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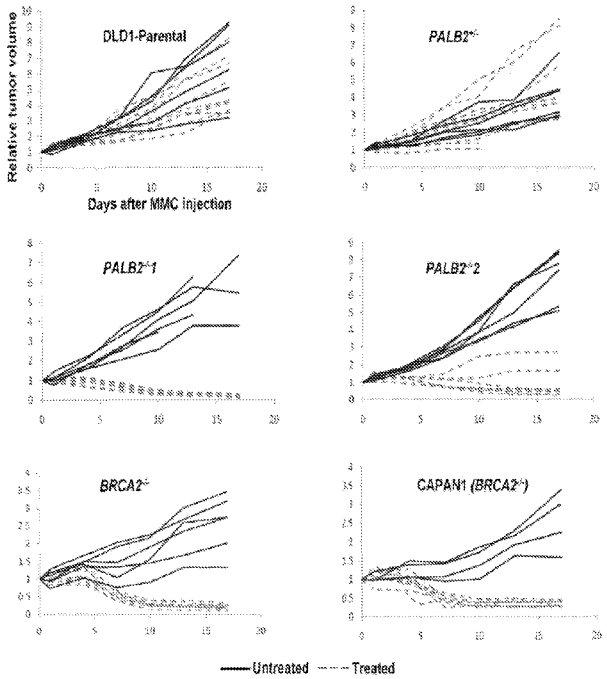
(54) **Title**: METHODS FOR TREATING CANCERS HAVING BRCA2 AND PALB2 MUTATIONS



(57) **Abstract**: The present invention provides a method for treating or preventing cancer in a subject wherein the subject is identified as having a BRCA2$^{-/-}$ and/or PALB2$^{-/-}$ mutation comprising: a) obtaining a sample from a subject; b) analyzing the sample for the presence of a BRCA2$^{-/-}$ and/or PALB2$^{-/-}$ mutation; c) administering to the subject a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof when the analysis of the sample of b) indicates the subject has a BRCA2$^{-/-}$ and/or PALB2$^{-/-}$ mutation. In addition, the use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof, and/or an aldehyde dehydrogenase inhibitor is also provided for treating cancer in a subject wherein the subject is identified as having a BRCA2 $^{-/-}$ and/or PALB2 $^{-/-}$ mutation. Also provided herein is an isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene, and methods for identifying a compound or small molecule that modulates a function of a cancer cell or population of cells genetically modified to have a biallelic disruption of the PALB2 gene are also provided.

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METHODS FOR TREATING CANCERS HAVING BRCA2 AND PALB2 MUTATIONS

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/727,428, filed on November 16, 2012, which is hereby incorporated by reference for all purposes as if fully set forth herein.

STATEMENT OF GOVERNMENTAL INTEREST

[0002] This invention was made with U.S. government support under grant nos. CA62924 and CA123483 awarded by the NIH. The U.S. government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 29, 2013, is named P11871-02_ST25.txt and is 1,710 bytes in size.

BACKGROUND OF THE INVENTION

[0004] Mutations in 16 genes are responsible for causing Fanconi anemia (FA), an autosomal recessive disease characterized by diverse clinical features that include congenital abnormalities, progressive born marrow failure, and cancer susceptibility. Mutations in FANCA, FANCC, and FANCG comprise 90% of FA patients, and FANCA alone, 70%. Genes of the Fanconi anemia pathway are often involved in human cancers. Biallellic germline mutations exist in FA patients, whereas heterozygous germline mutations, somatic mutations, and epigenetic silencing are found in various cancer types occurring in the non-FA patients.

[0005] FA is infrequent. Most FA patients develop life-threatening complications although some have long periods of subclinical disease. The estimated heterozygous mutation carrier frequency, however, can be high, (between 0.3-1%), with the higher frequencies seen among the Ashkenazi Jews and Afrikaners. This raises difficult issues regarding proper risk assessment in known carriers and in the general population, in which

the identity of FA carriers will generally be undetermined but whose risks must be considered nonetheless.

[0006] The rarity of some genotypes in human cancer impairs performing epidemiologic studies and therapeutic clinical trials for each genotype. The results of studying common genotypes might be extended to the rare genotypes to understand the latter better. For this purpose, engineered, ideally-matched human cancer syngeneic cell lines null for the FANCC, FANCG, and BRCA2 genes are currently employed. These lines form preclinical models illustrating the predictable functional similarities among differing FA pathway genotypes. Other naturally occurring lines, such as non-cancer FA lymphoblastoid lines, and FA-deficient cancers such as CAPAN1 are also used.

[0007] BRCA2 and PALB2 (Partner and Localizer of BRCA2) belong to the distal aspect of the FA pathway. PALB2 associates with BRCA2 to promote BRCA2 function in recombinational repair and in DNA damage responses. Non-cancer cells and lymphoma cells having natural PALB2 deficiency are reported to be functionally similar to cells lacking BRCA2. PALB2-null and heterozygous-mouse models are also reported. The homozygous mutant mice were embryonic lethal, similar to the BRCA2-null mice, but the PALB2-heterozygotes did not develop tumors in these studies.

[0008] Mutations in the Fanconi anemia genes are also clinically useful due to chemical hypersensitivities. Some patterns of hypersensitivity among FA genes are known, as in FANCC, FANCG, BRCA2, and PALB2 genes. New hypersensitivities can be discovered or even disproved, as when engineered BRCA2-null cells were found to lack hypersensitivity to the erroneously classified drug iniparib. Engineered cell lines (FANCC, FANCG, and BRCA2) are useful to compare pharmacogenetic advantages between compounds. Radiation sensitivity and drug sensitivity spectra in FA lymphoblastoid cells are reported. Sensitivities to UV radiation, mono- and bifunctional alkylating agents, crosslinking agents, and bleomycin helped to characterize the various FA complementation groups.

[0009] Syngeneic matched cells, engineered to be deficient or competent for individual FA pathway genes, permit numerical comparisons of drug sensitivities (the pharmacogenetic windows) between cells defective in the distal ($BRCA2^{-/-}$, $PALB2^{-/-}$) and proximal ($FANCC^{-/-}$, $FANCG^{-/-}$) aspects of the pathway. For example, a 15 to 25X difference existed with ICL agents in BRCA2-/- cells versus the parental cell line. $FANCC^{-/-/-}$ and $FANCG^{-/-}$ cells had 7 to 14X differences. $BRCA2^{-/-}$ had 6 and 10X differences, respectively, with the Topo II inhibitor etoposide and the Topo I inhibitor camptothecin, whereas $FANCC^{-/-/-}$ and $FANCG^{-/-}$

cells had no hypersensitivity to etoposide. Because not all null genotypes yield viable clones in all cell lines, differences among genotypes may reflect differences in cell lines and clonal variation in addition to the differing gene status; the same is true for real human tumors in a setting of personalized therapy.

SUMMARY OF THE INVENTION

[0010] In accordance with an embodiment, the present invention provides a method for treating or preventing cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation comprising: a) obtaining a sample from a subject; b) analyzing the sample for the presence of a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation; c) administering to the subject a therapeutically effective amount of an C_1-C_6 aldehyde or a prodrug or derivative thereof when the analysis of the sample of b) indicates the subject has a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

[0011] In accordance with an embodiment, the present invention provides a use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or a prodrug or derivative thereof, and a pharmaceutically acceptable carrier as a medicament for use in the prevention or treatment of cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

[0012] In accordance with an embodiment, the present invention provides a use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof, and at least one other therapeutically effective agent, and a pharmaceutically acceptable carrier as a medicament for use in the prevention or treatment of cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

[0013] In accordance with an embodiment, the present invention provides an isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene.

[0014] In accordance with an embodiment, the present invention provides a method for identifying a compound or small molecule that modulates a function of a cancer cell having a biallelic disruption of the PALB2 gene, comprising: a) measuring a function in at least one isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene ($PALB2^{-/-}$ cell); b) incubating said at least one isolated $PALB2^{-/-}$ cell with a

test compound or small molecule; c) measuring said function in said at least one PALB2$^{-/-}$ cell following incubation with said test compound or small molecule; and d) determining the difference in said function in said at least one PALB2$^{-/-}$ cell before and after said incubation with said test compound or small molecule; wherein a difference in function in said at least one PALB2$^{-/-}$ cell after said incubation as compared to before said incubation with said test compound or small molecule identifies said test compound or small molecule as a test compound or small molecule that modulates said function in cancer cells having a biallelic disruption of the PALB2 gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 illustrates the structural and functional evidence of PALB2 gene disruption. (A) Targeting scheme. Primer pairs CF+CR were used to confirm deletion of exon 8 in both alleles. Primer pairs LF+LR and RF+RR, left and right homology arm screening primers; LHA and RHA, left and right homology arms; SA, splice acceptor; IRES, Internal ribosomal entry sequence; neo, coding sequence of neomycin transferase; pA, polyadenylation signal sequence; solitary numbers label exons. All primer sequences are provided in supplementary material. (B) PCR detecting the wildtype and deleted alleles using genomic DNA as template. (C) Reverse-transcriptase PCR detecting the wildtype and truncated mRNA transcripts. (D) Western blot detecting the presence or absence of PALB2 protein. (E) Proliferation curves of parental, hemizygous, and PALB2-null cells, are compared. (F) Colony-formation assays following ionizing radiation. Each point represents the average of duplicate measurements for each cell line in a representative experiment (of two experiments).

[0016] Figure 2 shows cell cycle profiles 48 hours after MMC treatment. PALB2-deficient cells became arrested at the G2/M region of the cell cycle profile.

[0017] Figure 3 depicts the functional validation of *PALB2*-deficient cells by drug sensitivity. Cell population following treatment with ICL agents (melphalan, MMC, and cisplatin), a topoisomerase II inhibitor (etoposide), and a topoisomerase I inhibitor (camptothecin) at the indicated concentrations as compared with untreated cells. The IC_{50} ratios, comparing parental and FA-deficient cells, are indicated. Error bars represent standard error of the mean (SEM) of 6 replicate wells in a single experiment for each drug.

[0018] Figure 4 shows cell survival in CAPAN 1 cells. Cell survival following treatment of CAPAN1 cells (bold dotted lines) with MMC, KU0058498, and acetaldehyde at the indicated concentrations as compared with other cell lines tested (no matched control cells for CAPAN1). Error bars, SEM of 6 replicate wells in a single experiment for each drug.

[0019] Figure 5 shows *in vivo* treatment of xenografts with single dose of MMC. *PALB2*- and *BRCA2*- deficient xenografts (as indicated), parental, and *PALB2*- hemizygous xenografts were established and grown to an initial volume of 150-200 mm^3 before initiation of treatment. Final xenograft volume was expressed as relative to initial volume. Mice were treated with a single intraperitoneal dose (5 mg/kg) of MMC.

[0020] Figure 6 depicts morphologic depicts features of pancreatic cancer xenografts treated with MMC. Untreated xenografts and xenografts treated at 1, 4 and 7 days are shown. *PALB2*$^{+/-}$ is a cell line with a heterozygous *PALB2* mutation, and *PALB2*$^{-/-}$*1* has a homozygous *PALB2* deletion. Small arrows indicate apoptotic bodies/debris, the black block arrow a ballooned cell with disorganized chromosomes, and the white block arrow a multinucleated cell in *PALB2*$^{-/-}$*1*. By contrast, no morphologic changes were seen in *PALB2*$^{+/}$ cells.

[0021] Figure 7 shows chemical sensitivities among an FA-deficient cancer cell panel using compounds eliciting divergences among FA-null models. Cell populations following treatment with PARP I inhibitor KU0058948 and the biological metabolite acetaldehyde at indicated concentrations, as compared with untreated cells. The IC_{50} ratios, comparing parental and FA-deficient cells, are indicated. Error bars represent SEM of 6 replicate wells in a representative experiment.

[0022] Figure 8 shows metaphase chromosomes in PALB2-null cells following diepoxybutane (DEB) exposure.

[0023] Figure 9 depicts metaphase chromosomes in BRCA2-null cells following diepoxybutane (DEB) exposure.

[0024] Figure 10 depicts metaphase chromosomes following acetaldehyde exposure. Acetaldehyde exposure to PALB2$^{-/-}$ and BRCA2$^{-/-}$ cells promoted widespread chromosomal aberrations. Solid arrows, chromatid breaks; 'cb', chromosome breaks; '*', fragments; 'cg', chromosome gaps; arrows, dicentric chromosomes; 'R', rings; '3r', triradials; '3r-b', broken triradials; '4r', quadriradials; '4r-f', quadriradial with fragments.

DETAILED DESCRIPTION OF THE INVENTION

[0025] In accordance with one or more embodiments of the present invention, PALB2-deficient cells were generated and validated. Xenografts were used to explore multiple FA genotypes for rapid tumor regression upon MMC treatment, reflecting in vivo the large in vitro pharmacogenetic differences. These models can be informative for the rare genotypes, as their rarity makes it unlikely that clinical trials could be performed for each genotype. The ability to develop robust lessons from preclinical models of multiple FA pathway genotypes is an essential premise to explore. The cell lines of the present invention permit extrapolating among the multiple clinical genotypes. The findings herein reinforce this pathway-based strategy by comparing chemical hypersensitivities of matched syngeneic pairs of cell lines deficient in four of the five genes bearing inherited mutations in FA-deficient cancers in FA carriers, namely PALB2, BRCA2, FANCC, FANCG. The results have utility in genetic epidemiology, prevention strategies, and in exploration of novel therapeutic options.

[0026] In accordance with an embodiment, the present invention provides a method for treating or preventing cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation comprising: a) obtaining a sample from a subject; b) analyzing the sample for the presence of a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation; c) administering to the subject a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof when the analysis of the sample of b) indicates the subject has a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

[0027] As used herein, the term "treat," as well as words stemming therefrom, includes preventative as well as disorder remitative treatment. The terms "reduce," "suppress," "prevent," and "inhibit," as well as words stemming therefrom, have their commonly understood meaning of lessening or decreasing. These words do not necessarily imply 100% or complete treatment, reduction, suppression, or inhibition.

[0028] As used herein, the term "C_1-C_6 aldehyde or derivative thereof" means any alkane, alkene, alkyne, or aryl compound of 1 to 6 carbons in length, having at least one CHO functional group. This includes, for example, metabolites of various compounds having alcohol or carboxylic acid function groups that are either oxidized or reduced through enzymatic or chemical action in the body or the cell or population of cells of the subject. Examples of aldehydes useful in the present invention include, formaldehyde, acetaldehyde, glyoxal solution, acrolein, butyraldehyde, crotonaldehyde, benzaldehyde, glutaraldehyde,

phenylacetaldehyde, cinnamaldehyde, acetaldehyde dimethyl acetal, and aminoacetaldehyde dimethyl acetal.

[0029] The term "prodrug" as used herein, refers to a derivative of a drug molecule that requires one or more transformations, e.g., metabolism of the prodrug within the subject's body to cause the active drug to be formed. Prodrugs can be (though not necessarily) pharmacologically inactive until converted to the parent drug. In accordance with one or more embodiments, the prodrug can be a molecule which is converted into a C_1-C_6 aldehyde or derivative thereof through metabolism in the subject's body.

[0030] In accordance with an embodiment, the present invention provides a use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof, and a pharmaceutically acceptable carrier as a medicament for use in the prevention or treatment of cancer in a subject wherein the subject is identified as having a BRCA2 $^{-/-}$ and/or PALB2 $^{-/-}$ mutation.

[0031] In accordance with another embodiment, the present invention provides a use of a pharmaceutical composition wherein the pharmaceutical composition further comprises a therapeutically effective amount of an alcohol dehydrogenase inhibitor. In a preferred embodiment, the alcohol dehydrogenase inhibitor is disulfirum.

[0032] Accordingly, included within the compounds and derivatives of the present invention are the tautomeric forms of the disclosed compounds, isomeric forms including enantiomers, stereoisomers, and diastereoisomers, and the pharmaceutically-acceptable salts thereof. The term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases, such as those used to improve water solubility. Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid, and such organic acids as maleic acid, succinic acid and citric acid. Other pharmaceutically acceptable salts include salts with alkali metals or alkaline earth metals, such as sodium, potassium, calcium and magnesium, or with organic bases, such as dicyclohexylamine. Suitable pharmaceutically acceptable salts of the compounds of the present invention include, for example, acid addition salts which may, for example, be formed by mixing a solution of the compound according to the invention with a solution of a pharmaceutically acceptable acid, such as hydrochloric acid, sulphuric acid, methanesulphonic acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. All of these salts may

be prepared by conventional means by reacting, for example, the appropriate acid or base with the corresponding compounds of the present invention.

[0033] Salts formed from free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0034] For use in medicines, the salts of the compounds of the present invention should be pharmaceutically acceptable salts. Other salts may, however, be useful in the preparation of the compounds according to the invention or of their pharmaceutically acceptable salts.

[0035] In addition, embodiments of the invention include hydrates of the compounds of the present invention. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate and the like. Hydrates of the compounds of the present invention may be prepared by contacting the compounds with water under suitable conditions to produce the hydrate of choice.

[0036] Embodiments of the invention also include a process for preparing pharmaceutical products comprising the compounds. The term "pharmaceutical product" means a composition suitable for pharmaceutical use (pharmaceutical composition), as defined herein. Pharmaceutical compositions formulated for particular applications comprising the compounds of the present invention are also part of this invention, and are to be considered an embodiment thereof.

[0037] With respect to pharmaceutical compositions described herein, the pharmaceutically acceptable carrier can be any of those conventionally used, and is limited only by physico-chemical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. Examples of the pharmaceutically acceptable carriers include soluble carriers such as known buffers which can be physiologically acceptable (e.g., phosphate buffer) as well as solid compositions such as solid-state carriers or latex beads. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s), and one which has little or no detrimental side effects or toxicity under the conditions of use.

[0038] The carriers or diluents used herein may be solid carriers or diluents for solid formulations, liquid carriers or diluents for liquid formulations, or mixtures thereof.

[0039] Solid carriers or diluents include, but are not limited to, gums, starches (e.g., corn starch, pregelatinized starch), sugars (e.g., lactose, mannitol, sucrose, dextrose), cellulosic materials (e.g., microcrystalline cellulose), acrylates (e.g., polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

[0040] For liquid formulations, pharmaceutically acceptable carriers may be, for example, aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include, for example, water, alcoholic/aqueous solutions, cyclodextrins, emulsions or suspensions, including saline and buffered media.

[0041] Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, fish-liver oil, sesame oil, cottonseed oil, corn oil, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include, for example, oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0042] Parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include, for example, sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Formulations suitable for parenteral administration include, for example, aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0043] Intravenous vehicles include, for example, fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

[0044] In addition, in an embodiment, the compounds of the present invention may further comprise, for example, binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmelose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-

HCl, acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., cremophor, glycerol, polyethylene glycerol, benzlkonium chloride, benzyl benzoate, cyclodextrins, sorbitan esters, stearic acids), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g., hydroxypropyl cellulose, hyroxypropylmethyl cellulose), viscosity increasing agents (e.g., carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweetners (e.g., aspartame, citric acid), preservatives (e.g., thimerosal, benzyl alcohol, parabens), lubricants (e.g., stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g., colloidal silicon dioxide), plasticizers (e.g., diethyl phthalate, triethyl citrate), emulsifiers (e.g., carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g., ethyl cellulose, acrylates, polymethacrylates), and/or adjuvants.

[0045] The choice of carrier will be determined, in part, by the particular compound, as well as by the particular method used to administer the compound. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal and interperitoneal administration are exemplary, and are in no way limiting. More than one route can be used to administer the compounds, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0046] Suitable soaps for use in parenteral formulations include, for example, fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include, for example, (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0047] The parenteral formulations will typically contain from about 0.5% to about 25% by weight of the compounds in solution. Preservatives and buffers may be used. In order to

minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants, for example, having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include, for example, polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

[0048] The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0049] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., *Pharmaceutics and Pharmacy Practice*, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Trissel, 15th ed., pages 622-630 (2009)).

[0050] As used herein, the term "proliferative disease" includes cancer and other diseases such as neoplasias and hyperplasias. Cellular proliferative diseases include, for example, rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, leiomyomas, adenomas, lipomas, hemangiomas, fibromas, vascular occlusion, restenosis, artherosclerosis, a pre-neoplastic lesion, carcinoma in situ, oral hairy leukoplakia, or psoriasis. In accordance with one or more embodiments, the term cancer can include, for example cancers of the lung, liver, pancreas, prostate, breast and central nervous system, including glioblastomas and related tumors. In an embodiment, the term "administering" means that the compounds of the present invention are introduced into a subject, preferably a subject receiving treatment for a proliferative disease, and the compounds are allowed to come in contact with the one or more disease related cells or population of cells *in vivo*.

[0051] In accordance with an embodiment, the present invention provides a use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof, and at least one other therapeutically effective agent, and a pharmaceutically acceptable carrier as a medicament for use in the prevention or

treatment of cancer in a subject wherein the subject is identified as having a BRCA2 $^{-/-}$ and/or PALB2$^{-/-}$ mutation.

[0052] As used herein, the term "subject" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0053] The term “therapeutic agent” or “chemotherapeutic agent” as well as words stemming therefrom, as used herein, generally includes pharmaceutically or therapeutically active compounds that work by interfering with DNA synthesis or function in cancer cells. Based on their chemical action at a cellular level, chemotherapeutic agents can be classified as cell-cycle specific agents (effective during certain phases of cell cycle) and cell-cycle nonspecific agents (effective during all phases of cell cycle). Without being limited to any particular example, examples of chemotherapeutic agents can include alkylating agents, angiogenesis inhibitors, aromatase inhibitors, antimetabolites, anthracyclines, antitumor antibiotics, monoclonal antibodies, platinums, topoisomerase inhibitors, and plant alkaloids.

[0054] In a further embodiment, the compositions and methods of the present invention can be used in combination with one or more additional therapeutically active agents which are known to be capable of treating conditions or diseases discussed above. For example, the compositions of the present invention could be used in combination with one or more known therapeutically active agents, to treat a proliferative disease. Non-limiting examples of other therapeutically active agents that can be readily combined in a pharmaceutical composition with the compositions and methods of the present invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

[0055] In accordance with another embodiment of the present invention, it will be understood that the term “biological sample” or “biological fluid” includes, but is not limited to, any quantity of a substance from a living or formerly living patient or mammal. Such substances include, but are not limited to, blood, serum, plasma, urine, cells, organs, tissues,

bone, bone marrow, lymph, lymph nodes, synovial tissue, chondrocytes, synovial macrophages, endothelial cells, and skin.

[0056] In accordance with another embodiment of the present invention, it will be understood that the method of analysis of the sample for the presence of a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation is selected from the group consisting of: analysis of the DNA or RNA in the sample from the subject; detection of the expression product or lack thereof in the sample from the subject; and analysis of the family history of the subject.

[0057] By "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0058] The nucleic acids used as primers in embodiments of the present invention can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Sambrook et al. (eds.), *Molecular Cloning, A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York (2001) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, NY (1994). For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil,

beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0059] The nucleotide sequences used herein are those which hybridize under stringent conditions preferably hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C.

[0060] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0061] "Probe" as used herein may mean an oligonucleotide capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. Probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. There may be any number of base pair mismatches which will interfere with hybridization between the target sequence and the single stranded nucleic acids described herein. However, if the number of mutations is so great that no hybridization can occur under even the least stringent of hybridization conditions, the sequence is not a complementary target sequence. A probe may be single stranded or partially single and partially double stranded. The strandedness of the probe is dictated by the structure, composition, and properties of the target sequence. Probes may be directly labeled or indirectly labeled such as with biotin to which a streptavidin complex may later bind.

[0062] "Substantially complementary" used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides, or that the two sequences hybridize under stringent hybridization conditions.

[0063] "Substantially identical" used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

[0064] A probe is also provided comprising a nucleic acid described herein. Probes may be used for screening and diagnostic methods, as outlined below. The probes may be attached or immobilized to a solid substrate or apparatus, such as a biochip. The probes can be used to identify a subject as being $BRCA2^{-/-}$ and or $PALB2^{-/-}$.

[0065] In accordance with an embodiment, the present invention provides an isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene. It will be understood by those of ordinary skill in the art that the inventors have created a cell line that is useful for identifying compounds that modulate the growth of cancer cells having a biallelic disruption of the PALB2 gene.

[0066] In accordance with an embodiment, the present invention provides a method for identifying a compound or small molecule that modulates a function of a cancer cell having a biallelic disruption of the PALB2 gene, comprising: a) measuring a function in at least one isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene ($PALB2^{-/-}$ cell); b) incubating said at least one isolated $PALB2^{-/-}$ cell with a test compound or small molecule; c) measuring said function in said at least one $PALB2^{-/-}$ cell following incubation with said test compound or small molecule; and d) determining the difference in said function in said at least one $PALB2^{-/-}$ cell before and after said incubation with said test compound or small molecule; wherein a difference in function in said at least one $PALB2^{-/-}$ cell after said incubation as compared to before said incubation with said test compound or small molecule identifies said test compound or small molecule as a test compound or small molecule that modulates said function in cancer cells having a biallelic disruption of the PALB2 gene. The PALB2 gene was disrupted in the cancer cells of interest by serially deleting exon 8 in both alleles.

[0067] As defined herein, in another embodiment, the term "contacting" means that the one or more compounds of the present invention are introduced into a sample having at least one cancer cell and appropriate enzymes or reagents, in a test tube, flask, tissue culture, chip, array, plate, microplate, capillary, or the like, and incubated at a temperature and time sufficient to permit binding and uptake of the at least one compound to the cancer cell. Methods for contacting the samples with the compounds, and other specific binding components are known to those skilled in the art, and may be selected depending on the type of assay protocol to be run. Incubation methods are also standard and are known to those skilled in the art.

[0068] In a further embodiment, the compositions and methods of the present invention can be used in combination with one or more additional therapeutically active agents which are known to be capable of treating conditions or diseases discussed above. For example, the compositions of the present invention could be used in combination with one or more known therapeutically active agents, to treat a proliferative disease. Non-limiting examples of other therapeutically active agents that can be readily combined in a pharmaceutical composition with the compositions and methods of the present invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

[0069] Typically, an attending physician will decide the dosage of the composition with which to treat each individual subject, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, compound to be administered, route of administration, and the severity of the condition being treated. By way of example, and not intending to limit the invention, the dose of the compositions of the present invention can be about 0.001 to about 1000 mg/kg body weight of the subject being treated, from about 0.01 to about 100 mg/kg body weight, from about 0.1 mg/kg to about 10 mg/kg, and from about 0.5 mg to about 5 mg/kg body weight. In another embodiment, the dose of the compositions of the present invention can be at a concentration from about 1 nM to about 100 mM, preferably from about 10 μM to about 50 mM, more preferably from about 100 μM to about 5 mM.

EXAMPLES

[0070] Treatment of cells and cell quantitation. About 1000 and 2000 cells were plated per well for wild type and null cultures, respectively. After 24 hours, adherent cells were exposed to various drugs and chemicals for six days and quantified. Radiation exposure was as described (Gastroenterology 2006;130(7):2145-54).

[0071] Xenograft model. Six cell lines were injected (1 x 10^6 cells/injection for DLD1 parental, PALB2$^{+/-}$, PALB2$^{-/-}$1; PALB2$^{-/-}$2, and BRCA2$^{-/-}$; and 1.5 x 10^6 cells/injection for CAPAN1) subcutaneously into the two flanks of female athymic nude mice (Charles River Laboratories, 4-5 week old) with RPMI medium and Matrigel (BD, Pharmigen) (1-1.5 x 10^6 cells in 100 μl of RPMI medium and Matrigel (1:1)). Twelve mice were inoculated for each cell line. Tumors were treated when each reached a size of 150-200 mm^3, defined as "day 0"; four received no treatment. Care of animals was in accord with institutional guidelines. The term "chemotherapeutic agent" as well as words stemming therefrom, as used herein, generally includes pharmaceutically or therapeutically active compounds that work by interfering with DNA synthesis or function in cancer cells. Based on their chemical action at a cellular level, chemotherapeutic agents can be classified as cell-cycle specific agents (effective during certain phases of cell cycle) and cell-cycle nonspecific agents (effective during all phases of cell cycle). Without being limited to any particular example, examples of chemotherapeutic agents can include alkylating agents, angiogenesis inhibitors, aromatase inhibitors, antimetabolites, anthracyclines, antitumor antibiotics, monoclonal antibodies, platinums, topoisomerase inhibitors, and plant alkaloids.

[0072] Histopathologic examination. Harvested tumor tissue was fixed in buffered formalin until they were processed for histopathology.

[0073] Targeted disruption of PALB2 by homologous recombination. The PALB2 gene was disrupted according to the technique outlined by Rago et al. (Nat Protoc 2007;2(11):2734-46). The targeting construct excised exon 8 of the PALB2 gene such that a frameshift and a stop codon were created. A promoter-trap method was used in which the targeting construct contained the selection marker neomycin. The construct consisted of two homology arms flanking a central element, pSEPT, which contained a splice acceptor, an IRES, coding sequences of the neomycin transferase gene, and a polyadenylation signal sequence. The pSEPT element was flanked by LoxP sites. The homology arms were ligated to pAAV (Stratagene). The targeting construct was cotransfected with pRC and pHelper into HEK293 cells using Lipofectamine (Invitrogen). Virus was harvested from the HEK293 cells after 48 hours, and a DLD1 clone (#6, isolated by limiting dilution) was infected. Infected cells were distributed to 96-well plates by limiting dilution. Neomycin-resistant clones were screened after three weeks by PCR using primers located inside the selection cassette and outside the homology arms to detect homologous integrations. The selection cassette was removed from clones by Cre-mediated excision. A heterozygous PALB2 clone ($PALB2^{+/-}$), identified and confirmed by using primers on either side of the deleted region, was used when removing the second allele with the same exon 8-targeting construct. Clones having biallelic disruption of PALB2 were identified by PCR screening, as above. The neomycin cassette was again removed.

[0074] Plasmid purification, RNA and genomic DNA isolation, PCR, RT-PCR, and sequencing. Plasmid DNA was purified (Qiagen). Genomic DNA (crude) was released from cancer cells using either the Lyse-N-Go PCR Reagent (Pierce Biotechnology) or by purification (Qiagen). RNA was isolated (RNeasy Mini kit, Qiagen). Complementary DNA was made from RNA using Superscript III (Invitrogen). PCR products were resolved on agarose gels using lithium boric acid electrophoresis (Faster Better Media LLC). Automated sequencing was performed by Macrogen USA. PCR conditions and primer sequences are listed below.

[0075] Protein isolation and western blotting. Cells were washed once in PBS and lysed by radioimmune precipitation assay buffer supplemented with protease inhibitor mixture (Roche Applied Science, 1183617001), followed by sonication. Protein concentrations were determined by a DC protein assay (Bio-Rad, 500-011). Samples were boiled with loading

buffer and resolved by SDS-PAGE. Proteins were transferred onto a PVDF (Immobilon, Millipore) membrane and detected with anti-PALB2 antibody (Santa Cruz Biotechnology) and HRP-linked anti-rabbit secondary antibody (Santa Cruz Biotechnology). Membranes were developed using chemiluminescence (Millipore, WBKLS0500).

[0076] Cell cycle analysis. Cells (10^6) were treated with mitomycin C (MMC) for 48 hours, washed once in PBS, fixed in 70% ethanol at -20 °C for at least 30 minutes, washed again with PBS, and incubated in 0.2 ml of PI solution [0.1% Triton X-100, 1X PBS, 200 µg/ml RNase A (Sigma), 20 µg/ml propidium iodide (Sigma)] at 37 °C for 1 hour. The cells were diluted with 1 ml PBS for flow cytometry (FACScalibur, BD Biosciences). CellQuest (BD) software was used to interpret cell cycle profiles.

[0077] Treatment of cells and cell quantitation. About 1000 and 2000 cells were plated per well for wild type and null cultures, respectively. After 24 hours, adherent cells were exposed to various drugs and chemicals: melphalan, MMC, cisplatin, etoposide, camptothecin (Sigma-Aldrich), KU0058948 (synthesized as needed), and biologic metabolites (formaldehyde, acetaldehyde, glyoxal solution, acrolein, butyraldehyde, crotonaldehyde, benzaldehyde, glutaraldehyde, phenylacetaldehyde, cinnamaldehyde, acetaldehyde dimethyl acetal, and aminoacetaldehyde dimethyl acetal, Sigma-Aldrich). After six days, the cells were washed, lysed in 40 µl 0.03% SDS, and 0.5% Picogreen (Molecular Probes) was added. Fluorescence was measured and the relative cell numbers were calculated, defining the untreated samples as "1". Two to three independent experiments were performed per drug, with each graphed data point reflecting the average readings from six wells in a given representative experiment.

[0078] Synthesis of Ku0058948. The PARP inhibitor KU0058948 (4-(3-(1,4-diazepane-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one) was synthesized following a reported protocol (WO 2004/080976) in five steps. Briefly, 2-carboxy benzaldehyde was first converted in to 3-oxo-1,3-dihydro-isobenzofuranyl phosphonate dimethyl ester, which was then treated with 2-flouro-5-formyl benzonitrile to afford the 2-flouro-5-(3-oxo-1,3-dihydro isobenzofuranylidene methyl) benzonitrile intermediate as a mixture of E/Z isomers. This mixture was then refluxed with NaOH, followed by addition of hydrazine hydrate to replace the isobenzofuranyl ring with a dihydrophthazine moiety. The generated intermediate was then used for a tetramethyluronium hexaflourophosphate-mediated coupling with t-butyl homopiperazine carboxylate, which yielded the desired compound on acidic workup in an acceptable overall yield.

[0079] Irradiation of cells and colony-formation assay. Cells were plated, allowed to adhere, and exposed to ^{137}Cs gamma rays (1.3 Gy/min, Gammacell 40 Exactor; MDS Nordion, Inc.). Cells subsequently were incubated for 7 days or until colonies appeared, fixed, and stained (10% neutral buffered formalin, 1:500 crystal violet). All macroscopically visible colonies were counted. Experiments were performed twice. Each sample was tested at two different cell concentrations, each performed in duplicate. The number of cells plated was 10,000 and 100,000 for 0, 2, 4, 6, 8, and 10 Gy exposures.

[0080] Estimation of doubling time of cells. Cells were plated at a concentration of 2.5 x 10^4 cells in thirty 25 cm^2 flasks. Cells were washed with 1X PBS followed by trypsinization. Cells were disaggregated in 1 ml PBS and counted by hemocytometer. At each time point, two independent samples were counted. Counting was done every 6 hours, from 0 to 90 hours (total of 15 time points). The proliferation curves were plotted, and the doubling times of the parental and PALB2-null cells were estimated.

[0081] p53-luciferase reporter assays. p53 responses were assessed by a luciferase reporter assay, performed with the Steady-Glo Luciferase Assay System (Promega) according to the manufacturer's protocol. Briefly, cells were plated in triplicate, incubated with acetaldehyde (Sigma) for 18 hours, and then lysed for luciferase assay. A PerkinElmer Microbeta Trilux plate reader was used to measure luminescence.

[0082] Xenograft tumor samples for histologic examination. Twelve mice were inoculated for each of the six cell lines. Tumors were treated with MMC when each reached a size of 150-200 mm^3, defined as "day 0"; four received no treatment. One untreated tumor of each group was harvested on "day 1"; eight were treated with MMC, of which one each was harvested on days 1, 4, and 7. The drug was administered once intraperitoneally at 5 mg/kg on day 0. Length (L) and width (W) of tumors were measured with a caliper. Tumor volume (TV) was calculated as $TV=1/2 * length * width^2$.

[0083] PCR primers. Left homology arm-screening primers for determining integration into the desired locus (orientation is depicted in Fig. 1A), LF: AAC CTC CCC AGG CTC AGT AA (SEQ ID NO: 1); LR: AAA TCC TCC TCG TTT TTG GA (SEQ ID NO: 2). Right homology arm-screening primers for determining integration into the desired locus (orientation is depicted in Fig. 1A), RF: CAG GTT CAG GGG GAG GTG TG(SEQ ID NO: 3); RR: ATG TCT GGC TTC CAC CTC ACT AAC (SEQ ID NO: 4). Primers for genotyping parental and *PALB2*-null cells (orientation is depicted in Fig. 1A, PCR products in Fig. 1B), CF: CTT TAC ACA GAG GTG CCC AAT (SEQ ID NO: 5); CR: CTC CCA

GGT TCA AGC GAC T (SEQ ID NO: 6). Primers for RT-PCR (used for Fig. 1C), Forward: AGT GCC ATG TTT TGG GAA AG (SEQ ID NO: 7); Reverse: TCC ATC TTC TGC AAA CGT CA (SEQ ID NO: 8).

[0084] PCR conditions. For PCR reactions using the first three listed primers, touch-down PCR was used. Initial denaturation: 94 °C, 2 minutes. Addition of Taq polymerase: 78 °C x 4 cycles (Denaturation: 94 °C, 10 seconds. Annealing: 65 °C, 30 seconds. Extension: 72 °C, 1 minute, 45 seconds) x 4 cycles (Denaturation: 94 °C, 10 seconds. Annealing: 62 °C, 30 seconds. Extension: 72 °C, 1 minute, 45 seconds) x 4 cycles (Denaturation: 94 °C, 10 seconds. Annealing: 59 °C, 30 seconds. Extension: 72 °C, 1 minute, 45 seconds) x 30 cycles Denaturation: 94 °C, 10 seconds. Annealing: 56.5 °C, 30 seconds. Extension: 72 °C, 1 minute, 45 seconds) Extension: 72 °C, 7 minutes. Cool to 4 °C.

[0085] Chromosome breakage assay. $BRCA2^{-/-}$, $PALB2^{-/-}1$, and parental cells were treated with and without diepoxybutane (DEB, 0.1μg/ml), or with and without acetaldehyde (1mM, the IC_{50} concentration range in $BRCA2^{-/-}$, $PALB2^{-/-}$ lines) for three days, following which medium was changed. Cells were allowed to recover for one day, then treated with colcemid (1μg/ml) and harvested according to standard protocols. For each sample, ~30 metaphases (only 10 for acetaldehyde-treated due to extensive damage) were analyzed by the cytogenetics facility at The Kennedy Krieger Institute, Baltimore, MD. In untreated parental DLD1 cells and derived clones, a significant minority (<40%) of cells were hyperdiploid.

[0086] Statistical analyses. The mean (x), standard deviation (SD) and standard error of the mean (SEM) were computed for replicated experiments. The 90% confidence intervals of the fold-difference (X, pharmacogenetic window) between parental and null cells for three experimental replicates of the drug sensitivity assays were calculated using the formula ($x \pm 2.92 (SD/\sqrt{3})$).

EXAMPLE 1

[0087] Targeted disruption of PALB2. The PALB2 gene was disrupted in DLD1 cells by serially deleting exon 8 in both alleles (Fig 1A). We limited our attempts of generating PALB2-null cells to DLD1 parental cells (clone # 6) only, based on prior experience documenting a failure to isolate BRCA2-null clones in four other tested parental lines. We screened 80 neo-resistant clones after targeting the first allele and obtained six $PALB2^{+/-}$ clones. After targeting the second allele and screening 270 neo-resistant recombinants, we obtained two $PALB2^{-/-}$ clones, termed $PALB2^{-/-}1$ and $PALB2^{-/-}2$. PCR (Fig. 1B), RT-PCR

(Fig. 1C), and western blot analyses (Fig. 1D) confirmed the hemizygous and the homozygous gene disruptions. Of the 270 screened clones, we identified 22 clones that were integrated in the PALB2 locus, of which 20 clones had re-integrated the construct into the already-inactivated allele. The ratio of clones (10:1) integrated into the disrupted allele to the ones integrated into the wildtype allele suggested that PALB2, like BRAC2, was also a "lethal gene", or had loss-of-fitness, when null (Table 1).

Table 1: Lack of fitness' (lethality) implied for the *PALB2*$^{-/-}$ state

Intended genotype	No. of integrants screened	Integration into *PALB2*	Desired genotype obtained
PALB2$^{+/-}$	80	6	6
PALB2$^{-/-}$	270	22	2[1]

[1]The other 20 clones represented integration into the already-disrupted allele.

EXAMPLE 2

[0088] Functional validation of PALB2$^{-/-}$ cells. The two PALB2-null clones proliferated slowly as compared to the PALB2-hemizygous and parental cells. The doubling time was estimated at 27-28 hours for null clones and 19-20 hours for parental and hemizygous clones. The slow proliferation (Fig. 1E) of the null cells was a stable feature over successive passages. We assessed the effect of PALB2 deficiency on cell survival after ^{137}Cs γ-irradiation using colony-formation assays. We observed a two- to three-fold decrease in the survival of irradiated PALB2-null cells as compared to the parental line (Fig. 1F). Upon treatment with the crosslinker MMC for 48 hours, the PALB2-deficient cells became arrested at the G2/M phase of the cell division cycle. Using flow cytometry, we determined the fractions of cells at the different stages of the cell cycle 48 hours after treatment with MMC (Fig. 2). A pronounced G2/M arrest (>40% cells) was observed in PALB2$^{-/-}$ cells at the lower concentration of 20 nM, similar to the effect on parental cells at 200 nM (i.e., a pharmacogenetic window of 10X upon 48-hour observation).

EXAMPLE 3

[0089] Cell proliferation upon drug treatment. The role of PALB2 in DNA damage-response and in replication fork maintenance is indicated by the hypersensitivity of the PALB2-deficient cells to DNA crosslinking agents. We estimated cell survival following a six-day exposure of the parental, heterozygous and null cells to various relevant drugs. PALB2-/- cells had increased sensitivity to the ICL agents melphalan (20X), MMC (16X), and cisplatin (15X), (Fig. 3), and were hypersensitive to the tested topoisomerase inhibitors etoposide (8X) and camptothecin (6X) (Fig. 3). Three replicate experiments were done for MMC treatment and the 90% confidence intervals of the pharmacogenetic window (X) between the parental and null cells were found to be 13 to 22X for *PALB2*$^{-/-}$1 cells and 19 to 27X for *BRCA2*$^{-/-}$ cells. Naturally-derived *BRCA2*-null CAPAN1 cells[31] had intermediate hypersensitivities (Fig 4), consistent with the lower drug sensitivities previously noted for these cells.

EXAMPLE 4

[0090] In vivo confirmation of therapeutic validity. Xenograft models using PALB2$^{-/-}$ and BRCA2$^{-/-}$ cancer cell lines were explored due to the distal pathway genes commanding the greatest therapeutic and clinical interest. Mice were treated with a single dose of MMC (5mg/kg). The DLD1 parental cell line and PALB2$^{+/-}$ did not respond to MMC treatment (Fig. 5). In contrast, PALB2$^{-/-}$ cell lines showed sustained tumor regression to day 18-20 (Fig. 5). The xenograft responses to MMC mirrored the in vitro hypersensitivity patterns. We also tested two BRCA2-null cell lines, CAPAN1 (a naturally occurring BRCA2-mutated cell line) and an engineered cell line, BRCA2$^{-/-}$. These cell lines also had sustained tumor regression upon MMC treatment (Fig. 5).

EXAMPLE 5

[0091] Tumor histology. Histologic sections of xenografted tissues from untreated mice and mice 1, 4, and 7 days after treatment with mitomycin C were reviewed for extent of mitotic figures, apoptosis, confluent necrosis, hemorrhage and fibrosis. In all xenografts, tissues at Day 1 did not have appreciable differences from that seen in untreated tumors.

However, by day 4 all xenografts null for PALB2 ($PALB2^{-/-}$1, $PALB2^{-/-}$2) and BRCA2 (CAPAN1, $BRCA2^{-/-}$) had morphologic changes characterized by an increase in apoptotic bodies, cellular enlargement lacking an increase in nuclear-to-cytoplasmic ratio, prominent nucleoli, multinucleation, and enlarged eosinophilic cells containing chromosomes in disarray (Fig. 6). These changes were multifocal within a given xenograft at Day 4 and extensive at Day 7 after treatment. Fibrosis and confluent necrosis were not a feature of treatment in these xenografts, with only a single case (CAPAN1) having a hyalinized and inflamed stroma by Day 7. By contrast, no morphologic changes were observed in the wildtype cell line DLD1 or the heterozygous PALB2 mutant line $PALB2^{+/-}$ (Fig. 6).

EXAMPLE 6

[0092] High-magnitude hypersensitivities to PARP inhibition and acetaldehyde. Both $BRCA2^{-/-}$ and $PALB2^{-/-}$ cell lines had extreme hypersensitivities (≥1000X) to PARP inhibition (KU0058948) (Fig. 7). They also had 20 to 27 X hypersensitivity to acetaldehyde (Fig. 7) but no differential response with formaldehyde or glyoxal solution (Table 2). Three replicate experiments were done with KU0058948 and acetaldehyde, and the 90% confidence intervals of pharmacogenetic window (X) between the parental and null cells using KU0058948 were found to be 831 to 1057X for *PALB2*$^{-/-}$1 cells and 1066 to 1199X for *BRCA2*$^{-/-}$ cells. The 90% confidence intervals with acetaldehyde treatment were 15 to 29X for *PALB2*$^{-/-}$1 cells and 15 to 23X for *BRCA2*$^{-/-}$ cells. We used p53 reporter cells to explore whether acetaldehyde produced DNA strand breaks in cultured cells. Our results indicate that acetaldehyde induced a very weak p53 response in p53R cells, inferring that acetaldehyde may not exert its major action through DNA strand breaks. Both $BRCA2^{-/-}$ and $PALB2^{-/-}$ genotypes were tested using several other aldehydes (a two-carbon to nine-carbon series, Table 2), including acrolein, butyraldehyde, crotonaldehyde, benzaldehyde, glutraldehyde, phenylacetaldehyde, cinnamaldehyde, acetaldehyde dimethyl acetal, and aminoacetaldehyde dimethyl acetal. While we noted a hypersensitivity of $BRCA2^{-}$ and $PALB2^{-}$ null lines to acetaldehyde, its small size and high volatility deems it an undesirable agent for in vivo studies and further evaluation. In an attempt to identify a lead candidate with better pharmacologic properties we decided to explore a small structure-activity-relationship study centered around acetaldehyde. A series of aliphatic and aromatic aldehydes (1-9 carbons) were examined in the hope that a higher molecular weight might lead to a less volatile lead

candidate with a similar biological response. As aldehydes are also well known to be unstable and exceptional crosslinking agents, we also tried to replace the "active" aldehyde moiety with a protected aldehyde synthon (acetaldehyde dimethyl acetal, and aminoacetaldehyde dimethyl acetal) capable of generating the active functionality on degradation. None of these treatments elicited differential responses between wildtype and null cells (Table 2). They were either uniformly toxic or benign to all cell types. We tested the sensitivities of the proximal genes FANCC and FANCG with the PARP inhibitor KU0058948 and with the biological metabolites acetaldehyde and formaldehyde, there was a 3X difference with PARP inhibition (Fig. 7), and 2X and 3X differences with acetaldehyde (Fig. 7), and formaldehyde (Table 2), respectively.

[0093] TABLE 2: Relative chemical sensitivities of differing FA-null genotypes

Treatment	Class	Pharmacogenetic windows (IC_{50} of null cells)*			
		PALB2$^{-/-}$	*BRCA2*$^{-/-}$	*FANCC*$^{-/-/-}$	*FANCG*$^{-/-}$
Melphalan	Crosslinker	20X (0.25μM)	25X†	14X†	14X†
Mitomycin C	Crosslinker	17X (10nM)	23X†	13X†	12X†
Cisplatin	Crosslinker	15X (0.6-0.7μM)	16X†	9X†	7X†
KU0058948	PARP inhibitor	1000X (0.075μM)	1133X (0.022μM)	3X	3X (17μM)
Etoposide	Topo II inhibitor	8X (50-60nM)	10X†	- †	- †
Camptothecin	Topo I inhibitor	6X (0.625nM)	6X†	ND	ND
Formaldehyde	1-C aldehyde	-	-	2.8X (2μM)	2.8X (2μM)
Acetaldehyde	2-C aldehyde	22X (0.7mM)	19X (~1mM)	-	2.4X (5.3mM)

Other tested aldehydes ‡	2-9C aldehydes	-	-	ND	ND

*Data, where available. '-', less than 2-fold window. Windows were defined as the ratio of the IC_{50} values of parental cells and gene-knockout cells; data here are from the graphical results of figures 5 and 2. ND, Not done. Parentheses contain IC_{50} values of the gene-null cells.

†Data from our prior reports (Gastroenterology 2006, 130:2145-2154; Cancer Res 2008, 68:5023-5030)

‡See text

EXAMPLE 7

[0094] Chromosomal breakage and instability. *BRCA2*$^{-/-}$ and *PALB2*$^{-/-}$ cells displayed increased chromosomal instability, which was further enhanced after treatment with DEB and acetaldehyde. Treatment-induced breakage serves as a conventional diagnostic test for the FA syndrome. The number of breaks/gaps scored per cell was 0.1, 0.5 and 1.3, respectively, for the untreated parental, *BRCA2*$^{-/-}$, and *PALB2*$^{-/-}$1 cells. The number of breaks and gaps per cell scored upon DEB treatment were 0.03, 5.2 and 4.2, respectively, for the parental, *BRCA2*$^{-/-}$, and *PALB2*$^{-/-}$1 cells (Figs. 8, 9A and B). For acetaldehyde-treated cells, there were no breaks in parental cells but breaks too many to count in the *BRCA2*$^{-/-}$ and *PALB2*$^{-/-}$1 cells (Fig. 10). Numerous fragments were seen in these cell lines along with rings, broken or intact triradials, quadriradials, and other complex radials, indicating breakage and rejoining of chromatids and/or chromosomes (Fig. 10). *BRCA2*$^{-/-}$ and *PALB2*$^{-/-}$ cells therefore displayed much higher rates of induced chromosomal aberrations than parental cells.

EXAMPLE 8

[0095] Multi-gene cancer panels for preclinical studies provide novelty and cancer modeling. The multi-gene panel of the present invention, comprising engineered FANCC^{-}, FANCG-, BRCA2/FANCD1-, and PALB2/FANCN-null human cancer cell lines had the shared phenotypes typical of FANC pathway defects, including hypersensitivities to the ICL agents and γ-radiation in vitro, and comparable tumor regression in xenograft models when

treated with a single dose of MMC. The same panel had divergences of hypersensitivities for some chemicals, however, including the epidemiologically important ethanol metabolite, acetaldehyde, the PARP inhibitor KU0058948, and the topo II inhibitor, etoposide. High-magnitude pharmacogenetic windows were observed. These were distinguished from prior work in avian and noncancer mammalian cells, which had low-magnitude (<5X) changes. A panel of cancer cells, also novel in being tumorigenic, now enables chemotherapeutic animal trials of pharmacogenetically-targeted agents and could aid preclinical therapeutic explorations.

EXAMPLE 9

[0096] FANC-null status is insufficient to convey every FANC-related hypersensitivity to cancer cells. The large numerical discrepancies found in the current work clearly challenges a loosely held but conventional dogma that FANC- null cells might largely share a set of common chemical hypersensitivities currently used to make some clinical decisions. Our results advance the idea that knowledge of divergent phenotypes may be useful to dissect differing functions in proximal and distal FANC genes and in anticipating rational treatment of patients with differing FANC genotypes. The high-magnitude divergences of hypersensitivities seen for acetaldehyde, a PARP inhibitor, and etoposide may be gene-dependent, affected by clonal variation, or by the choice of the tumor cells under study. Notably, the same considerations apply to real human tumors in a setting of personalized therapy. Thus, panel-based strategies comparing the sensitivities of matched, engineered genotypes to differing chemical probes provides insights for genetic epidemiology, for prevention strategies, and for exploration of novel therapeutic options due to the discrepant hypersensitivity patterns being unambiguous, emerging in multiple knockout clones, and remaining stable in these models.

EXAMPLE 10

[0097] Comparison of formaldehyde and acetaldehyde in human cancers. Both formaldehyde and acetaldehyde are natural human metabolites known to damage DNA and to elicit hypersensitive responses in FANC-null cells. In the model of the present invention, the formaldehyde pharmacogenetic window was 2-3X while that of acetaldehyde could be a log larger, at 19-22X. These pharmacogenetic windows show that the BRCA2 and PALB2 genes

in our syngeneic matched cell line pairs protected against ⅓ to ½ of the toxicity caused by formaldehyde, but near 96% of the toxicity caused by acetaldehyde. This suggests that acetaldehyde may have had the larger role in driving the evolutionary selection operating on the BRCA2 and PALB2 genes. Intriguingly, studies of inherited syndromes and cancer epidemiology imply that the FANC gene system may be adequately optimized to protect certain organs like ductal epithelia of pancreas and breast, oral cavity, and upper aerodigestive tract (among others), whose risk of cancer are elevated by both ethanol intake and by FANC gene mutations. The consequences of acetaldehyde damage might unite those two types of observation.

EXAMPLE 11

[0098] Acetaldehyde-induced DNA damage and novel therapeutic options. Acetaldehyde, an intermediate metabolite of ethanol breakdown, is carcinogenic. Genotoxicity was also reported: when cells from an FA patient were exposed to acetaldehyde, chromosomal aberrations appeared under conditions where cells of a healthy person experienced little effect. The best-studied DNA adduct from acetaldehyde is N2-ethyl-2'-deoxyguanosine, which is increased in liver DNA obtained from ethanol-treated rodents and in white blood cells from human alcohol abusers. The carcinogenic potential of this product is unclear. A different adduct, 1,N2-propano-2'-deoxyguanosine (PdG), formed from acetaldehyde in the presence of histones and other basic molecules, may be responsible for some mutagenic and genotoxic effects of acetaldehyde.

[0099] Prior literature suggested that acetaldehyde produced ICLs and implicated a role of the FANC DNA-damage response network in protecting cells against these crosslinks. Our finding of widely differing pharmacogenetic windows (19 to 22X in cells null for some FA genes; 2X, for others) may dampen support for the ICL theory somewhat as a major mechanism of action of acetaldehyde, given that all FANC defects in our panel had similar levels of hypersensitivity when tested using other ICL agents. Nonetheless, our cytogenetic observations support a mechanism producing widespread chromosomal breakage in $BRCA2^{-/-}$ and $PALB2^{-/-}$ cancer cells exposed to acetaldehyde.

[0100] The IC_{50} for acetaldehyde in our PALB2- and BRCA2-null cells was ≥700uM. This concentration is not physiologic in untreated humans. Data from human volunteers, however, indicate that local salivary acetaldehyde concentrations could reach 450 μM at high

blood alcohol concentrations, far above the genotoxic threshold. It is possible that ethanol, acetaldehyde, or ethanol-disulfirum administration could generate acetaldehyde levels sufficient for producing differential effects on FANC-null cells arising in carriers of FANC-gene mutations, chronic effects which could occur far below the IC_{50} concentration.

[0101] Implications for cancer epidemiology and prevention. In accordance with the present invention, cancer risks from ethanol can be stratified better in epidemiologic studies by determining the BRCA2 and PALB2 mutational gene status of individuals in studied populations. Cancer risks of BRCA2 and PALB2 carriers can be further stratified by metrics gauging the exposure to acetaldehyde and ethanol and by examining gene polymorphisms of the ADH and ALDH2 genes. The polymorphisms govern the tissue concentrations of acetaldehyde and, indirectly, the tendencies to ingest alcohol.

[0102] Potential interventions to reduce cancer mortality in the carrier population are also provided. FA patients and carriers might suffer increased risks of cancer upon consumption of alcohol or foods containing high levels of acetaldehyde. Reports in the literature suggest the use of aldehyde antagonists or aldehyde dehydrogenase agonists.

[0103] In accordance with some embodiments, the present invention provides chemical prophylaxis or even aldehyde-based chemotherapy for FANC-null neoplasms which pose an alternative at least as attractive as the current use of prophylactic surgery in BRCA2 mutation carriers. Given the levels of acetaldehyde achieved in humans in voluntary and anecdotal settings, the methods of the present invention can be used for treating PALB2- and BRCA2-deficient precursor neoplasm or cancers with either local infusion of acetaldehyde or systemic ethanol/disulfirum. Conversely, chronic exposure to acetaldehyde can also selectively harm FA patients or $FANC^-$ mutation carriers.

[0104] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0105] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely

intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0106] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

Claims:

1. A method for treating or preventing cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation comprising:

a) obtaining a sample from a subject;

b) analyzing the sample for the presence of a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation;

c) administering to the subject a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof when the analysis of the sample of b) indicates the subject has a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

2. The method of claim 1, wherein the sample is obtained from the blood, tissue or tumor of the subject.

3. The method of claim 1 or 2, wherein the method of analysis of the sample for the presence of a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation is selected from the group consisting of: analysis of the DNA or RNA in the sample from the subject; detection of the expression product or lack thereof in the sample from the subject; and analysis of the family history of the subject.

4. The method of any of claims 1 to 3, wherein the C_1-C_6 aldehyde or prodrug or derivative thereof is acetaldehyde.

5. The method of any of claims 1 to 4, wherein the aldehyde is the result of pretreatment of the subject with ethanol.

6. The method of any of claims 1 to 5, wherein the method further comprises administering to the subject a therapeutically effective amount of an alcohol dehydrogenase inhibitor.

7. The method of claim 6, wherein the alcohol dehydrogenase inhibitor is disulfiram.

8. The method of either of claims 6 or 7, wherein the alcohol dehydrogenase inhibitor is administered before administration of a C_1-C_6 aldehyde or prodrug or derivative thereof.

9. The method of any of claims 1 to 8, wherein the method further comprises administering to the subject a therapeutically effective amount at least one additional chemotherapeutic agent.

10. The method of claim 9, wherein the at least one additional chemotherapeutic agent is selected from the group consisting of alkylating agents, angiogenesis inhibitors, aromatase inhibitors, antimetabolites, anthracyclines, antitumor antibiotics, monoclonal antibodies, platinums, topoisomerase inhibitors, monoclonal antibodies, PARP enzyme inhibitors and plant alkaloids.

11. Use of a pharmaceutical composition comprising a therapeutically effective amount of an C_1-C_6 aldehyde or prodrug or derivative thereof, and a pharmaceutically acceptable carrier as a medicament for use in the prevention or treatment of cancer in a subject wherein the subject is identified as having a $BRCA2^{-/-}$ and/or $PALB2^{-/-}$ mutation.

12. The use of claim 11, wherein the C_1-C_6 aldehyde or prodrug or derivative thereof is acetaldehyde.

13. The use of either of claim 11 or 12, wherein the pharmaceutical composition further comprises a therapeutically effective amount of an alcohol dehydrogenase inhibitor.

14. The use of any of claims 11 to 13, wherein the pharmaceutical composition further comprises a therapeutically effective amount at least one additional chemotherapeutic agent.

15. The use of any of claims 11 to 14, wherein the at least one additional chemotherapeutic agent is selected from the group consisting of alkylating agents, angiogenesis inhibitors, aromatase inhibitors, antimetabolites, anthracyclines, antitumor antibiotics, monoclonal antibodies, platinums, topoisomerase inhibitors, monoclonal antibodies, PARP enzyme inhibitors and plant alkaloids.

16. An isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene.

17. A method for identifying a compound or small molecule that modulates a function of a cancer cell having a biallelic disruption of the PALB2 gene, comprising:

a) measuring a function in at least one isolated human adenocarcinoma cell line genetically modified to have a biallelic disruption of the PALB2 gene ($PALB2^{-/-}$ cell);

b) incubating said at least one isolated $PALB2^{-/-}$ cell with a test compound or small molecule;

c) measuring said function in said at least one $PALB2^{-/-}$ cell following incubation with said test compound or small molecule; and

d) determining the difference in said function in said at least one $PALB2^{-/-}$ cell before and after said incubation with said test compound or small molecule;

wherein a difference in function in said at least one $PALB2^{-/-}$ cell after said incubation as compared to before said incubation with said test compound or small molecule identifies said test compound or small molecule as a test compound or small molecule that modulates said function in cancer cells having a biallelic disruption of the PALB2 gene.

FIGURE 1

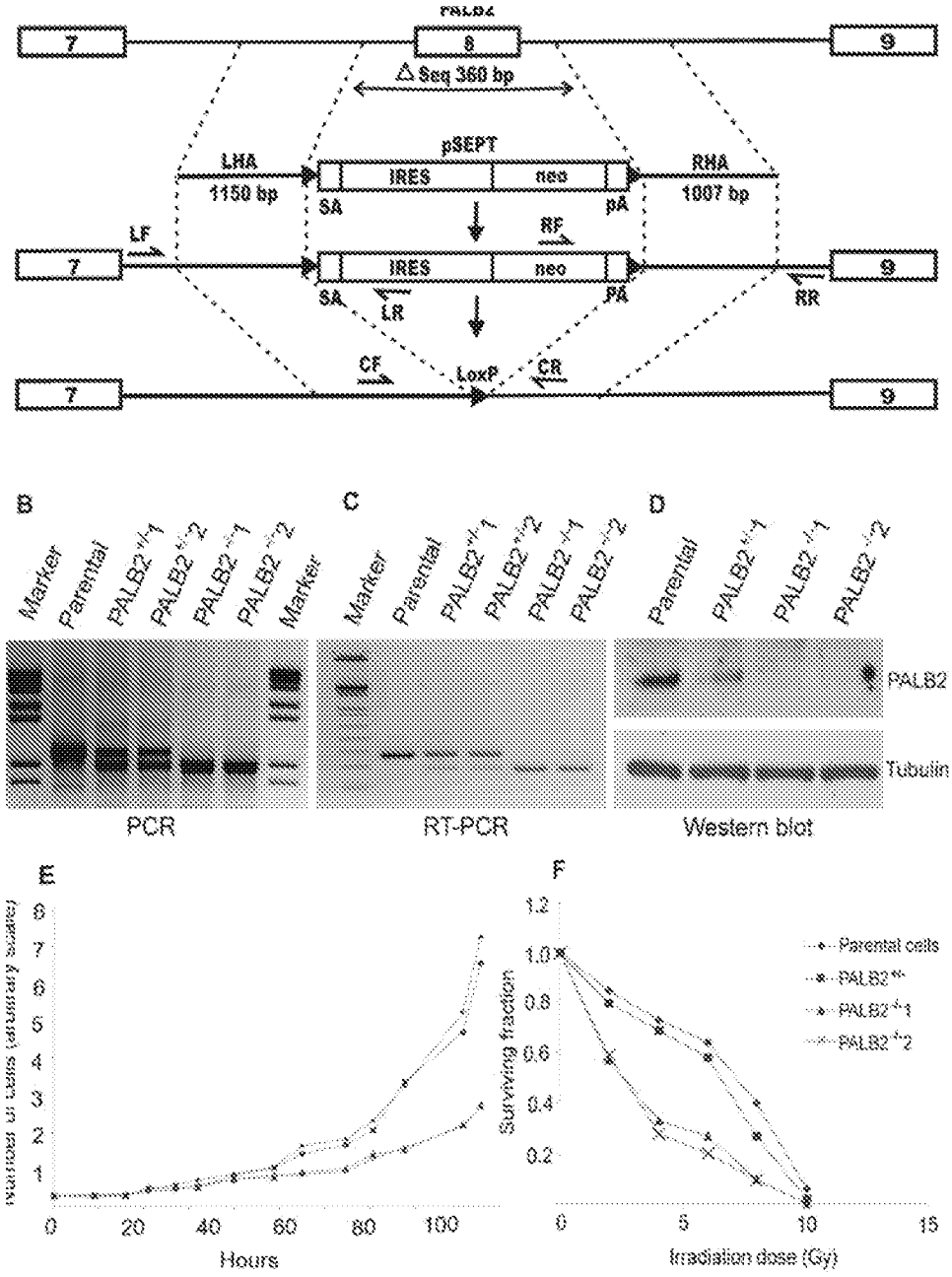

PALB2
7
8
9
Δ Seq 360 bp
pSEPT
LHA
1150 bp
IRES
neo
SA
pA
RHA
1007 bp
LF
RF
IRES
neo
SA
LR
PA
RR
CF
LoxP
CR
B
C
D
Marker
Parental
PALB2 +/-1
PALB2 +/-2
PALB2 -/-1
PALB2 -/-2
Marker
PALB2
Tubulin
PCR
RT-PCR
Western blot
E
F
Hours
Irradiation dose (Gy)
Surviving fraction
Parental cells
PALB2 -/-
PALB2 +/-1
PALB2 +/-2

FIGURE 2

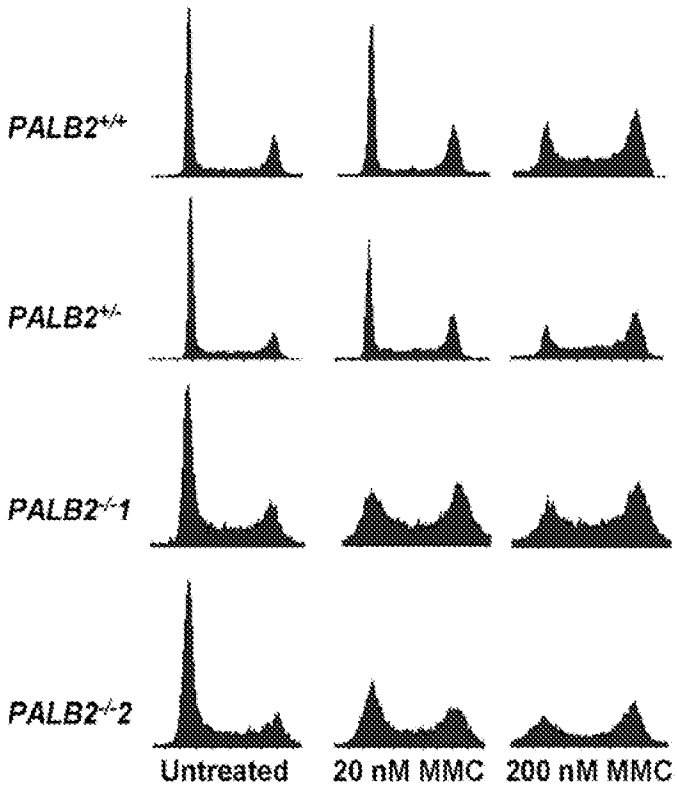


FIGURE 3

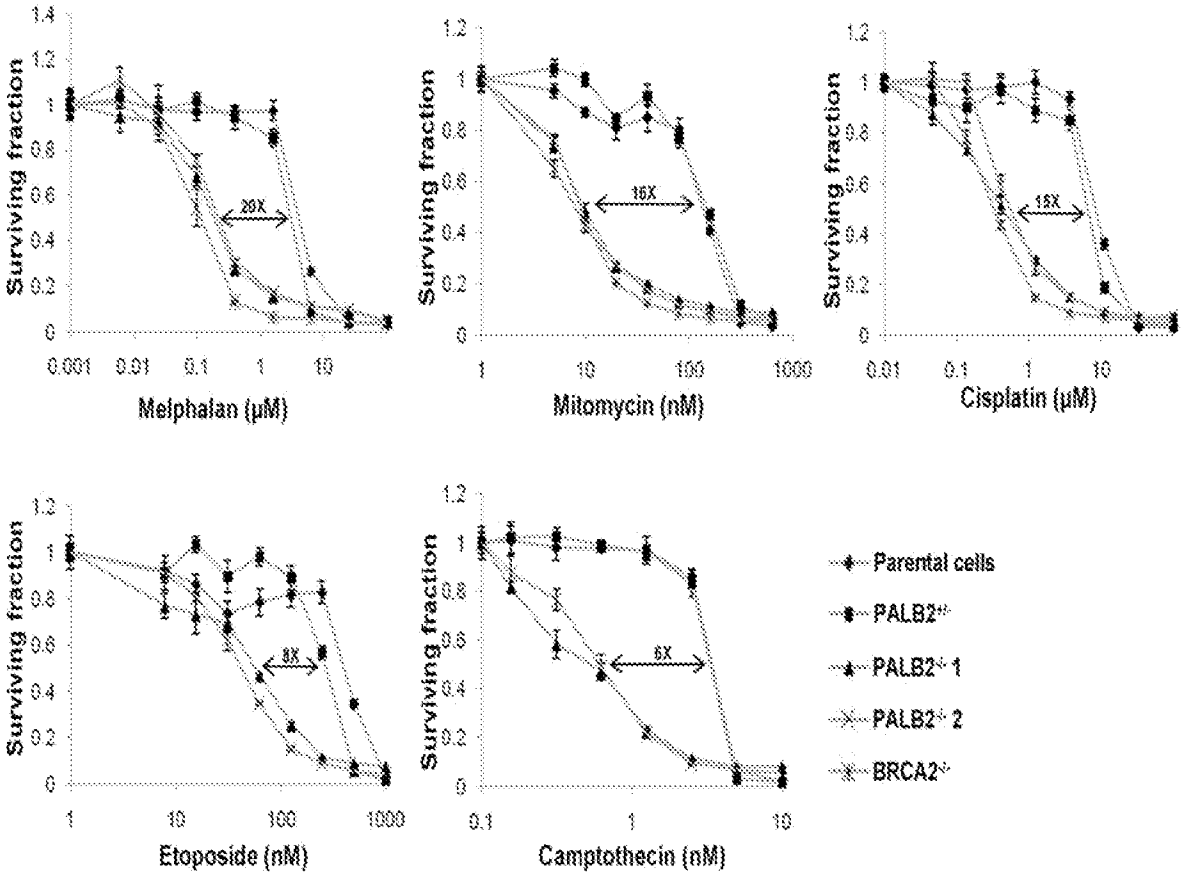

Surviving fraction
Melphalan (μM)
20X
Mitomycin (nM)
16X
Cisplatin (μM)
15X
Etoposide (nM)
8X
Camptothecin (nM)
6X
Parental cells
PALB2-/-
PALB2-/- 1
PALB2-/- 2
BRCA2-/-

FIGURE 4

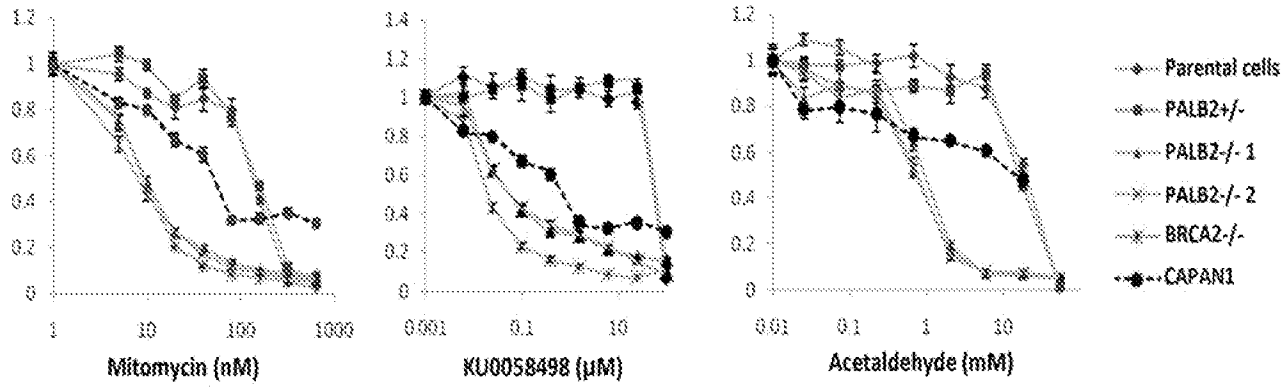

1.2
1
0.8
0.6
0.4
0.2
0
1
10
100
1000
Mitomycin (nM)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.1
10
KU0058498 (μM)
1.2
1
0.8
0.6
0.4
0.2
0
0.01
0.1
1
10
Acetaldehyde (mM)
Parental cells
PALB2+/-
PALB2-/- 1
PALB2-/- 2
BRCA2-/-
CAPAN1

FIGURE 5

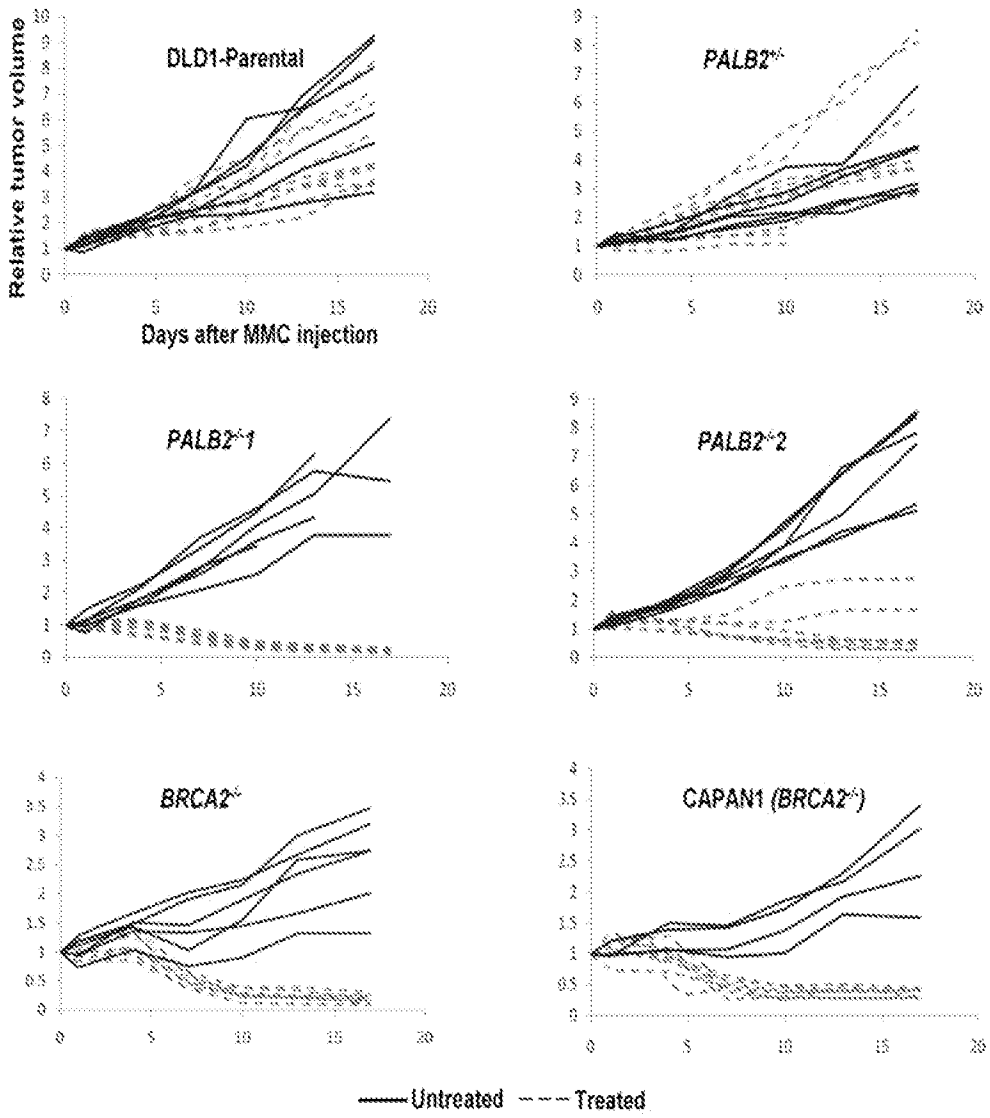

DLD1-Parental
PALB2-/-
PALB2-/-1
PALB2-/-2
BRCA2-/-
CAPAN1 (BRCA2-/-)
Relative tumor volume
Days after MMC injection
Untreated
Treated

FIGURE 6

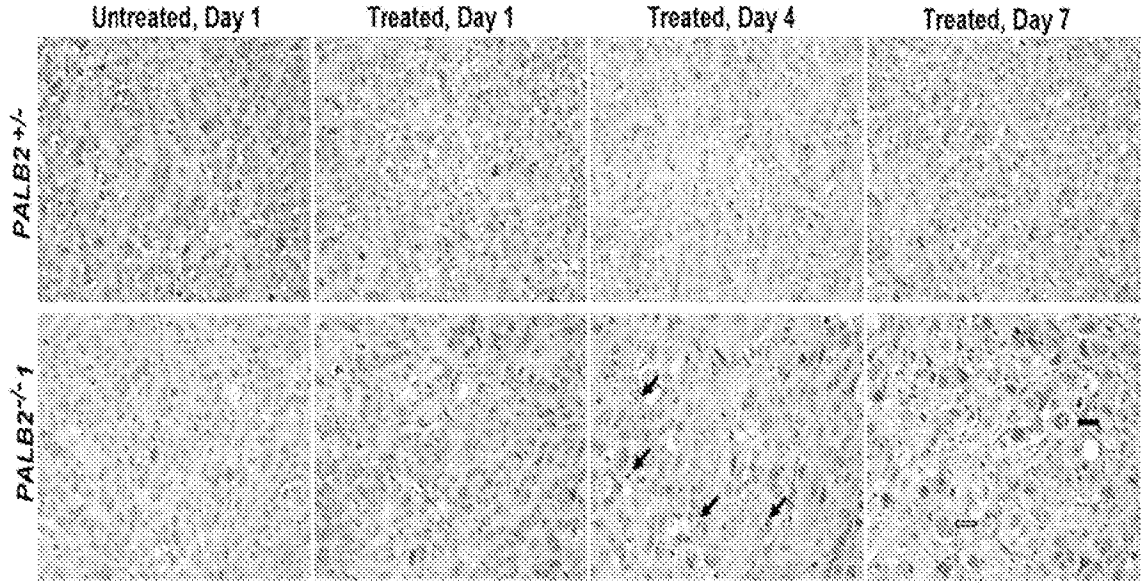

Untreated, Day 1
Treated, Day 1
Treated, Day 4
Treated, Day 7
PALB2 +/-
PALB2 -/- 1

FIGURE 7

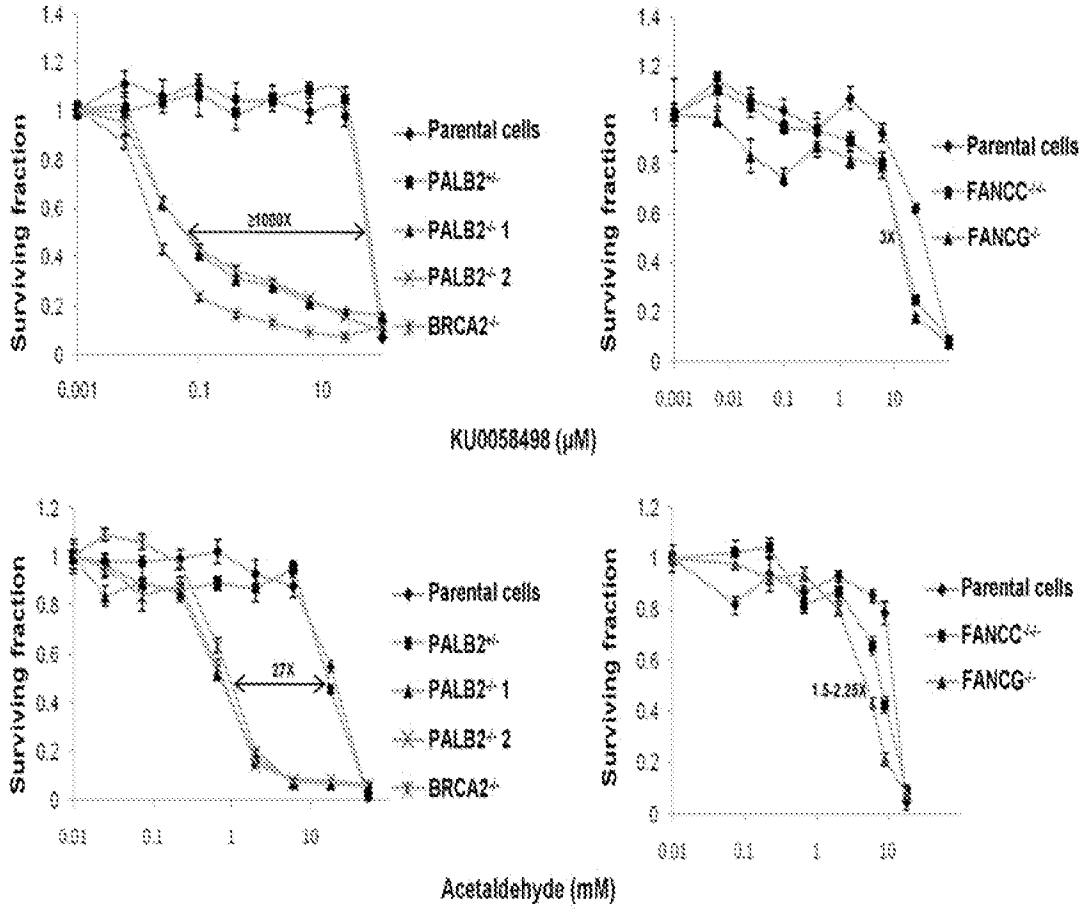

Surviving fraction
Parental cells
PALB2-/-
PALB2-/- 1
PALB2-/- 2
BRCA2-/-
>1000X
FANCC-/-
FANCG-/-
3X
KU0058498 (μM)
27X
1.5-2.25X
Acetaldehyde (mM)

FIGURE 8

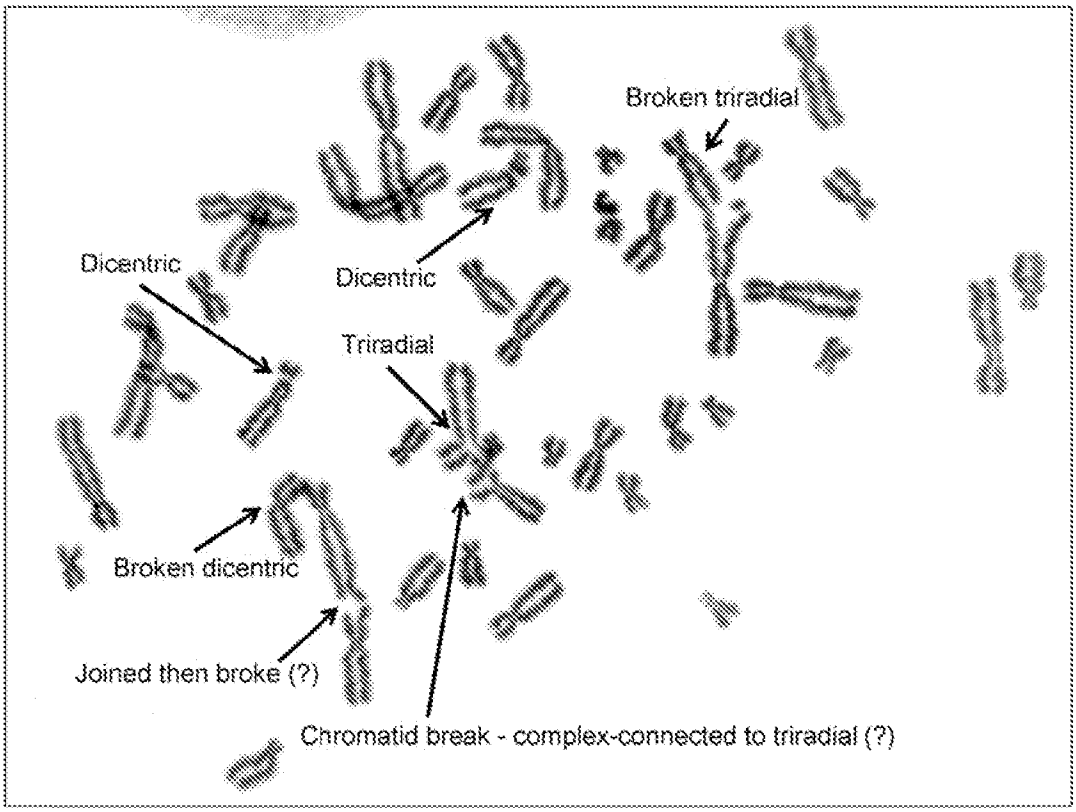

Broken triradial
Dicentric
Dicentric
Triradial
Broken dicentric
Joined then broke (?)
Chromatid break - complex-connected to triradial (?)

FIGURE 9

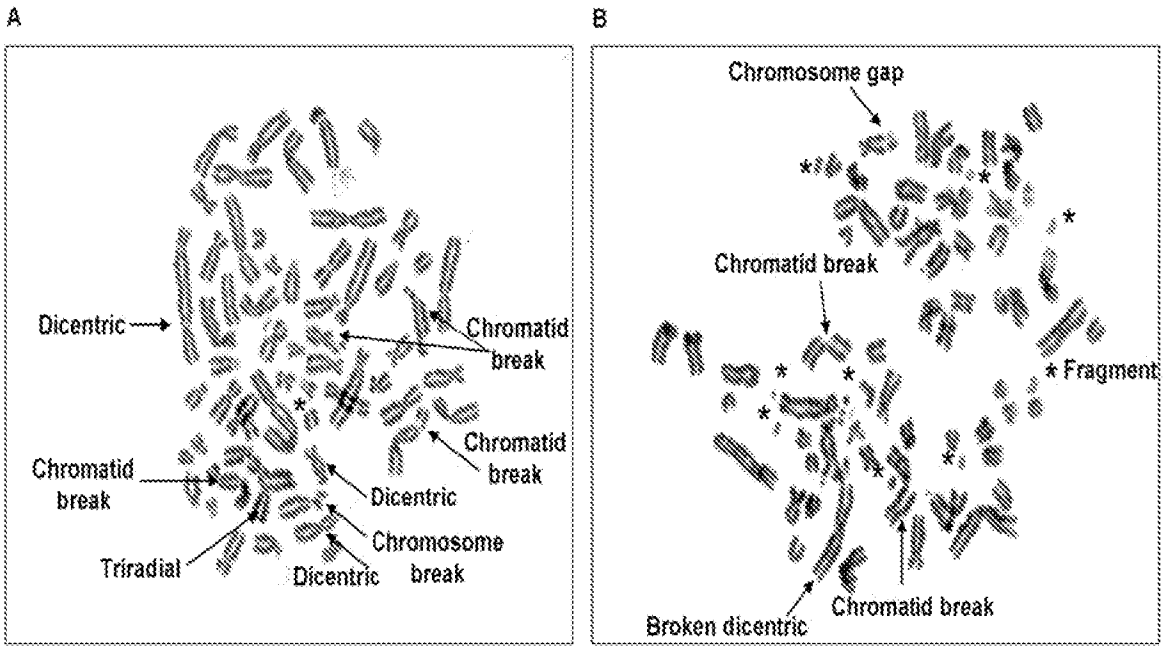
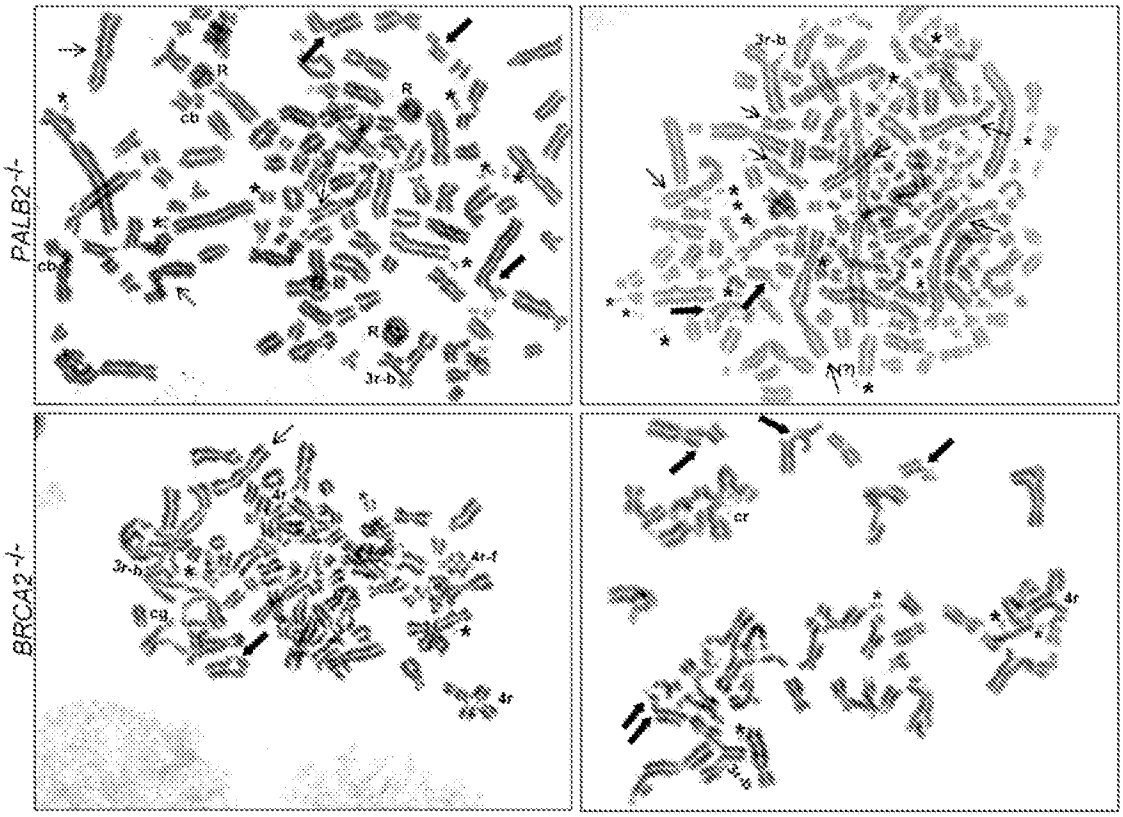

A
Dicentric
Chromatid break
Chromatid break
Chromatid break
Dicentric
Chromosome break
Triradial
Dicentric
B
Chromosome gap
Chromatid break
Fragment
Broken dicentric
Chromatid break

FIGURE 10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/070257

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/11(2006.01)i, A61P 35/00(2006.01)i, G01N 33/574(2006.01)i, G01N 33/48(2006.01)i

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 31/11; A61P 35/00; G01N 33/574; G01N 33/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: BRCA2, PALB2, biallelic mutation, aldehyde, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RIDPATH, J. R. et al., `Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde`, Cancer Research, 2007, Vol. 67, No. 23, pages 11117-11122. See abstract; figure 1; and pages 11117-11118.	11-13
A		16-17
X	VILLARROEL, M. C. et al., `Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer`, Molecular Cancer Therapeutics, 2011, Vol. 10, No. 1, pages 3-8. See abstract and pages 3-6.	16-17
A	MARX, J., `Possible new role for BRCA2`, Science, 2004, Vol. 305, page 1691. See page 1691.	11-13,16-17
A	REID, S. et al., `Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer`, Nature Genetics, 2007, Vol. 39, No. 2, pages 162-164. See the whole document.	11-13,16-17

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 2014 (28.01.2014)	**28 January 2014 (28.01.2014)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/070257

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEN, D. et al., `Disulfiram, a clinically used anti-alcoholism drug and copper-binding agent, induces apoptotic cell death in breast cancer cultures and xenografts via inhibition of the proteasome activity`, Cancer Research, 2606, Vol. 66, No. 21, pages 10425-10433. See the whole document.	11-13,16-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/070257

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-10
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-10 pertain to a method for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☒ Claims Nos.: 7, 10
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 7 and 10 are unclear, since they refer to one of claims which are not drafted in accordance with PCT Rule 6.4(a) (PCT Article 6).

3. ☒ Claims Nos.: 4-6, 8-9, 14-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/070257

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
None			