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(54) Title: METHODS OF INCREASING THE SENSITIVITY OF CANCER CELLS TO DNA DAMAGE

(57) Abstract: The present invention relates to the finding that cells which have a kinase-deficient phenotype have increased sensi-
tivity to DNA damage promoting agents, in particular PARP inhibitors. Methods of treating cancers with a kinase-deficient phenotype
using DNA damage promoting agents and methods of treating cancers with a combination of DNA damage promoting agents and
kinase inhibitors are provided, along with screening methods for identifying new therapeutics for use in combination with DNA
damage promoting agents.



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Methods of Increasing the Sensitivity of Cancer Cells to DNA Damage

This invention relates to the induction of cellular lethality in cancer cells, in particular cancer cells with kinase-deficient phenotypes.

Mammalian cells which are deficient in homologous recombination (HR) dependent DNA DSB repair, in particular cells which are BRCA1 or BRCA2 deficient, have been shown to be extremely sensitive to the inhibition of Poly(ADP-Ribose) polymerase (PARP), which is a component of the Base Excision Repair pathway which repairs single strand DNA damage. (Farmer H et al. Nature 2005; 434:917-21, McCabe et al Cancer Biology & Therapy 2005 4:9, 934-936, Bryant et al, Nature 2005; 434: 913-917). Recent data also suggests that defects in other components of the HR pathway e.g. ATR, ATM, NBS1, RAD51, RAD54, DSS1, CHK1, CHK2, FANCD2, FANCA, FANCC also leads to sensitivity to PARP inhibition (McCabe et al, Cancer Res (2006), and WO05/053662).

The present inventors have now discovered that cells that are deficient in kinases which have no known role in HR dependent DNA DSB repair are also extremely sensitive to the inhibitors of poly (ADP-ribose) polymerase (PARP) and other agents which increase the amount of DNA damage in a cell. This has important implications in the treatment of cancer conditions.

An aspect of the invention provides a method of treating an individual with a cancer condition having a kinase-deficient phenotype, comprising;

administering a DNA damage-promoting agent to said individual.

Related aspects of the invention provide the use of a DNA damage promoting agent for the manufacture of a medicament for use in the treatment of a cancer condition in an individual, wherein said cancer condition has a kinase-deficient phenotype; and, a DNA damage

promoting agent for use in the treatment of a cancer condition in an individual, wherein said cancer condition has a kinase-deficient phenotype.

5 A DNA damage promoting agent is an compound or entity (such as a small organic molecule, peptide or nucleic acid) which increases the amount of DNA damage in a cell, either directly or indirectly, for example through inhibition of DNA repair. The DNA damage promoting agent is often a small organic molecule compound.

10 Suitable DNA damage promoting agents include agents which damage DNA in a cell (i.e. DNA damaging agents), for example alkylating agents such as methyl methanesulfonate (MMS), temozolomide, dacarbazine (DTIC), cisplatin, oxaliplatin, carboplatin, cisplatin-doxorubicin-
15 cyclophosphamide, carboplatin-paclitaxel, cyclophosphamide, nitrogen mustard, melphalan, chlorambucil, busulphan, etoposide, teniposide, amsacrine, irinotecan, topotecan and rubitecan and nitrosoureas, topoisomerase-1 inhibitors like Topotecan, Irinotecan, Rubitecan, Exatecan, Lurtotecan, Gimetecan, Diflomotecan (homocamptothecins); as
20 well as 7-substituted non-silatecans; the 7-silyl camptothecins, BNP 1350; and non-camptothecin topoisomerase-I inhibitors such as indolocarbazoles, topoisomerase-II inhibitors like Doxorubicin, Danorubicin, and other rubicins, the acridines (Amsacrine, m-AMSA), Mitoxantrone, Etoposide, Teniposide and AQ4, dual topoisomerase-I and
25 II inhibitors like the benzophenazines, XR 11576/MLN 576 and benzopyrindolindoles, and antimetabolites such as gemcitabine, antifolates such as fluoropyrimidines like 5 fluorouracil and tegafur, raltitrexed, methotrexate, cytosine arabinoside, and hydroxyurea, and arsenic trioxide.

30 Suitable DNA damage promoting agents include agents which inhibit DNA repair in cells (i.e. DNA repair inhibitors), for example inhibitors of the Base Excision Repair pathway. In some preferred embodiments,

the DNA damage promoting agent is an inhibitor of poly (ADP-ribose) polymerase (PARP).

Another aspect of the invention provides a method of treating an individual with a cancer condition having a kinase-deficient phenotype, comprising;

administering a poly (ADP-ribose) polymerase (PARP) inhibitor to said individual.

Related aspects of the invention provide the use of a PARP inhibitor for the manufacture of a medicament for use in the treatment of a cancer condition in an individual, wherein said cancer condition has a kinase-deficient phenotype; and, a PARP inhibitor for use in the treatment of a cancer condition in an individual, wherein said cancer condition has a kinase-deficient phenotype.

In some embodiments, the DNA damage promoting agent is ionising radiation (IR). The use of IR to induce DNA damage in cancer cells is well known in the art and any suitable technique may be used to irradiate cancer cells with a kinase-deficient phenotype as described herein.

IR includes external beam therapy, such as X-rays, gamma rays and electrons. Suitable regimes include fractionated palliative and curative regimes involving accelerated- and hyper-fractionation as appropriate and all geometric forms, conventional, 3D, 3D conformal, IMRT (intensity modulated radiotherapy), 4D and adaptive radiotherapy. (Bucci MK et al [2005] CA Cancer J Clin 55; 117-134, Haustermans et al (2004) Rays 29(3):231-6).

IR includes local/targeted therapies, such as radio active seeds or wires surgically implanted as part of a brachytherapy regime (Dale et al [1998] B J Radiol 71; 465-483); radioimmunotherapy, where a radioactive emitter is linked to an immunologic molecule such as a

monoclonal antibody e.g. ibritumomab (Zevalin) (Blum KA, Bartlett NL [2004] Expert Opin Biol Ther. 4(8):1323-31); and non-immunological targeting such as radioactive microspheres delivered by injection e.g. SIR-Spheres® (Ho S et al (2001) Journal of Nuclear Medicine 42(10):1587-1589). Non-immunological targeting may also be accomplished with targeted peptide receptor therapy. For example, radiolabelled somatostatin analogues (¹¹¹In-Octreotide, ⁹⁰Y-OctreoTher™, ¹⁷⁷Lu-Octreotate) or other peptide ligands, such as Bombesin and NPY(y₁) analogues (Krenning et al [2004] Ann NY Acad Sci. 1014(2): 234-245)

An individual having a cancer condition may comprise one or more cancer cells. Cancer cells in general are characterised by abnormal proliferation relative to normal cells and typically form clusters or tumours in an individual having a cancer condition. The cancer cells may possess a phenotype which characterises the cancer condition. For example, an individual with a cancer condition with a kinase-deficient phenotype may comprise one or more cancer cells which possess the kinase-deficient phenotype. A kinase-deficient phenotype may be selected from the group consisting of: a cyclin-dependent kinase 5 (CDK5) deficient phenotype, a mitogen-activated protein kinase 12 (MAPK12) deficient phenotype, a polo-like kinase 3 (PLK3) deficient phenotype, a polynucleotide kinase 3'-phosphatase (PNKP) deficient phenotype, a serine/threonine kinase 36 (STK36) deficient phenotype, or a serine/threonine kinase 22C (STK22C) deficient phenotype (STK22C is also known as testis-specific serine kinase 3; TSSK3).

In a cancer cell with a kinase-deficient phenotype, the activity of a cellular pathway which is mediated by the kinase is reduced or abrogated i.e. the ability of the pathway to perform its cellular function is lost or reduced relative to control cells with a normal phenotype, leading to a loss or reduction in the effects or consequences of the pathway within the cell. For example, reduction or abrogation of the activity of a kinase-mediated cellular pathway may

lead to a reduction or loss in the amount of the final product produced by the pathway.

A kinase-mediated cellular pathway is a cellular pathway which requires active kinase in order to function (i.e. a pathway in which the kinase is a non-redundant component). The kinase-mediated cellular pathway may be selected from the group consisting of a CDK5 mediated pathway, a MAPK12 mediated pathway, a PLK3 mediated pathway, a PNKP mediated pathway, a STK36 mediated pathway, and a STK22C mediated pathway.

In some embodiments, the cancer cells may have a CDK5 deficient phenotype i.e. there may be a reduction or loss of function of a CDK5 mediated cellular pathway in the cancer cells. CDK5-mediated pathways include the axon guidance pathway (Kyoto Encyclopaedia of Genes and Genomes (KEGG) database reference hsa04360). Examples of components of a CDK5-mediated pathway are shown in the database entry. Other examples include CDK5R1 (National Center for Biotechnology Information (NCBI) Protein Entrez database accession number NP_003876.1), CDK5R2 (NP_003927.1), CDK5RAP1 (NP_057492.2), CABLES2 (NP_112492.1), FSD1 (NP_077309.1), CABLES1 (NP_612384.1), CCND2 (NP_001750.1), CCNG1, CTNNB (NP_001895.1), GAK (NP_005246.1), NES (NP_006608.1), PCNA (NP_002583.1), STX1A (NP_004594.1) and STXBP1 (NP_001027392.1).

A cancer cell with a CDK5 deficient phenotype may be deficient in a component of a CDK5 mediated pathway i.e. expression or activity of a component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in CDK5 i.e. expression or activity of CDK5 may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of CDK5 (Mendelian Inheritance in Man (MIM) database entry number: 123831, NCBI GeneID reference: 1020) has the NCBI Protein entrez database reference NP_004926.1 and the GeneInfo (GI) identifier gi4826675. The nucleic acid sequence of CDK5 has the database reference NM_004935.2 GI: 38454327.

In some embodiments, the cancer cells may have a MAPK12 deficient phenotype i.e. there may be a reduction or loss of function of a MAPK12 mediated cellular pathway in the cancer cells. MAPK12-mediated pathways include the MAPK signalling pathway (KEGG database reference hsa04010), Toll-like receptor signalling pathway (KEGG database reference hsa04620), Fc epsilon RI signalling pathway (KEGG database reference hsa04664) and the Leukocyte transendothelial migration pathway (KEGG database reference hsa04670). Examples of components of MAPK12-mediated pathways are shown in the relevant database entries. Other examples include DUSP1 (NP_004408.1), GRB2 (NP_002077.1), MPKAPK5 (NP_620777.1) and SNTB1 (NP_066301.1).

A cancer cell with a MAPK12 deficient phenotype may be deficient in a component of a MAPK12 mediated pathway i.e. expression or activity of a component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in MAPK12 i.e. expression or activity of MAPK12 may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of MAPK12 (MIM: 602399 GeneID: 6300) has the NCBI database reference NP_002960.2 GI: 48255970. The nucleic acid sequence of MAPK12 has the NCBI database reference NM_002969.3 GI: 48255969.

In some embodiments, cancer cells may have a PLK3 deficient phenotype i.e. the activity of a PLK3 mediated cellular pathway may be reduced or abolished in the cancer cells. PLK3-mediated pathways include the axon guidance pathway (KEGG database reference hsa04360). Examples of components of a PLK3 mediated cellular pathway are shown in the database entry. Other examples include CDC25C (NP_001781.1), CHK2 (NP_001005735.1), CIB1 (NP_006375.1), and TP53 (NP_000537.1).

A cancer cell with a PLK3 deficient phenotype may be deficient in a component of a PLK3 mediated pathway i.e. expression or activity of a

component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in PLK3 i.e. expression or activity of PLK3 may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of PLK3 (MIM: 602913 GeneID: 1263) has the NCBI database references NP_004064.2 GI: 41872374. The nucleic acid sequence of PLK3 has the NCBI database references NM_004073.2 GI: 41872373.

10 In some embodiments, the cancer cells may have a PNKP deficient phenotype i.e. the activity of a PNKP mediated cellular pathway may be reduced or abolished in the cancer cells. PNKP possesses dual DNA processing activities as a 5' DNA-kinase and a 3'-phosphatase and is the principal enzyme responsible for restoring the preferred termini at DNA breaks. PNKP-mediated pathways include Non-Homologous End Joining (NHEJ). Examples of components of PNKP mediated pathways include Ku70/80, XRCC1 (NP_006288.1), XRCC4 (NP_003392.1), NEIL1 (NP_078884.1) and NEIL2 (NP_659480.1). A deficiency in a PNKP mediated pathway may be monitored by observing genomic instability in the cancer cell.

A cancer cell with a PNKP deficient phenotype may be deficient in a component of a PNKP mediated pathway i.e. expression or activity of a component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in PNKP i.e. expression or activity of PNKP may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of PNKP (MIM: 605610, GeneID: 11284) has the NCBI database references NP_009185.2 GI: 31543419. The nucleic acid sequence of PNKP has the NCBI database references NM_007254.2 GI: 31543418.

In some embodiments, the cancer cells may have a STK36 deficient phenotype i.e. the activity of a STK36 mediated cellular pathway may

be reduced or abolished in the cancer cells. STK36-mediated pathways include the Hedgehog signalling pathway (KEGG database entry hsa04340). Examples of components of STK36 mediated cellular pathways are shown in the database entry. Other examples include CDK9
5 (NP_001252.1), GLI1 (NP_005260.1), GLI2 (NP_084655.1), GLI3 (P10071), MAST2 (AAH65499.1) and SUFU (NP_057253.2).

A cancer cell with a STK36 deficient phenotype may be deficient in a component of a STK36 mediated pathway i.e. expression or activity of a
10 component of the pathway may be reduced or abolished in the cancer cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in STK36 i.e. expression or activity of STK36 may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of STK36 (MIM: 607652 GeneID:
15 27148) has the NCBI database references NP_056505.1 GI: 24308123. The nucleic acid sequence of STK36 has the NCBI database references NM_015690.2 GI: 34222107.

In some embodiments, the cancer cells may have a STK22C deficient
20 phenotype i.e. the activity of a STK22C mediated cellular pathway may be reduced or abolished in the cancer cells. STK22C mediated pathways include male germ cell development or mature sperm function signalling. SMAD4 (NP_005350.1) is an example of a component of a STK22C mediated pathway.

A cancer cell with a STK22C deficient phenotype may be deficient in a component of a STK22C mediated pathway i.e. expression or activity of a component of the pathway may be reduced or abolished in the cancer
25 cell relative to control cells. In some preferred embodiments, the cancer cell may be deficient in STK22C i.e. expression or activity of STK22C may be reduced or abolished in the cancer cell relative to control cells. The amino acid sequence of STK22C (MIM: 607660 GeneID: 81629) has the NCBI database reference NP_443073.1 GI: 16418343. The
30

nucleic acid sequence of STK22C has the NCBI database reference NM_052841.3 GI: 61744443.

5 A cancer cell may be deficient in a kinase or other component of a
kinase-mediated cellular pathway through either the absence of the
component (component-null), reduction in amount of the component, or
dysfunction of the component, for example by means of mutation or
polymorphism in the encoding nucleic acid, or by means of mutation or
polymorphism in a gene encoding a regulatory factor. Kinase-deficient
10 cells may, for example, be heterozygous or homozygous for mutations or
polymorphisms in the nucleic acid encoding a kinase, or its regulatory
elements, which reduce expression or activity.

A cancer cell which is deficient in a component of a kinase-mediated
15 cellular pathway, such as a kinase set out above, may possess a level
or activity of the component which is less than 50%, less than 40%,
less than 30%, less than 20% or less than 10% of the normal population
level of the active component protein (e.g. in a non-deficient cell).
Deficient cells include null cells which contain no active component
20 or substantially no active component i.e. the activity of a component
of a kinase-mediated cellular pathway, such as a kinase selected from
the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C, is
abolished or substantially abolished in null cells.

25 Preferably, only cancer cells from the individual display the kinase-
deficient phenotype and non-cancer cells from the individual do not
display the kinase-deficient phenotype i.e. healthy cells from the
individual have a non-kinase-deficient phenotype. This allows the
sensitivity of cancer cells to PARP inhibition to be increased
30 relative to non-cancer cells, using the present methods.

A kinase-deficient phenotype may be displayed by any type of solid or
non-solid cancer or malignant lymphoma and especially leukaemia,
sarcomas, skin cancer, bladder cancer, breast cancer, uterine cancer,

ovarian cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, oesophageal cancer, pancreatic cancer, renal cancer, stomach cancer and cerebral cancer. In some preferred embodiments, the cancer condition may be breast, ovary, pancreas or prostate cancer. Cancers may be familial or sporadic.

A individual suitable for treatment as described herein may include a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), a murine (e.g. a mouse), a canine (e.g. a dog), a feline (e.g. a cat), an equine (e.g. a horse), a primate, such as a simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, gibbon), or a human.

The term "treatment", as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

In some embodiments, a cancer condition may have been previously identified as having a kinase-deficient phenotype, before treatment as described herein. In other embodiments, a method as described herein may comprise the step of identifying a cancer condition in an individual as having a kinase-deficient phenotype. The identification of a cancer condition in an individual as having a kinase-deficient phenotype is described in detail below.

Other aspects of the invention relate to the methods for identifying a cancer condition in an individual as having a kinase-deficient

phenotype and therefore suitable for treatment with a DNA damage promoting agent, or for predicting or assessing the effect of treatment with a DNA damage promoting agent on an individual having a cancer condition. In some preferred embodiments, the DNA damage promoting agent is a PARP inhibitor.

A cancer condition having a kinase-deficient phenotype may be identified at the protein level. A method of identifying an individual having a cancer condition which is suitable for treatment with a DNA damage promoting agent, for example an inhibitor of DNA repair, such as a PARP inhibitor, may comprise:

determining the activity of a kinase-mediated cellular pathway in cancer cells obtained from the individual,

wherein reduced activity of the pathway relative to controls, for example less than 50%, is indicative that the individual has a cancer condition which is suitable for treatment with the DNA damage promoting agent.

Suitable cancer cell(s) for use in the described methods may be obtained from an individual in a tissue sample for example a biopsy from a cancerous tissue. Control cell(s) may be obtained from non-cancerous tissue from the same or a different individual. Suitable controls include non-cancer cells from the same tissue or lineage. In some embodiments, the control values may have been pre-determined and the value from the cancer cell(s) tested compared to such pre-determined (e.g. historical or archived) control values.

Kinase-mediated cellular pathways include CDK5-, MAPK12-, PLK3-, PNKP-, STK36- and STK22C-mediated pathways, which are described in more detail above.

The activity of a kinase-mediated cellular pathway may be determined using any suitable technique. For example, the loss or reduced amount of one or more components of the pathway may be detected, the phosphorylation profile of the pathway may be monitored, or the

cellular effects of the pathway, such as apoptosis or cell migration, may be determined.

The phosphorylation profile of a pathway may be conveniently monitored
5 by immunological techniques, such as Western blotting, using phosphospecific antibodies which are directed to epitopes of substrates within the pathway which only appear when the pathway is active.

10 In some embodiments, the activity of a kinase-mediated cellular pathway may be determined by determining the amount or activity of a component of the pathway. A component of a kinase-mediated cellular pathway may include a member of a CDK5-, MAPK12-, PLK3-, PNKP-, STK36- or STK22C- mediated cellular pathway, as described above. Preferably,
15 the component of a kinase-mediated cellular pathway is a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C, as described above. The amount or activity of a component of a kinase-mediated cellular pathway, for example a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and
20 STK22C, may be determined using any suitable technique.

The activity of a kinase-mediated cellular pathway or a component thereof may be determined relative to normal (i.e. non-cancer) cells, preferably from the same tissue. Reduced activity of the pathway or
25 component in the one or more cancer cells, for example less than 50%, less than 40%, less than 30%, less than 20% or less than 10%, relative to the activity of the pathway in normal cells (i.e. cells with a non-kinase-deficient phenotype), is indicative that the cancer has an kinase-deficient phenotype. Zero activity in the one or more cancer
30 cells relative to the activity in normal cells, is indicative that the cancer has a kinase null phenotype.

The amount of a component of a kinase-mediated cellular pathway, such as CDK5, MAPK12, PLK3, PNKP, STK36 or STK22C, may be determined

relative to control cells, preferably from the same tissue. Reduced amounts of the component in the one or more cancer cells, for example less than 50%, less than 40%, less than 30%, less than 20% or less than 10%, relative to the amount of the component in control cells (i.e. cells with a non-kinase-deficient phenotype), is indicative that the cancer has an kinase-deficient phenotype. Zero or undetectable amounts of the component in the one or more cancer cells relative to the amount in normal cells is indicative that the cancer has a kinase null phenotype. Many suitable methods are available in the art for determining the amount of a target protein in a cell including, for example, Western blot analysis, immunohistochemistry (Angèle S et al (2000) Clin. Cancer Res. 6, 3536-3544) and immunoassay (Butch AW et al (2004) Clinical Chemistry 50, 2303-2308). Antibodies to CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C suitable for use in immunoassays may be produced using standard techniques or obtained from commercial sources (for example, Abnova Corp, TW; Bethyl Laboratories Inc, USA; Abagent Inc, USA; Abcam Ltd, UK)

In some embodiments, the presence or amount of a component of the kinase-mediated cellular pathway in a cell may be determined by contacting a sample comprising one or more cancer cells with a specific binding member directed against the component, and determining binding of the specific binding member to the sample. The presence or amount of binding of the specific binding member is indicative of the presence or amount of the component of the pathway in a cell within the sample. Numerous techniques and formats for determining the presence or amount of a target polypeptide using a binding member, such as an antibody, or an antibody fragment or derivative, are well-known in the art.

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A cancer condition having a kinase-deficient phenotype may be identified at the nucleic acid level. A method of identifying an individual having a cancer condition which is suitable for treatment

with a DNA damage promoting agent, such as a PARP inhibitor may comprise;

determining the level or amount of nucleic acid, for example mRNA, encoding a component of a kinase-mediated cellular pathway in a cancer cell obtained from the individual,

wherein a reduced amount of said nucleic acid relative to controls is indicative that the individual has a cancer condition which is suitable for treatment with the DNA damage promoting agent.

The level or amount of encoding nucleic acid in a cancer cell may be determined for example by detecting the amount of transcribed encoding nucleic acid in the cell. This may be performed using standard techniques such as Northern blotting or RT-PCR.

Another method of identifying an individual having a cancer condition which is suitable for treatment with a DNA damage promoting agent, such as a PARP inhibitor, may comprise:

determining the presence of one or more sequence variations, for example, polymorphisms, mutations or regions of hypermethylation, in a nucleic acid encoding a component of a kinase-mediated cellular pathway in a cancer cell from the individual,

wherein said one or more sequence variations reduce or abolish the expression or activity of the component, and

wherein the presence of one or more variations relative to controls is indicative that the individual has a cancer condition which is suitable for treatment with the DNA damage promoting agent.

A component of a kinase-mediated cellular pathway may be a component of a CDK5-, MAPK12-, PLK3-, PNKP-, STK36- or STK22C- mediated cellular pathway, as described above. Preferably, the component of a kinase-mediated cellular pathway is a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C, as described above.

The level or activity of a component of a kinase-mediated cellular pathway, the amount of a nucleic acid encoding such a component, or the presence or absence of variation in a nucleic acid encoding such a component may be determined relative to normal (i.e. non-cancer) cells, preferably from the same tissue.

Sequence variations, such as mutations and polymorphisms, which reduce or abolish the expression or activity may include a deletion, insertion or substitution of one or more nucleotides, relative to the wild-type nucleotide sequence, a gene amplification or an increase or decrease in methylation, for example hypermethylation. The one or more sequence variations may be in a coding or non-coding region of the nucleic acid sequence. Mutations in the coding region of the gene encoding the component may prevent the translation of full-length active protein i.e. truncating mutations, or allow the translation of full-length but inactive or impaired function protein i.e. mis-sense mutations. Mutations or epigenetic changes, such as methylation, in non-coding regions of the gene encoding the component, for example, in a regulatory element, may prevent transcription of the gene. A nucleic acid comprising one or more sequence variations may encode a variant polypeptide which has reduced or abolished activity or may encode a wild-type polypeptide which has little or no expression within the cell, for example through the altered activity of a regulatory element. A nucleic acid comprising one or more sequence variations may have one, two, three, four or more mutations or polymorphisms relative to the wild-type sequence.

The presence of one or more sequence variations in a nucleic acid may be determined by detecting the presence of the variant nucleic acid sequence in one or more cells of a test sample or by detecting the presence of the variant polypeptide which is encoded by the nucleic acid sequence. Preferred nucleic acid sequence variation detection techniques include ARMS[™]-allele specific amplification, OLA, ALEX[™], COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR

and FRET techniques. Preferred polypeptide sequence variation techniques include immunoassays, which are well known in the art e.g. A Practical Guide to ELISA by D M Kemeny, Pergamon Press 1991; Principles and Practice of Immunoassay, 2nd edition, C P Price & D J Newman, 1997, published by Stockton Press in USA & Canada and by Macmillan Reference in the United Kingdom.

Numerous suitable methods for determining the amount of a nucleic acid encoding a component of a kinase-mediated cellular pathway, or the presence or absence of sequence variation in a nucleic acid encoding such a component, in a cancer cell obtained from an individual, are available in the art (see for example Molecular Cloning: a Laboratory Manual: 3rd edition, Sambrook & Russell (2001) Cold Spring Harbor Laboratory Press NY; Current Protocols in Molecular Biology, Ausubel et al. eds. John Wiley & Sons (1992); DNA Cloning, The Practical Approach Series (1995), series eds. D. Rickwood and B.D. Hames, IRL Press, Oxford, UK and PCR Protocols: A Guide to Methods and Applications (Innis, et al. 1990. Academic Press, San Diego, Calif.)).

In general, the detection of sequence variation requires a discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 4 lists a number of mutation detection techniques, some based on PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in table 5. Further amplification techniques are listed in table 6. Many current methods for the detection of sequence variation are reviewed by Nollau et al., Clin. Chem. 43, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

In some embodiments, nucleic acid or an amplified region thereof may be sequenced to identify or determine the presence of polymorphism or

mutation therein. A polymorphism or mutation may be identified by comparing the sequence obtained with the known sequence of the component of the kinase-mediated cellular pathway, for example as set out in sequence databases. Alternatively, it can be compared to the sequence of the corresponding nucleic acid from normal cells. In particular, the presence of one or more polymorphisms or mutations that cause abrogation or loss of function may be determined.

Sequencing may be performed using any one of a range of standard techniques. Sequencing of an amplified product may, for example, involve precipitation with isopropanol, resuspension and sequencing using a TaqFS+ Dye terminator sequencing kit (e.g. from GE Healthcare UK Ltd UK). Extension products may be electrophoresed on an ABI 377 DNA sequencer and data analysed using Sequence Navigator software.

Having sequenced nucleic acid of an individual or sample, the sequence information can be retained and subsequently searched without recourse to the original nucleic acid itself. Thus, for example, scanning a database of sequence information using sequence analysis software may identify a sequence alteration or mutation.

In some embodiments, the presence of one or more variations in a nucleic acid may comprise hybridising one or more (e.g. two) oligonucleotides to nucleic acid obtained from a sample, for example genomic DNA, RNA or cDNA. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. The binding of the oligonucleotide to target nucleic acid may then be determined. The oligonucleotide may comprise a nucleotide sequence which binds specifically to a nucleic acid sequence which contains one or more mutations or polymorphisms and does not bind specifically to the nucleic acid sequence which does not contain the one or more mutations or polymorphisms, or vice versa.

Binding of a probe to target nucleic acid (e.g. DNA) may be measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other methods not employing labelling of probe include examination of restriction fragment length polymorphisms, amplification using PCR, RNase cleavage and allele specific oligonucleotide probing. Probing may employ the standard Southern blotting technique. For instance, DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a suitable filter (e.g. nitrocellulose). Labelled probe may be hybridised to the DNA fragments on the filter and binding determined.

Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on.

Suitable selective hybridisation conditions for oligonucleotides of 17 to 30 bases include hybridization overnight at 42°C in 6X SSC and then washing in 6X SSC at a series of increasing temperatures from 42°C to 65°C. For example, probes may be washed in 6xSSC at 42 °C for 30 minutes then 6xSSC at 50°C for 45 mins then 2xSSC for 45 mins at 65°C. Other suitable conditions and protocols are described in Molecular Cloning: a Laboratory Manual: 3rd edition, Sambrook & Russell (2001) Cold Spring Harbor Laboratory Press NY and Current Protocols in Molecular Biology, Ausubel et al. eds. John Wiley & Sons (1992).

In some embodiments, a specific amplification reaction such as PCR using one or more pairs of primers may conveniently be employed to amplify the region of interest within the nucleic acid sequence, for example, the portion of the sequence suspected of containing mutations

or polymorphisms. The amplified nucleic acid may then be sequenced as above, and/or tested in any other way to determine the presence or absence of a mutation or polymorphism which reduces or abrogates the expression or activity of the component of the kinase mediated cellular pathway. Suitable amplification reactions include the polymerase chain reaction (PCR) (reviewed for instance in "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic Press, New York, Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR technology, Stockton Press, NY, 1989, and Ehrlich et al, Science, 252:1643-1650, (1991)).

In some embodiments, the presence of one or more variations in a nucleic acid may be detected by allele discrimination techniques such as allele-specific amplification. In such techniques, PCR is performed with allele-specific oligonucleotide primers capable of discriminating between the different bases at a particular allele. Such as using amplification refractory mutation system (ARMS™-allele specific amplification).

ARMS™-allele specific amplification (EP-B-332435, US 5,595,890 and Newton et al. Nucleic Acids Research, 17, 2503; 1989)), relies on the complementarity of the 3' terminal nucleotide of the primer and its template. The 3' terminal nucleotide of the primer being either complementary or non-complementary to the specific mutation, allele or polymorphism to be detected. There is a selective advantage for primer extension from the primer whose 3' terminal nucleotide complements the base mutation, allele or polymorphism. Those primers which have a 3' terminal mismatch with the template sequence severely inhibit or prevent enzymatic primer extension. Polymerase chain reaction or unidirectional primer extension reactions therefore result in product amplification when the 3' terminal nucleotide of the primer complements that of the template, but not, or at least not efficiently, when the 3' terminal nucleotide does not complement that of the template.

PCR may be performed using one or more fluorescently labelled probes or using one or more probes which include a DNA minor groove binder.

5 In some embodiments, sequence variations, such as mutations and polymorphisms, in a gene encoding a component of the kinase-mediated cellular pathway may be detected by detecting the presence of a polypeptide having a variant amino acid sequence (i.e. a mutant or allelic variant with reduced activity). A method of identifying a
10 cancer cell in a sample from an individual as having a kinase-deficient phenotype may, for example, comprise contacting a sample with a specific binding member directed against a variant (e.g. a mutant) component of a kinase-mediated cellular pathway, such as a kinase, and determining binding of the specific binding member to the
15 sample. Binding of the specific binding member to the sample may be indicative of the presence of the variant component of the pathway in a cell within the sample.

Preferred specific binding molecules for use in aspects of the present
20 invention include antibodies and fragments or derivatives thereof ('antibody molecules').

The binding of a specific binding member such as an antibody on normal and variant components of a kinase-deficient pathway may be determined
25 by any appropriate means and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

A treatment regimen comprising administration of a PARP inhibitor may
30 be designed for an individual identified as having a cancer condition with a kinase-deficient phenotype and thus being suitable for treatment using a method described herein. For example, a suitable PARP inhibitor may be selected and the dosage and schedule of administration established for the individual using appropriate

medical criteria. In some embodiments, a method may include the step of administering the PARP inhibitor to the individual. In an analogous way, an appropriate treatment regimen employing any other DNA damage promoting agent may be designed for the individual.

5 The methods described herein may be particularly useful in identifying cohorts of cancer patients, for example for clinical trials of DNA damaging therapeutic agents, for example, agents which inhibit PARP.

10 Another aspect of the invention provides a method of identifying a population of individuals having a cancer condition suitable for treatment with a DNA damage promoting agent, such as a PARP inhibitor, comprising,

15 identifying a sample of individuals having a cancer condition, assessing the activity of a kinase mediated cellular-pathway in one or more cancer cells obtained from each of the individuals in said sample,

20 identifying a population of individuals within the sample who have a cancer condition with a kinase-deficient phenotype, the individuals of said population being suitable for treatment with the DNA damage promoting agent.

A DNA damage promoting agent, such as a PARP inhibitor, may be administered to the individuals of said population and the efficacy of the PARP inhibitor in treating the cancer condition determined in the individuals.

Another aspect of the invention provides a method of treating an individual with a cancer condition, comprising;

30 administering a DNA damage promoting agent and an inhibitor of a kinase-mediated cellular pathway to said individual.

Related aspects of the invention provide the use of a DNA damage promoting agent and an inhibitor of a kinase-mediated cellular pathway

for the manufacture of a medicament for the treatment of a cancer condition in an individual, and a DNA damage promoting agent and an inhibitor of a kinase-mediated cellular pathway for use in the treatment of a cancer condition in an individual.

5 ,
An inhibitor of a kinase-mediated cellular pathway may be a compound or entity, such as a small organic molecule, peptide or nucleic acid, which inhibits a component of a kinase-mediated cellular pathway selected from the group consisting of a cyclin-dependent kinase 5
10 (CDK5) mediated pathway, a mitogen-activated protein kinase 12 (MAPK12) mediated pathway, a polo-like kinase 3 (PLK3) mediated pathway, a polynucleotide kinase 3'-phosphatase (PNKP) mediated pathway, a serine/threonine kinase 36 (STK36) mediated pathway (STK22C is also known as testis-specific serine kinase 3; TSSK3), and a
15 serine/threonine kinase 22C (STK22C) mediated pathway. Components of these pathways are described elsewhere herein. A suitable inhibitor induces a kinase-deficient phenotype in a cell i.e. it inhibits, reduces or abolishes the activity of the kinase-mediated cellular pathway. In some particular embodiments, the inhibitor of a kinase-
20 mediated cellular pathway is an inhibitor of a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C.

In particular embodiments, a kinase inhibitor is specific for the target kinase and shows no inhibition or substantially no inhibition
25 of other kinases, in particular related kinases. For example, a specific inhibitor may show at least 10 fold' at least' 100 fold, or at least 1000 fold greater inhibition of the target kinase than other kinases. A specific CDK5 inhibitor may display no inhibition or substantially no inhibition of other members of the CDC2/CDKX protein
30 subfamily, such as CDKs1-4 and CDKs6-9, a MAPK12 inhibitor may display no inhibition or substantially no inhibition of other members of the MAPK subfamily, such as APK3 (ERK1), MAPK8 (JNK1), or MAPK14 (p38), a PLK3 inhibitor may display no inhibition or substantially no inhibition of other members of the CDC5/POLO subfamily or proteins

containing POLO box domains, such as PLK1, PLK2 or PLK4, a PNKP inhibitor may display no inhibition or substantially no inhibition of other DNA 3' phosphatases, a STK36 inhibitor may display no inhibition or substantially no inhibition of members of the ser/thr protein kinase family, such as STK22C, Akt, and p70S6K and a STK22C inhibitor may display no inhibition or substantially no inhibition of members of the ser/thr protein kinase family such as STK36, Akt, and p70S6K.

Kinase inhibitors may be identified using standard techniques, for example, by determining the phosphorylation of a substrate. For example, CDK5 inhibitors may be identified by determining a decrease in the phosphorylation of a substrate such as p35, p39, PAK1, SRC, CABLES, B-CATENIN, TAU, MAP1B, NUDEL, NFH/NFM, SYNAPSIN1, MUNC18, AMPHYPHYSIN1, BAPP, DARPP32, PP1 INHIBITOR, PGAMMA, ERBB or PRB in the presence of the inhibitor, MAPK12 inhibitors may be identified by a decrease in the phosphorylation of a PDZ domain containing protein, such as SAP90 and SAP97. PLK3 inhibitors may be identified by a decrease in the phosphorylation of CHK2 and PNKP inhibitors may be identified by a decrease in the phosphorylation of DNA. Suitable kinase substrates are known in the art and are commercially available (Biaffin GmbH & Co KG, Germany, BioMol International LP, Exeter UK; Upstate, Hampshire UK).

Suitable DNA damage promoting agents are described above. In some embodiments, the DNA damage promoting agent is a PARP inhibitor. PARP inhibitors suitable for use as described herein include any compound or entity, such as a small organic molecule, peptide or nucleic acid, which induces a PARP deficient phenotype in a cell e.g. it inhibits, reduces or abolishes the activity of PARP. Suitable PARP inhibitors include small molecule ATP-competitive kinase inhibitors which inhibit PARP in an ATP-competitive manner.

The term 'PARP' as used herein refers to PARP1 (EC 2.4.2.30, Genbank No: M32721.1 GI: 190266, D'Amours et al, (1999) Biochem. J. 342: 249-

268, Ame et al BioEssays (2004) 26 882-893) and/or PARP2 (Ame et al J. Biol. Chem. (1999) 274 15504-15511; Genbank No: AJ236912.1 GI: 6688129), unless context dictates otherwise.

5 PARP inhibition may be determined using conventional methods, including for example dot blots (Affar EB et al Anal Biochem. 1998; 259(2):280-3), and BER assays that measure the direct activity of PARP to form poly ADP-ribose chains for example by using radioactive assays with tritiated substrate NAD or specific antibodies to the polymer
10 chains formed by PARP activity (K.J. Dillon et al, Journal of Biomolecular Screening, 8(3): 347-352 (2003). Examples of suitable methods for determining PARP activity are described below.

Examples of compounds which are known PARP inhibitors and which may be
15 used in accordance with the invention include:

1. Nicotinamides, such as 5-methyl nicotinamide and O-(2-hydroxy-3-piperidino-propyl)-3-carboxylic acid amidoxime, and analogues and derivatives thereof.
20
2. Benzamides, including 3-substituted benzamides such as 3-aminobenzamide, 3-hydroxybenzamide, 3-nitrosobenzamide, 3-methoxybenzamide and 3-chloroprocainamide, and 4-aminobenzamide, 1, 5-di[(3-carbamoylphenyl)aminocarbonyloxy] pentane, and analogues and
25 derivatives thereof.
3. Isoquinolinones and Dihydroisoquinolinones, including 2H-isoquinolin-1-ones, 3H-quinazolin-4-ones, 5-substituted dihydroisoquinolinones such as 5-hydroxy dihydroisoquinolinone, 5-methyl dihydroisoquinolinone, and 5-hydroxy isoquinolinone, 5-amino
30 isoquinolin-1-one, 5-dihydroxyisoquinolinone, 3, 4 dihydroisoquinolin-1(2H)-ones such as 3, 4 dihydro-5-methoxy-isoquinolin-1(2H)-one and 3, 4 dihydro-5-methyl-1(2H)isoquinolinone, isoquinolin-1(2H)-ones, 4,5-dihydro-imidazo[4,5,1-ij]quinolin-6-ones, 1, 6,-naphthyridine-5(6H)-
35 ones, 1,8-naphthalimides such as 4-amino-1,8-naphthalimide,

isoquinolinone, 3, 4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2H)-
isoquinolinone, 2, 3-dihydrobenzo[de]isoquinolin-1-one, 1,11b-dihydro-
[2H]benzopyrano[4, 3, 2-de]isoquinolin-3-one, and tetracyclic lactams,
including benzpyranoisoquinolinones such as benzopyrano[4,3,2-de]
5 isoquinolinone, and analogues and derivatives thereof

4. Benzimidazoles and indoles, including benzoxazole-4-carboxamides,
benzimidazole-4-carboxamides, such as 2-substituted benzoxazole 4-
carboxamides and 2-substituted benzimidazole 4-carboxamides such as 2-
10 aryl benzimidazole 4-carboxamides and 2-cycloalkylbenzimidazole-4-
carboxamides including 2-(4-hydroxyphenyl) benzimidazole 4-carboxamide,
quinoxalinecarboxamides, imidazopyridinecarboxamides, 2-phenylindoles,
2-substituted benzoxazoles, such as 2-phenyl benzoxazole and 2-(3-
methoxyphenyl) benzoxazole, 2-substituted benzimidazoles, such as 2-
15 phenyl benzimidazole and 2-(3-methoxyphenyl) benzimidazole, 1, 3, 4, 5
tetrahydro-azepino[5, 4, 3-cd]indol-6-one, azepinoindoles and
azepinoindolones such as 1, 5 dihydro-azepino[4, 5, 6-cd]indolin-6-one
and dihydrodiazapinoindolinone, 3-substituted
dihydrodiazapinoindolinones, such as 3-(4-trifluoromethylphenyl)-
20 dihydrodiazapinoindolinone, tetrahydrodiazapinoindolinone and 5,6,-
dihydroimidazo[4, 5, 1-j, k][1, 4]benzodiazopin-7(4H)-one, 2-phenyl-
5,6-dihydro-imidazo[4,5,1-jk][1,4]benzodiazepin-7(4H)-one and 2, 3,
dihydro-isoindol-1-one, and analogues and derivatives thereof

25 5. Phthalazin-1(2H)-ones and quinazolinones, such as 4-
hydroxyquinazoline, phthalazinone, 5-methoxy-4-methyl-1(2)
phthalazinones, 4-substituted phthalazinones, 4-(1-piperazinyl)-
1(2H)-phthalazinone, tetracyclic benzopyrano[4, 3, 2-de]
phthalazinones and tetracyclic indeno [1, 2, 3-de] phthalazinones and
30 2-substituted quinazolines, such as 8-hydroxy-2-methylquinazolin-4-
(3H) one, tricyclic phthalazinones and 2-aminophthalhydrazide, and
analogues and derivatives thereof.

6. Isoindolinones and analogues and derivatives thereof

7. Phenanthridines and phenanthridinones, such as 5[H]phenanthridin-6-one, substituted 5[H] phenanthridin-6-ones, especially 2-, 3-substituted 5[H] phenanthridin-6-ones and sulfonamide/carbamide derivatives of 6(5H)phenanthridinones, thieno[2, 3-c]isoquinolones such as 9-amino thieno[2, 3-c]isoquinolone and 9-hydroxythieno[2, 3-c]isoquinolone, 9-methoxythieno[2, 3-c]isoquinolone, and N-(6-oxo-5, 6-dihydrophenanthridin-2-yl)-2-(N,N-dimethylamino)acetamide, substituted 4,9-dihydrocyclopenta[lmn]phenanthridine-5-ones, and analogues and derivatives thereof.

8. Benzopyrones such as 1, 2-benzopyrone, 6-nitrosobenzopyrone, 6-nitroso 1, 2-benzopyrone, and 5-iodo-6-aminobenzopyrone, and analogues and derivatives thereof.

9. Unsaturated hydroximic acid derivatives such as O-(3-piperidino-2-hydroxy-1-propyl)nicotinic amidoxime, and analogues and derivatives thereof.

10. Pyridazines, including fused pyridazines and analogues and derivatives thereof.

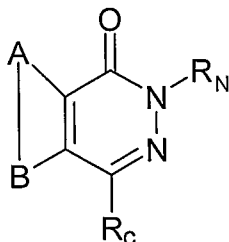
11. Other compounds such as caffeine, theophylline, and thymidine, and analogues and derivatives thereof.

Additional PARP inhibitors are described for example in WO2006078503 WO2006078711, DE102004050196, WO2006024545, WO2006003148, WO2006003147, WO2006003146, PCT/JP03/14319, WO2005123687, WO2005097750, WO2005058843, WO2005054210, WO2005054209, WO2005054201, US 2005054631, WO2005012305, WO2004108723, WO2004105700, US2004229895, WO2004096793, WO2004096779, WO2004087713, WO2004048339, WO2004024694, WO2004014873, US6,635,642, US5,587,384, WO2003080581, WO2003070707, WO2003055865, WO2003057145, WO2003051879, US6514983, WO2003007959, US6426415, WO2003007959, WO 2002036599,

WO2002094790, WO2002068407, US6476048, WO2001090077, WO2001085687,
 WO2001085686, WO2001079184, WO2001057038, WO2001023390, WO2001021615,
 WO2001016136, WO2001012199, WO9524379, Banasik et al. J. Biol. Chem.,
 267:3, 1569-75 (1992), Banasik et al. Molec. Cell. Biochem. 138:185-97
 5 (1994)), Cosi (2002) Expert Opin. Ther. Patents 12 (7), and Southan &
 Szabo (2003) Curr Med Chem 10 321-340 and references therein.

Other examples of compounds which are known PARP inhibitors includes
 the hydrochloride salt of *N*-(-oxo-5,6-dihydro-phenanthridin-2-yl)-*N,N*-
 10 dimethylacetamide and other analogues or similar compounds, such as
 INO-1001 that show PARP inhibition.

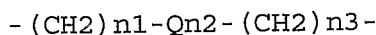
One preferred class of PARP inhibitors includes phthalazinones such as
 1(2H)-phthalazinone and derivatives thereof, as described in
 15 WO02/36576, which is incorporated herein by reference. In particular,
 a PARP inhibitor may be a compound of the formula (I):



or an isomer, salt, solvate, chemically protected form, or prodrug
 25 thereof, wherein:

A and B together represent an optionally substituted, fused aromatic
 ring;

R_C is represented by -L-RL, where L is of formula:



30 wherein n₁, n₂ and n₃ are each selected from 0, 1, 2 and 3, the sum of
 n₁, n₂ and n₃ is 1, 2 or 3 and Q is selected from O, S, NH, C(=O) or -
 CR₁R₂-, where R₁ and R₂ are independently selected from hydrogen,
 halogen or optionally substituted C₁-7 alkyl, or may together with the
 carbon atom to which they are attached form a C₃-7 cyclic alkyl group,

which may be saturated (a C3-7 cycloalkyl group) or unsaturated (a C3-7 cycloalkenyl group), or one of R1 and R2 may be attached to an atom in RL to form an unsaturated C3-7 cycloalkenyl group which comprises the carbon atoms to which R1 and R2 are attached in Q, $-(CH_2)_n-$ (if present) and part of RL;

and RL is optionally substituted C5-20 aryl; and

RN is selected from hydrogen, optionally substituted C1-7 alkyl, C3-20 heterocyclyl, and C5-20 aryl, hydroxy, ether, nitro, amino, amido, thiol, thioether, sulfoxide and sulfone.

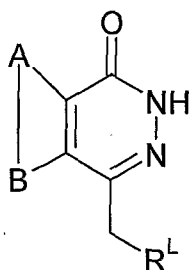
For example, a preferred compound may have the formula (I) wherein: A and B together represent an optionally substituted, fused aromatic ring;

RC is $-CH_2-RL$;

RL is optionally substituted phenyl; and

RN is hydrogen.

Other examples of suitable PARP inhibitors are described in WO 03/093261, which is incorporated herein by reference, and have the formula (II):

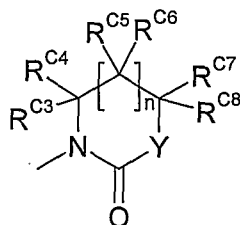


or an isomer, salt, solvate, chemically protected form, or prodrug thereof, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

RL is a C5-7 aryl group substituted in the meta position by the group R2, and optionally further substituted; wherein R2 is selected from:

(a)

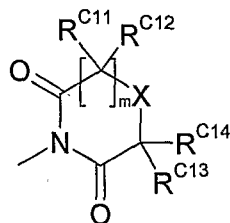


wherein:

n is 0 or 1;

Y is selected from NRN1 and CRC1RC2;

- 5 RN1 is selected from H, optionally substituted C1-10 alkyl, optionally substituted C5-6 aryl and optionally substituted C1-10 alkylacyl;
 RC1, RC2, RC3, RC4, RC5, RC6, RC7 and RC8 are independently selected from H, R, SR and NHC(=O)OR, where R is optionally substituted C1-10 alkyl or optionally substituted C5-6 aryl;
 10 RC4 and RC6, RC6 and RC8 or RC8 and RC2 may optionally together form a double bond;
 RC1 and RC2, RC5 and RC6 or RC7 and RC8 together with the carbon atom to which they are attached may optionally form a spiro-fused C5-7 carbocyclic or heterocyclic ring; and
 15 RC5 and RC7 or RC7 and RC1 together with the carbon atoms to which they are attached form an optionally substituted ring system;
 b)



20 wherein

m is 0 or 1;

X is selected from NRN2 and CRC9RC10;

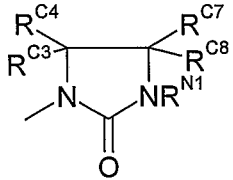
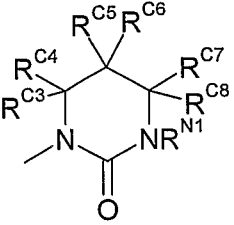
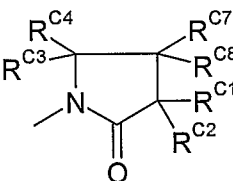
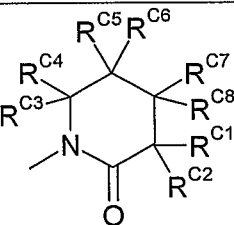
- RN2 is selected from H, optionally substituted C1-10 alkyl, optionally substituted C5-6 aryl and optionally substituted C1-10 alkylacyl;
 25 RC9, RC10, RC11, RC12, RC13 and RC14 are independently selected from H, R, SR and NHC(=O)OR, where R is as defined above;

RC12 and RC10 or RC10 and RC14 may optionally together form a double bond;

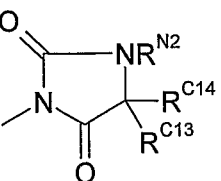
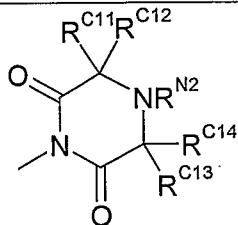
RC11 and RC12, RC9 and RC10 or RC13 and RC14 together with the carbon atom to which they are attached may optionally form a spiro-fused C5-7 carbocyclic or heterocyclic ring; and

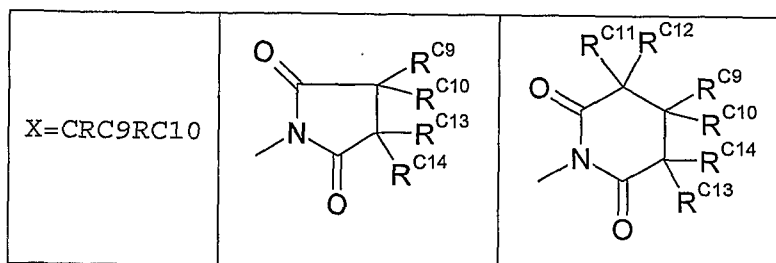
RC11 and RC9 or RC9 and RC13 together with the carbon atoms to which they are attached may form an optionally substituted ring system.

The options for the structure of R2 under a) above when n is 0 or 1 and Y is NRN1 or CRC1RC2 are as follows:

	n=0	n=1
Y= NRN1		
Y= CRC1RC2		

The options for the structure of R2 under b) above when m is 0 or 1 and X is NRN2 or CRC9RC10 are as follows:

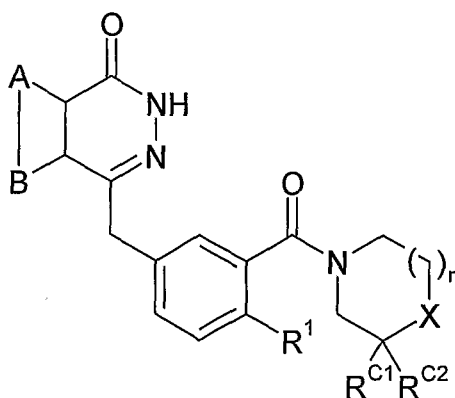
	m=0	m=1
X= NRN2		



In some preferred embodiments, 1-[2-Fluoro-5-(4-oxo-3,4-dihydro-

5 ([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one or an isomer, salt, solvate, chemically protected form, or prodrug thereof, may be used to inhibit PARP.

Other examples of suitable PARP inhibitors are described in WO
10 2004/080976, which is incorporated herein by reference, and may have the formula (III):



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof

15 wherein:

A and B together represent an optionally substituted, fused aromatic ring;

X can be NRX or CRXRY;

if X = NRX then n is 1 or 2 and if X = CRXRY then n is 1;

20 RX is selected from the group consisting of H, optionally substituted C1-20 alkyl, C5-20 aryl, C3-20 heterocyclyl, amido, thioamido, ester, acyl, and sulfonyl groups;

RY is selected from H, hydroxy, amino;

or RX and RY may together form a spiro-C3-7 cycloalkyl or heterocyclyl group;

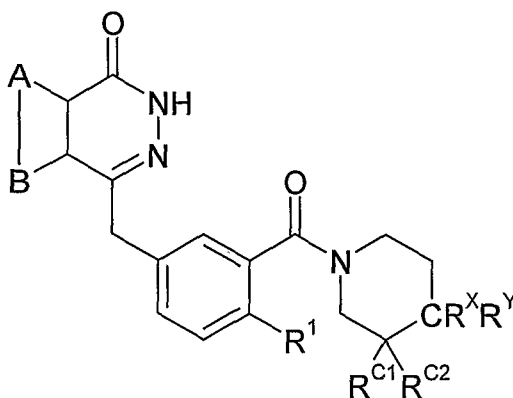
RC1 and RC2 are both hydrogen, or when X is CRXRY, RC1, RC2, RX and

5 RY, together with the carbon atoms to which they are attached, may form an optionally substituted fused aromatic ring; and

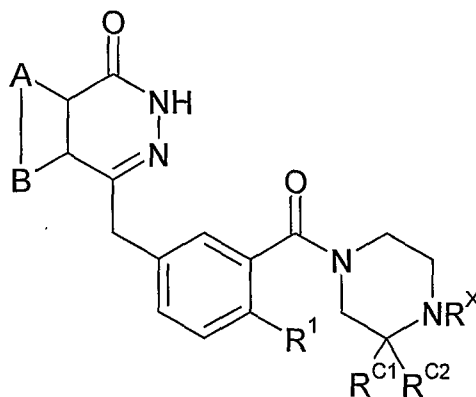
R1 is selected from H and halo.

Therefore, if X is CRXRY, then n is 1, the compound is of formula

10 (IV):

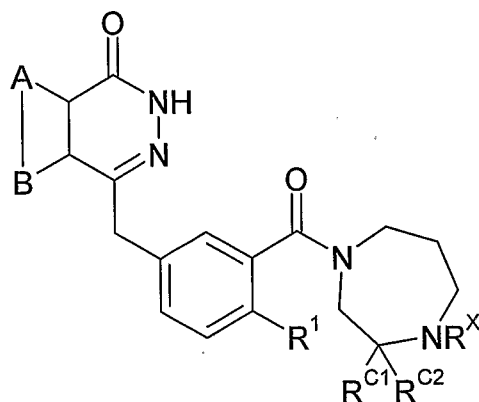


If X is NRX, and n is 1, the compound is of formula (V):



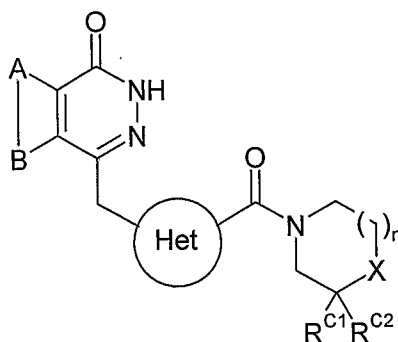
15

If X is NRX, and n is 2, the compound is of formula (VI):



In some preferred embodiments, 4-[3-(4-Cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one or an isomer, salt, solvate, chemically protected form, or prodrug thereof, may be used to inhibit PARP.

Other examples of suitable PARP inhibitors are described in WO2006/021801, which is incorporated herein by reference, and have the formula (VII):



and isomers, salts, solvates, chemically protected forms, and prodrugs thereof

wherein:

A and B together represent an optionally substituted, fused aromatic ring;

X can be NRX or CRXRY;

if X = NRX then n is 1 or 2 and if X = CRXRY then n is 1;

RX is selected from the group consisting of H, optionally substituted C1-20 alkyl, C5-20 aryl, C3-20 heterocyclyl, amido, thioamido, ester, acyl, and sulfonyl groups;

RY is selected from H, hydroxy, amino;

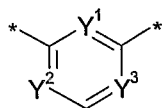
5 or RX and RY may together form a spiro-C3-7 cycloalkyl or heterocyclyl group;

RC1 and RC2 are independently selected from the group consisting of hydrogen and C1-4 alkyl, or when X is CRXRY, RC1, RC2, RX and RY, together with the carbon atoms to which they are attached, may form an
10 optionally substituted fused aromatic ring;

R1 is selected from H and halo; and

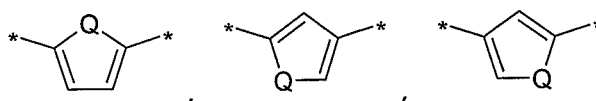
Het is selected from:

(i)



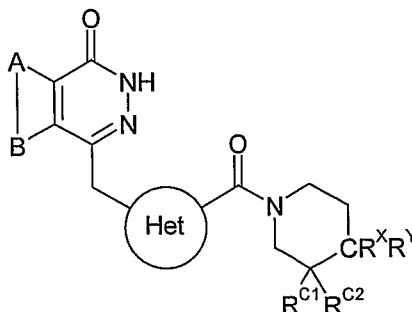
15 where Y1 is selected from CH and N, Y2 is selected from CH and N, Y3 is selected from CH, CF and N, where only one or two of Y1, Y2 and Y3 can be N; and

(ii)

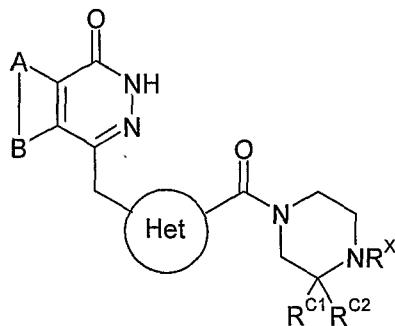


20 where Q is O or S.

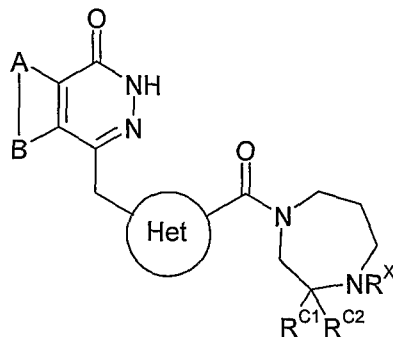
Therefore, if X is CRXRY, then n is 1 and the compound is of formula (VIII):



If X is NRX, and n is 1, the compound is of formula (IX):



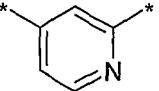
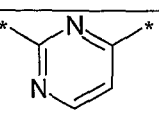
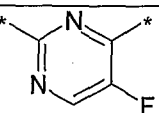
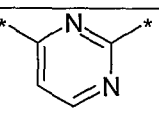
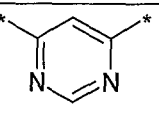
If X is NRX, and n is 2, the compound is of formula (X):

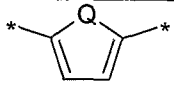
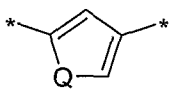
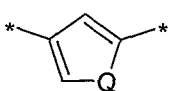
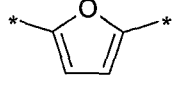
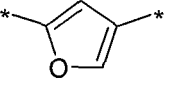
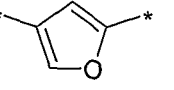
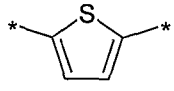
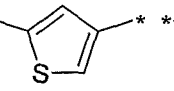
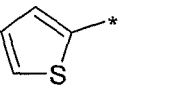


5

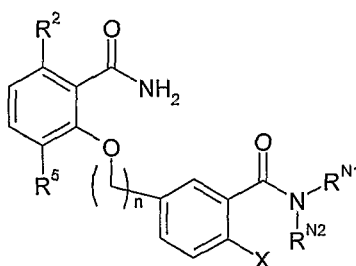
The possibilities for Het are:

Formula				Group
	Y1	Y2	Y3	
	N	CH	CH	
	N	CH	CF	
	CH	N	CH	
	CH	N	CF	

	CH	CH	N	
	N	N	CH	
	N	N	CF	
	N	CH	N	
	CH	N	N	

  	Q			
	O			
	S			

Other examples of suitable PARP inhibitors are described in
 5 WO2006/067472, which is incorporated herein by reference, and have the
 formula (XI):



and isomers, salts, solvates, chemically protected forms, and prodrugs
 thereof, wherein:

R2 is selected from the group consisting of H, C1-7 alkoxy, amino, halo or hydroxy;

R5 is selected from the group consisting of H, C1-7 alkoxy, amino, halo or hydroxy;

5 n is 1 or 2;

X is H, Cl or F;

RN1 and RN2 are independently selected from H and R, where R is

optionally substituted C1-10 alkyl, C3-20 heterocyclyl and C5-20 aryl;

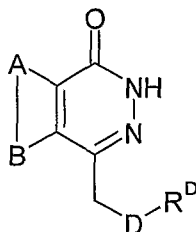
or RN1 and RN2, together with the nitrogen atom to which they are

10 attached form an optionally substituted 5-7 membered, nitrogen containing, heterocyclic ring.

Other examples of suitable PARP inhibitors are described in

GB0521373.1 which is incorporated herein by reference, and have the

15 formula (XII):



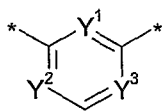
and isomers, salts, solvates, chemically protected forms, and prodrugs thereof

wherein:

20 A and B together represent an optionally substituted, fused aromatic ring;

D is selected from:

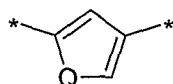
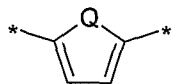
(i)



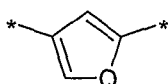
, where Y1 is selected from CH and N, Y2 is selected from

25 CH and N, Y3 is selected from CH, CF and N; and

(ii)

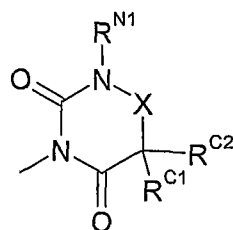


and



, where Q is O or S;

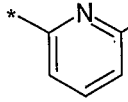
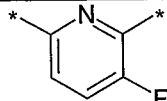
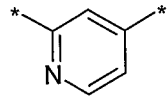
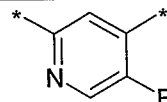
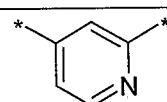
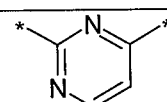
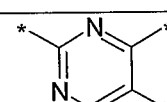
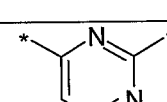
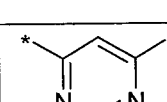
RD is:

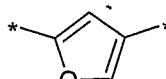
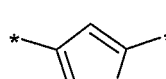
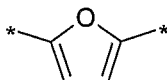
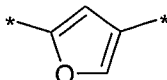
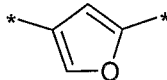
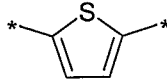
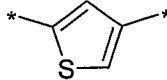
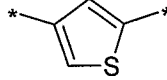


- 5 wherein
- RN1 is selected from H and optionally substituted C1-10 alkyl;
- X is selected from a single bond, NRN2 and CRC3RC4;
- RN2 is selected from H and optionally substituted C1-10 alkyl;
- RC1 and RC2 are independently selected from H, R, C(=O)OR, where R is
- 10 optionally substituted C1-10 alkyl, optionally substituted C5-20 aryl or optionally substituted C3-20 heterocyclyl;
- RC1 and RC12 together with the carbon atom to which they are attached may form an optionally substituted spiro-fused C5-7 carbocyclic or heterocyclic ring; and
- 15 when X is a single bond RN1 and RC2 may together with the N and C atoms to which they are bound, form an optionally substituted C5-7 heterocyclic ring;
- and when X is CRC3RC4, RC2 and RC4 may together form an additional bond, such that there is a double bond between the atoms substituted
- 20 by RC1 and RC3.

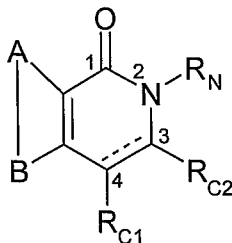
The possibilities for D are:

Formula				Group
	Y1	Y2	Y3	
	CH	CH	CH	
	CH	CH	CF	

	N	CH	CH	
	N	CH	CF	
	CH	N	CH	
	CH	N	CF	
	CH	CH	N	
	N	N	CH	
	N	N	CF	
	N	CH	N	
	CH	N	N	

  	Q			
	O			
	S			

One preferred class of PARP inhibitors includes isoquinolinones and derivatives thereof, as described in WO 02/090334, which is incorporated herein by reference. Suitable PARP inhibitors may be compounds of the formula (XIII):

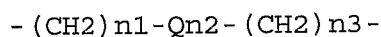


and isomers, salts, solvates, chemically protected forms, and prodrugs thereof, wherein:

A and B together represent an optionally substituted, fused aromatic ring;

the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;

at least one of R_{C1} and R_{C2} is independently represented by -L-RL, and if one of R_{C1} and R_{C2} is not represented by -L-RL, then that group is H, where L is of formula:



wherein n₁, n₂ and n₃ are each selected from 0, 1, 2 and 3, the sum of n₁, n₂ and n₃ is 1, 2 or 3 and each Q (if n₂ is greater than 1) is selected from O, S, NR₃, C(=O), or -CR₁R₂-, where R₁ and R₂ are independently selected from hydrogen, halogen or optionally

substituted C₁-7 alkyl, or may together with the carbon atom to which they are attached form a C₃-7 cyclic alkyl group, which may be saturated (a C₃-7 cycloalkyl group) or unsaturated (a C₃-7 cycloalkenyl group), or one of R₁ and R₂ may be attached to an atom in RL to form an unsaturated C₃-7 cycloalkenyl group which comprises the carbon atoms to which R₁ and R₂ are attached in Q, -(CH₂)_{n3}- (if present) and part of RL, and where R₃ is selected from H or C₁-7 alkyl; and

RL is selected from optionally substituted C₃-20 heterocyclyl, C₅-20 aryl and carbonyl; and

RN is selected from hydrogen, optionally substituted C1-7 alkyl, C3-20 heterocyclyl, C5-20 aryl, hydroxy, ether, nitro, amino, thioether, sulfoxide and sulfone.

5

For example, a preferred PARP inhibitor may have the formula (XIII) wherein:

A and B together represent an optionally substituted, fused aromatic ring;

10 the dotted line between the 3 and 4 positions indicates the optional presence of a double bond;

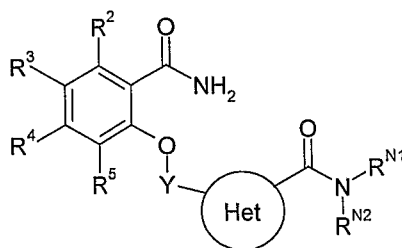
one of Rc1 and Rc2 is -CH₂-RL, and the other of Rc1 and Rc2 is H;

RL is optionally substituted phenyl; and

RN is hydrogen.

15

Other examples of suitable PARP inhibitors are described in US60/804848, which is incorporated herein by reference, and have the formula (XIV):



20 and pharmaceutically acceptable salts thereof, wherein:

R², R³, R⁴ and R⁵ are independently selected from the group consisting of H, C₁₋₇ alkoxy, amino, halo or hydroxy;

Y is -CR^{C1}R^{C2}-(CH₂)_m-, where m is 0 or 1, R^{C1} is selected from CH₃ and CF₃, and R^{C2} is selected from H and CH₃, or R^{C1} and R^{C2} together with the

25 carbon atom to which they are attached form the 1,1-cyclopropylene group:



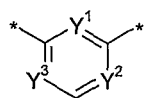
R^{N1} and R^{N2} are independently selected from H and R, where R is optionally substituted C₁₋₁₀ alkyl, C₃₋₂₀ heterocyclyl and C₅₋₂₀ aryl;

or R^{N1} and R^{N2} , together with the nitrogen atom to which they are attached form an optionally substituted 5-7 membered, nitrogen containing, heterocyclic ring;

5

Het is selected from:

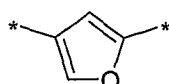
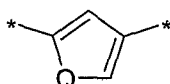
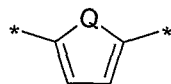
(i)



, where Y^1 and Y^3 are independently selected from CH and N, Y^2 is selected from CX and N and X is H, Cl or F; and

10

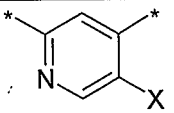
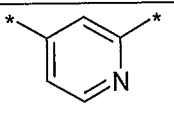
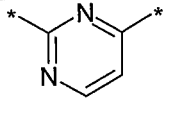
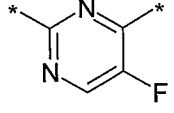
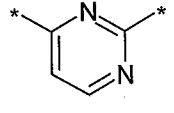
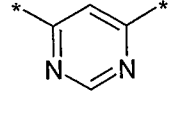
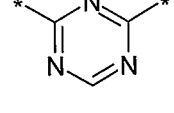
(ii)

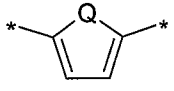
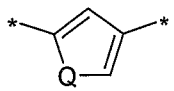
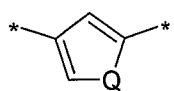
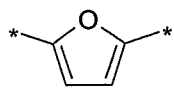
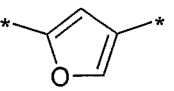
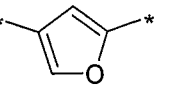
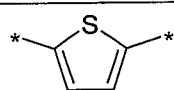
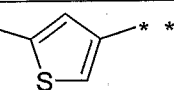
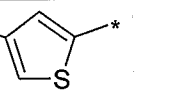


where Q is O or S.

The possibilities for Het are:

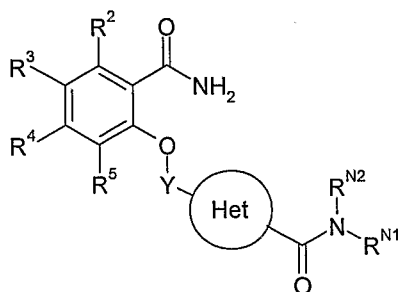
Formula				Group
	Y^1	Y^2	Y^3	
	CH	CH	CH	
	CH	CX	CH	
	N	CH	CH	
	N	CX	CH	
	CH	CH	N	

	CH	CX	N	
	CH	N	CH	
	N	CH	N	
	N	CX	N	
	N	N	CH	
	CH	N	N	
	N	N	N	

  	Q	
	O	  
	S	  

Other examples of suitable PARP inhibitors are described in US60/804849, which is incorporated herein by reference, and have the

5 formula (XV):



and pharmaceutically acceptable salts thereof, wherein:

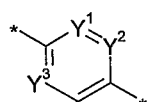
R^2 , R^3 , R^4 and R^5 are independently selected from the group consisting of H, C_{1-7} alkoxy, amino, halo or hydroxy;

- 5 Y is $-CR^{C1}R^{C2}-(CH_2)_m-$, where m is 0 or 1, R^{C1} is selected from H, CH_3 and CF_3 , and R^{C2} is selected from H and CH_3 , or R^{C1} and R^{C2} together with the carbon atom to which they are attached form the 1,1-cyclopropylene group:



- 10 R^{N1} and R^{N2} are independently selected from H and R, where R is optionally substituted C_{1-10} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl; or R^{N1} and R^{N2} , together with the nitrogen atom to which they are attached form an optionally substituted 5-7 membered, nitrogen containing, heterocyclic ring;

- 15 Het is:

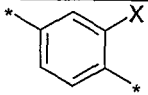
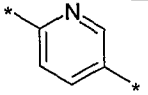
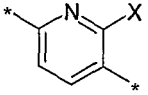
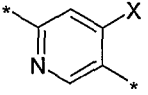
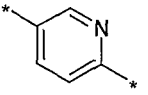
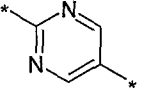
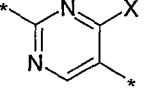
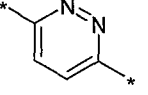
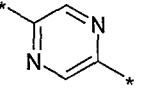
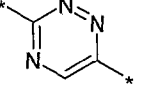


, where Y^1 and Y^3 are independently selected from CH and N, Y^2 is selected from CX and N and X is H, Cl or F.

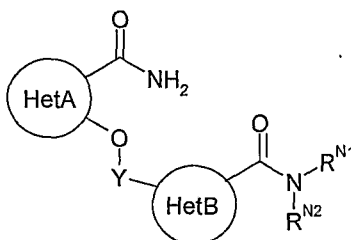
The possibilities for Het are:

20

Formula				Group
	Y^1	Y^2	Y^3	
	CH	CH	CH	

	CH	CX	CH	
	N	CH	CH	
	CH	CH	N	
	N	CX	CH	
	CH	CX	N	
	CH	N	CH	
	N	CH	N	
	N	CX	N	
	N	N	CH	
	CH	N	N	
	N	N	N	

Other examples of suitable PARP inhibitors are described in
 US60/804851, which is incorporated herein by reference, and have the
 5 formula (XVI):



and pharmaceutically acceptable salts thereof, wherein:

HetA is a C₅ arylene group, wherein the two substituent groups are on adjacent ring atoms, and where the group is further optionally

5 substituted by one halo, amino or C₁₋₇ alkoxy group;

Y is -CR^{C1}R^{C2}-(CH₂)_m-, where m is 0 or 1, R^{C1} is selected from H, CH₃ and CF₃, and R^{C2} is selected from H and CH₃, or R^{C1} and R^{C2} together with the carbon atom to which they are attached form the 1,1-cyclopropylene group:



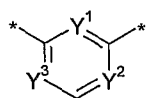
10

R^{N1} and R^{N2} are independently selected from H and R, where R is optionally substituted C₁₋₁₀ alkyl, C₃₋₂₀ heterocyclyl and C₅₋₂₀ aryl; or R^{N1} and R^{N2}, together with the nitrogen atom to which they are attached form an optionally substituted 5-7 membered, nitrogen

15 containing, heterocyclic ring;

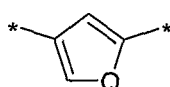
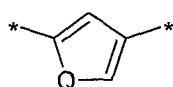
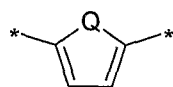
HetB is selected from:

(i)



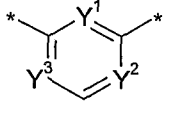
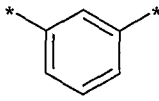
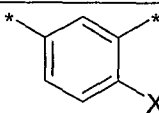
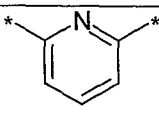
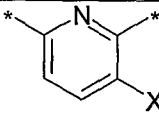
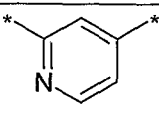
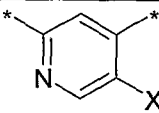
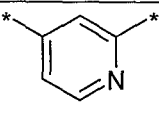
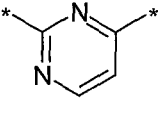
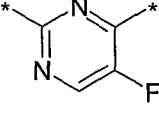
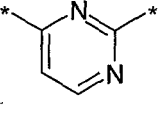
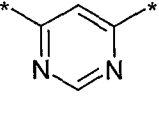
, where Y¹ and Y³ are independently selected from CH and N, Y² is selected from CX and N and X is H, Cl or F; and

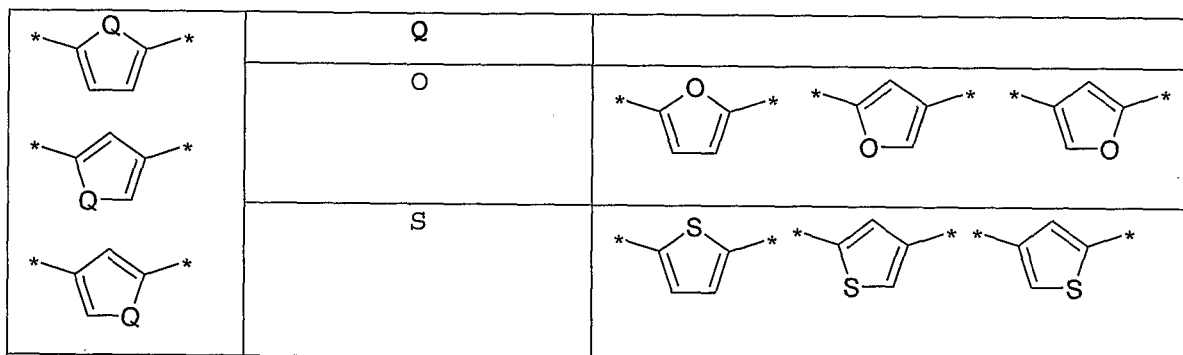
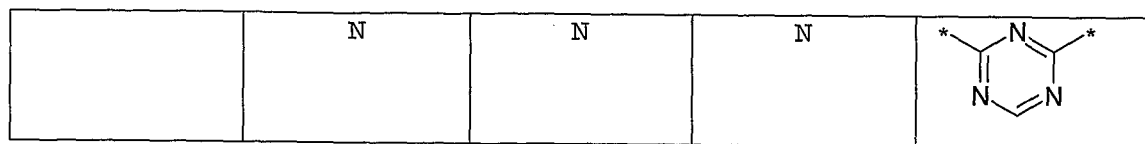
20 (ii)



where Q is O or S.

The possibilities for HetB are:

	Formula			Group
	Y ¹	Y ²	Y ³	
	CH	CH	CH	
	CH	CX	CH	
	N	CH	CH	
	N	CX	CH	
	CH	CH	N	
	CH	CX	N	
	CH	N	CH	
	N	CH	N	
	N	CX	N	
	N	N	CH	
	CH	N	N	



PARP inhibitors currently in clinical trials include INO-1001

- 5 (Inotek/Genentech), AG-0014699 (Pfizer), and BSI-201 (BiPar Sciences) and PARP inhibitors in preclinical trials include BSI-401 and BSI-101 (BiPar Sciences).

In some preferred embodiments, the PARP inhibitor may be a compound
 10 selected from the group consisting of: 3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-imidazolidine-2,4-dione; 3-[3-(5,8-difluoro-4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-imidazoline-2,4-dione; 5-chloro-2-{1-[3-
 ([1,4]diazepane-1-carbonyl)-4-fluoro-phenyl]-ethoxy}-benzamide; 2-{3-
 15 [2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-2,4-dioxo-imidazolidin-1-yl}-acetamide; 4-[3-(4-Cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one; 3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5,5-dimethyl-1-[2-(4-methyl-piperazin-1-yl)-2-oxo-ethyl]-imidazoline-2,4-dione; 8-fluoro-2-(4-methylaminomethyl-phenyl)-
 20 1,3,4,5-tetrahydro-azepino[5,4,3-cd]indol-6-one, INO-1001, AG-0014699, BSI-201, BSI-401 and BSI-101.

In some preferred embodiments, the PARP inhibitor may have a greater potency than the potency of 3-aminobenzamide ($IC_{50} \sim 20\mu M$), preferably 5-fold or greater, 10-fold or greater, 50-fold or greater, 100 fold or greater or 1000-fold or greater than the potency of 3-aminobenzamide.

5

Suitable PARP inhibitors are either commercially available or may be synthesized by known methods from starting materials that are known (see, for example, Suto et al. *Anticancer Drug Des.* 6:107-17 (1991)).

10 Peptide fragments of the PARP sequence may be used to inhibit PARP. Similarly, peptide fragments of a component of a kinase-mediated cellular pathway may be used to inhibit the component and thus, the pathway itself. For example, a peptide fragment of a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and
15 STK22C may be used to inhibit the kinase. Peptide fragments may be generated wholly or partly by chemical synthesis using the published sequences, for example the published PARP sequence (Acc No: NM_001618.1), or the published sequence of a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C, as set
20 out above. Peptide fragments can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, *Solid Phase Peptide Synthesis*, 2nd edition, Pierce Chemical Company, Rockford, Illinois
25 (1984), in M. Bodanzsky and A. Bodanzsky, *The Practice of Peptide Synthesis*, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first
30 completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof. In some

embodiments, peptides comprising modified or D-amino acids may be employed.

Other candidate compounds for inhibiting an enzyme, such as PARP or a
5 kinase described herein, may be based on modelling the 3-dimensional
structure of the enzyme and using rational drug design to provide
candidate compounds with particular molecular shape, size and charge
characteristics. A candidate inhibitor, for example, may be a
"functional analogue" of a peptide fragment or other compound which
10 inhibits the enzyme. A functional analogue has the same functional
activity as the peptide or other compound in question, i.e. it may
interfere with the interactions or activity of the enzyme. Examples
of such analogues include chemical compounds which are modelled to
resemble the three dimensional structure of the enzyme in an area
15 which contacts another enzyme or factor, and in particular the
arrangement of the key amino acid residues as they appear.

Another class of suitable enzyme inhibitors includes nucleic acid
encoding part or all of the amino acid sequence of the enzyme, for
20 example PARP (Acc No: NM_001618.2 GI: 11496989) or a kinase selected
from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and
STK22C, or the complement thereof, which inhibit activity or function
by down-regulating production of active enzyme.

25 For instance, expression of an enzyme may be inhibited using anti-
sense or RNAi technology. The use of these approaches to down-regulate
gene expression is now well-established in the art.

Anti-sense oligonucleotides may be designed to hybridise to the
30 complementary sequence of nucleic acid, pre-mRNA or mature mRNA,
interfering with the production of the base excision repair pathway
component so that its expression is reduced or completely or
substantially completely prevented. In addition to targeting coding
sequence, anti-sense techniques may be used to target control

sequences of a gene, e.g. in the 5' flanking sequence, whereby the anti-sense oligonucleotides can interfere with expression control sequences. The construction of anti-sense sequences and their use is described for example in Peyman and Ulman, Chemical Reviews, 90:543-584, (1990) and Crooke, Ann. Rev. Pharmacol. Toxicol. 32:329-376, (1992).

Oligonucleotides may be generated *in vitro* or *ex vivo* for administration or anti-sense RNA may be generated *in vivo* within cells in which down-regulation is desired. Thus, double-stranded DNA may be placed under the control of a promoter in a "reverse orientation" such that transcription of the anti-sense strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the sense strand of the target gene. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein (see for example, Applied Antisense Oligonucleotide Technology C A. Stein (1998) Wiley & Sons).

The complete sequence corresponding to the coding sequence in reverse orientation need not be used. For example fragments of sufficient length may be used. It is a routine matter for the person skilled in the art to screen fragments of various sizes and from various parts of the coding or flanking sequences of a gene to optimise the amount of anti-sense inhibition. It may be advantageous to include the initiating methionine ATG codon, and perhaps one or more nucleotides upstream of the initiating codon. A suitable fragment may have about 14-23 nucleotides, e.g. about 15, 16 or 17.

An alternative to anti-sense is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression; Angell & Baulcombe (1997) The EMBO Journal 16,12:3675-3684; and Voinnet & Baulcombe (1997) Nature 389: pg 553).

Small RNA molecules may also be employed to regulate gene expression. These include targeted degradation of mRNAs by small interfering RNAs (siRNAs), post transcriptional gene silencing (PTGs), developmentally regulated sequence-specific translational repression of mRNA by micro-
5 RNAs (miRNAs) and targeted transcriptional gene silencing.

Double-stranded RNA (dsRNA)-dependent post transcriptional silencing, also known as RNA interference (RNAi), is a phenomenon in which dsRNA complexes can target specific genes of homology for silencing in a
10 short period of time. It acts as a signal to promote degradation of mRNA with sequence identity. A 20-nt siRNA is generally long enough to induce gene-specific silencing, but short enough to evade host response. The decrease in expression of targeted gene products can be extensive with 90% silencing induced by a few molecules of siRNA.

15 These RNA sequences are termed "short or small interfering RNAs" (siRNAs) or "microRNAs" (miRNAs) depending in their origin. Both types of sequence may be used to down-regulate gene expression by binding to complementary RNAs and either triggering mRNA elimination
20 (RNAi) or arresting mRNA translation into protein. siRNA are derived by processing of long double stranded RNAs and when found in nature are typically of exogenous origin. Micro-interfering RNAs (miRNA) are endogenously encoded small non-coding RNAs, derived by processing of short hairpins. Both siRNA and miRNA can inhibit the translation of
25 mRNAs bearing partially complementary target sequences without RNA cleavage and degrade mRNAs bearing fully complementary sequences.

The siRNA ligands are typically double stranded and, in order to optimise the effectiveness of RNA mediated down-regulation of the
30 function of a target gene, it is preferred that the length of the siRNA molecule is chosen to ensure correct recognition of the siRNA by the RISC complex that mediates the recognition by the siRNA of the mRNA target and so that the siRNA is short enough to reduce a host response.

miRNA ligands are typically single stranded and have regions that are partially complementary enabling the ligands to form a hairpin. miRNAs are RNA genes which are transcribed from DNA, but are not translated into protein. A DNA sequence that codes for a miRNA gene is longer than the miRNA. This DNA sequence includes the miRNA sequence and an approximate reverse complement. When this DNA sequence is transcribed into a single-stranded RNA molecule, the miRNA sequence and its reverse-complement base pair to form a partially double stranded RNA segment. The design of microRNA sequences is discussed on John et al, PLoS Biology, 11(2), 1862-1879, 2004.

Typically, the RNA ligands intended to mimic the effects of siRNA or miRNA have between 10 and 40 ribonucleotides (or synthetic analogues thereof), more preferably between 17 and 30 ribonucleotides, more preferably between 19 and 25 ribonucleotides and most preferably between 21 and 23 ribonucleotides. In some embodiments of the invention employing double-stranded siRNA, the molecule may have symmetric 3' overhangs, e.g. of one or two (ribo)nucleotides, typically a UU or dTdT 3' overhang. The skilled person can readily design suitable siRNA and miRNA sequences, for example using resources such as Ambion's siRNA finder, see http://www.ambion.com/techlib/misc/siRNA_finder.html. siRNA and miRNA sequences can be synthetically produced and added exogenously to cause gene downregulation or produced using expression systems (e.g. vectors).

Longer double stranded RNAs may be processed in the cell to produce siRNAs (see for example Myers (2003) Nature Biotechnology 21:324-328). The longer dsRNA molecule may have symmetric 3' or 5' overhangs, e.g. of one or two (ribo)nucleotides, or may have blunt ends. The longer dsRNA molecules may be 25 nucleotides or longer. Preferably, the longer dsRNA molecules are between 25 and 30 nucleotides long. More preferably, the longer dsRNA molecules are between 25 and 27

nucleotides long. Most preferably, the longer dsRNA molecules are 27 nucleotides in length. dsRNAs 30 nucleotides or more in length may be expressed using the vector pDECAP (Shinagawa et al., Genes and Dev., 17, 1340-5, 2003).

5

siRNA molecules, longer dsRNA molecules or miRNA molecules may be made recombinantly by transcription of a nucleic acid sequence, preferably contained within a vector. In some embodiments, the siRNA, longer dsRNA or miRNA is produced endogenously (within a cell) by transcription from the vector. The vector may be introduced into the cell in any of the ways known in the art. Optionally, expression of the RNA sequence can be regulated using a tissue specific promoter. In other embodiments, the siRNA, longer dsRNA or miRNA is produced exogenously (in vitro) by transcription from the vector.

15

A vector may comprise a nucleic acid sequence as described herein in both the sense and antisense orientation, such that, when expressed as RNA, the sense and antisense sections will associate to form a double stranded RNA. In other embodiments, the sense and antisense sequences may be provided on different vectors. Alternatively, siRNA molecules may be synthesized using standard solid or solution phase synthesis techniques which are known in the art.

20

Modified nucleotide bases may be used in addition to naturally occurring bases, and may confer advantageous properties on siRNA molecules containing them. For example, modified bases may increase the stability of the siRNA molecule, thereby reducing the amount required for silencing. The provision of modified bases may also provide siRNA molecules which are more, or less, stable than unmodified siRNA.

30

Methods relating to the use of RNAi to silence genes are well known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes

Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619, and Elbashir S M, et al., 2001 Nature 411:494-498).

Other aspects of the invention relate to methods of screening for agents useful in increasing the sensitivity of cancer cells to DNA damage promoting agents, such as PARP inhibitors. Methods may be *in vivo* cell-based methods, or *in vitro* non-cell-based methods

An increase in sensitivity of a cancer cell to a DNA damage promoting agent such as a PARP inhibitor (also referred to as 'sensitisation' or 'hypersensitisation') is an increase in the therapeutic index of the agent against the cancer cell.

A method of screening for an agent which increases the sensitivity a cancer cell to a DNA damage promoting agent may comprise;

contacting a component of a kinase-mediated cellular pathway or a fragment or variant thereof, with a test compound, and determining the interaction of the component with the test compound.

Preferred DNA damage promoting agents are described elsewhere herein and include PARP inhibitors.

The interaction of the component with the test compound may be determined by determining the presence or amount of binding of the test compound to the component. The presence of binding may be indicative that the test compound is an inhibitor of the kinase-mediated cellular pathway and may be useful in increasing the sensitivity of the cell to the DNA damage promoting agent.

The interaction of the component with the test compound may be determined by determining the activity of the component in the presence of the test compound. A decrease in activity in the presence
5 relative to the absence of test compound is indicative that the test compound is an inhibitor of the kinase-mediated cellular pathway and may be useful in increasing the sensitivity of the cell to the DNA damage promoting agent.

10 Kinase-mediated cellular pathways and components thereof are described elsewhere herein. In some preferred embodiments, the component is a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C or a fragment or variant of any of these. The activity of a kinase may be determined, for example, by determining
15 the phosphorylation of a suitable kinase substrate in the presence of the test compound. A compound which is found to inhibit kinase activity and therefore the activity of a kinase-mediated cellular pathway, may be useful in sensitising a cell to the DNA damage promoting agent, for example in the treatment of cancer.

20 The component of the kinase-mediated cellular pathway may be an isolated polypeptide or may be a polypeptide comprised in a cell. The polypeptide may be naturally expressed in the cell or not naturally expressed in the cell i.e. heterologous.

25 A fragment or variant of the wild-type sequence of a component of a kinase-mediated cellular pathway, such as a kinase, may differ from the wild-type sequence by the addition, deletion, substitution and/or insertion of one or more amino acids, provided kinase activity is
30 retained, for example up to ten, up to twenty or up to 30 amino acids or more. In particular, suitable variants include natural allelic variant forms of a wild-type polypeptide.

A variant of a wild-type Cdk5 polypeptide may include the kinase-defective cdk5 isoform Cdk5i (Moorthamer et al Biochem Biophys Res Commun. 1998 Dec 18; 253(2):305-10).

5 A polypeptide which is a variant of a wild-type sequence may comprise an amino acid sequence which shares greater than about 60% sequence identity with the wild-type sequence, greater than about 70%, greater than about 80%, greater than about 90% or greater than about 95%. The sequence may share greater than about 60% similarity with the wild-
10 type kinase sequence, greater than about 70% similarity, greater than about 80% similarity or greater than about 90% similarity.

Sequence similarity and identity are commonly defined with reference to the algorithm GAP (Genetics Computer Group, Madison, WI). GAP uses
15 the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of
20 Altschul et al. (1990) J. Mol. Biol. 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) PNAS USA 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) J. Mol Biol. 147: 195-197), or the TBLASTN program, of Altschul et al. (1990) supra, generally employing default parameters. In particular, the
25 psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used. Sequence identity and similarity may also be determined using GenomequestTM software (Gene-IT, Worcester MA USA).

Sequence comparisons are preferably made over the full-length of the
30 relevant sequence described herein.

Similarity allows for "conservative variation" where one amino acid is substituted for another amino acid of similar chemical structure and may have no effect on the protein function, e.g. substitution of one

hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine.

5

The effect of amino acid substitution on a protein function depends on the role of the particular residue in protein activity. Using established techniques, such as *in vitro* mutagenesis, it is routine to test whether particular amino acids are necessary for protein

10 function.

Determining the activity of a component of a kinase-mediated cellular pathway may include detecting the presence of activity, detecting the presence of activity above a threshold value and/or measuring the

15 amount of activity.

As described above, polypeptide fragments which retain all or part of the activity of the full-length protein may be generated and used in the methods described herein, whether *in vitro* or *in vivo*. Suitable

20 ways of generating fragments include recombinant techniques and chemical synthesis techniques which are well known in the art.

A fragment of a full-length sequence may consist of fewer amino acids than the full-length sequence. For example a fragment may consist of

25 at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the full length sequence but 800 or less, 700 or less, 600 or less, 500 or less, 250 or less or 200 or less amino acids of the full length sequence.

Another aspect of the invention provides a method of screening for an agent which increases the sensitivity a cancer cell to a DNA damage promoting agent, such as a PARP inhibitor, comprising:

30 contacting a cell with a test compound, and

determining the activity of a kinase-mediated cellular pathway in the presence of the test compound.

A decrease in pathway activity in the presence relative to the absence of test compound is indicative that the test compound is useful in sensitising a cell to a DNA damage promoting agent.

Suitable kinase-mediated cellular pathways are described above. Techniques for determining the activity of kinase-mediated cellular pathways are well-known in the art and any suitable technique may be employed. For example, the loss or reduced amount of one or more components of the pathway may be detected, the phosphorylation profile of the pathway may be monitored, or the cellular effects of the pathway, such as apoptosis or cell migration, may be determined.

Another aspect of the invention provides a method of screening for an agent which increases the sensitivity of a cancer cell to a DNA damage promoting agent, such as a PARP inhibitor, comprising:

contacting a cell with a test compound, and
determining the expression of a gene encoding a component of a kinase-mediated cellular pathway.

A decrease in expression of the gene encoding the component in the presence relative to the absence of test compound is indicative that the test compound may be useful in inhibiting a kinase-mediated cellular pathway and thereby increasing the sensitivity of a cell to the a DNA damage promoting agent.

Kinase-mediated cellular pathways and components thereof are described elsewhere herein. In some preferred embodiments, the expression of a gene encoding a kinase selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C may be determined. Methods of determining the level of expression of a target gene are well known in the art and are described elsewhere herein.

Methods of screening may comprise testing the test compound further. For example, a method described above may further comprise the step of contacting a cell with a DNA damage promoting agent, such as a PARP inhibitor, in the presence and absence of the test compound and the effect of the agent on the growth and/or proliferation of the cell determined. Reduced growth and/or proliferation in the presence relative to the absence of test compound may be indicative that the test compound increases the sensitivity of a cell to the agent. A compound which is found to increase the sensitivity of a cell to a DNA damage promoting agent, such as a PARP inhibitor, may be useful in the treatment of a cancer condition, in combination with the agent.

Another aspect of the invention provides a method of screening for a DNA damage promoting agent, comprising:

contacting a test compound with a cell having a kinase-deficient phenotype and a control cell not having a kinase-deficient phenotype, and;

determining the sensitivity of cell having the kinase-deficient phenotype to the test compound relative to the control cell ,

wherein an increase in the sensitivity of the cell having the kinase-deficient phenotype relative to the control cell is indicative that the test compound is a DNA damage promoting agent.

The sensitivity of a cell to a test compound may be determined by measuring cell growth or cell death in the presence relative to the absence of the test compound. The amount by which cell death is increased or by which cell growth is reduced in the presence of the test compound relative to its absence is indicative of the level of sensitivity of the cells to the test compound.

Another aspect provides the use of a cell with a kinase-deficient phenotype in a method of screening for DNA damage promoting compounds, for example a method described above.

DNA damage promoting agents and cells with a kinase-deficient phenotype are described above.

5 In some embodiments, a cell with a kinase-deficient phenotype may be a naturally occurring cell, for example a cell which has one or more mutations which reduce or abrogate the activity of a kinase-mediated cellular pathway, for example a pathway selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C mediated
10 cellular pathways.

In other embodiments, a cell with a kinase-deficient phenotype may be generated by administering an inhibitor of a component of a kinase-mediated cellular pathway, for example an inhibitor of a kinase
15 selected from the group consisting of CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C. Suitable kinase inhibitors are described elsewhere herein and include sense or anti-sense nucleic acid molecules as described herein.

20 The precise format for performing the methods described herein may be varied by those of skill in the art using routine skill and knowledge.

Compounds which may be screened using the methods described herein may be natural or synthetic chemical compounds used in drug screening
25 programmes. Extracts of plants, microbes or other organisms which contain several characterised or uncharacterised components may also be used.

Combinatorial library technology provides an efficient way of testing
30 a potentially vast number of different compounds for ability to modulate an interaction. Such libraries and their use are known in the art, for all manner of natural products, small molecules and peptides, among others. The use of peptide libraries may be preferred in certain circumstances.

The amount of test compound or compound which may be added to a method of the invention will normally be determined by serial dilution experiments. Typically, from about 0.001 nM to 1 mM or more of putative inhibitor compound may be used, for example from 0.01 nM to 100µM, e.g. 0.1 to 50 µM, such as about 10 µM.

A method may comprise identifying the test compound as an inhibitor of a kinase-mediated cellular pathway. Such a compound may, for example, be useful in the sensitising a cell to PARP inhibition, for example in the treatment of cancer, as described herein.

A test compound identified using one or more initial screens as having ability to sensitise a cell to a DNA damage promoting agent, such as a PARP inhibitor, may be assessed further using one or more secondary screens. A secondary screen may, for example, involve testing for a biological function such as an effect on tumour growth, proliferation or metastasis in an animal model in combination with a DNA damage promoting agent.

20

The test compound may be isolated and/or purified or alternatively, it may be synthesised using conventional techniques of recombinant expression or chemical synthesis. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals, in combination with a DNA damage promoting agent, such as a PARP inhibitor, for the treatment of a cancer condition. Methods of the invention may thus comprise formulating the test compound in a pharmaceutical composition with a pharmaceutically acceptable excipient, vehicle or carrier for therapeutic application, as discussed further below.

30

Following identification of a compound which inhibits a kinase-mediated cellular pathway and may therefore be useful in increasing

the sensitivity of cancer cells to a DNA damage promoting agent, such as a PARP inhibitor, a method may further comprise modifying the compound to optimise the pharmaceutical properties thereof.

5 The modification of a 'lead' compound identified as biologically active is a known approach to the development of pharmaceuticals and may be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, e.g. peptides are not well suited as active agents for
10 oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Modification of a known active compound (for example, to produce a mimetic) may be used to avoid randomly screening large number of molecules for a target property.

15 Modification of a 'lead' compound to optimise its pharmaceutical properties commonly comprises several steps. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid
20 residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled
25 according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR.

Computational analysis, similarity mapping (which models the charge
30 and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional structure of the compound which inhibits the kinase-mediated cellular pathway is

modelled. This can be especially useful where the compound changes conformation, allowing the model to take account of this in the optimisation of the lead compound.

5 A template molecule is then selected, onto which chemical groups that mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the modified compound is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade in vivo, while
10 retaining the biological activity of the lead compound. The modified compounds found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Modified compounds include mimetics of the lead compound.

15 Further optimisation or modification can then be carried out to arrive at one or more final compounds for in vivo or clinical testing.

As described above, a compound identified and/or obtained using the present methods may be formulated into a pharmaceutical composition.

20 While it is possible for an active compound (e.g. an inhibitor of PARP or a kinase-mediated cellular pathway) to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising at least one active compound, as defined
25 above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

30 Pharmaceutical compositions comprising a PARP inhibitor and/or a kinase-mediated cellular pathway inhibitor as defined above, for example, an inhibitor admixed or formulated together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants,

stabilisers, or other materials, as described herein, may be used in the methods described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well-known in the art of pharmacy. Such methods include the step of bringing the active compound into association with a carrier which may constitute one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

The inhibitor(s) or pharmaceutical composition comprising the inhibitor(s) may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site

of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; 5 vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular, intraarticular, subarachnoid, and intrasternal; by implant of a depot, 10 for example, subcutaneously or intramuscularly.

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a 15 powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

A tablet may be made by conventional means, e.g., compression or 20 molding, optionally with one or more accessory ingredients.

Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g., povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); 25 fillers or diluents (e.g., lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, silica); disintegrants (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p- 30 hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled

release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for parenteral administration (e.g. by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 µg/ml, for example, from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

It will be appreciated that appropriate dosages of the active compounds, and compositions comprising the active compounds, can vary from patient to patient. Determining the optimal dosage will

generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration in vivo can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

In general, a suitable dose of the active compound is in the range of about 100 μ g to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

Aspects of the present invention will now be illustrated with reference to the accompanying figures described below and experimental exemplification, by way of example and not limitation. Further

aspects and embodiments will be apparent to those of ordinary skill in the art.

5 All documents mentioned in this specification and the sequences and other contents of database entries recited in this specification are hereby incorporated herein by reference.

10 Unless otherwise stated, database entries for sequences refer to the National Center for Biotechnology Information (NCBI) Protein Entrez or Nucleotide entrez databases. A 'GenInfo Identifier' (GI) sequence identification number is a series of digits assigned consecutively to each sequence record processed by NCBI and is unique to a particular sequence. KEGG pathway references refer to the Kyoto Encyclopaedia of Genes and Genomes database references. GeneID reference numbers refer to the unique identifier assigned by NCBI. MIM reference numbers refer to the identifier assigned to human genes and phenotypes by the Online Mendelian Inheritance in Man database. All these identifiers are commonly used and understood in the art. References to database entries herein refer to the contents of the database entry which are current on the filing date of the application, unless otherwise stated.

25 Figure 1 shows the surviving fraction of CAL51 cells were transfected with siRNA against siCON, BRCA1, and BRCA2 after 5 days treatment 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one at various doses.

30 Figure 2 shows the correlation of the effect of siRNA on cell growth in vehicle treated plates from two replicates of the screen.

Figure 3 shows the correlation of PARPi sensitivity Z scores from two replicates of the screen.

Figure 4 shows a scatter plot of averaged Z scores from PARP inhibitor sensitivity screen with 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one 1 μ M carried out in duplicate. The dashed line indicates -2 averaged Z score significance threshold.

5

Figure 5 shows revalidation of initial hits from the screen * - $p < 0.0227$ compared to siCON one sided T test, Error bars represent SEM.

Figure 6 shows the effect of siRNA on cell growth in vehicle alone plates as percentage of growth in siCON transfected wells.

10

Figure 7 shows the results of clonogenic assays for PARP inhibitor sensitivity in the 6 revalidated hits from the initial screen (CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C). FOLD - fold increase in sensitivity compared to siCON. Error bars represent SEM of 3 independent experiments.

15

Figure 8 shows the level of silencing for each siRNA compared to siCON transfected cells.

20

Experiments

Materials and Methods

PARP Inhibition

In order to assess the ability of a compound to inhibit PARP, the following assay may be used to determine IC₅₀ values or percentage inhibition at a given concentration.

25

Mammalian PARP may be isolated from Hela cell nuclear extract and incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM MgCl₂ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK). Varying concentrations of a test compound may be added. Compounds may be diluted in DMSO to give a final assay concentration of between 10

30

and 0.01 μM , with the DMSO being at a final concentration of 1% per well. The total assay volume per well may be 40 μl .

After 10 minutes incubation at 30°C, reactions may be initiated by the addition of a 10 μl reaction mixture, containing NAD (5 μM), ^3H -NAD and 30mer double stranded DNA-oligos. Designated positive and negative reaction wells may be done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates may then be shaken for 2 minutes and incubated at 30°C for 45 minutes.

Following the incubation, the reactions may be quenched by the addition of 50 μl 30% acetic acid to each well. The plates may then be shaken for 1 hour at room temperature.

The plates may then be transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded are counts per minute (cpm) following a 30 second counting of each well.

The % enzyme activity for each compound may then be calculated using the following equation:

$$\% \text{ Inhibition} = 100 - \left(100 \times \frac{(\text{cpm of unknowns} - \text{mean negative cpm})}{(\text{mean positive cpm} - \text{mean neagative cpm})} \right)$$

IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited) may be calculated, which are determined over a range of different concentrations, normally from 10 μM down to 0.001 μM . Such IC_{50} values are used as comparative values to identify increased compound potencies.

The Potentiation Factor (PF_{50}) for compounds is calculated as a ratio of the IC_{50} of control cell growth divided by the IC_{50} of cell growth +

PARP inhibitor. The test compounds were used at a fixed concentration of 0.5 micromolar.

Cell growth may be assessed using the sulforhodamine B (SRB) assay
5 (Skehan, P., et al., (1990) J. Natl. Cancer Inst. 82, 1107-1112.).
2,000 HeLa cells may be seeded into each well of a flat-bottomed 96-
well microtiter plate in a volume of 100 μ l and incubated for 6 hours
at 37°C. Cells may be either replaced with media alone or with media
containing PARP inhibitor at a final concentration of 0.5, 1 or 5 μ M.
10 Cells treated with PARP inhibitor may be used to assess the growth
inhibition by the PARP inhibitor.

Cells may be left for a further 16 hours before replacing the media
and allowing the cells to grow for a further 72 hours at 37°C. The
15 media may be removed and the cells fixed with 100 μ l of ice cold 10%
(w/v) trichloroacetic acid. The plates may then be incubated at 4°C
for 20 minutes and then washed four times with water. Each well of
cells may then be stained with 100 μ l of 0.4% (w/v) SRB in 1% acetic
acid for 20 minutes before washing four times with 1% acetic acid.
20 Plates may then be dried for 2 hours at room temperature. The dye
from the stained cells may then be solubilized by the addition of
100 μ l of 10mM Tris Base into each well. Plates may then be gently
shaken and left at room temperature for 30 minutes before measuring
the optical density at 564nm on a Microquant microtiter plate reader.

25

High-Throughput Screen (HTS) method

3500 CAL51 cells were plated per well in 96 well plates. CAL51 cells
were transfected with siRNA using Oligofectamine (Invitrogen)
according to manufacturer's instructions. In total, cells were
30 transfected with siRNAs targeting 779 kinases and kinase-related
genes. Each 96 well plate contained 80 experimental siRNA (Dharmacon
SMARTpool), 4 wells of non-targeting siCONTROL #1 (siCON; Cat no
D001210-01-05 Dharmacon Corp, CO, USA), and 2 wells siRNA directed
against BRCA1 as positive control. Each plate was trypsinised and

split into 6 identical daughter plates, half treated with DMSO vehicle alone and half with PARP inhibitor (PARPi) 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one (compound 4:

WO2004/080976) at 1 μ M. Cell viability was assessed after 5 days drug

5 treatment with luminescent ATP assay (CellTiter-Glo Luminescent Cell Viability Assay; Promega). For each siRNA, the effect of the siRNA on

cell growth was calculated by dividing mean luminescence in the 3 vehicle alone (0.01% DMSO) plates by mean luminescence of the wells transfected with siCON. Sensitivity to PARP inhibitor for each siRNA

10 was assessed by calculating the surviving fraction following PARP inhibitor as log₂ ratio of mean luminescence in wells treated with PARP inhibitor divided by mean luminescence in wells treated with vehicle.

15 *Real time RT-PCR*

CAL51 cells were transfected with siRNA in 96 well plates. After 48hrs cells were harvested and total RNA isolated using Trizol (Invitrogen) according to manufacturer's instructions. cDNA was made with

SuperScript III First Strand Synthesis System for RT-PCR (Invitrogen)

20 according to manufacturer's instructions. Assay-on-Demand primer/probe sets were purchased from Applied Biosystems for each gene and

endogenous control β -Glucuronidase (4310888E). Real-Time qPCR was performed on the 7900HT Fast Real-Time PCR System (Applied

Biosystems). HeLa cDNA was used to calculate reaction efficiency. All

25 expression values were normalised to the expression of β -Glucuronidase.

Results

Validation of HTS

30 The HTS method was validated by transfecting CAL51 cells with siRNA against siCON, BRCA1, and BRCA2 and measuring the surviving fraction after 5 days treatment with 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one at various doses. The results showed that

the HTS method can demonstrate sensitivity to PARP inhibitor following silencing of either BRCA1 or BRCA2 (Figure 1).

5 The reproducibility of the HTS method was investigated by measuring the growth in siCON wells as percentage of growth in siCON wells in vehicle treated plates from two replicates of the whole screen. The results are shown in Figure 2. The PARPi sensitivity Z scores from two replicates of the whole screen were also correlated and the results set out in Figure 3. The screen was found to be highly
10 reproducible between two replicates.

Initial PARP inhibitor sensitivity screen

779 kinases or kinase-related genes were screened for an effect on the sensitivity of CAL51 cells to PARP inhibition, using siCON and BRCA1
15 siRNA as controls. Each of the 779 genes in the library (Dharmacon human kinase siARRAY) was represented by a Dharmacon SmartPOOL™. Each gene-specific SmartPOOL™ was contained within one well of a 96 well plate and consisted of 4 different siRNA species each targeting a different sequence within the gene transcript. Standard deviation was
20 calculated from the combined Protein Kinase and DNA Repair library screens, not including the BRCA1 siRNA controls. A scatter plot of averaged Z scores from PARP inhibitor sensitivity screen with 1µM 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one carried out in duplicate is shown in Figure 4. siRNA directed against
25 Ataxia Telangiectasia and Rad3 Related protein (ATR: NP_001175.1 GI:4502325) and PNKP are indicated in this figure. The dashed line indicates -2 averaged Z score significance threshold. The Z factor of the screen was 0.34, representing a highly efficient screen.

30 31 hits were identified from the siRNA screen, 20 of which are shown in Table 1 in Z score order, with siRNA target gene, PARPi sensitivity Z score, and the effect of siRNA on cell growth in vehicle alone plates. The mean of BRCA1 siRNA control wells presented as a comparison.

Revalidation of Putative Sensitising siRNA

The PARP inhibitor sensitivity assay was repeated in triplicate with original siRNA pool, and separately with each individual siRNA species
5 from the SmartPOOL™, in order to revalidate the initial hits. ATR, ATM and CHEK1 (hits, 3, 17 and 19 in table 1) have been shown to sensitise to PARP inhibitors, and have well documented roles in DNA damage response pathways, so these were not revalidated.

10 Genes were taken as revalidated when the SMARTpool sensitised to PARP inhibitor, and at least 2 of the 4 individual siRNAs from the SmartPOOL™. Surviving fractions following PARP inhibition with the 4 individual siRNA per gene, BRCA1 control, and siCON were measured and the results shown in Figure 5. CDK5 was found to revalidate with all 4
15 siRNAs; PNKP with 3 siRNAs and MAPK12, PLK3, STK36 and STK22C with 2 siRNAs. None of the other hits from table 1 was successfully revalidated.

The effect of the siRNA on cell growth in vehicle alone plates was
20 also measured as percentage of growth in siCON transfected wells and the results are shown in Figure 6.

PARP inhibitor sensitivity was assessed by clonogenic assay in the 6 revalidated hits from the screen (CDK5, MAPK12, PLK3, PNKP, STK36 and
25 STK22C). CAL51 cells were transfected with siRNA (SMARTpool targeting each gene, with the exception of MAPK12 that was targeted with MAPK12-3), split 48 hrs after transfection into 6 wells plates, and exposed continuously to various doses of 4-[3-([1,4]diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one starting at 60hrs post
30 transfection. The colonies were fixed, counted at 10-14 days post transfection, and surviving fraction for each dose of 4-[3-([1,4]Diazepane-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one assessed. Data was plotted and analysed using GraphPad PRISM 4, and

SF50 estimated from survival curve (SF50 - dose giving 50% survival). The results are shown in Figure 7 and Table 2.

Gene-specific silencing by all the siRNA that sensitise cells to PARP inhibition was confirmed by real time RT-PCR (qPCR). For each gene, gene silencing was examined for each individual siRNA and siCON transfected cells 48hrs following transfection. The level of silencing for each siRNA was compared to siCON transfected cells and the results are shown in Figure 8. All sensitising siRNA were found to silence the corresponding gene mRNA greater than 50% with $p < 0.05$.

Sensitivity of revalidated Hits to different cytotoxic agents in clonogenic assay

The sensitivity of the revalidated hits to Cisplatin (1hr exposure 72 hrs post transfection), Camptothecin (24hrs exposure 60hrs post transfection) and Docetaxel (24hrs exposure, 60hrs post transfection) was assessed by clonogenic formation assay after transfection of siRNA SMARTpool. Genes were targeted with SMARTpool siRNA with the exception of MAPK12 that was targeted with siRNA MAPK12-3. Each drug treatment was repeated in three independent experiments. Data was plotted using GraphPad PRISM version 4 and SF50 estimated from survival curve. Fold increase in sensitivity in comparison to siCON is shown in Table 3. The revalidated hits from the screen were found to sensitise the CAL51 cells to other DNA damaging agents. Knockdown of CDK5, PLK3 or PNKP was found to sensitise to Camptothecin and Cisplatin, knockdown of MAPK12 was found to sensitise to Cisplatin and knockdown of STK22C was found to sensitise to Camptothecin.

None of the siRNA Hits from the screen was found to sensitise cells to Docetaxel. This finding indicates that the sensitivity to PARP inhibitors, and other DNA damaging agents, is not a general increase in sensitivity to cellular insults but is specific to the DNA damage response.

RANK	siRNA	PARPi Z SCORE	siRNA GROWTH
	MEAN BRCA1	-7.23	45%
1	IMPK	-6.54	96%
2	PNKP	-6.45	54%
3	ATR	-5.71	106%
4	TTBK1	-4.95	60%
5	STK36	-4.32	65%
6	PLK1	-4.04	6%
7	STK35	-4.00	77%
8	CDC2	-3.28	55%
9	ADRA1A	-3.26	56%
10	PIP5K2B	-3.22	48%
11	PLK3	-3.14	73%
12	ITGB1BP1	-3.09	86%
13	STK22C	-3.02	87%
14	AKAP1	-2.86	48%
15	FLJ34389	-2.63	63%
16	GSK3A	-2.61	78%
17	CHEK1	-2.56	42%
18	CDK5	-2.50	106%
19	ATM	-2.45	92%
20	MAPK12	-2.44	59%

Table 1

siRNA	SF50 nM	FOLD
■ CDK5	66	49
△ MAPK12	32	102
▽ PLK3	131	25
◇ PNKP	52	63
● STK36	71	46
▣ STK22C	336	10
▼ BRCA1	182	18
▽ BRCA2	6	527
◇ siCON	3269	-

Table 2

siRNA	Camptothecin		Cisplatin		Docetaxel	
	SF50 nM	FOLD	SF50 μ M	FOLD	SF50 nM	FOLD
CDK5	11.3	3.0	6.4	2.2	0.9	1.3
MAPK12-3			8.0	1.7	1.4	0.8
PLK3	17.2	1.9	9.0	1.5	0.9	1.2
PNKP	15.6	2.2	10.0	1.4	1.0	1.1
STK36	20.4	1.6	16.3	0.8	1.3	0.8
STK22C	8.5	3.9	23.4	0.6	0.9	1.2
BRCA1	7.5	4.5	7.6	1.8	0.7	1.5
BRCA2	5.7	5.9	1.1	13.0	0.7	1.6
siCON	33.5	1.0	13.8	1.0	1.1	1.0

Table 3

	General:	DNA sequencing, Sequencing by hybridisation
5	Scanning:	PTT, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage
	Hybridisation	
	Solid phase:	Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips).
10	Solution phase:	Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi et al (1996), Nature Biotechnology, 14 , 303; WO 95/13399 (Public Health Inst., New York)
15	Extension Based:	ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS (Gibbs et al (1989), Nucleic Acids Research, 17 , 2347).
20	Incorporation Based:	Mini-sequencing, APEX
	Restriction Based:	RFLP, Restriction site generating PCR
	Ligation Based:	OLA
25	Other:	Invader assay

Table 4

Fluorescence: FRET, Fluorescence quenching, Fluorescence
polarisation - United Kingdom Patent No. 2228998
(Zeneca Limited)

5

Other: Chemiluminescence, Electrochemiluminescence, Raman,
Radioactivity, Colorimetric, Hybridisation protection
assay, Mass spectrometry

10

Table 5

Amplification Methods:

SSR

NASBA

15

LCR

SDA

b-DNA

20

Table 6

Protein variation detection methods:

Immunoassay

Immunohistology

Peptide sequencing

25

Table 7

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
Bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
ELISA	Enzyme Linked ImmunoSorbent Assay
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Claims:

1. A method of treating an individual with a cancer condition having
5 a kinase-deficient phenotype, comprising;
administering a PARP inhibitor to said individual.
2. A method according to claim 1 wherein the PARP inhibitor is a
compound selected from the group consisting of nicotinamides,
10 benzamides, isoquinolinones, dihydroisoquinolinones, benzimidazoles,
indoles, phthalazin-1(2H)-ones, quinazolinones, isoindolinones,
phenanthridines, benzopyrones, salicylamides, unsaturated hydroximic
acid derivatives, caffeine, theophylline, and thymidine, and analogues
and derivatives thereof.
- 15 3. A method according to claim 2 wherein said PARP inhibitor is a
phthalazin-1(2H)-one or an analogue or derivative thereof.
4. A method according to claim 2 wherein said PARP inhibitor is an
20 isoquinolinone or an analogue or derivative thereof.
5. A method according to claim 2 wherein the PARP inhibitor is a
compound selected from the group consisting of: 3-[2-fluoro-5-(4-oxo-
3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-imidazolidine-2,4-
25 dione; 3-[3-(5,8-difluoro-4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-
phenyl]-5-methyl-imidazoline-2,4-dione; 5-chloro-2-{1-[3-
([1,4]diazepane-1-carbonyl)-4-fluoro-phenyl]-ethoxy}-benzamide; 2-{3-
[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-
methyl-2,4-dioxo-imidazolidin-1-yl}-acetamide; 4-[3-(4-
30 cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-
phthalazin-1-one; 3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-
ylmethyl)-phenyl]-5,5-dimethyl-1-[2-(4-methyl-piperazin-1-yl)-2-oxo-
ethyl]-imidazoline-2,4-dione; 8-fluoro-2-(4-methylaminomethyl-phenyl)-

1,3,4,5-tetrahydro-azepino[5,4,3-cd]indol-6-one, INO-1001, AG-0014699, BSI-201, BSI-401 and BSI-101.

6. A method according to any one of claims 1 to 5 wherein the cancer
5 condition has a phenotype selected from the group consisting of: a cyclin-dependent kinase 5 (CDK5) deficient phenotype, a mitogen-activated protein kinase 12 (MAPK12) deficient phenotype, a polo-like kinase 3 (PLK3) deficient phenotype, a polynucleotide kinase 3'-phosphatase (PNKP) deficient phenotype, a serine/threonine kinase 36
10 (STK36) deficient phenotype, and a serine/threonine kinase 22C (STK22C) deficient phenotype.

7. A method according to claim 6 wherein the cancer comprises one or
more cancer cells which are deficient in a kinase selected from the
15 group consisting of: CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C.

8. A method according to any one of claims 1 to 7 comprising the
step of identifying the individual as having cancer condition with a
kinase-deficient phenotype.
20

9. Use of a PARP inhibitor for the manufacture of a medicament for
use in a method according to any one of claims 1 to 8.

10. A PARP inhibitor for use in a method according to any one of
25 claims 1 to 8.

11. A method of treating an individual with a cancer condition,
comprising:

administering a PARP inhibitor and an inhibitor of a kinase-
30 mediated cellular pathway to said individual.

12. A method according to claim 11 wherein the PARP inhibitor is
selected from the group consisting of: nicotinamides, benzamides,
isoquinolinones, dihydroisoquinolinones, benzimidazoles, indoles,

phthalazin-1(2H)-ones, quinazolinones, isoindolinones, phenanthridines, benzopyrones, salicylamides, unsaturated hydroximic acid derivatives, caffeine, theophylline, and thymidine, and analogues and derivatives thereof.

5

13. A method according to claim 12 wherein said PARP inhibitor is a phthalazin-1(2H)-one or an analogue or derivative thereof.

14. A method according to claim 12 wherein said PARP inhibitor is an
10 isoquinolinone or an analogue or derivative thereof.

15. A method according to claim 12 wherein the PARP inhibitor is a compound selected from the group consisting of: 3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-imidazolidine-2,4-
15 dione; 3-[3-(5,8-difluoro-4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-imidazoline-2,4-dione; 5-chloro-2-{1-[3-([1,4]diazepane-1-carbonyl)-4-fluoro-phenyl]-ethoxy}-benzamide; 2-{3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5-methyl-2,4-dioxo-imidazolidin-1-yl}-acetamide; 4-[3-(4-
20 cyclopropanecarbonyl-piperazine-1-carbonyl)-4-fluoro-benzyl]-2H-phthalazin-1-one; 3-[2-fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)-phenyl]-5,5-dimethyl-1-[2-(4-methyl-piperazin-1-yl)-2-oxo-ethyl]-imidazoline-2,4-dione; 8-fluoro-2-(4-methylaminomethyl-phenyl)-1,3,4,5-tetrahydro-azepino[5,4,3-cd]indol-6-one, INO-1001, AG-0014699,
25 BSI-201, BSI-401 and BSI-101.

16. A method according to any one of claims 11 to 15 wherein the kinase-mediated cellular pathway is a CDK5-mediated cellular pathway, a MAPK12-mediated cellular pathway, a PLK3-mediated cellular pathway,
30 a PNKP-mediated cellular pathway, a STK36-mediated cellular pathway or a STK22C-mediated cellular pathway.

17. A method according to claim 16 wherein the inhibitor of the kinase-mediated cellular pathway inhibits CDK5, MAPK12, PLK3, PNKP, STK36 or STK22C.

5 18. Use of a PARP inhibitor and an inhibitor of a kinase-mediated cellular pathway for the manufacture of a medicament for use in a method according to any one of claims 11 to 17.

10 19. A PARP inhibitor and an inhibitor of a kinase-mediated cellular pathway for use in a method according to any one of claims 11 to 18.

20. A method of screening for an agent which increases the sensitivity of a cell to PARP inhibition comprising;
contacting a component of a kinase mediated cellular pathway with
15 a test compound, and
determining the activity of said component,
wherein a decrease in activity in the presence relative to the absence of test compound is indicative that the compound sensitises a cell to PARP inhibitor.

20 21. A method of screening for an agent which increases the sensitivity of a cell to PARP inhibition comprising;
contacting a cell with a test compound, and
determining the expression of a gene encoding a component of a
25 kinase mediated cellular pathway,
wherein a decrease in the expression in the presence relative to the absence of test compound is indicative that the compound sensitises a cell to PARP inhibitor.

30 22. A method of screening for an agent which increases the sensitivity of a cell to PARP inhibitor comprising;
contacting a cell with a test compound, and
determining the activity of a kinase-mediated cellular pathway in said cell,

wherein a decrease in the activity of said pathway in the presence relative to the absence of test compound is indicative that the compound sensitises a cell to PARP inhibitor.

23. A method according to any one of claims 20 to 22 wherein the kinase-mediated cellular pathway is a CDK5-mediated cellular pathway, a MAPK12-mediated cellular pathway, a PLK3-mediated cellular pathway, a PNKP-mediated cellular pathway, a STK36-mediated cellular pathway or a STK22C-mediated cellular pathway.

24. A method according to any one of claims 20 to 23 wherein the component of the kinase-mediated cellular pathway is CDK5, MAPK12, PLK3, PNKP, STK36 or STK22C.

25. A method according to any one of claims 20 to 24 comprising identifying the test compound as an agent which increases the sensitivity of a cell to PARP inhibition

26. A method of identifying an individual having a cancer condition which is suitable for treatment with a PARP inhibitor comprising;

determining the activity of a kinase-mediated cellular pathway in a cancer cell obtained from the individual,

wherein reduced activity of the pathway relative to controls may be indicative that the individual has a cancer condition which is suitable for treatment with a PARP inhibitor.

27. A method according to claim 26 wherein the activity of a kinase-mediated cellular pathway is determined by determining the amount or activity of a component of the kinase-mediated cellular pathway in said cancer cell.

28. A method according to claim 26 wherein the activity of a kinase-mediated cellular pathway is determined by determining the level of a

nucleic acid encoding a component of the kinase-mediated cellular pathway in said cell.

29. A method according to claim 26 wherein the activity of a kinase-mediated cellular pathway is determined by determining the presence of one or more sequence variations in a gene encoding a component of the kinase-mediated cellular pathway in said cell.

30. A method according to any one of claims 26 to 29 wherein the kinase-mediated cellular pathway is a CDK5-mediated cellular pathway, a MAPK12-mediated cellular pathway, a PLK3-mediated cellular pathway, a PNKP-mediated cellular pathway, a STK36-mediated cellular pathway or a STK22C-mediated cellular pathway.

31. A method according to any one of claims 26 to 30 wherein the component of the kinase-mediated cellular pathway is CDK5, MAPK12, PLK3, PNKP, STK36 or STK22C.

32. A method of screening for a DNA damage promoting agent comprising; comprising:

contacting a test compound with a cell having a kinase-deficient phenotype and a control cell not having a kinase-deficient phenotype, and;

determining the sensitivity of cell having the kinase-deficient phenotype to the test compound relative to the control cell ,

wherein an increase in the sensitivity of the cell having the kinase-deficient phenotype relative to the control cell is indicative that the test compound is a DNA damage promoting agent.

33. A method according to claim 32 wherein the cell has a phenotype selected from the group consisting of: a cyclin-dependent kinase 5 (CDK5) deficient phenotype, a mitogen-activated protein kinase 12 (MAPK12) deficient phenotype, a polo-like kinase 3 (PLK3) deficient phenotype, a polynucleotide kinase 3'-phosphatase (PNKP) deficient

phenotype, a serine/threonine kinase 36 (STK36) deficient phenotype, and a serine/threonine kinase 22C (STK22C) deficient phenotype.

34. A method according to claim 33 wherein the cell is deficient in a
5 kinase selected from the group consisting of: CDK5, MAPK12, PLK3, PNKP, STK36 and STK22C.

35. A method according to any one of claims 32 to 34 comprising identifying the test compound as a DNA damage promoting agent.

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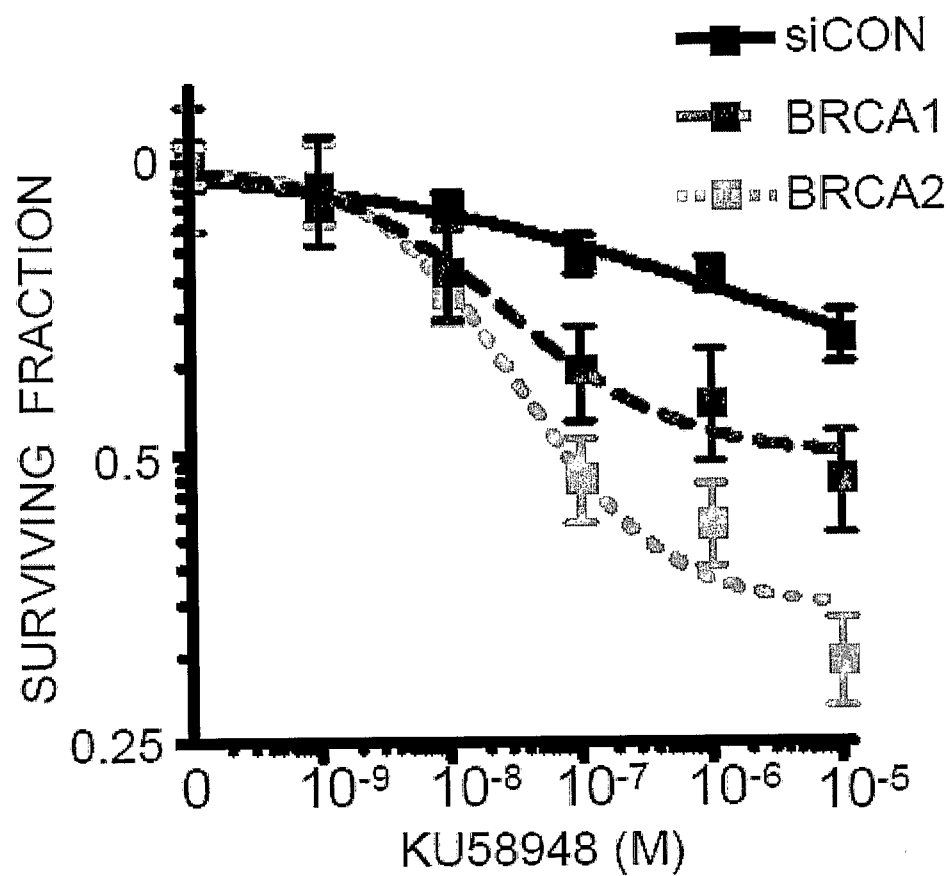


Figure 1

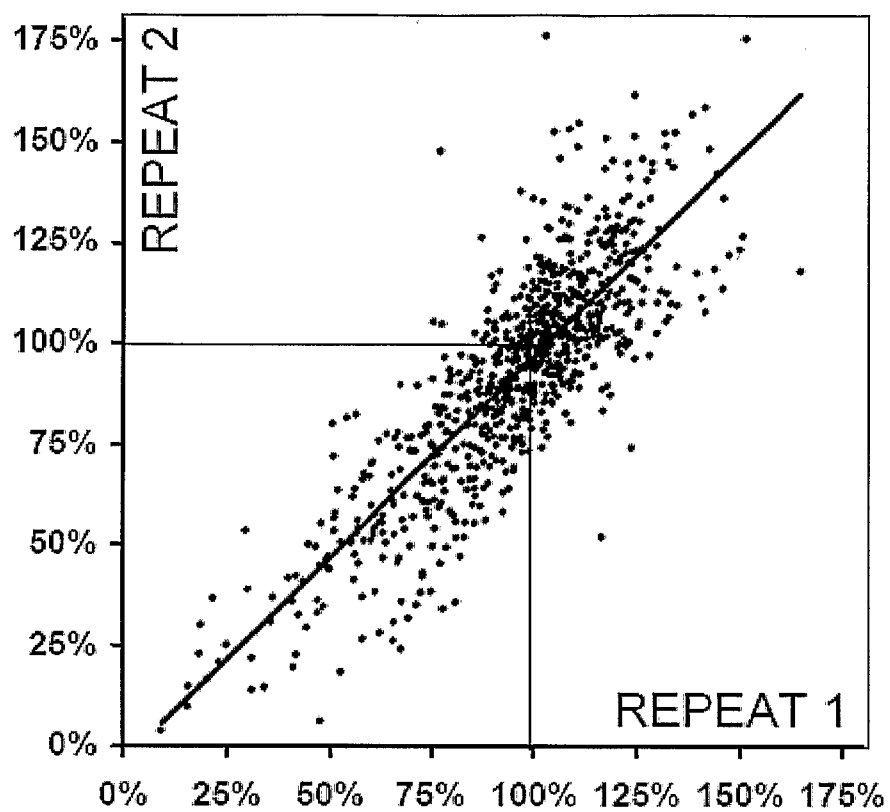


Figure 2

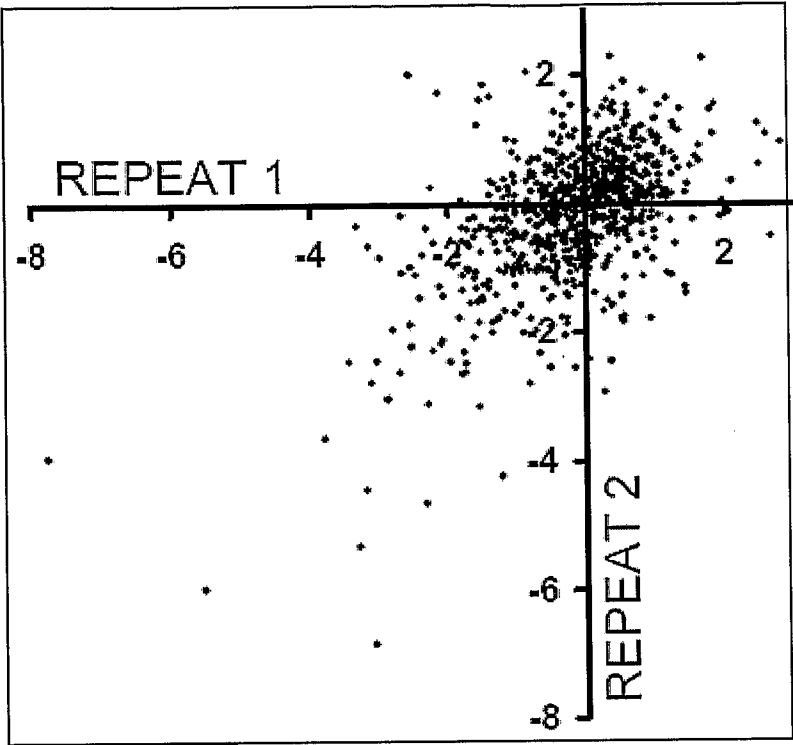


Figure 3

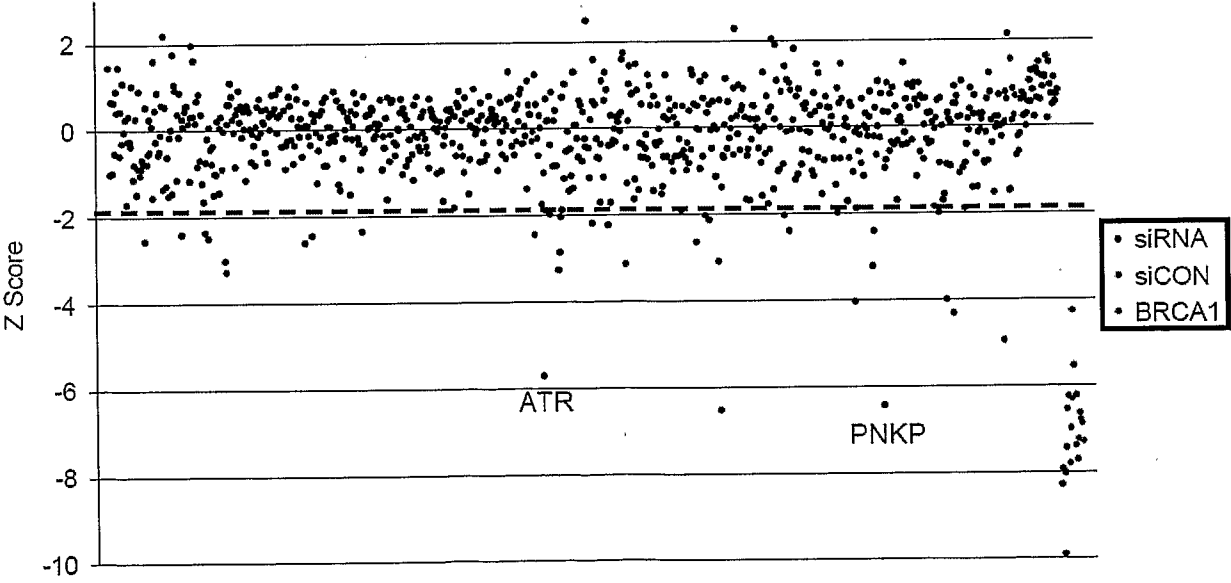


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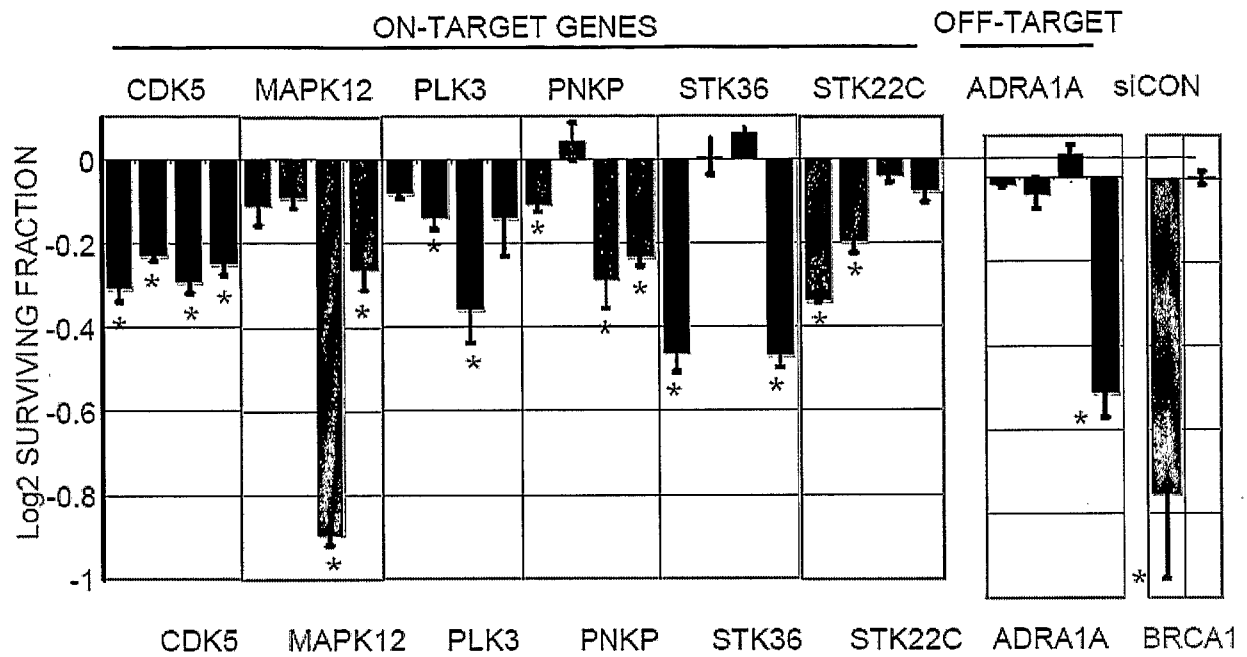


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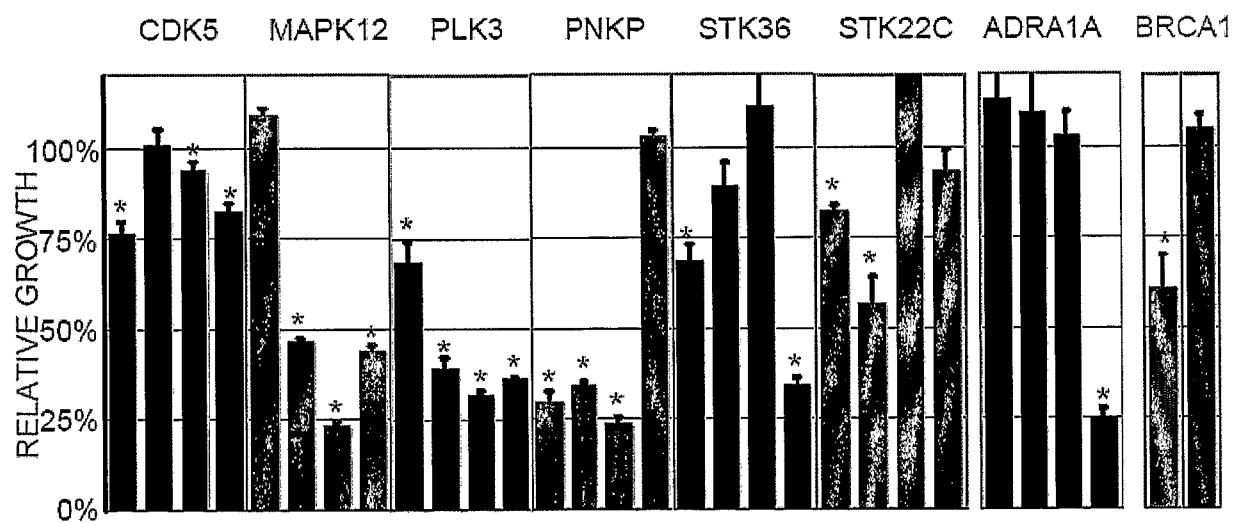


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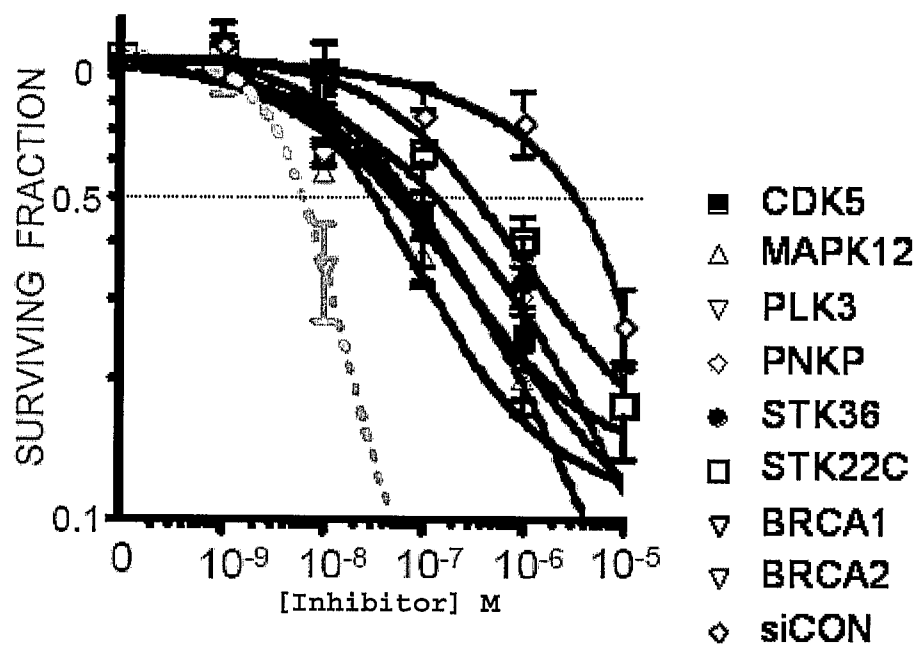


Figure 7

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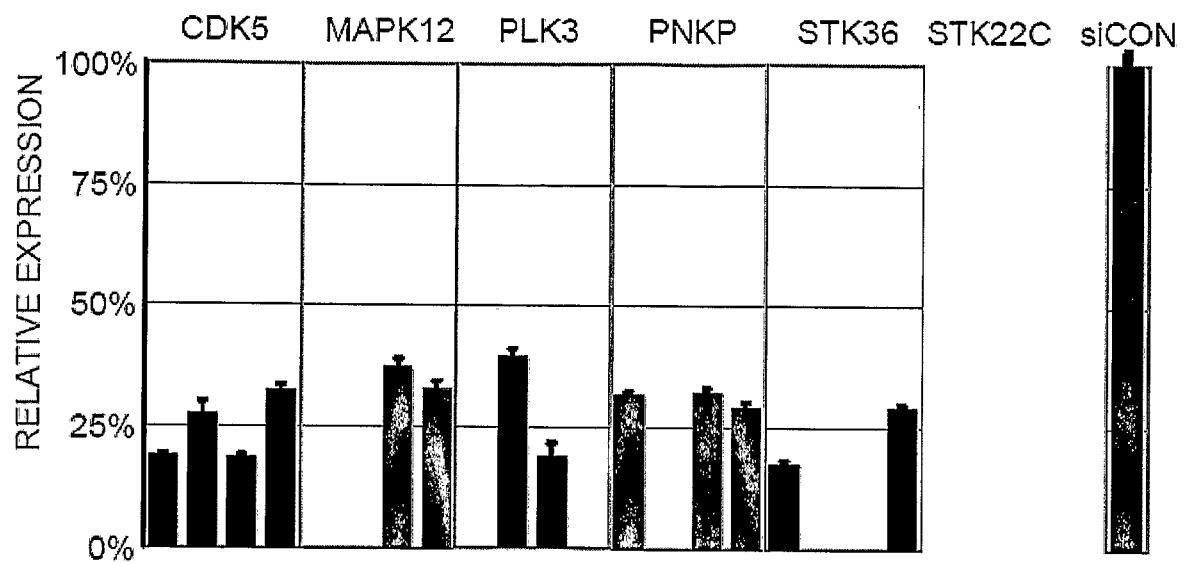


Figure 8