression were analyzed by qPCR in EnzR1 and EnzS1 cells.

[0023] FIG. 7. Schematic depiction of Enz/ARv7/SPHKAP/S1P pathway Enz treatment suppress SPHKAP protein level through miRNA. In consequence, SPHK1 activity was induced after Enz treatment, which can enhance S1P:Ceramide ration to promote Enz resistance. To conquer EnzR, different compounds were applied (Fingolimod and SKI2 to reduce S1P level, THC and CBD to reduce S1P and increase ceramide level) to interrupt the abnormal S1P-ceramide rheostat, which could induce methuosis. Methuosis could further suppress the EnzR cells proliferation.

Detailed Description of the Invention

[0024] 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.

[0025] 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)

[0026] The mechanism by which CRPC patients received Enz develop EnzR remains not fully understood.

[0027] ADT with Enz treatment can further suppress the CRPC cell growth to extend patients survivals, yet eventually Enz-resistance may still be developed. Recent studies demonstrated several mechanisms that could confer to the Enz resistance. The first mechanism that was reported to drive the Enz resistance is activation of GR signaling. (Arora, *et al.* **2013** *Cell* 155, 1309-1322.) By inhibiting AR activity with ADT-Enz, GR level is up regulated in a subset of PCa cells due to relief of AR-mediated feedback repression of GR expression. The activated GR could replace some AR roles during development of Enz resistance. Importantly,

Arora *et al.* found the GR agonist dexamethasone was sufficient to confer Enz resistance, whereas a GR antagonist could restore Enz sensitivity. By overexpressing IL6, Liu *et al.* found that LNCaP cells might acquire the Enzalutamide resistance that may involve the activation STAT3 signaling to promote the AR transactivation and knocking down STAT3 might then result in increasing the Enzalutamide sensitivity in the LNCaP cells. Importantly, adding the STAT3 inhibitor AG490 may then reverse the Enzalutamide resistant in Enzalutamide resistant LNCaP cells, and adding Niclosamide to suppress the STAT3-AR axis could also suppress Enzalutamide resistant cells invasion and migration. Among all of the possible mechanisms, the increase of ARV7 is most convincing one to confer to Enz resistance. Results from *in vitro* and *in vivo* mouse model using the CRPC VCaP cells revealed that addition of Enz increased the expression of constitutively active ARv7. Importantly, the Enz-enhanced ARv7 could then transactivate AR target genes to promote CRPC progression in an androgen-independent manner that Enz is no longer able to function as AR antagonist. The clinical survey also suggested that patients with ARv7 might have poor response to ADT-Enz treatment, and ADT-Enz treatment might enhance ARv7 expression. (Yemelyanov, *et al.* **2012** *Cell Cycle* 11, 395-406; Sharifi **2014** *The New England journal of medicine* 370, 970-971; Liu, *et al.* **2014** *The Prostate* 74, 201-209; Hu, *et al.* **2009** *Cancer Res* 69, 16-22; Lai, *et al.* **2013** *The American journal of pathology* 182, 460-473; Antonarakis, *et al.* **2014** *The New England journal of medicine* 371, 1028-1038.)

[0028] To overcome the Enz resistance, targeting ARV7 is one of the best strategies. Wang *et al.* found Enz might be able to function *via* altering the lncRNA-*Malat1* expression to better interact with splicing factor SRSF1 to increase the ARv7 expression. Knocking-down lncRNA-*Malat1* with *Malat1*-siRNA suppressed the Enz-induced ARv7 expression in the Enz-resistant PCa cells, and adding *Malat1* could then induce ARv7 that can then enhance the development of Enz resistance. By applying the AR degradation enhancer ASC-J9, Yamashita *et al.* showed that ARV7 expression in 22RV1 cells can be significantly suppressed, which suggested that using ASC-J9 is a potential therapeutic to overcome the Enz resistance. Actually, Wang *et al.* found out that ASC-J9 do result in a significant reduction of Enz-resistant CRPC cell growth. Furthermore, results from Chou *et al.* also found that Cisplatin (and its analog Carboplatin (with less side effects)) could also function *via* degrading ARv7 (and AR) to further suppress the Enz resistant CRPC cell growth. Liu *et al.* showed that another FDA approved drug, Niclosamide,

can degrade ARV7 expression and re-sensitize the Enz-R cells to Enz treatment. Although in preclinical studies, all of these compounds or treatments showed the significant effect to reduce the Enz resistance, none of them have been approved by FDA to be applied in clinical use. Thus, effectively overcoming Enz resistance remains an unmet medical need. (Wang, *et al.* “Anti-androgen Enzalutamide promotes expression of Androgen receptor splicing variant 3 through Malat1 to accelerate the development of Enzalutamide-resistance” (submitted 2015). Yamashita, *et al.* **2012** *Neoplasia* 14, 74-83; Liu, *et al.* **2014** *Clinical cancer research : an official journal of the American Association for Cancer Research* 20, 3198-3210.)

[0029] The impact of S1P-ceramide axis to the Enz resistance is not yet fully studied. Recent reports showed that sphingosine 1-phosphate (S1P)-ceramide axis might play key roles in the tumor progression, with S1P promoting cell proliferation and maintain the cell survival, and ceramide playing the opposite role to induce the cell apoptosis and autophagy. Brizuela *et al.* also found that targeting the S1P with decreasing the S1P lyase could suppress PCa cell growth, and using the SPHK1 inhibitor-Fingolimod to target the S1P could alter the radiation therapy sensitivity to further suppress PCa growth. In contrast, targeting ceramide with up-regulating the ceramidase could then alter the radiation sensitivity to suppress the PCa. (Maceyka, *et al.* *Trends in cell biology* 22, 50-60 (2012); Subei, *et al.* *CNS drugs* 29, 565-575 (**2015**); Morad, *et al.* *Cancer* 13, 51-65 (**2013**); Saddoughi, *et al.* *Advances in cancer research* 117, 37-58 (**2013**); An, *et al.* *The Journal of biological chemistry* 275, 288-296 (**2000**); Calise, *et al.* *Biochimica et biophysica acta* 1823, 439-450 (**2012**); Pettus, *et al.* *Biochimica et biophysica acta* 1585, 114-125 (2002); Yabu, *et al.* *Cell death and differentiation* 22, 258-273 (2015); Pattingre, *et al.* *Autophagy* 5, 558-560 (2009); Brizuela, *et al.* *Molecular cancer therapeutics* 11, 1841-1851 (**2012**). Pchejetski, *et al.* *Cancer research* 70, 8651-8661 (**2010**). Mahdy, *et al.* *Molecular therapy : the journal of the American Society of Gene Therapy* 17, 430-438 (**2009**).

[0030] The results disclosed herein indicated that the interruption of the S1P-Ceramide biostat can result in the resistance to the Enz treatment. The mechanistic study revealed for the first time that Enz could control the S1P-Ceramide biostat through degrading SPHKAP and activating SPHK1 activity. By re-balancing the S1-ceramide biostat, the autophagy and apoptosis can be triggered after Enz treatment and reduce the Enz-R cell survival. Importantly, our study showed that by using certain compounds, some of which are FDA approved, such as fingolimod

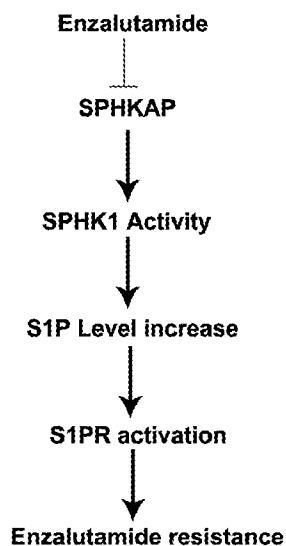
and THC, it is possible to re-sensitize the Enz-R cells to Enz. Such results provide an immediate and convenient strategy to prevent the patients' death from Enz resistant prostate cancer.

[0031] S1P is the key sphingolipid that maintains survival of cancer cells. Ceramide is another important sphingolipid that show the counter effect to S1P. The rheostat (biostat) between S1P and ceramide controls the cell proliferation and death. In prostate cancer, studies have shown that regulation S1P-ceramide by changing the different enzymes could be a potential strategy to reduce the tumor growth. In particular, in irradiation resistant prostate cancer, by decreasing S1P level or increasing ceramide level, the cells can be re-sensitized to IR. Such evidence suggested that S1P-ceramide biostat is also important to different kinds of treatment. In prostate cancer, however, there has been no evidence that showed S1P-ceramide biostat also contributes to the resistance to the anti-androgen treatment. The disclosed study is the first to reveal that targeting this pathway can be a good strategy to reverse the Enz resistance.

Significantly, SPHK1 inhibitors are available for clinical use or are under clinical investigation.

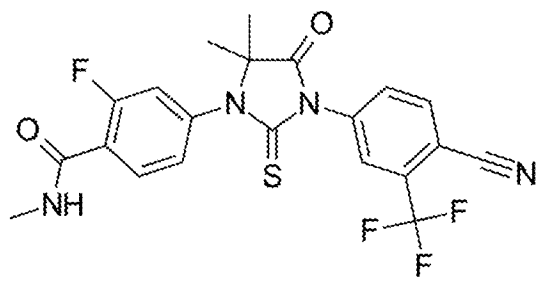
[0032] Additionally, THC, which is the active component of marijuana, can enhance the ceramide level to re-balance the S1P-ceramide biostat. By using these FDA approved compounds and others under development, a promising and practical approach to overcome Enz resistance has been discovered.

Scheme 1. S1P & Enz-R



[0033] Thus, the present invention enables a novel approach 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 SPHK1 inhibitor or an activator of ceramide (*e.g.*, THC) with 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 SPHK1 inhibitor or an activator of ceramide (*e.g.*, THC) at or after confirmation of EnzR (*e.g.*, when decreased PSA start to rise again).

[0034] In one aspect, the invention generally relates to 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 selected from an inhibitor of SPHK and an activator of ceramide, 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.

[0035] In certain embodiments, each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in reducing, reversing or delaying the onset of or delaying the development of drug resistance.

[0036] 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. 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.

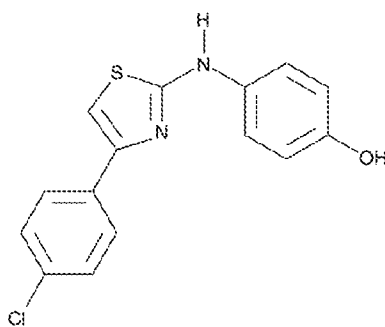
[0037] The first and second compounds may be present in the pharmaceutical composition at any suitable ratios. For example, the weight ratio of the first compound to the second compound is from about 10 : 1 to about 1 : 10 (*e.g.*, from about 7 : 1 to about 1 : 7, from about 5 : 1 to about 1 : 5, from about 3 : 1 to about 1 : 3, from about 2 : 1 to about 1 : 2, about 1 : 1).

[0038] 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.

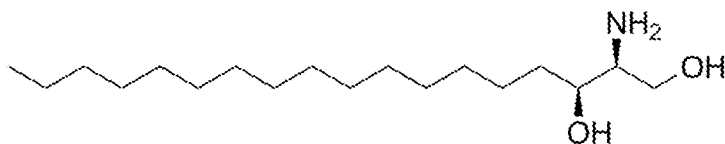
[0039] The second compound may be present in the pharmaceutical composition in any suitable amount, for example in a unit dosage of, in the range of about 0.1 mg to about 1,000 mg, about 1 mg to about 500 mg, about 1 mg to about 10 mg, about 10 mg to about 250 mg, about 10 mg to about 125 mg, about 10 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.

[0040] In certain embodiments, the pharmaceutical composition is suitable for one or more of oral administration, intravenous, intramuscular, and subcutaneous administration.

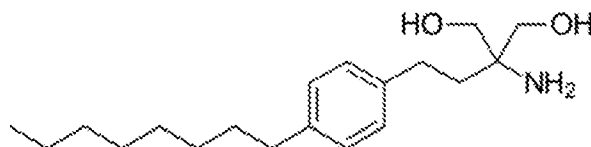
[0041] In certain embodiments, the second compound is selected from the group consisting of SKI2, Safingol, Fingolimod and tetrahydrocannabinol.



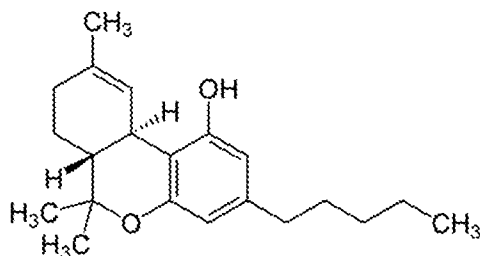
SKI2



Safingol



Fingolimod



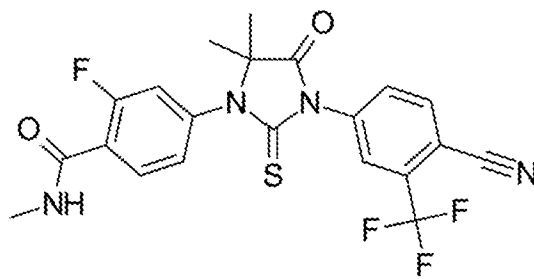
Tetrahydrocannabinol

[0042] In another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition disclosed herein.

[0043] In certain embodiments, the unit dosage form is 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.

[0044] In yet another aspect, the invention generally relates to a method for treating prostate cancer, or a related disease or condition thereof, comprising administering to a subject in need thereof a pharmaceutical composition disclosed herein.

[0045] In yet another aspect, the invention generally relates to 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 SPHK or an activator of ceramide, 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.

[0046] In certain embodiments, the second compound is administered simultaneously with the first compound, or subsequently after the administration of the first compound. In certain embodiments, the second compound is administered simultaneously with the first compound. In certain embodiments, the second compound is administered subsequently after the administration of the first compound.

[0047] In certain embodiments, the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.

[0048] In certain embodiments, the second compound is selected from the group consisting of SKI2, Safingol, Fingolimod and tetrahydrocannabinol. In certain embodiments, the second compound is SKI2. In certain embodiments, the second compound is Safingol. In certain embodiments, the second compound is Fingolimod. In certain embodiments, the second compound is tetrahydrocannabinol.

[0049] In certain embodiments, the prostate cancer is castration resistant prostate cancer.

[0050] In certain embodiments, the method further includes administering to the subject one or more other anti-cancer agents.

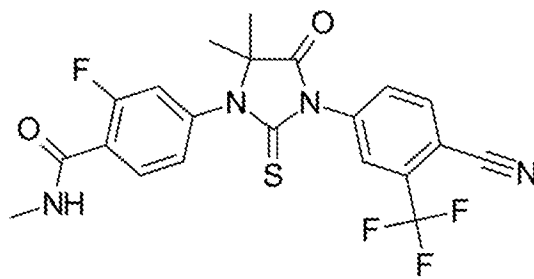
[0051] In certain embodiments, the one or more other anti-cancer agents comprise a chemotherapeutic agent.

[0052] 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), Lonaferon (SCH 66336), Sorafenib (BAY43-9006, Bayer Labs), and Gefitinib (IRESSA®, AstraZeneca), AG1478, AG1571 (SU 5271; Sugen), alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; 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 trimethylmelamine; 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 ranimustine; 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, carabacin, caminomycin, carzinophilin, chromomycins, 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, mepitiothane, 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, Schaumburg, 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.

[0053] In yet another aspect, the invention generally relates to 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 selected from an inhibitor of an inhibitor SPHK and an activator of ceramide, 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.

[0054] 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.

[0055] 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.

[0056] In certain embodiments, the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.

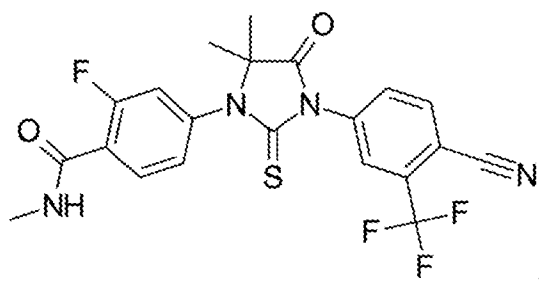
[0057] In certain embodiments, the prostate cancer is castration resistant prostate cancer.

[0058] In certain embodiments, the second compound is SKI2, Safingol, Fingolimod or tetrahydrocannabinol. In certain embodiments, the second compound is SKI2. In certain embodiments, the second compound is Safingol. In certain embodiments, the second compound is Fingolimod. In certain embodiments, the second compound is tetrahydrocannabinol.

[0059] In yet another aspect, the invention generally relates to 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 SPHK or an activator of ceramide, 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.

[0060] In certain embodiments, the resistance is to a compound having Formula (I)



(I)

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

[0061] In certain embodiments, the compound is SKI2, Safingol, Fingolimod or tetrahydrocannabinol.

[0062] The following examples are meant to be illustrative of the practice of the invention, and not limiting in any way.

Examples

SIP-Ceramide axis is altered significantly in EnzR CRPC cells

[0063] As ARv7 has promising human clinical data to support its contribution to the development of Enz resistance, several new therapeutic approaches *via* direct targeting the ARv7, including ASC-J9, Resveratrol, Galeterone, UT-155, Niclosamide and ARv-771 have been developed and tested in human clinical trials. However, some of them already failed in their clinical trials and others still need more clinical trials before they can be used to treat the EnzR CRPC patients.

[0064] It was decided to search those FDA-approved drugs that could target the ARv7 downstream targets instead of direct targeting the ARv7. First, the RNAseq assay was applied to compare the whole-transcriptome between EnzS1-C4-2 and EnzR1-C4-2 cells, and picked up those genes whose expression are significantly altered in EnzR1 cells (Fold log₂>1.8) (**FIG. 1A**). Next, signal pathway analysis was performed based on the database of Pathways common

pathway, and found 21 genes linked to the sphingosine-1-phosphate (S1P) signals, with most of them are S1P downstream genes that belong to the S1P-receptor-1 (S1PR1) sub-pathways, suggesting that S1P signals are activated in EnzR1 cells (**FIG. 1B**).

[0065] To confirm these initial key findings, next performed was the GSEA analysis, and results also found that in the EnzR cells, the sphingolipid pathway is highly activated (**FIG. 1C-1D**). As early studies suggested that the S1P signals could act as enhancer to increase the cell proliferation, and in contrast, ceramide, which is precursor of S1P, is the key metabolite to promote the cell death, it was therefore hypothesize that the balance between S1P and ceramide (named as S1P-ceramide rheostat) may play key roles to control the cell fate.

[0066] The above bioinformatic results were further confirmed *via* direct assaying the concentration of S1P and ceramide. The results revealed that higher S1P concentration was detected in the EnzR1-C4-2 cells as compared to the parental EnzS1-C4-2 cells. In contrast, little change was found for the ceramide concentration in the EnzR1-C4-2 cells *vs* EnzS1-C4-2 cells (**FIG. 1E**). Similar results were also obtained when EnzR1-C4-2/EnzS1-C4-2 cells were replaced with EnzR4-C4-2B/EnzR4-C4-2B cells (**FIG. 1E**). To link higher level of S1P to the Enz-induced Enz resistance in the EnzR cells, the potential impacts of altering the S1P concentration to the Enz sensitivity were then examined. The results revealed that adding 1uM S1P led to decrease the Enz sensitivity in the EnzS1-C4-2 cells (**FIG. 1F**).

[0067] Together, results from **FIG. 1A-1F** suggest that altered S1P-ceramide rheostat with higher S1P concentration was found in the EnzR1 cells.

[0068] Human clinical sample survey to confirm that S1P-ceramide rheostat is significantly altered in the CRPC patients received Enz treatment. As shown in **FIG. 1G**, the ADT, including abiraterone and Enz, increased the S1P levels in patients' plasma. Interestingly, although there are only 4 Enz treated patients' samples, the S1P level show most significantly increase in these samples.

Table 1. S1P downstream genes are highly activated in Enz-R cells

gene	sample_1	sample_2	status	value_1	value_2	log2	p_value
IGFBP3	MDV-R	C4-2_DMSO	OK	137.951	0.808658	-7.41441	0
IGFBP1	MDV-R	C4-2_DMSO	OK	0.663961	0.03473	-4.25683	0.000636
SGK1	MDV-R	C4-2_DMSO	OK	37.5044	2.1524	-4.12304	2.84E-09
SH3GL2	MDV-R	C4-2_DMSO	OK	1.25523	0.073714	-4.08986	3.37E-13
CCL2	MDV-R	C4-2_DMSO	OK	4.63409	0.305077	-3.92504	1.50E-10
AKAP5	MDV-R	C4-2_DMSO	OK	4.32974	0.332146	-3.70439	0.004069
ETS1	MDV-R	C4-2_DMSO	OK	5.06085	0.405684	-3.64095	4.44E-16
TIAM1	MDV-R	C4-2_DMSO	OK	1.06102	0.088181	-3.58884	0.001662
PLD1	MDV-R	C4-2_DMSO	OK	3.05819	0.349476	-3.12941	0.000216
BMP7	MDV-R	C4-2_DMSO	OK	36.4789	4.20691	-3.11623	0
DUSP5	MDV-R	C4-2_DMSO	OK	6.60501	0.936791	-2.81776	0
HMOX1	MDV-R	C4-2_DMSO	OK	119.197	17.0347	-2.8068	0
LGALS1	MDV-R	C4-2_DMSO	OK	61.0068	12.2747	-2.31328	0
HMGCS2	MDV-R	C4-2_DMSO	OK	152.89	32.0491	-2.25414	0
CHRD1	MDV-R	C4-2_DMSO	OK	2.85056	0.6141	-2.2147	1.28E-10
CYFIP2	MDV-R	C4-2_DMSO	OK	26.2638	5.82591	-2.17252	0.00033
ODC1	MDV-R	C4-2_DMSO	OK	1267.46	284.046	-2.15775	0
GRB14	MDV-R	C4-2_DMSO	OK	12.1828	2.76257	-2.14076	0.000414
BATF3	MDV-R	C4-2_DMSO	OK	5.54632	1.25808	-2.14031	4.22E-06
CDKN2A	MDV-R	C4-2_DMSO	OK	21.3282	5.26463	-2.01836	5.04E-06
EDN2	MDV-R	C4-2_DMSO	OK	15.5975	4.068	-1.93892	1.44E-11

Re-sensitize the Enz-resistance in the EnzR cells via reversing the S1P-Ceramide rheostat through reducing the S1P concentration with Fingolimod or SKI2

[0069] To test whether targeting the Enz-altered S1P-ceramide rheostat in EnzR cells can lead to reverse Enz resistance, FDA-approved Fingolimod was applied which could suppress the S1P production and S1PR activation to confirm the above results, and results revealed that suppressing S1P led to increase significantly the Enz sensitivity in the EnzR1-C4-2 cells (**FIG. 2A**). Similar results were also obtained when Fingolimod was replaced with another sphingosine kinase (SPHK) inhibitor SKI2, showing suppressing S1P also led to re-sensitize the Enz-resistance to further suppress the EnzR1-C4-2 cell growth (**FIG. 2B**). As expected, similar

results were also obtained when EnzR1-C4-2 cells were replaced with the EnzR4-C4-2B cells (**FIG. 2c**).

[0070] Interestingly, it was found that both Fingolimod and SKI2 could only suppress the cell growth in the EnzR1-C4-2 and EnzR3-CWR22Rv1, and not in the EnzS1-C4-2 cells (**FIG. 2E-2F**), suggesting that suppressing S1P signals with these two inhibitors is rather specific to the CRPC cells that already developed the Enz resistance.

Re-sensitize the Enz-resistance via reversing the S1P-ceramide rheostat through increasing the ceramide concentration/reducing S1P level with marijuana-CBD/THC

[0071] In addition to alter S1P-ceramide rheostat *via* reducing the S1P concentration, S1P-ceramide rheostat can also be altered *via* increasing the ceramide concentration. The consequences of both approaches can all lead to reduce the “ratio of S1P to ceramide”, which may then lead to alter the cell fate, including Enz sensitivity.

[0072] First found was that direct adding the ceramide (to reduce the S1P-ceramide ratio) led to increase the Enz-sensitivity to further suppress the EnzR1 cell growth (**FIG. 2G**). Similar results were also obtained when the EnzR1-C4-2 cells were replaced with the EnzR4-C4-2B cells (**FIG. 2H**).

[0073] Instead of adding ceramide, cannabidiol (CBD) or tetrahydrocannabinol (THC) was added. The two active components of marijuana can increase the ceramide concentration in various tumors, to the EnzR1-C4-2 and EnzR4-C4-2B cells and results revealed that adding CBD or THC in these two EnzR cells also led to re-sensitize the Enz-resistance (**FIG. 2I-2K**). Similar results were also obtained when MTT growth assay was replaced with Brdu proliferation assay showing adding CBD or THC or Fingolimod could also lead to re-sensitize the Enz-resistance in the EnzR1-C4-2 and EnzR4-C4-2B cells.

[0074] Interestingly, in addition to function *via* increasing ceramide level with activating the Cannabinoid receptor (CB1 and CB2) to increase ceramide to alter the ratio of S1P to ceramide, it was uncovered that adding CBD or THC could also function *via* suppressing SPHK1 activity (**FIG. 2N**) to further reduce S1P level in EnzR cells.

[0075] Together, results from **FIG. 2** suggest that adding marijuana-CBD/THC can also re-sensitize the Enz-resistance *via* increasing ceramide levels/reducing S1P levels to reduce the ratio of S1P to ceramide to further suppress EnzR cell growth.

Targeting the S1P-ceramide axis can also delay the development of Enz resistance in the parental EnzS1-C4-2 cells

[0076] In addition to suppress the CRPC cells that already developed Enz resistance, it was also interesting to see if targeting the S1P-ceramide axis can also delay the Enz development in CRPC cells that are still sensitive to Enz treatment. The EnzS1-C4-2 cells were pretreated with 5 uM Enz for 1 month, and assayed their Enz sensitivity. The results revealed that these cells sensitivity to 10 uM Enz decreased from 50% to 17%, suggesting that long term treatment of Enz could reduce the PCa cells sensitivity to Enz. (**FIG. 2P**)

[0077] Next, the Enz was combined with marijuana-CBD or SKI2 to pretreat the EnzS1-C4-2 cells for 1 month, and then assayed their sensitivity to Enz. The results revealed that adding marijuana-CBD or SKI2 to alter the S1P-ceramide axis can significantly increase the EnzS1-C4-2 cells sensitivity to Enz as compared to the long-term treatment of Enz only (SKI2-40%, CBD-35%) (**FIG. 2Q-2R**).

[0078] Together, results from (**FIG. 2P-2R**) suggest that that targeting the S1P-ceramide axis with either marijuana-CBD or SKI2 can also delay the development of Enz resistance in the parental EnzS1-C4-2 cells

Mechanism dissection of why Enz can alter the S1P-ceramide rheostat via increasing S1P: via increasing the SPHK1 activity in EnzR cells

[0079] To dissect the mechanism of how Enz can alter the S1P-ceramide rheostat *via* increasing S1P, several key enzymes that are involved in the sphingolipid's metabolism *via* the RNAseq were searched for. It was found that the mRNA expression of ACER1, SPHK1/2 and CERK increased significantly in EnzR1 cells. However, the expression of these four proteins (ACER1, CERK and SPHK1/2) failed to increase in EnzR1 cells (**FIG. 3A**). The SPHKs activity became the focus since it is critical for the S1P generation. As shown in **FIG. 3B**, the SPHK activity enhanced in the EnzR1 cells. To study if Enz can impact the SPHKs activity, EnzS1 cells

were treated with Enz for 10 days, and found long term treatment with Enz enhanced the SPHKs activity (**FIG. 3C**), suggesting that Enz can alter SPHKs activity to increase S1P levels that led to alter the S1P-ceramide rheostat.

[0080] SPHKs (including SPHK1 and SPHK2) can phosphorylate sphingosine to generate S1P⁹ to increase PCa cell growth and survival, and higher SPHK1 can also alter the radiation sensitivity to suppress PCa growth. The S1PRs modulator Fingolimod treatment can suppress SPHK1/S1P/S1PR1 axis and can sensitize PCa cells to Radiation. To explore which SPHK (1 or 2) can play the key function to alter the Enz sensitivity, either the SPHK1 inhibitor (PF543) or SPHK2 inhibitor (ABC294640) with Enz was added to assay their impact on the Enz sensitivity to suppress the EnzR1-C4-2 cell growth. The results revealed that only PF543 could increase the Enz sensitivity to better suppress the EnzR1-C4-2 cell growth (**FIG. 3D**), suggesting that SPHK1 is the key enzyme to impact the Enz sensitivity.

[0081] The SPHK1 serine 225 phosphorylation in EnzS1 and EnzR1 cells was also analyzed since early reports showed that the p-S225 level is an indicator for SPHK1 activity, and found the SPHK1 p-S225 levels increased in EnzR1 cells, suggesting that SPHK1 activity is increased in EnzR1 cells. (**FIG. 3E**). Finally, it was found that suppress SPHK1 activity *via* adding SPHK1-shRNA could increase significantly Enz sensitivity in EnzR1-C4-2 cells (**FIG. 3F**).

[0082] Together, the results of **FIG. 3A-3F** suggest that increase SPHK1 activity is the key step to increase the S1P concentration to promote the Enz resistance in the EnzR cells.

Mechanism dissection of why Enz can increase the SPHK1 activity in the EnzR cells: via suppressing the SPHKAP expression

[0083] To further study how Enz can alter the activity of SPHK1, searches were done for any factors that can alter the SPHK1 activity, and found the SPHK1 Interactor, AKAP Domain Containing protein (SPHKAP) was decreased significantly in EnzR cells and adding Enz can decrease SPHKAP expression in EnzS1 cells (**FIG. 4A-4B**). To further confirm this key finding, the human PDX PCa samples (PDX-133-4C) were then implanted into SCID mice and treated the tumor bearing mice with Enz for 20 days. The results also confirm the *in vitro* cell lines results showing adding Enz can suppress dramatically the SPHKAP expression in the PDX tumors (**FIG. 4C**).

[0084] Since SPHKAP was identified as a SPHK1 co-regulator, which can suppress SPHK1 activity, studies were performed to see if Enz treatment can alter SPHKAP to change SPHKs activity. Results showed that suppressing the SPHKAP in EnzS1 cells resulted in higher SPHKs activity, with increase the S1P levels (FIG. 4D-4E).

[0085] Importantly, it was found that increased the SPHKAP expression *via* adding the SPHKAP-cDNA in EnzR1-C4-2 cells also led to increase the Enz sensitivity to suppress the EnzR1-C4-2 cell growth (FIG. 4F). In contrast, decreased SPHKAP expression *via* adding SPHKAP-shRNA in the parental EnzS1-C4-2 cells led to significantly decreased the Enz-sensitivity to suppress the cell growth (FIG. 4G).

[0086] As the previous results showed that CBD or Fingolimod can increase Enz sensitivity in EnzR1 & EnzR4 cells, it was tested whether both compounds have the same effects on EnzS1-shSPHKAP cells and results showed that treating the EnzS1-shSPHKAP cells with CBD or Fingolimod can all enhance Enz sensitivity (FIG. 4H).

[0087] Together, results from FIG. 4A-4H suggest that Enz can function *via* suppressing SPHKAP expression to increase the SPHK1 activity to enhance the S1P concentration, which may then lead to increase the Enz resistance.

Human clinical sample survey to support the SPHKAP negative roles to promote the PCa progression.

[0088] To further support the above *in vitro* cell lines results to the human clinical relevance, clinical survey *via* TCGA database analysis was performed. The results revealed that SPHKAP mRNA expression is negatively correlated with Gleason score and metastasis status (FIG. 4I). Results from The Human Protein Atlas also suggested that SPHKAP protein expression is lower in PCa samples than in the normal prostate tissues (FIG. 4J).

[0089] Results from our human tissue micro-array also demonstrated that SPHKAP protein expression is negatively correlated with Gleason score (FIG. 4K).

[0090] Together, results from multiple human clinical sample surveys (FIG. 4I-4K) all suggest that SPHKAP may play negative roles to promote the PCa progression.

[0091] In summary, the results from multiple *in vitro* cell lines, *in vivo* mouse model and human clinical sample surveys all suggest that Enz may function *via* altering the

SPHKAP/SPHK1 axis to change the S1P level to impact the S1P-ceramide rheostat to increase the Enz resistance.

Mechanism dissection of how Enz can suppress SPHKAP expression: via increasing the ARv7 expression

[0092] To further understand the mechanism of how Enz can alter SPHKAP expression, key factors were assayed first, including ARF876L, ARv7, and GR that have been linked to development of Enz-resistance. The WB data showed that only ARv7 could significantly reduce SPHKAP expression in EnzS cells (**FIG. 5A**) and knocking down ARv7 can increase SPHKAP expression in EnzR cells (**FIG. 5B**). The results from the Elisa assay also demonstrated that only adding ARv7 could increase the S1P production (**FIG. 5C**) and in contrast, suppressing ARv7 in EnzR1-C4-2 cells can significantly decrease S1P concentration (**Fig. 5D**). The Enz-induced ARv7 has the most convincing human clinical evidence, which shows CRPC patients who have higher ARv7 have more resistance to Enz therapy and CRPC patients who received Enz had higher ARv7 expression⁸.

[0093] Together, results from **FIG. 5A-5D** suggest that Enz may function *via* increasing the ARv7 to suppress SPHKAP expression.

Mechanism dissection of how Enz-increased ARv7 can suppress SPHKAP expression: via altering the miRNA expression

[0094] To further study how Enz-increased ARv7 can suppress SPHKAP expression, it was first noticed that SPHKAP mRNA expression is not altered significantly in the EnzR1-C4-2 cells, suggesting that Enz may regulate mainly the SPHKAP expression at the protein level (**FIG. 5E**). Focus was placed on the miRNAs, since recent studies indicated that miRNAs might play important roles to regulate the gene expression at the protein level.

[0095] By Ago2 IP assay, it was found that in EnzR1-C4-2 cells, the binding of SPHKAP mRNA and Ago2 increased, suggesting that some miRNAs may be involved in the SPHKAP is downregulated SPHKAP in the EnzR1-C4-2 cells (**FIG. 5F**).

[0096] To explore which miRNAs regulate SPHKAP expression, the potential miRNAs were screened to check which can target SPHKAP from 3 miRNA databases and then compared

their expression in EnzS1/4 and EnzR1/4 cells (**FIG. 5G**). Among all candidates, miR-6731-3p and miR-6832-3p both increase in EnzR (1&4) cells (**FIG. 5H**). Overexpression of ARv7 in EnzS1 cells can induce both miR-6731-3p and miR-6832-3p.

Mechanism dissection of how Enz/ARv7/miRNA/SPHKAP/SPHK1 axis altered S1P-ceramide rheostat can lead to increase the Enz sensitivity: via inducing the methuosis

[0097] To dissect the mechanism of how Enz/ARv7/SPHKAP/SPHK1 axis altered S1P-ceramide rheostat can lead to increase the Enz sensitivity, attention was paid to those related cell proliferation and cell death to the Enz treatment. It was noticed that adding Marijuana-CBD/THC, or SKI2/Fingolimod to increase the Enz sensitivity also led to an significant morphology changes in the EnzR1-C4-2 cells vs EnzS1-C4-2 cells: it was noticed an significant inducing cell swelling and visible vacuoles accumulation occurred only in the EnzR1-C4-2, and not in the EnzS1-C4-2 cells (**FIG. 6A**). Importantly, also observed were similar morphology changes in the EnzR4-C4-2B vs EnzS4-C4-2B cells (**FIG. 6A**).

Together, results from **FIG. 6A-B** suggest that some selective cell death⁴¹ may be involved.

[0098] To further study what kind of the cell death is induced *via* altering the S1P-ceramide rheostat in EnzR cells, the inhibitors were applied for apoptosis (zVAD), autophagy (chloroquine), and macropinocytosis (EIPA), and found only the EIPA could block the SKI2-induced cell death in EnzR cells, suggesting that methuosis may be the cell death induced by SKI2. (**FIG. 6C**). However, it was also found that all three inhibitors can partially suppress the Marijuana-THC induced cell deaths, suggesting that besides methuosis, THC may also function *via* multiple signals to repress the EnzR cell growth.

[0099] Importantly, electronic microscope was applied to confirm our findings, and results revealed that combination of Marijuana-THC or SKI2 with Enz could significantly increase the number of macropinosomes, which agrees with above results (**FIG. 6A-6C**) and suggest that Marijuana-THC or SKI2 may function *via* altering the methuosis to increase the Enz sensitivity to suppress cell growth in EnzR cells.

[00100] Lucifer yellow (LY) absorption assay was applied in EnzR cells, since the fast absorption the LY is the hallmark of the methuosis, and results revealed that treating with SKI2, Fingolimod, and marijuana-THC all led to enhance the LY absorption (**FIG. 6E**). Furthermore,

the fluorescent microscopic images also showed that LY located inside the vacuoles, suggesting these vacuoles are macropinosomes (**FIG. 6F**). The GFP-Rab7-EnzR1 cells were also established and after treating the cells with SKI2, GFP-Rab7 located on the membrane of the vacuoles was observed, which confirm these vacuoles derived from macropinosomes, since the early reports showed that Rab7 is the key protein to assemble the macropinosome's membrane (**FIG. 6G**).

[00101] Together, results from **FIG. 6A-6G** suggest that targeting S1P-ceramide rheostat with marijuana-THC or SKI2, Fingolimod can lead to increase the Enz sensitivity *via* inducing the methuosis.

Mechanism dissection of how targeting the Enz/ARv7/SPHKAP/SPHK1 axis altered S1P-ceramide rheostat S1P-Ceramide can induce cell methuosis

[00102] Finally, to dissect the mechanism of how targeting the Enz/ARv7/SPHKAP/SPHK1 axis altered S1P-ceramide rheostat can induce cell methuosis in the EnzR cells, first checked was the downstream signals which can be regulated by S1P. As shown in **FIG. 6H**, in EnzR1 cells, Rac1 activity was increased significantly, and several Rac1 target genes expression were upregulated in EnzR1 cells (**FIG. 6I**). Interestingly, early reports also indicated that Rac1 is the key protein to regulate the maturation of macropinosomes, and interruption of Rac1 activity would induce methuosis.

[00103] To further investigate the role of Rac1, EnzR1 cells were treated with Marijuana-THC, SKI2 or Fingolimod and found out Rac1 activity was suppressed in EnzR cells, which is consistent with our results. Furthermore, increasing Rac activity can then block/reverse the Marijuana-THC- or SKI2-induced cell methuosis.

[00104] Together, results from **FIG. 6h-k** suggested that targeting S1P-ceramide rheostat could reduce the Rac1 activity and promote methuosis.

Experimental

Cell culture

[00105] C4-2, HEK293T and PC-3, 22RV1 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in RPMI 1640 with 10% FBS. C4-2 parental and C4-2 MDVR cell lines were generated. Enz-resistant PCa cells (named C4-2MDVR) via chronic culture of CRPC C4-2 cells in media containing increasing Enz (from 10 nM to 30 nM). All cells were maintained in a humidified 5% CO₂ environment at 37°C. All cell lines were cultured in a 5% (v/v) CO₂ humidified incubator at 37°C. All cell lines have been detected and authenticated as mycoplasma and bacteria free following ATCC's instructions.

Cell Proliferation Assays

[00106] 10000 C4-2, 22RV1 and C4-2 MDVR cells were plated into each well of 24 well-plates, on 0, 2, 4, 6 days, cells were collected, and then MTT assay was performed: 100 µl of 5 mg/ml MTT was added to each well. One set of wells with MTT were included but no cells (control), then incubated for 3 hours at 37 °C in a culture hood, 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

[00107] Sh-ARV7 was constructed into the pLKO.1 lentiviral vector as reported previously. The pLKO.1 sh-SPHKAP together with package and envelope plasmids, psPAX2 and pMD2G, were co-transfected into 293T cells for 48 hours to produce the SPHKAP shRNA lentivirus particle soup. Lentivirus soup was then collected and frozen at -80°C for later use in transduction of PCa cells.

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

[00108] 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, Grand Island, NY). qRT-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.

Western blot analysis

[00109] 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, HRP-conjugated secondary antibodies, and visualized using ECL system (Thermo Fisher Scientific, Rochester, NY). AR, GAPDH, tubulin antibodies were from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). SPHKAP antibody was purchased from GeneTex.

Chromatin Immunoprecipitation Assay (ChIP)

[00110] Cells were crosslinked 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 designed to amplify a target sequence within human SPHKAP promoter; PCR products were analyzed by agarose gel electrophoresis.

Luciferase assay

[00111] PCa cells were plated in 24-well plates and transfected with MMTV-luc containing ARE sequence using Lipofectamine (Invitrogen, Grand Island, NY). After transfection, regular media were added with various DHT concentrations, 0 (ethanol as vehicle control), 1 nM, and 10 nM, and incubated for 48 hrs. pRL-TK was used as internal control. Luciferase activity was measured by Dual-Luciferase Assay (Promega, Madison, WI) according to the manufacturer's manual.

Sphingolipid measurement by HPLC-Mass spectrum

[00112] The sphingolipids in EnzR1, R4 and EnzS1, S4 cells were extracted as previously described. The measurements were performed by HPLC-Mass spectrum by UPMC core-facility.

SPHK1 activity assay

[00113] The SPHKs assay kit was purchased from Echelon Bio, Inc. The procedure was followed by the instructions.

In vivo tumorigenesis assay

[00114] C4-2 parental or C4-2 MDVR cells (4 million) were mixed with Matrigel (1:1) and injected into the prostates of 6- to 7-week old male SCID mice. Tumor-bearing mice were randomized into four groups and treated as follows: (1) vehicle control (5% Tween 80 and 5% ethanol in PBS, i.p.), (2) enzalutamide (25 mg/kg, p.o.), (3) Fingolimod (10 mg/kg, i.p.), (4) enzalutamide (25 mg/kg, p.o.) + Fingolimod (10 mg/kg, i.p.). (5) CBD, (6) Enz + CBD. Tumors were measured using calipers twice a week and tumor volumes were calculated using $\text{length} \times \text{width}^2 / 2$. Tumor tissues were harvested after 3 weeks of treatment.

[00115] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[00116] 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

[00117] 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.

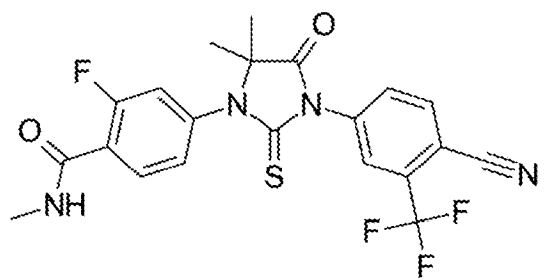
Equivalents

[00118] 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

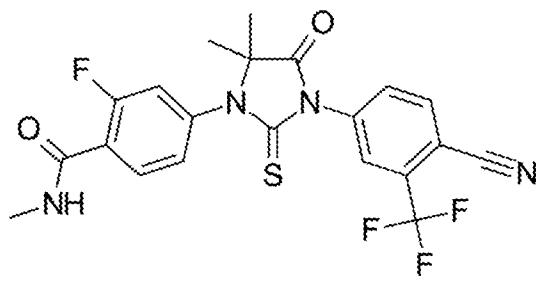
1. A pharmaceutical composition comprising:
a first compound of Formula (I)



(I)

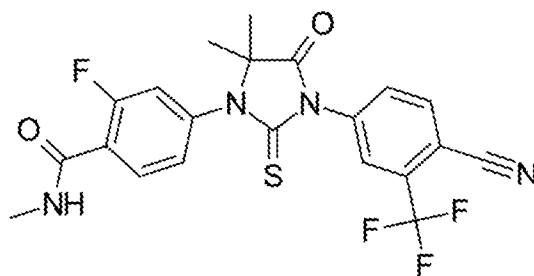
- or a pharmaceutically acceptable salt, ester or pro-drug thereof, and
a second compound selected from an inhibitor of SPHK or an activator of ceramide,
- wherein each of the first and second compounds is present in an amount such that the pharmaceutical composition is effective in the treatment of 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 reducing 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 reversing 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 delaying the onset of 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 delaying the development of 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 selected from the group consisting of SKI2, Safingol, Fingolimod and tetrahydrocannabinol.
9. The pharmaceutical composition of any of claims 1-8, wherein the second compound is selected from the group consisting of SKI2, Safingol, Fingolimod and tetrahydrocannabinol.
10. The pharmaceutical composition of claim 9, wherein the second compound is SKI2.
11. The pharmaceutical composition of claim 9, wherein the second compound is Safingol.
12. The pharmaceutical composition of claim 9, wherein the second compound is Fingolimod.
13. The pharmaceutical composition of claim 9, wherein the second compound is tetrahydrocannabinol.
14. A unit dosage form comprising the pharmaceutical composition of any of claims 1-13.
15. The unit dosage form of claim 14, 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.
16. 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 1-15.
17. 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 SPHK or an activator of ceramide, 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.
18. The method of claim 17, wherein the second compound is administered simultaneously with the first compound.
 19. The method of claim 17, wherein the second compound is administered subsequently after the administration of the first compound.
 20. The method of any of claims 17-19, wherein the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.
 21. The method of any of claims 17-20, wherein the weight ratio of the first compound to the second compound is from about 10 : 1 to about 1 : 10.
 22. The method of any of claims 17-21, wherein the second compound is selected from the group consisting of SKI2, Safingol, Fingolimod and tetrahydrocannabinol.
 23. The method of claim 22, wherein the second compound is SKI2.
 24. The method of claim 22, wherein the second compound is Safingol.
 25. The method of claim 22, wherein the second compound is Fingolimod.
 26. The method of claim 22, wherein the second compound is tetrahydrocannabinol.
 27. The method of any of claims 17-26, further comprising administering to the subject one or more other anti-cancer agents.
 28. The method of claim 27, wherein the one or more other anti-cancer agents comprise a chemotherapeutic agent.
 29. The method of any of claims 17-28, wherein the prostate cancer is castration resistant prostate cancer.
 30. 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 selected from an inhibitor of an inhibitor SPHK and an activator of ceramide, 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.

31. The method of claim 30, 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.

32. The method of claim 30, 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

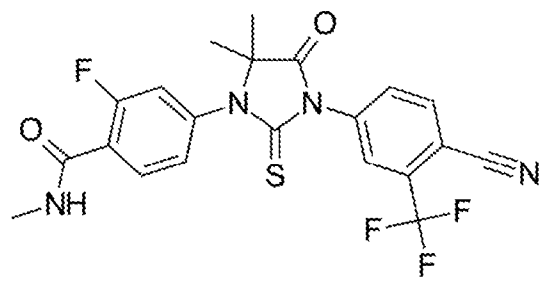
upon the subject being detected of a substantial reduction or disappearance of drug resistance, re-starting the administration of the first compound.

33. The method of any of claims 30-32, wherein the first and/or the second compound is administered orally, intravenously, intramuscularly, or subcutaneously.

34. The method of any of claims 30-33, wherein the prostate cancer is castration resistant prostate cancer.

35. The method of any of claims 30-34, wherein the second compound is SKI2, Safingol, Fingolimod or tetrahydrocannabinol.

36. 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 SPHK or an activator of ceramide, 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.
37. The method of claim 36, wherein the resistance is to a compound having Formula (I)

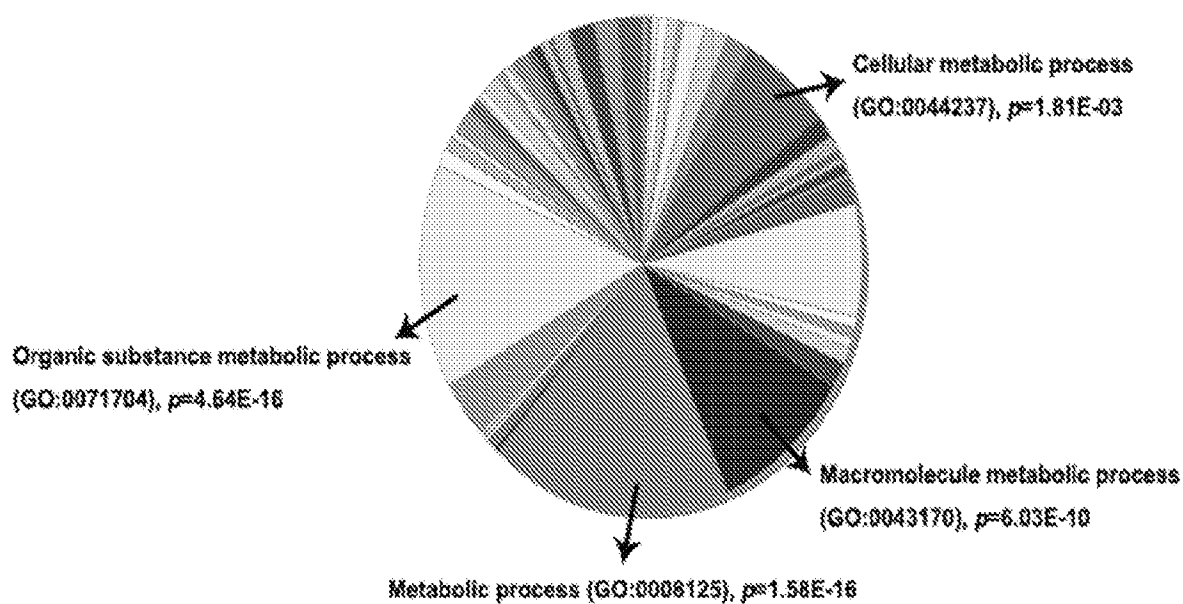


(I)

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

38. The method of claim 36 or 37, wherein the compound is SKI2, Safingol, Fingolimod or tetrahydrocannabinol.

A



B

Database: Pathway Commons Pathway;
Name: Sphingosine 1-phosphate (S1P) pathway;
ID:08_ID:1635
C=1311; D=21; E=8.68; R=2.42; rawP=0.0002; adjP=0.0007

FIG. 1

C

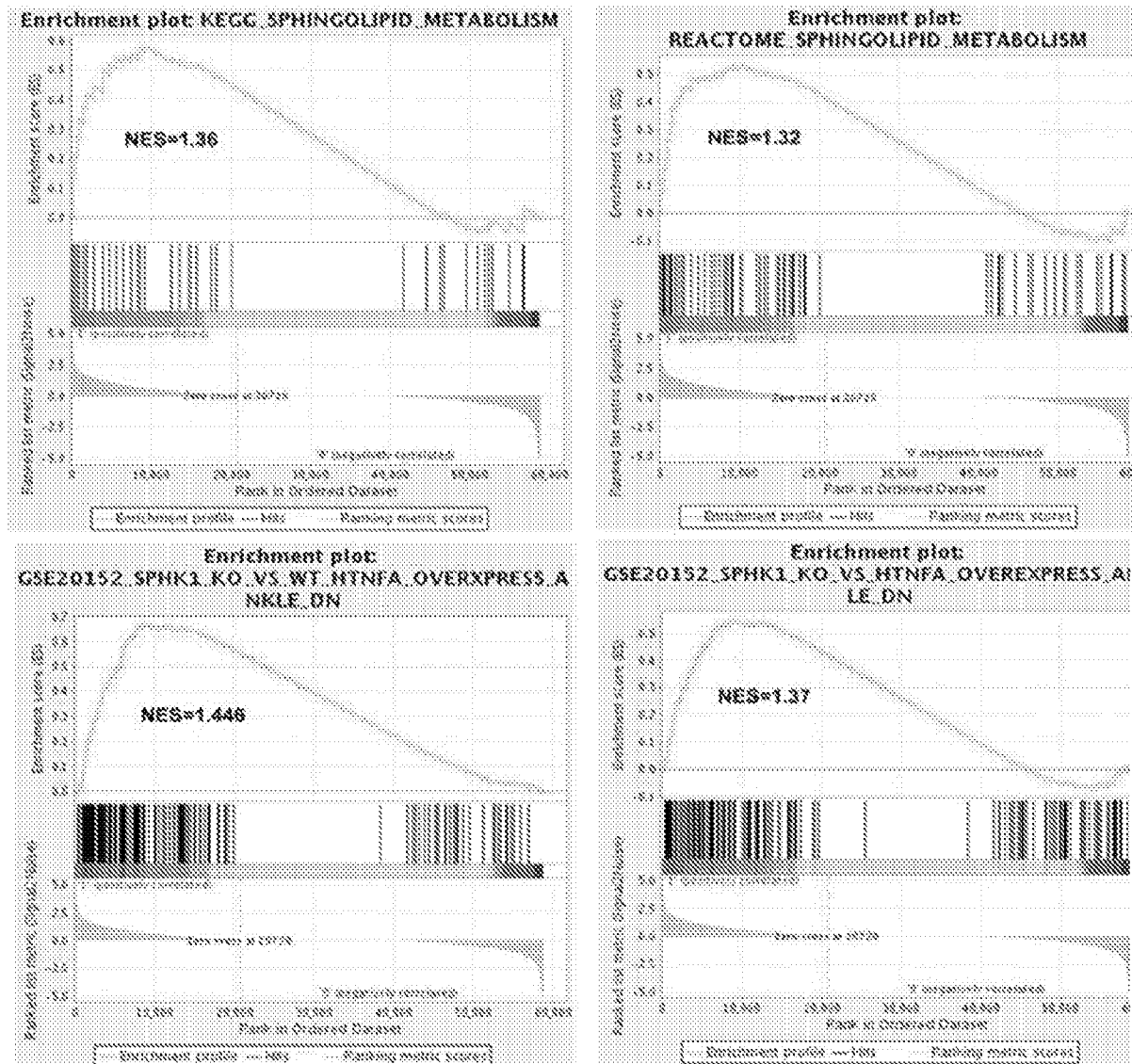
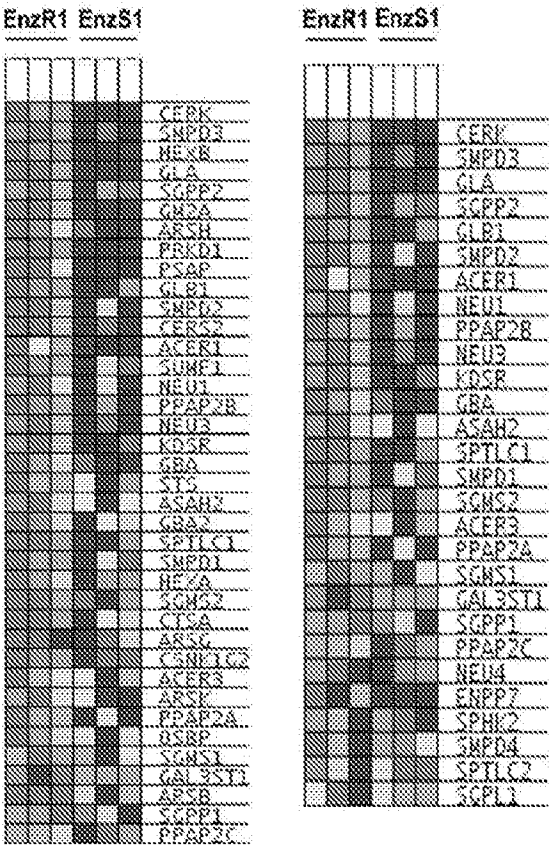


FIG. 1 (Cont'd)

D



E

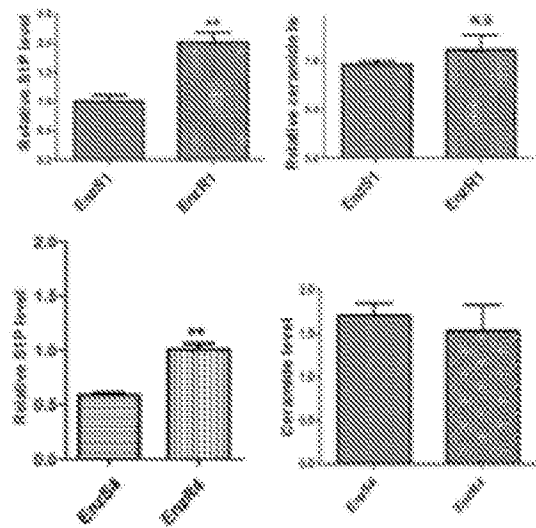
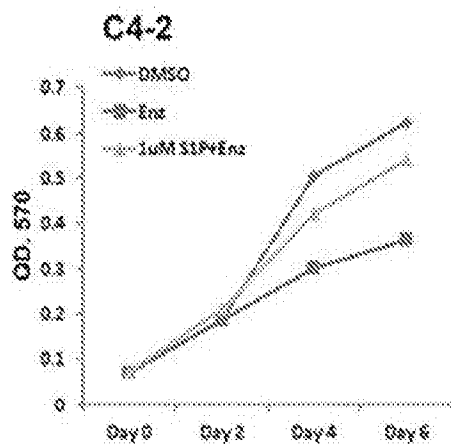


FIG. 1 (Cont'd)

F



G

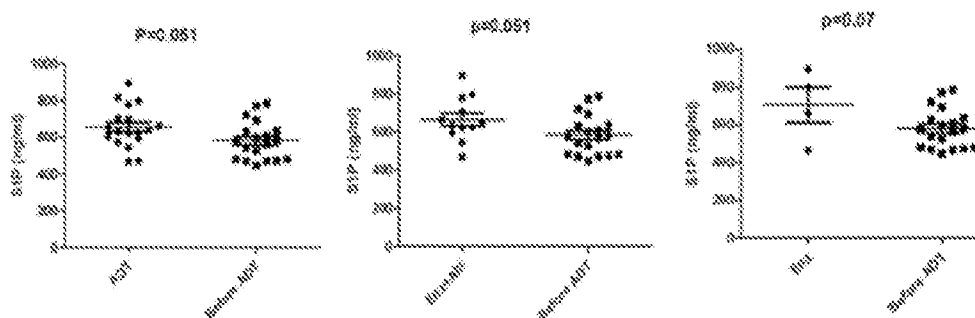


FIG. 1 (Cont'd)

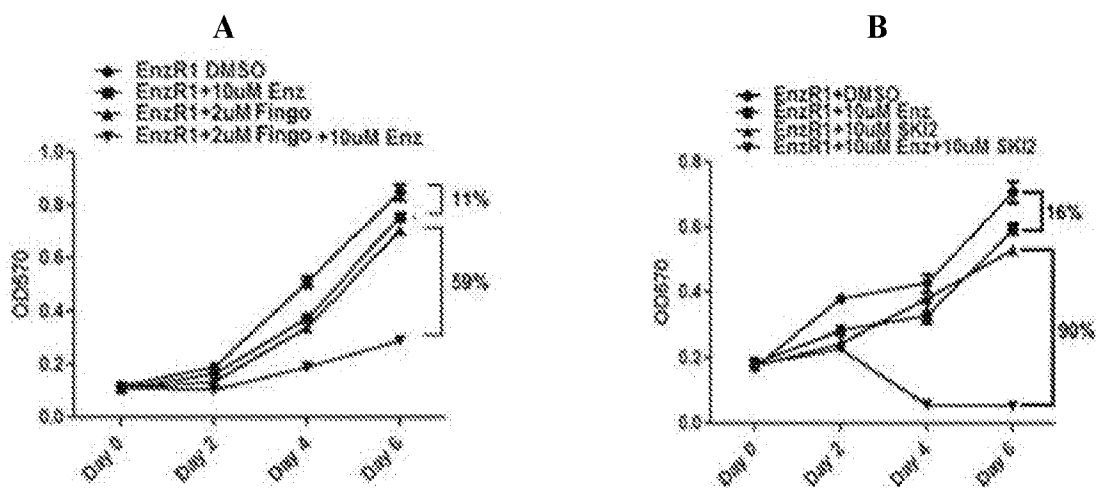


FIG. 2

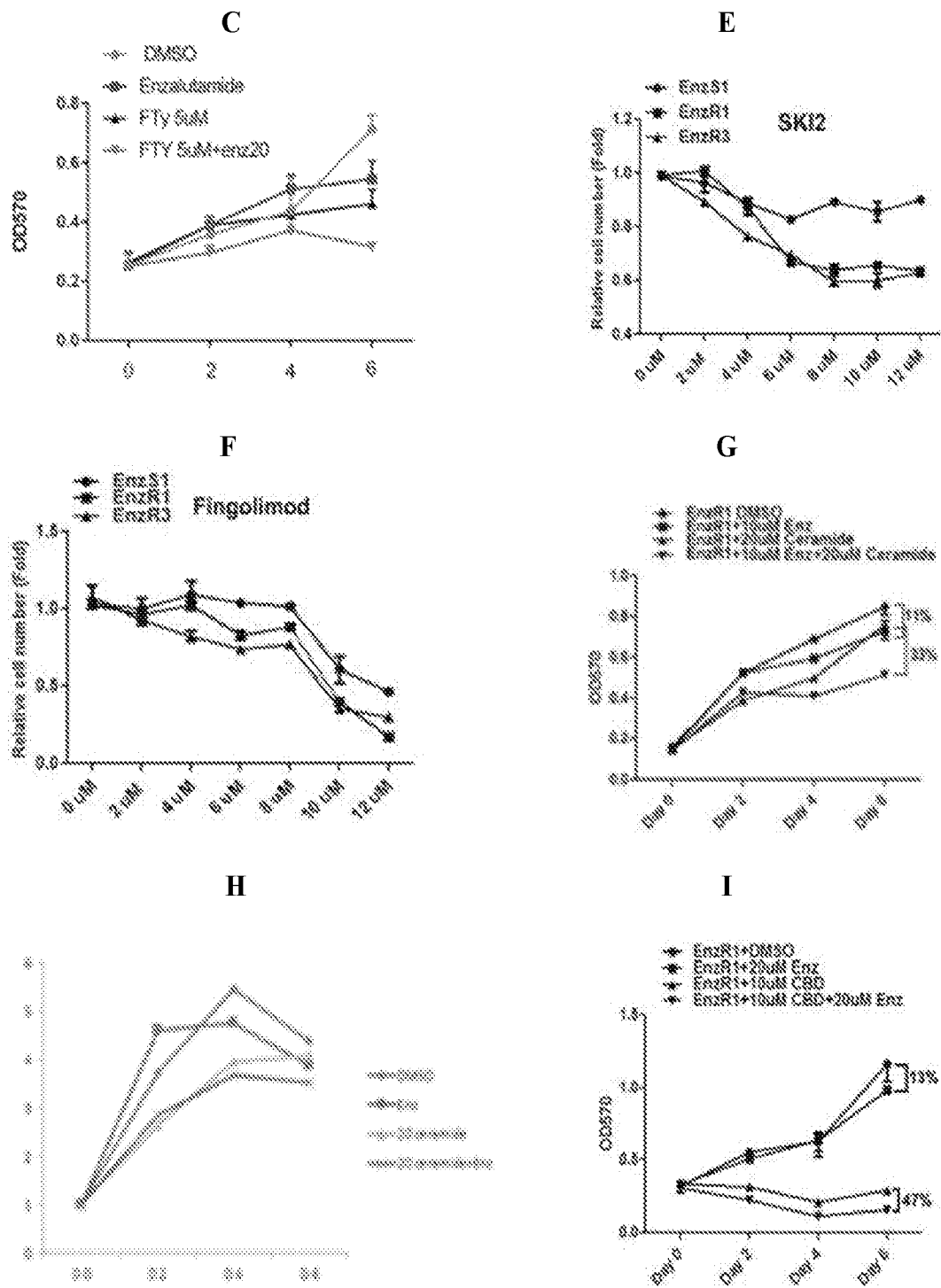


FIG. 2 (Cont'd)

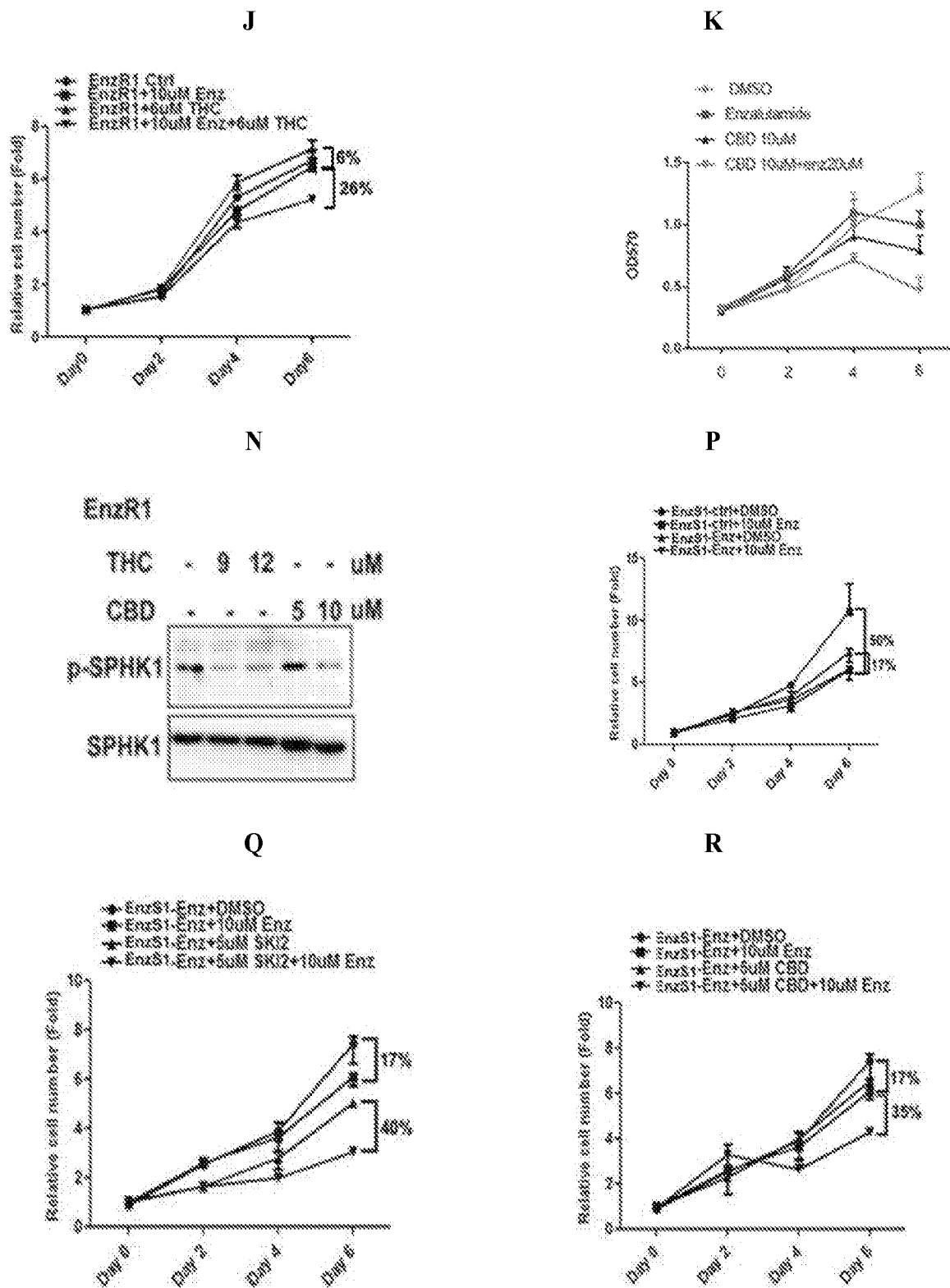


FIG. 2 (Cont'd)

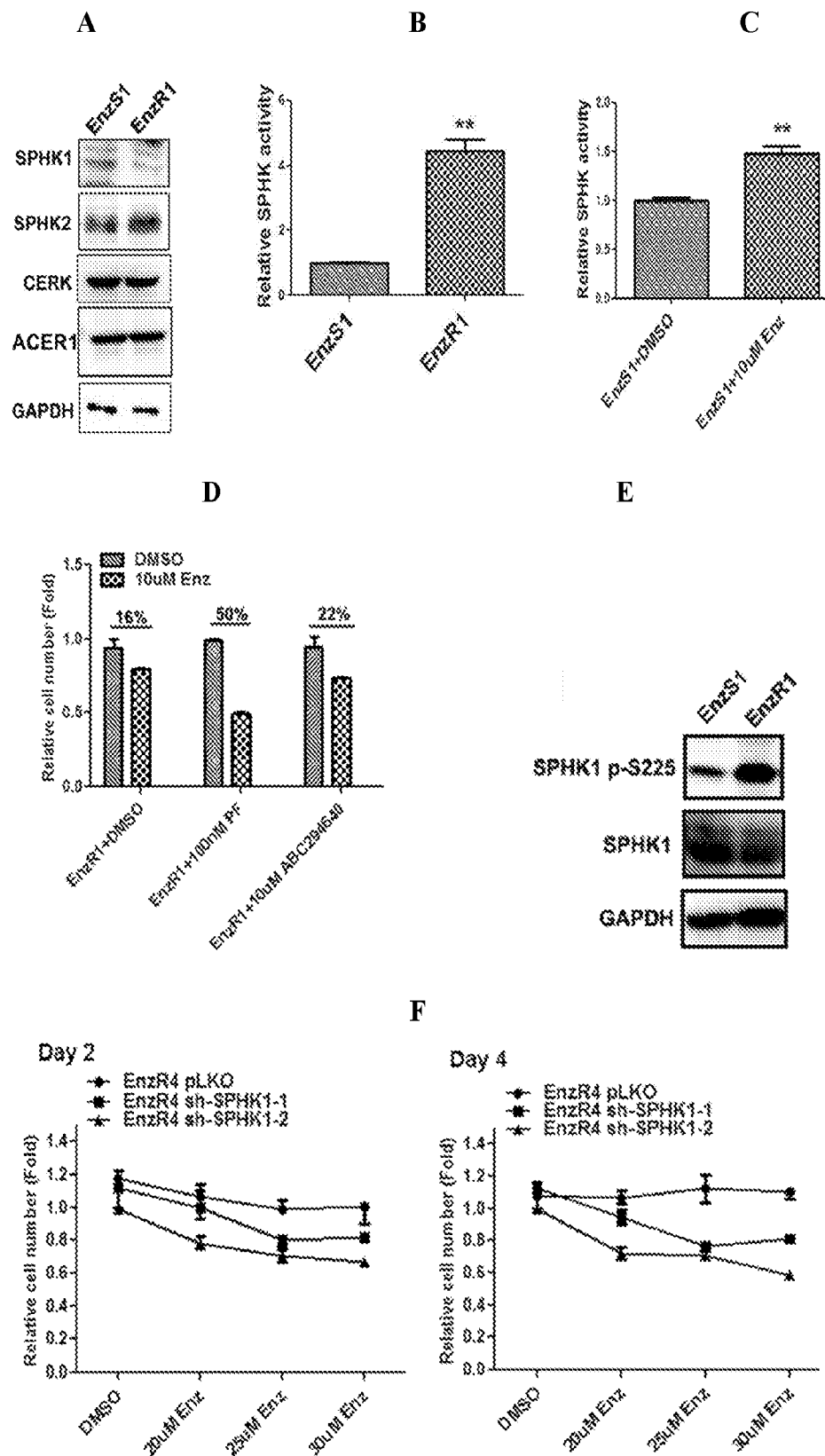


FIG. 3

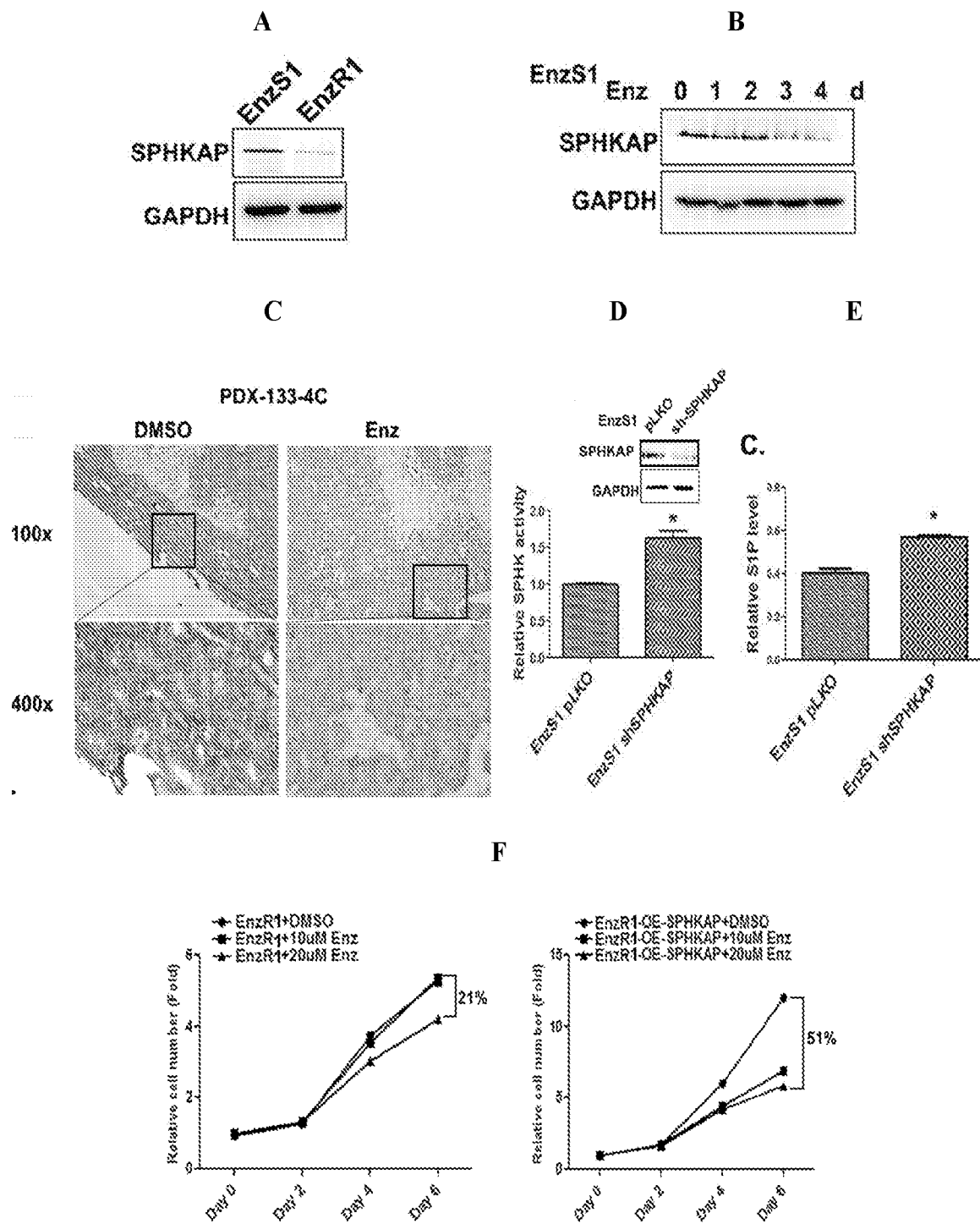
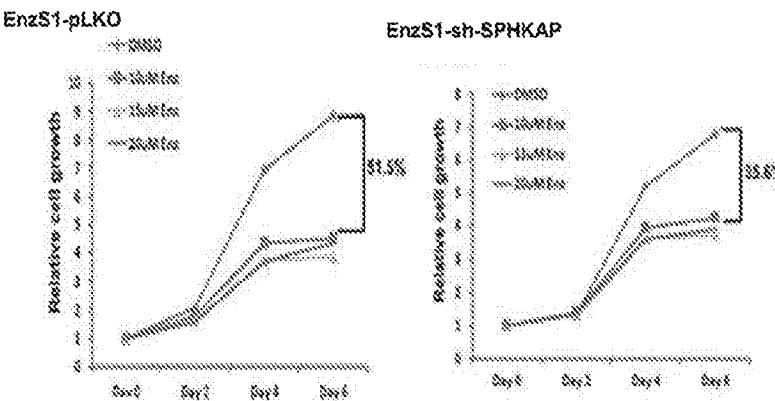
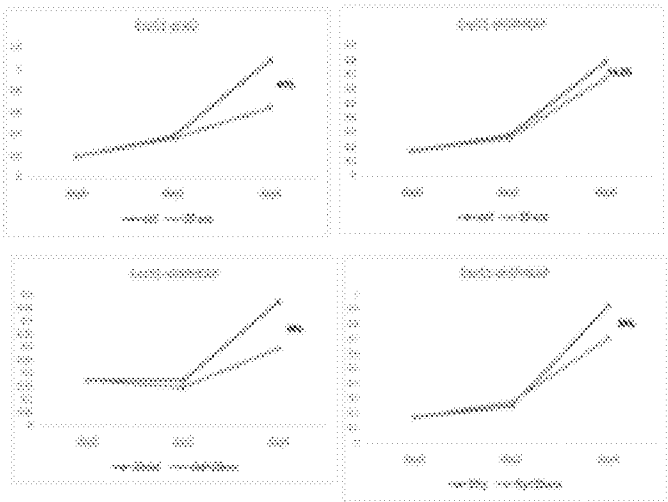


FIG. 4

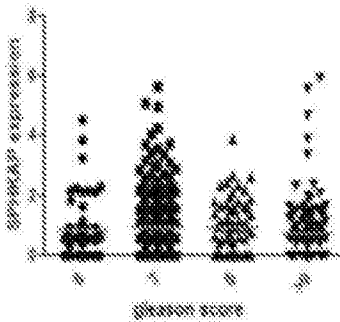
G



H



I



J

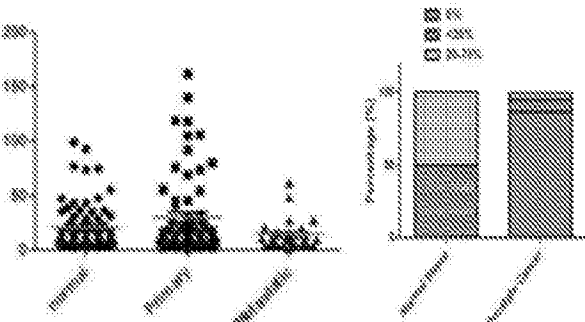


FIG. 4 (Cont'd)

K

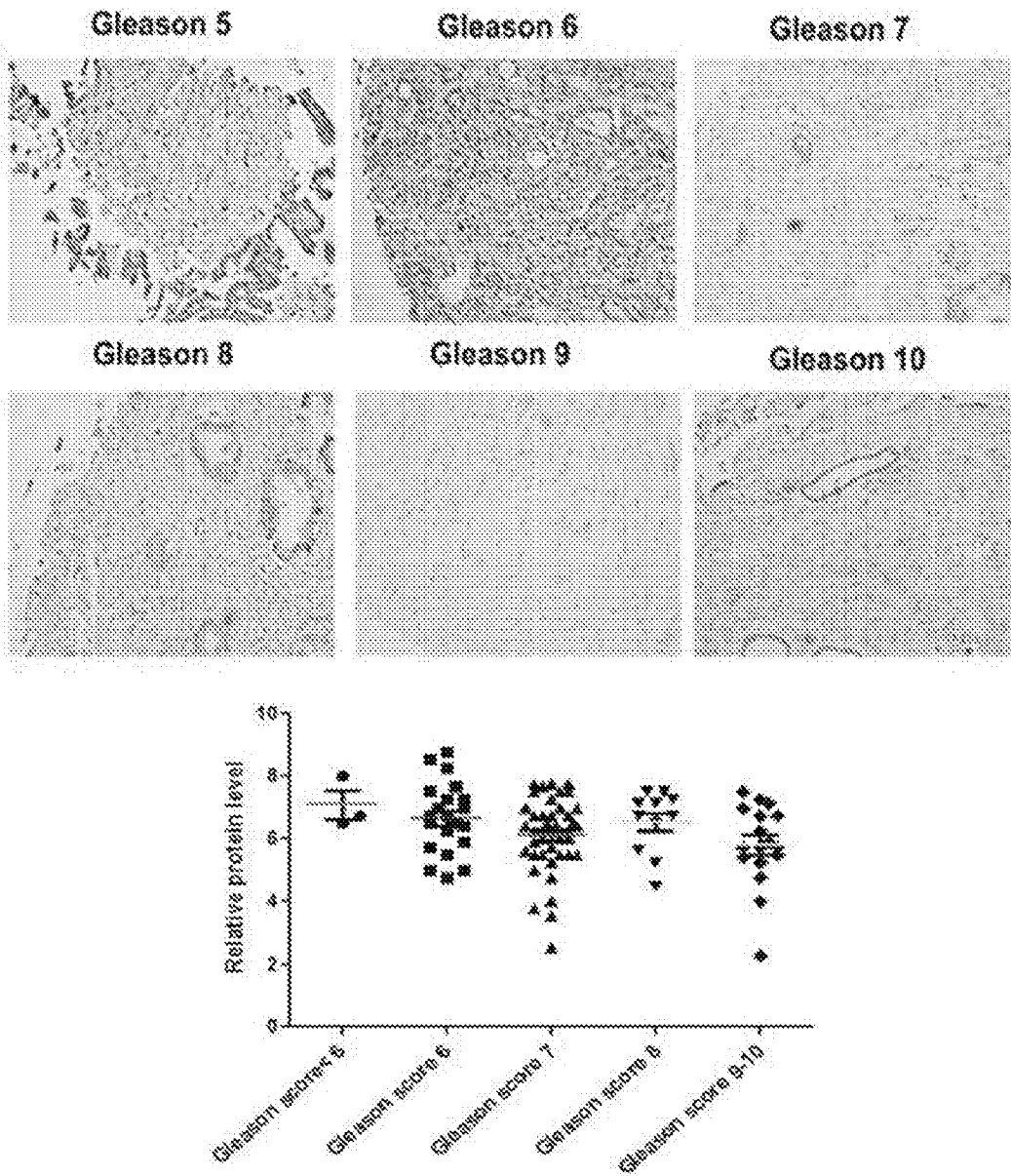


FIG. 4 (Cont'd)

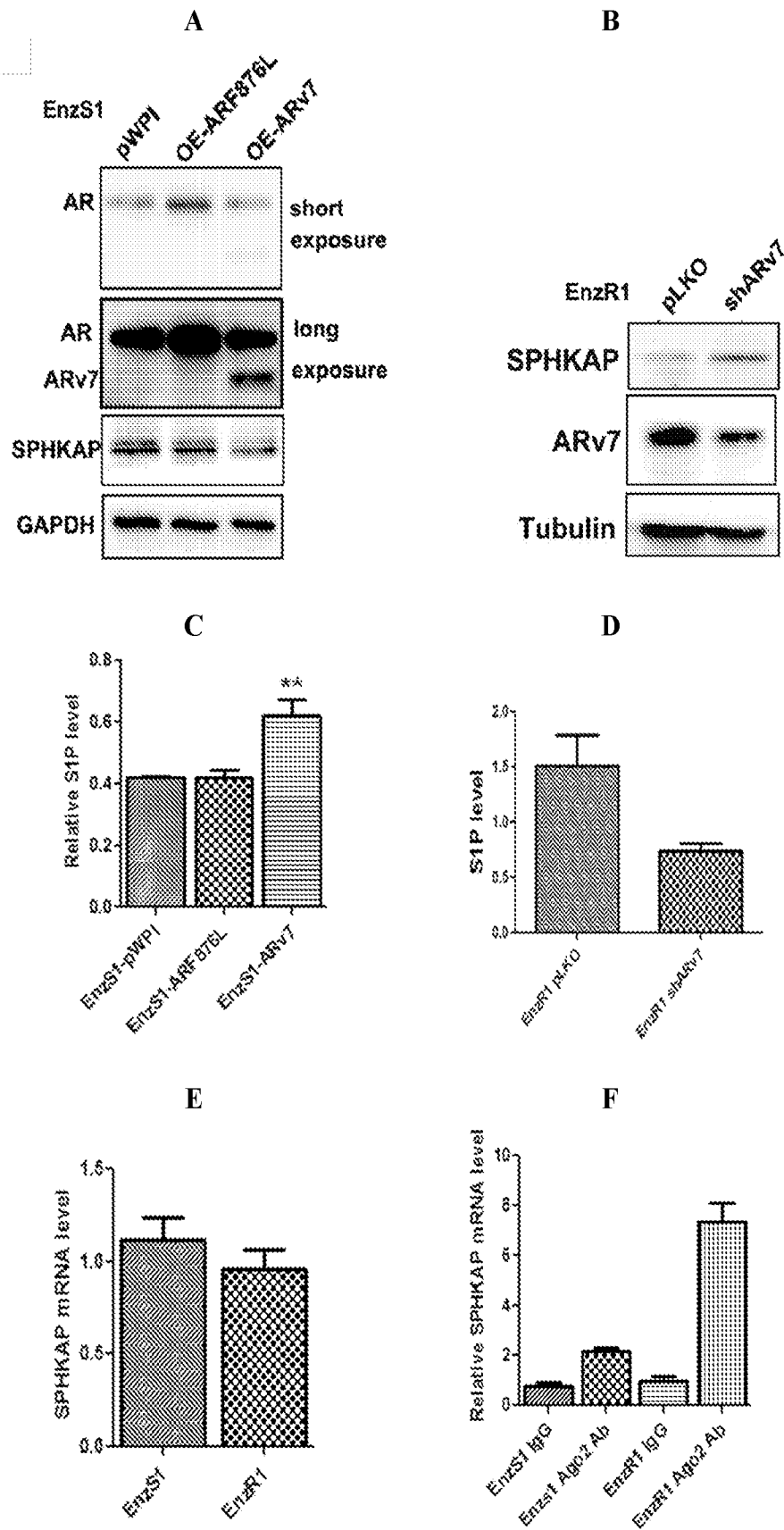


FIG. 5

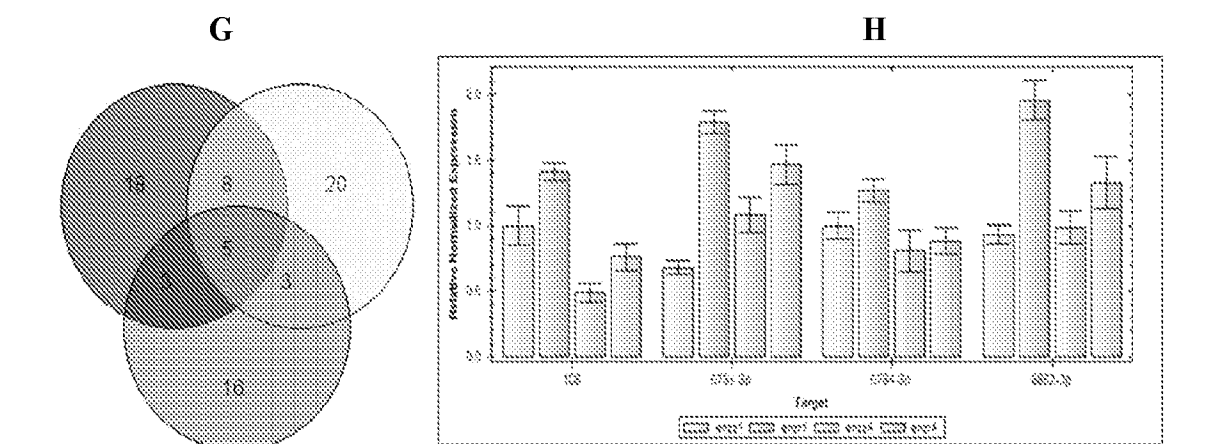


FIG. 5 (Cont'd)

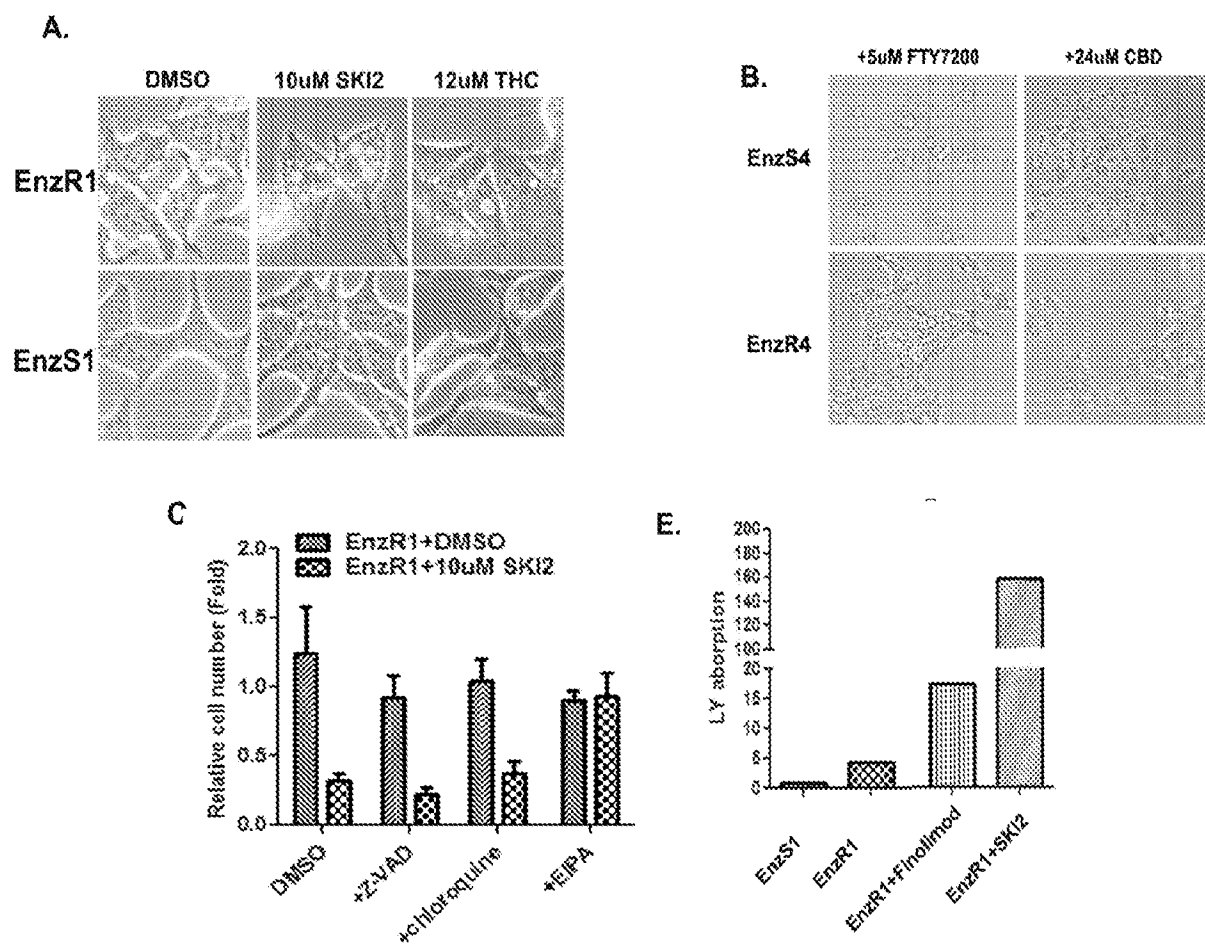


FIG. 6

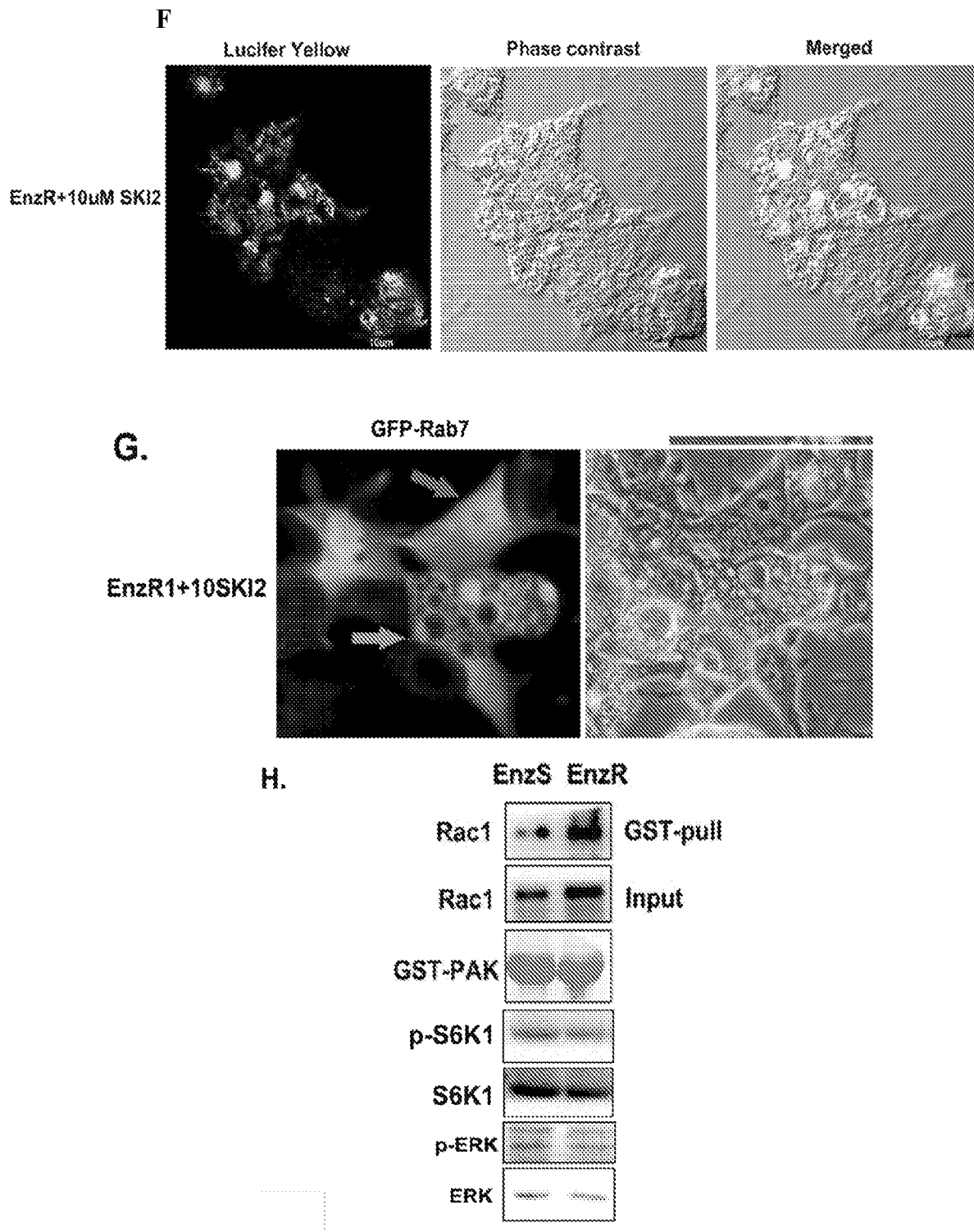


FIG. 6 (Cont'd)

I.

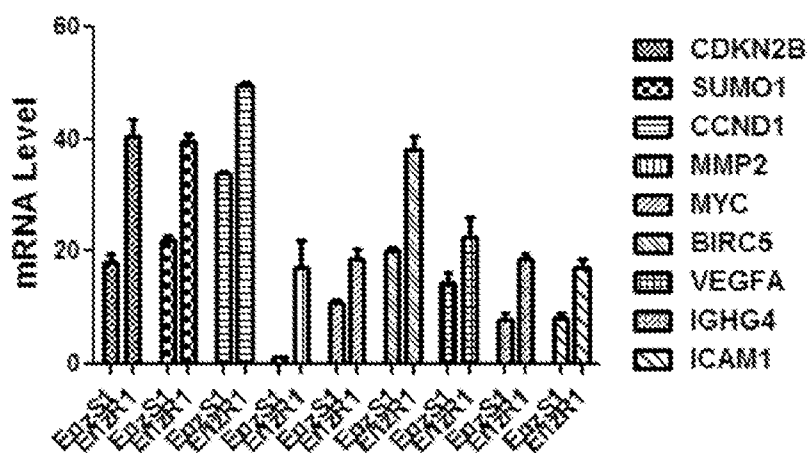


FIG. 6 (Cont'd)

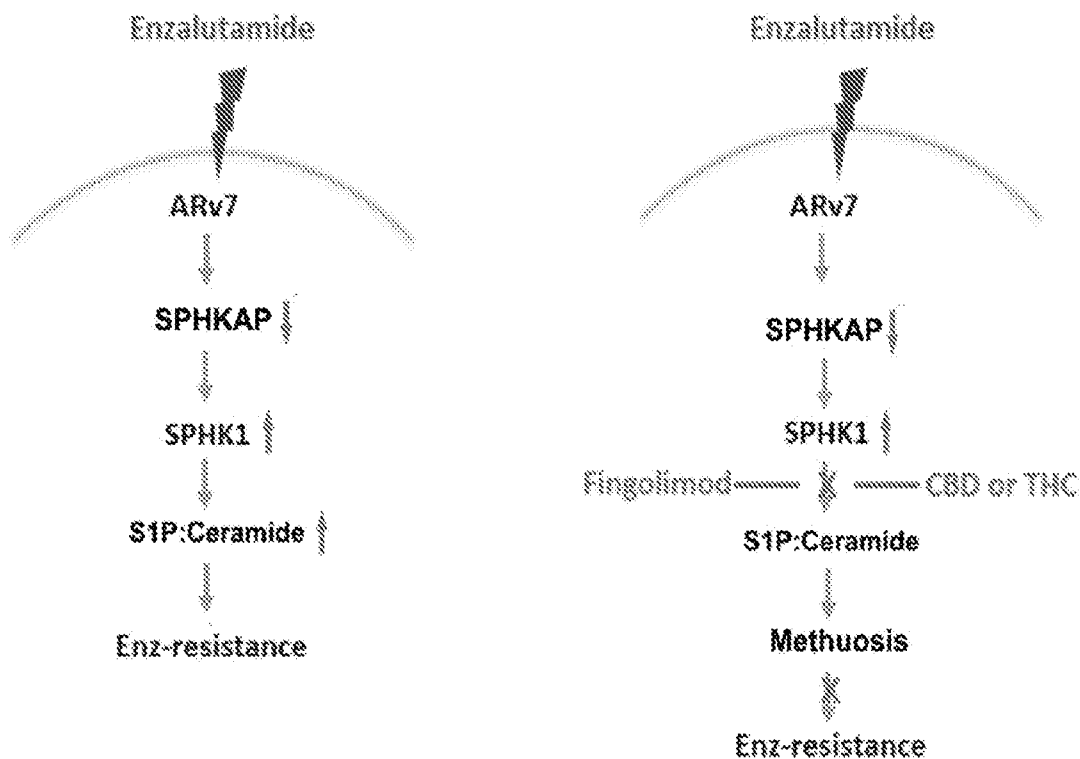


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/015130

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K31/4166 A61K31/133 A61K31/137 A61K31/164 A61K31/352	
	A61K31/426 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, CHEM ABS Data, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PCHEJETSKI DIMITRI ET AL: "Sphingosine kinase-1 as a chemotherapy sensor in prostate adenocarcinoma cell and mouse models", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 24, 15 December 2005 (2005-12-15), pages 11667-11675, XP002496979, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-05-2702	36
Y	title, abstract ----- -/--	1-15, 22-26, 35,38
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 6 May 2020		Date of mailing of the international search report 18/05/2020
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Dahse, Thomas

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/015130

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. PCHEJETSKI ET AL: "Chemosensitizing effects of sphingosine kinase-1 inhibition in prostate cancer cell and animal models", MOLECULAR CANCER THERAPEUTICS, vol. 7, no. 7, 1 July 2008 (2008-07-01), pages 1836-1845, XP055451872, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-07-2322	36
Y	title, abstract	1-15, 22-26, 35,38
X	----- Y. ZHU ET AL: "Antiandrogens Inhibit ABCB1 Efflux and ATPase Activity and Reverse Docetaxel Resistance in Advanced Prostate Cancer", CLINICAL CANCER RESEARCH, vol. 21, no. 18, 15 September 2015 (2015-09-15), pages 4133-4142, XP055328512, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-15-0269	1-7
Y	title, abstract, Fig. 2B, Fig. 3	1-15, 22-26, 35,38
Y	----- ROBERT J. VAN SOEST ET AL: "Targeting the Androgen Receptor Confers In Vivo Cross-resistance Between Enzalutamide and Docetaxel, But Not Cabazitaxel, in Castration-resistant Prostate Cancer", EUROPEAN UROLOGY, 1 December 2014 (2014-12-01), XP055183387, ISSN: 0302-2838, DOI: 10.1016/j.eururo.2014.11.033 title, abstract; see p. 983, col. 2	1-15, 22-26, 35,38
Y	----- TONELLI FRANCESCA ET AL: "FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells", CELLULAR SIGNALLING, vol. 22, no. 10, 2010, pages 1536-1542, XP029502205, ISSN: 0898-6568, DOI: 10.1016/J.CELLSIG.2010.05.022 p. 1540, last three sentences in section 3.4.; first of summary	1-15, 22-26, 35,38
	----- -/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/015130

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/095460 A1 (FATHI REZA [US] ET AL) 6 April 2017 (2017-04-06) [0221], table -----	1-15, 22-26, 35,38
Y	JIN XU ET AL: "Current opinion and mechanistic interpretation of combination therapy for castration-resistant prostate cancer", ASIAN JOURNAL OF ANDROLOGY, vol. 21, no. 3, 1 January 2019 (2019-01-01), page 270, XP55691681, US ISSN: 1008-682X, DOI: 10.4103/aja.aja_10_19 title, abstract; p. 273, section "Taxanes plus anti-androgen agents"; -----	1-15, 22-26, 35,38
Y,P	WO 2019/090255 A1 (UNIV VIRGINIA PATENT FOUNDATION [US]) 9 May 2019 (2019-05-09) claims 35 and 39; Fig. 4B -----	1-15, 22-26, 35,38

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/015130

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2017095460	A1	06-04-2017	AU 2016336133 A1 15-03-2018 BR 112018003232 A2 25-09-2018 CA 2997671 A1 13-04-2017 CN 108136207 A 08-06-2018 EP 3359255 A2 15-08-2018 JP 2018533560 A 15-11-2018 KR 20180058716 A 01-06-2018 RU 2018109902 A 07-11-2019 US 2017095460 A1 06-04-2017 US 2018125831 A1 10-05-2018 US 2020113881 A1 16-04-2020 WO 2017060771 A2 13-04-2017
WO 2019090255	A1	09-05-2019	NONE