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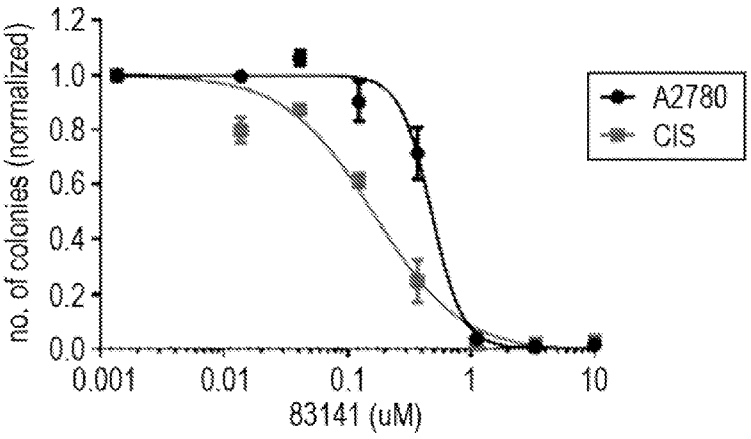
(54) Title: METHODS FOR EXPLOITING SYNTHETIC LETHALITY AND CHEMO-SENSITISATION IN DNA DAMAGE RESPONSE (DDR) PATHWAYS



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IC50	0.4781	0.1633

Fig. 17

(57) Abstract: The present invention relates to methods for exploiting synthetic lethality and chemosensitisation in tumours defective in DNA damage response pathways or that are resistant to platinum-based chemotherapies. In particular the invention relates to the ubiquitin hydrolase protein Ubiquitin Specific Protease 11 (USPll) and its association with DNA damage response pathways and / or use in the treatment of tumours that are drug-resistant to platinum-based chemotherapy. The invention further relates to methods of screening subjects for suitability of treatment with USPll inhibitors and the use of USPll inhibitors in the treatment of cancer. Further, the invention relates to the use of USPll inhibitors with anti-tumour therapeutic agents in treating cancer, as a sensitising agent.

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Methods for exploiting synthetic lethality and chemo-sensitisation in DNA Damage Response (DDR) Pathways

The present invention relates to materials and methods for exploiting synthetic lethality and chemo-sensitisation in DNA damage response (DDR) pathways. In particular the invention relates to the ubiquitin hydrolase protein Ubiquitin Specific Protease 11 (USP11) and its association with DDR pathways and / or use in the treatment of tumours that are drug-resistant to platinum-based chemotherapy. The invention further relates to use of USP11 inhibitors in the treatment of cancer alone or in combination with additional anti-tumour therapeutic agents and methods of screening patients for suitability for treatment with USP11 inhibitors. Further, biomarkers for the identification of suitable patients are disclosed.

Background to the Invention

DDR refers to a range of processes by which cells sense, signal and correct damage to genetic material. DNA damage has numerous sources such as chemotherapy, radiotherapy, UV light, replication errors and alkylating agents resulting in millions of lesions to the human genome every day. DNA repair processes continually keep the genome protected against the impact of propagating lesions that can have deleterious consequences. During tumourigenesis, genomic instability leads to mutations in key regulatory proteins including those involved in DDR/DNA repair, causing cells to become reliant on remaining DNA repair pathways. This reliance is known to vary between tumour types.

The concept of synthetic lethality was first derived from genetic studies in model organisms, where mutation of a particular gene results in lethality only through mutation or loss of another gene. The application of synthetic lethality to cancer biology has led to the discovery of novel therapeutic avenues to treat cancer. For instance, targeting DDR pathways that tumours have become reliant on has led to new approaches to selectively kill cancer cells with little toxicity to normal cells. In recent years, more rational approaches have been undertaken that are supported by the increased understanding of the type of pathways that are differentially defective in cancer versus normal cells (Nijman SMB *et al*, Science, 342, 809-811).

The synthetic lethality approach to the treatment of cancer offers the possibility of selectively killing cancer cells by targeting pathways that the cell exclusively relies on for survival. This approach offers a significant advantage over cytotoxic chemotherapy and radiotherapy, and potentially represents a

safer and more targeted treatment. However the characterisation of the specific dependencies based on this approach requires further understanding to enable the development of effective drugs useful in targeted cancer therapy.

Tumour cells often become resistant to existing chemotherapy treatment and re-sensitisation or selective killing of drug-resistant disease is also a valid strategy for treating cancer. Indeed, it is the resistant disease that accounts for cancer mortality and represents an unmet need across a broad range of tumour types. For instance, the majority of Type II ovarian tumours initially respond to platinum-based chemotherapies but ultimately become resistant, with palliative care then becoming the only option. Re-sensitising or selectively killing the resistant tumours would thus target the individuals with the highest unmet need. Such an approach may involve the development of anti-cancer agents which specifically target cancer cells which have become resistant to chemotherapy treatments such as platinum-based drugs, and therefore, provide an effective second-line treatment to patients with resistant or refractory cancer cells.

Platinum-based chemotherapies such as carboplatin, cisplatin and oxaliplatin are broadly-used anti-cancer agents applicable to a wide range of tumour types including lung, ovarian, bladder, colorectal, oesophageal, head and neck, testicular, breast, cervix and gastric cancers. Such agents work by causing DNA breaks and triggering cell death in tumour cells. Although the clinical utility of platinum therapies is established, treatment often results in resistance and ultimately therapeutic failure. This can be caused by a variety of mechanisms including restoration or up-regulation of cellular DNA repair processes that allow DNA damage to be efficiently removed before engagement of cytotoxic responses, thus enabling tumour cell survival. To date, most efforts at circumventing platinum resistance have focussed on identifying further platinum analogues that have distinct pharmacological properties to the earlier compounds such as cisplatin, an approach that led to the development of oxaliplatin. However, more recent platinum-based therapies such as picoplatin and satriplatin have yet to show any proven advantages over cisplatin, carboplatin and oxaliplatin. This highlights the need for the development of new strategies and drugs that overcome platinum-based chemotherapy resistance and provide longer term benefit to patients suffering from drug-resistant cancers.

The USP11 gene was initially identified as localised on the X chromosome, at a locus involved in retinal disorders (Swanson *et al*, Human Molecular genetics, 1996, Apr; 5(4), 533-8). The same locus is also linked to several types of cancer, including ovarian cancer. Several interaction partners have

been identified for USP11 that can link it to several pathways associated with cancer development such as RanBPM in microtubule nucleation (Ideguchi *et al*, Biochem J. 2002, Oct 1; 367(Pt1); 87-95) and BRCA2 in the double-strand break repair pathways (Schoenfeld *et al* AR, Mol Cell Biol. 2004, Sep; 24(17), 7444-55), however USP11 does not directly deubiquitylate BRCA2. Additional interaction partners include IKKalpha in the TNF-alpha pathway ((Yamaguchi *et al*, J Biol Chem. 2007, Nov 23; 282(47) 33943-8 and Sun W *et al*, Cell Signal. 2010 Mar; 22(3): 386-94) , the major transforming protein of human papillomaviruses HPV-16E7(Lin CH *et al*, J Biol Chem. 2008, Jin 6; 283(23): 15681-8), the polycomb complexes at the Ink4A/Arf locus (Maertens GN *et al*, EMBO J. 2010, Aug 4; 29(15): 2553-65), and finally the type I TGF-beta receptor (ALK5) (Al-Salihi MA *et al*, Open Biol. 2012 Jun; 2(6): 120063). A number of reports have described USP11 as a predictive and prognostic biomarker for various cancers such as breast cancer (Bayraktar S *et al*, Cancer J. 2013 Jan-Feb; 19(1): 10-17)

In addition, USP11 was identified as a component of the homologous recombination double strand-break repair pathway in an siRNA screen for PARP inhibitor sensitisers (Wiltshire TD *et al,* J Biol Chem. 2010 May 7; 285 (19): 14565-71). Given the encouraging efficacy of PARP inhibitors in treating pancreatic cancer efforts have been initiated to discover USP11 inhibitors as anti-cancer agents Burkhart RA et al (Mol Cancer Res. 2013 Aug; 11(8):901-11). However in view of the limited function of USP11 in DDR pathways there is a need in the art to further understand its mechanisms, synergy and interactions with other regulatory proteins and associated DDR pathways.

Summary of the Invention

The inventors have identified DDR pathways which have not previously been associated with USP11 function and have shown that inhibition of USP11 is an effective strategy for the treatment of tumours deficient in particular DDR pathways. Specifically they have found that USP11 compensates for deficiencies or defects in BRCA2, ATM and ATR proteins. USP11 is thus essential for cell survival in cancers having a deficiency in one or more of these proteins and associated DDR pathways. These cancers are thus hyper-reliant or hyper-dependent upon USP11. This discovery has significance in the development of USP11 inhibitors which have use in the treatment of tumours characterised by a deficiency in one or more of these pathways and provides for the pre-screening of patients to identify those who would benefit from USP11 inhibitor treatment. Thus, the use of a USP11 inhibitor can enable treatment of relevant cancers without the requirement for further or additional anti-tumour or anti-cancer agents. The USP11 inhibitor will selectively kill cancer cells, due to a synthetic lethal pairing with defective BRCA2, ATM or ATR and/or the resistance to one or more

platinum-based chemotherapies. In such a synthetic-lethal pairing, normal (non-cancerous) cells are spared from the effect of the USP11 inhibitor.

In addition, the inventors have also identified combination therapies for USP11 inhibitors with the following chemotherapeutic agents: platinums, PARP inhibitors, cross-linking agents, topoisomerase I poisons, topoisomerase II poisons, alkylating agents and radiomimetics. The use of USP11 inhibitors offer an advantage over conventional chemotherapy treatment allowing the selective targeting of cancer cells thereby reducing the side effects known to be associated with chemotherapeutic drugs. These combined therapies can be used for any cancer cell type, regardless of the status of the DDR pathways. Thus, the use of a USP11 inhibitor can sensitise a cancer cell to treatment with another anti-tumour or anti-cancer therapeutic agent. This may be sensitising for a chemical anti-tumour agent or for a radiation-based anti-tumour agent.

The inventors have also found that inhibition of USP11 is effective in selectively killing cancer cells that are resistant to platinum-based chemotherapy treatment. In particular the results demonstrate that USP11 inhibition preferentially kills cancer cells which are resistant when compared to cells from the same patient that are sensitive to platinum-based treatment. These findings have significance and potential in the development of new drugs and treatment regimens for patients that have developed resistance to platinum-based chemotherapy and/or have cancer cells deficient in DDR pathways.

According to a first aspect of the invention there is provided a USP11 inhibitor for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

In an embodiment, the cancer comprises cells deficient in one or more of DDR pathways selected from: base excision repair, single strand break repair, mismatch repair, nucleotide excision repair, transcription-coupled repair, non-homologous end joining repair, translesion synthesis and double strand break repair processes, including homologous recombination repair; double strand break (DSB) signalling and DNA inter-strand cross-link repair.

According to a further aspect of the invention, there is provided a USP11 inhibitor and an anti-tumour therapeutic agent for use in the treatment of cancer.

In an embodiment, the anti-tumour therapeutic agent and the USP11 inhibitor are used separately, sequentially, simultaneously or in combination.

Thus, the use of a USP11 inhibitor can sensitise a cancer cell to treatment with a further anti-tumour therapeutic agent, including chemical anti-tumour therapeutic agents and radiological anti-tumour therapeutic agents.

In one embodiment, the anti-tumour therapeutic agent may be an inhibitor of ATR, ATM or BRCA2.

According to a further aspect of the invention there is provided a method of screening for agents of USP11 suitable for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

According to a further aspect of the invention, there is provided a use of cancer cells deficient in one or more DNA damage response (DDR) pathways or cancer cells resistant to platinum-based chemotherapy for the screening of agents as inhibitors of USP11.

According to a further aspect of the invention there is provided a method of determining the responsiveness of a subject having a cancer to a USP11 inhibitor, the method comprising determining whether the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, wherein the presence of said deficiency or resistance indicates that the subject is responsive to a USP11 inhibitor.

Thus, a subject may be screened to determine whether therapy with a USP11 inhibitor will be effective and/or appropriate.

According to a further aspect of the invention, there is provided a method of selecting subjects having a cancer for treatment with a USP11 inhibitor, the method comprising determining whether the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, and selecting said subjects showing one or more defective DDR pathways or resistance to platinum-based chemotherapy for treatment.

According to a further aspect of the invention there is provided a method of treating a subject having a cancer comprising cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, the method comprising administering to the subject a therapeutically effective amount of a USP11 inhibitor.

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According to a further aspect, the present invention provides a method of treating a subject having cancer, said method comprising administering to the subject a therapeutically effective amount of a USP11 inhibitor in combination with one or more anti-tumour therapeutic agents.

10 According to a further aspect of the invention there is provided a pharmaceutical composition comprising an agent for use in the in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways and / or cells resistant to platinum-based chemotherapy, and wherein said agent is a USP11 agent.

15 According to a further aspect, there is provided the use of the presence of a mutated *BRCA2* gene, mutated BRCA2 protein, mutated *ATR* gene, mutated ATR protein, mutated *ATM* gene or mutated ATM protein in a sample of cancer cells as a biomarker for determining the sensitivity of said cancer with treatment with a USP11 inhibitor.

20 In an embodiment, the biomarker can determine the sensitivity of said cancer to treatment with a USP11 inhibitor alone or in combination with a further anti-tumour therapeutic agent.

Optional features are defined in the dependent claims. Further advantages are described below.

25 **Brief description of the Figures**

Figure 1 provides an image of FLAG-USP11 expressed and purified from mammalian cells. FLAG-purified protein or the indicated concentrations of Bovine Serum Albumin (BSA) were separated by SDS-PAGE and stained with Imperial Protein Stain (Pierce Biotechnology).

30 Figure 2 is a graph showing the result of a USP11 assay for high throughput screening of compounds using an isopeptidase linked substrate. The proteolytic activity of purified FLAG-USP11 is measured using a fluorescence polarisation assay. Various volumes of purified USP11 as indicated were incubated with a TAMRA labelled peptide linked to ubiquitin via an iso-peptide bond. The graph shows fluorescence polarisation (measured in mP) versus time in minutes.

Figure 3 shows that siRNA knockdown of USP11 sensitises tumour cells to the platinating agent carboplatin. Cal51 breast carcinoma, HeLa cervical carcinoma or HCT116 colorectal carcinoma cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (siUSP11_4 or siUSP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with carboplatin for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 3a is a graph showing normalised cell survival (%) for Cal51 cells transfected with siRNA versus does of carboplatin administered. Figure 3b is a graph showing normalised cell survival (%) for HeLa cells transfected with siRNA versus does of carboplatin administered. Figure 3c is a graph showing normalised cell survival (%) for HCT116 cells transfected with siRNA versus does of carboplatin administered.

Figure 4 shows siRNA knockdown of USP11 sensitises tumour cells to the topoisomerase I poison camptothecin. Cal51 breast carcinoma or HeLa cervical carcinoma cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (siUSP11_4 and siUSP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with camptothecin for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 4a is a graph showing normalised cell survival (%) for Cal51 cells transfected with siRNA versus does of Camptothecin administered. Figure 4b is a graph showing normalised cell survival (%) for HeLa cells transfected with siRNA versus does of Camptothecin administered.

Figure 5 shows siRNA knockdown of USP11 sensitises tumour cells to the alkylating agent temozolomide. HeLa cervical carcinoma cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (siUSP11_4 and siUSP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with temozolomide for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 5 is a graph showing normalised cell survival (%) for HeLa cells transfected with siRNA versus does of Temozolomide administered.

Figure 6 shows siRNA knockdown of USP11 sensitises tumour cells to the topoisomerase II poison etoposide. HeLa cervical carcinoma cells were transfected with control (siLuc) or USP11-specific

siRNAs targeting different USP11 regions (siUSP11_4, siUSP11_5 and siUSP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with etoposide for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 6 is a graph showing normalised cell survival (%) for HeLa cells transfected with siRNA versus does of Etoposide administered.

Figure 7 shows siRNA knockdown of USP11 sensitises tumour cells to the DNA cross-linking agent Mitomycin C (MMC). HCT116 colorectal carcinoma cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (siUSP11_4 and siUSP11_10) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with MMC for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 7 is a graph showing normalised cell survival (%) for HCT116 cells transfected with siRNA versus does of MMC administered

Figure 8 shows siRNA knockdown of USP11 sensitises tumour cells to the radiomimetic bleomycin. HCT116 colorectal carcinoma cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (siUSP11_4 and siUSP11_10) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before treatment with bleomycin for a further 72h. The cells were fixed and then stained with SRB. Cell number was estimated by measuring absorbance at 560nm using the Pherastar plate reader. Figure 8 is a graph showing normalised cell survival (%) for HCT116 cells transfected with siRNA versus does of Bleomycin administered

Figure 9 shows the results of a colony forming assay showing selective killing of DDR -deficient cells compared to isogenic DDR-wild-type cells. Control or DDR-deficient HeLa cervical carcinoma cells were transfected with control (siLuc) or USP11-specific siRNA (siUSP11_6 and siUSP11_10) for 16h before re-seeding into multi-well plates. The cells were incubated for 10 days before the colonies were fixed in Giemsa stain and counted. The results demonstrate the synthetic lethal interaction between knockdown of USP11 and deficiencies in homologous recombination repair. Figure 9 is a graph showing normalised cell survival (%) for various cells types – control (isogenic DDR wild-type), ATR-deficient, ATM-deficient or BRCA2 deficient cells.

Figure 10 shows the on-target activity of a USP11 small molecule inhibitor. U2OS osteosarcoma cells were treated for 1 hour with various concentrations of a USP11 inhibitor as indicated or with DMSO. Subsequently, cells were washed and lysed. Protein lysates were incubated in the absence or presence of an ubiquitin-VME probe. Proteins were separated by SDS-PAGE electrophoresis, and Western blotting on a nitrocellulose membrane and immune detection was performed using anti-USP11 antibodies. USP11 activity was determined via covalent binding to the ubiquitin-VME, giving rise to a shifted (active) form of USP11. USP11 activity was determined via measurement of the shifted (active, Ub-USP11) relative to the non-shifted (inactive, USP11) form of USP11 as indicated by Western blotting with an anti-USP11 antibody. The figure shows the results of the Western blot.

Figure 11 depicts the results of a growth assay to show selective killing of DDR -deficient cells compared to isogenic DDR-wild-type cells using a small molecule inhibitor of USP11. Control or DDR-deficient HeLa cervical carcinoma cells were seeded into multi-well plates and incubated overnight. The cells were treated with serial dilutions of a USP11 inhibitor then monitored using the IncuCyte FLR until the vehicle-treated cells were confluent. Confluence measurements were taken and expressed as percentage of vehicle-treated cells. This demonstrates that USP11 inhibitors are synthetic lethal with homologous recombination deficiency. Figure 11 is a graph showing normalised cell survival (%) for each of the cell types depicted versus does of USP11 inhibitor administered.

Figure 12 depicts the results of a proliferation assay showing that knockdown of USP11 with specific siRNAs in combination with carboplatin treatment sensitises cells derived from a relapsed ovarian tumour (PEO4) selectively compared to cells derived from a treatment-sensitive tumour (PEO1) from the same individual. Cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (Hs_USP11_3, Hs_USP11_5 and Hs_USP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated overnight before being treated with serial dilutions of carboplatin then incubated for a further 72h before staining with Cell Titer Glo (CTG). Cell number was estimated by measuring luminescence on the Clariostar plate reader. The results demonstrate that siRNA targeting USP11 preferentially sensitises platinum resistant cancer cells to carboplatin. Figure 12 is a graph of luminescence using CTG versus cells tested .

Figure 13 depicts the results of a Cell Titer Glo assay showing that knockdown of USP11 with specific siRNAs kills *in vitro*-derived platinum resistant cancer cells (CIS) preferentially as compared to platinum-sensitive cancer cells (A2780). Cells were transfected with control (siLuc) or USP11-specific

siRNAs targeting different USP11 regions (Hs_USP11_3, Hs_USP11_4, Hs_USP11_5 and Hs_USP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated for a 5 days before staining with Cell Titer Glo. Cell number was estimated by measuring luminescence on the Clariostar plate reader. The results demonstrate that siRNA targeting USP11 preferentially kills platinum resistant cancer cells. Figure 13 is a graph of luminescence relative to control using CTG versus cells tested.

Figure 14 depicts the results of a growth assay which shows selective killing of *in vitro*-derived platinum resistant cancer cells (CIS) as compared to platinum-sensitive cancer cells (A2780) using a small molecule inhibitor of USP11. A2780 or A2780cis cells were seeded into multi-well plates and incubated overnight. The cells were treated with serial dilutions of a USP11 inhibitor then proliferation measured by colony formation assay and by cell imaging on the IncuCyte FLR. Figure 14 is a graph which depicts the number of colonies versus dose of USP11 inhibitor.

Figure 15 depicts the results of a growth assay which shows selective killing of BRCA2 deficient cells as compared to a control using a small molecule USP11 inhibitor. Control (SiX control) or BRCA2 deficient cells (SiX BRCA2) were seeded into multi-well plates and incubated overnight. The cells were treated with serial dilutions of a USP11 inhibitor then proliferation measured by colony formation assay and by cell imaging on the IncuCyte FLR. Figure 15 is a graph which depicts the number of colonies versus dose of USP11 inhibitor.

Figure 16 depicts the results of a proliferation assay showing that knockdown of USP11 with specific siRNAs selectively kills cells derived from a relapsed ovarian tumour (PEO4) compared to cells derived from a treatment-sensitive tumour (PEO1) from the same individual. Cells were transfected with control (siLuc) or USP11-specific siRNAs targeting different USP11 regions (Hs_USP11_3, Hs_USP11_5 and Hs_USP11_6) for 16h before re-seeding into multi-well plates. The cells were incubated for 6 days before staining with Cell Titer Glo (CTG). Cell number was estimated by measuring luminescence on the Clariostar plate reader. The results demonstrate that siRNA targeting USP11 preferentially kills platinum resistant cancer cells. Figure 16 is a graph of luminescence relative to control using CTG versus cells tested.

Figure 17 depicts the results of a growth assay which shows selective killing of *in vitro*-derived platinum resistant cancer cells (CIS) as compared to platinum-sensitive cancer cells (A2780) using a small molecule inhibitor of USP11 (using a different inhibitor to Figure 14). A2780 or A2780cis cells

were seeded into multi-well plates and incubated overnight. The cells were treated with serial dilutions of a USP11 inhibitor then proliferation measured by colony formation assay and by cell imaging on the IncuCyte FLR. Figure 17 is a graph which depicts the number of colonies versus dose of USP11 inhibitor.

Figure 18 depicts the results of a growth assay which shows selective killing of BRCA2 deficient cells as compared to a control using a small molecule USP11 inhibitor (using a different inhibitor to Figure 15). Control (SiX control) or BRCA2 deficient cells (SiX BRCA2) were seeded into multi-well plates and incubated overnight. The cells were treated with serial dilutions of a USP11 inhibitor then proliferation measured by colony formation assay and by cell imaging on the IncuCyte FLR. Figure 18 is a graph which depicts the number of colonies versus dose of USP11 inhibitor.

Brief Description of the Sequences

SEQ ID NO: 1 is the nucleic acid sequence of human USP11.

SEQ ID NO: 2 is the sequence of USP11_3 siRNA

SEQ ID NO: 3 is the sequence of USP11_4 siRNA

SEQ ID NO: 4 is the sequence of USP11_5 siRNA

SEQ ID NO: 5 is the sequence of USP11_6 siRNA

SEQ ID NO: 6 is the sequence of USP11_10 siRNA

Detailed Description of certain embodiments

According to a first aspect of the invention there is provided a USP11 inhibitor for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

In another aspect there is provided use of a USP11 inhibitor in the manufacture of a medicament for the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

In the Examples, it is shown that use of siRNA to knockdown USP11 function in isogenic cell lines which were DDR wild-type or DDR deficient resulted in reduced cell viability in cells with mutations in DDR pathways. In particular, it has been shown that ATM, ATR or BRCA2 deficient cells have reduced viability when siRNA is used to knockdown UPS11 function. USP11 is thus essential for cell survival in cancers having a mutation in one or more DDR pathways.

References herein to "USP11"refer to ubiquitin-specific protease 11 polypeptide, variants, mutants and fragments thereof. USP11 is a member of the ubiquitin-specific processing protease superfamily and functions as a deubiquitylating enzyme by removal of the ubiquitin moiety from ubiquitin-fused precursors and ubiquitylated proteins.

The USP11 polypeptide may be in its native or wild type form which refers to a USP11 polypeptide having an amino acid sequence corresponding to that found in nature. Alternatively the USP11 polypeptide may be a variant having at least 80% sequence amino acid identity to the native amino acid sequence. The nucleic acid sequence of human USP11 is provided in SEQ ID NO 1 (table 2).

USP11 may be derived from any source, for example a eukaryotic source or a prokaryotic source. In one embodiment USP11 is mammalian and derived from rat, mouse, rabbit, dog, or non-human primate. In another embodiment USP11 is avian and derived from chicken or turkey. In another embodiment USP11 is human. In a further embodiment USP11 may be derived from human tissue types or from recombinant synthetic methods.

The USP11 polypeptide may be a mutant polypeptide with a different genotype to the wild type or native form of USP11. Such a mutant may also result in a different phenotypic difference. Mutants of USP11 may be produced by any suitable method where each amino acid of the native USP11 protein is independently changed to a different amino acid residue. Molecular biological techniques for cloning and engineering genes and cDNAs, for mutating DNA, and for expressing polypeptides from polynucleotides in host cells are well known in the art as exemplified "Molecular cloning, a laboratory manual", third edition, Sambrook, J. & Russell, D.W. (eds), Cold Spring Harbor Laboratory press, Cold Spring Harbor, NY, incorporated herein by reference.

For the avoidance of doubt, "derived from" means that the cDNA or gene was originally obtained from the defined source and the USP11 protein may be expressed in any suitable host cell. For example USP11 derived from a eukaryotic source may be expressed in a prokaryotic host cell such as *E. coli*.

References to "deubiquitylating activity" refer to the catalytic or enzymatic activity of USP11 and the removal of ubiquitin from target proteins.

For the avoidance of doubt, "deficient in one or more DNA damage response (DDR) pathways" means that the DDR pathway is dysfunctional or defective such that normal function is impaired either partially or completely. One or more DDR pathway can be made dysfunctional or defective by a single deficient or defective pathway component / protein.

References to a "USP11 inhibitor" refer to an agent capable of binding to USP11 and causing a reduction in functional activity of USP11, for example a decrease in catalytic or enzymatic activity which may be partial or complete. "USP11 inhibitor" also refers to agents that do not affect the intrinsic activity of USP11, but impair the ability of USP11 to bind to its substrate or co-factor. Partial or complete reduction in the functional activity of USP11 may cause cell death or apoptosis of cancer cells which are defective in one or more DDR pathways and are reliant or hyper-dependent on USP11 mediated repair, thus allowing selective targeting and killing of cancer cells whilst normal replicating cells survive.

A USP11 inhibitor useful in the present invention may be a known USP11 inhibitor, a polypeptide, polynucleotide, antibody, peptide, small molecule compound, an inhibitory (RNAi) molecule or any other suitable chemical. In one embodiment a USP11 inhibitor is an inhibitory (RNAi) molecule or a small molecule compound.

The USP11 inhibitor or inhibitors may be specific or selective for USP11.

References to "antibody" include but are not limited to monoclonal, human, humanized or chimaeric antibodies. The antibody may be an antibody fragment including a VH or VL domain or a single chain antibody molecule (scFv). In one embodiment the antibody is human.

References to "RNA interference (RNAi)" refer to a technique used to "knock down" gene function through the introduction of double stranded RNA (RNAi) into the cell resulting in the inhibition of mRNA complementary to one of the RNAi sequences.

Known USP11 inhibitors such as the one described in Burkhart RA *et al* are of use according to the present invention and are incorporated herein. In this paper, the inhibitors described include Mitoxantrone, Sennoside A, Sennoside B, tetracholoroisophtalonitrile, Epirubicin and Rutoside.

Reference to "DNA damage response (DDR) pathway" refers to a range of processes by which cells sense, signal and correct damage to genetic material. DNA damage can be caused by chemotherapy, radiotherapy, UV light, replication errors and alkylating agents, for example. Any DDR pathway or pathways are contemplated in the uses, methods and biomarkers of the present invention.

DDR pathways according to the invention may be selected from one or more of, base excision repair, single strand break repair, mismatch repair, nucleotide excision repair, transcription-coupled repair, non-homologous end joining repair, translesion synthesis and double strand break repair processes, including homologous recombination repair; double strand break (DSB) signalling and DNA inter-strand cross-link repair.

In one embodiment DDR pathways are selected from one or more of single strand break repair, base excision repair or double strand break repair processes, including homologous recombination repair; double strand break (DSB) signalling and DNA inter-strand cross-link repair.

In a particular embodiment, the DDR pathways is a double strand break repair process, including homologous recombination repair; double strand break (DSB) signalling and DNA inter-strand cross-link repair.

Deficiencies in DDR pathways may be due to mutations in, the absence of or defective expression of a gene encoding a protein involved in one or more of homologous recombinational repair, base excision repair, single strand break repair, mismatch repair, nucleotide excision repair, transcription-coupled repair, non-homologous end joining repair, translesion synthesis or DNA inter-strand cross-link repair.

Deficiencies in DDR pathways may be due to mutations in, the absence of or defective expression of a gene encoding proteins selected from one or more of BRCA2, ATM and ATR.

For the avoidance of doubt reference to cancer or tumour cells deficient in one or more proteins involved in DDR pathways, for example BRCA2, ATM or ATR, refers to mutations in, defective / reduced expression or the absence of such proteins.

In one embodiment an increase in DNA damage burden caused by DDR deficiency are due to mutations in, the absence of, or defective expression of a gene encoding proteins selected from one or more of BRCA2, ATM and ATR. Thus, the *BRCA2*, *ATM* or *ATR* genes may be mutated or absent.

In a further embodiment cells deficient in one or more DDR pathways have increased sensitivity to DNA damaging agents such as chemotherapeutic agents.

Reference to "ATM" refers to Ataxia telangiectasia mutated which is a serine/threonine protein kinase belonging to the PI3KK subfamily of kinases (PI3K-related kinases) that is recruited and activated by DNA double-strand breaks. It phosphorylates several key proteins that initiate activation of the DNA damage checkpoint, leading to cell cycle arrest, DNA repair or apoptosis. Several of these targets, including p53, CHK2 and H2AX are tumour suppressors. The protein gets its name from the disorder Ataxia telangiectasia caused by mutations of ATM. ATM is frequently mutated or down-regulated in tumours. Pathways that rely upon ATM signalling are thus particularly important in the repair of double-stranded DNA breaks.

Reference to "BRCA2" refers to breast cancer type 2 susceptibility protein. Carriers of heterozygous germ-line mutations in the *BRCA1* or *BRCA2* genes are strongly predisposed to cancer of the breast, ovary, and other organs. BRCA1 and BRCA2 proteins are critically important for the repair of double strand breaks by homologous recombination and loss of the wild-type *BRCA1* or *BRCA2* allele in tumours likely fosters cancer progression by promoting genome instability and mutation.

Reference to "ATR" refers to the Ataxia-Telangiectasia and Rad3-related which is a serine/threonine protein kinase belonging to the PI3KK subfamily of kinases (PI3K-related kinases) that is recruited and activated by the persistent presence of single stranded DNA indicating DNA damage, such as single-strand DNA damage, replication fork defects and maintenance of fragile site in the genome. Single stranded DNA is an intermediate in several DDR pathways, including but not limited to nucleotide excision repair, mismatch repair, base excision repair and homologous recombination. This kinase has been shown to phosphorylate checkpoint kinase CHK1, checkpoint proteins RAD17, and RAD9, as well as tumour suppressor protein BRCA1. Mutations of this gene are associated with Seckel syndrome.

Reference to "PARP" refers to Poly-(ADP-ribose) polymerase enzymes that play a role in the repair of single stranded DNA (ssDNA) breaks. The PARP family comprises 17 members, all having a role in DNA repair and cell death. PARP-1 and PARP-2 are both activated by DNA single stranded breaks.

Reference to "resistant to platinum-based chemotherapies" refers to tumour cells that do not effectively respond to treatment with platinum-based chemotherapy. Such cells have either intrinsic or acquired cellular mechanisms that allow continued survival in the presence of platinum chemotherapeutic agents compared to tumour cells sensitive to platinum chemotherapeutic agents and as such a higher dose is required to effect cell killing. Tumours that are refractory to platinum-based treatment i.e. do not show even a partial response to platinum-based chemotherapy or that initially respond to platinum-based chemotherapy but then recur within a short time following completion of the treatment can be defined as resistant. This time may be defined differently for various tumour types but for ovarian cancer this time has been determined by the Gynaecologic Oncology Group as a period of 6 months. Thus, a cancer that recurs within 6 months is likely to be resistant to platinum-based drugs.

Resistance to platinum-based chemotherapy occurs in a wide range of cancers, including but not limited to lung, ovarian, bladder, colorectal, oesophageal, head and neck, testicular, breast, cervix and gastric cancers.

Platinum-based chemotherapy agents /treatments are known to those skilled in the art and include, for example carboplatin, cisplatin, oxaliplatin, picoplatin and satriplatin.

The inventors have found that tumour cells deficient in BRCA2, ATR, and/or ATM or cells resistant to platinum-based treatment are selectively killed in the presence of USP11 inhibition. Thus, tumours with reduced levels of BRCA2, ATR and /or ATM or cells resistant to platinum-based treatment or PARP inhibitors are hyper-dependent on USP11. Tumour cells that have a deficiency in one or more of BRCA2, ATR and ATM and demonstrate resistance to platinum-based treatment are selectively killed in the presence of USP11 inhibition and are hyper-dependent on USP11.

Furthermore it is recognised that inhibition of USP11 may selectively kill tumour cells with reduced activity of double-strand break repair proteins by further reducing the levels of these activities.

Cancer cells having mutations in, the absence of or defective expression of a gene encoding a protein involved in double-strand break repair processes are selectively killed in the presence of USP11 inhibition.

Particularly, it is recognised that inhibition of USP11 may selectively kill tumour cells with reduced homologous recombination repair by further reducing the levels of these activities. Cancer cells having mutations in, the absence of or defective expression of a gene encoding a protein involved in homologous recombination repair processes are selectively killed in the presence of USP11 inhibition.

In a further embodiment there is provided a USP11 inhibitor for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, wherein such DDR deficiencies are due to mutations in, the absence of or defective expression of a gene encoding proteins selected from one or more of BRCA2, ATR and ATM.

In one embodiment there is provided a USP11 inhibitor for use in the treatment of cancer, the cancer comprising cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapies wherein cells deficient in one or more DNA damage response (DDR) pathways are hyper-dependent on USP11 associated pathways for survival.

In another aspect, there is provided a USP11 inhibitor and an anti-tumour therapeutic agent for use in the treatment of cancer.

The USP11 inhibitor according to any aspect of the invention may be combined with an additional anti-tumour therapeutic agent, for example chemotherapeutic drugs or inhibitors of other regulatory proteins. In one embodiment the additional anti-tumour therapeutic agent is selected from a PARP (poly ADP ribose polymerase) inhibitor, a BRCA2 inhibitor, an ATR inhibitor and an ATM inhibitor. In another embodiment the PARP (poly ADP ribose polymerase) inhibitor is an inhibitory RNA (RNAi) molecule (PARPi). In a further embodiment PARP inhibitors may be selected from one or more of iniparib (BSI 201), olaparib (AZD-2281), rucaparib (AG014699, PF-01367338) and veliparib (ABT-888), MK-4827, CEP-9722, E7016(GPI-21016), LT-673, MP-124, NMS-P118. In another embodiment the ATR inhibitor may be selected from one or more of AZD6738 and VE-822. In a further embodiment, the additional anti-tumour agent may be an ATM inhibitor, such as 2-

Morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU 55933), (2R,6S-rel)-2,6-Dimethyl-N-[5-[6-(4--morpholinyl)-4-oxo-4H-pyran-2-yl]-9H-thioxanthen--2-yl-4-morpholineacetamide (KU 60019) or KU59403. Furthermore, the additional anti-tumour agent may be a BRCA2 inhibitor, such as siRNA targeting BRCA2. In one embodiment, the anti-tumour therapeutic agent may be an inhibitor of ATR, ATM or BRCA2.

In a further embodiment the additional anti-tumour agent is a chemotherapeutic agent. Chemotherapeutic agents may be selected from olaparib, mitomycin C, cisplatin, carboplatin, oxaliplatin, picoplatin, satriplatin, ionizing radiation (IR), bleomycin, camptothecin, irinotecan, topotecan, temozolomide, taxanes, 5-fluoropyrimidines, gemcitabine, etoposide and doxorubicin.

The additional anti-tumour therapeutic agent may be a platinum-based chemotherapy. Suitable platinum based chemotherapies include: carboplatin, cisplatin, oxaliplatin, picoplatin, nedaplatin, triplatin and satriplatin.

The additional anti-tumour therapeutic agent may be a chemotherapeutic agent that acts as a cross-linking agent. Suitable cross-linking reagents include cisplatin, Mitomyocin C, melphalan and cyclophosphamide.

The USP11 inhibitor may be combined with an additional anti-tumour therapeutic agent that is a topoisomerase I chemotherapeutic agent or a topoisomerase II chemotherapeutic agent. Chemotherapeutic agents may include poisons and inhibitors of toposimerase I and/or II. Poisons may be a compound that targets the topoisomerase-DNA complex, rather than inhibits the topoisomerase. Suitable topoisomerase I chemotherapeutic agents include camptothecin, irinotecan, topotecan and lamellarin D. Suitable topoisomerase II chemotherapeutic agents include etoposide (VP-16), teniposide, doxorubicin, daunorubicin, mitoxanthine, amsacrine, ellipticines, aurintricarboxylic acid and HV-331.

The additional anti-tumour therapeutic agent may be a chemotherapeutic alkylating agent. Suitable alkylating agents include bendamorphine, busulfan, chlorambucil, carboplatin, cisplatin, oxaliplatin, isofamide, temozolomide and cyclophosphamide.

The additional anti-tumour therapeutic agent may be a chemotherapy agent that acts as a radiomimetic. Suitable radiomimetics include bleomycin, dactinomycin and streptonigrin.

The additional anti-tumour therapeutic agent may be a chemotherapy agent that acts as a spindle poison or anti-mitotic. Suitable spindle poisons include taxanes (including paclitaxel) mebendazole, colchicine, griseofulvin, and vinca alkaloids.

The additional anti-tumour therapeutic agent may be a chemotherapeutic agent that acts as an anti-metabolic agent. Suitable anti-metabolic agents include fluropyrimidines, azathioprine and mercaptopurine.

The additional anti-tumour therapeutic agent may be a chemotherapeutic agent that acts as a nucleoside analogue or nucleobase analogue. Suitable analogues include vidarabine, cytarabine and gemcitabine.

In certain embodiments, the additional anti-tumour therapeutic agent is selected from the list comprising ionizing radiation (IR) or a chemotherapeutic agent selected from bleomycin, etoposide, carboplatin, camptothecin, or olaparib, or a combination thereof.

The USP11 inhibitor can act to sensitise a tumour or cancer to one or more further anti-tumour therapeutic agents.

References to “cancer” or “tumour” include but are not limited to breast, ovarian, prostate, lung, kidney, gastric, colorectal, testicular, head and neck, pancreatic, brain, melanoma, bone, oesophageal, bladder, cervical, endometrial or other cancers of tissue organs and cancers of the blood cells such as lymphomas and leukaemias. Cancer or tumour and the cells therefrom also can relate to secondary cancers or tumours due to metastasis.

In one embodiment USP11 inhibitors are useful in the treatment of cancer, wherein the cancer is at least partially responsive to treatment with an anti-tumour therapeutic agent or anti-cancer agent. For example the anti-tumour therapeutic agent or anti-cancer agent may be a chemotherapeutic agent selected from olaparib, cisplatin, carboplatin, picoplatin, satriplatin and ionizing radiation. In a further embodiment the anti-tumour therapeutic agent or anti-cancer agent is a PARP inhibitor, for example olaparib, or an inhibitory RNA (RNAi) molecule against PARP1 or PARP2.

According to a further aspect of the invention there is provided a method of screening for agents of USP11 suitable for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapies.

Agents of USP11 may be inhibitors, antagonists or inverse agonists of USP11 such that the activity of USP11 is reduced, for example a polypeptide, polynucleotide, antibody, peptide, small molecule compound, an inhibitory (RNAi) molecule or any other suitable chemical. The reduction may be partial or complete. Agents may function such that the intrinsic activity of USP11 is not affected but the ability of USP11 to bind to its substrate or co-factor is impaired.

In one embodiment the USP11 agent is an inhibitor or antagonist.

The method of screening for agents of USP11 may comprise the steps of:

a) Obtaining purified or recombinant USP11, preferably from mammalian cells.

b) Contacting the isolated USP11 complex with one or more test agents and the appropriate substrate for USP11.

c) Selecting those agents that demonstrate a reduction in USP11 activity.

d) Using cellular activity probe assays to select compounds that demonstrate on-target activity.

e) Testing USP11 inhibitors in cellular models for synthetic lethality.

In one embodiment the agent identified in step c) has a binding affinity in the nanomolar (nM) range. In another embodiment the agent demonstrates specific binding to USP11 such that binding to other proteins is significantly reduced or absent.

Reference is made to Examples 2 and 10, which are incorporated by reference, which relate to methods of screening according to this aspect of the invention.

Methods used to identify agents to purified or recombinant USP11 will be appreciated by those skilled in the art and are incorporated herein by reference (Burkhart RA et al)

In one embodiment the reduction in USP11 activity is a reduction in catalytic activity.

In one embodiment a reduction in activity of USP11 is measured *in vitro* by a reduction in cell proliferation of cells deficient in at least one DDR pathway. In another embodiment the reduction in cell proliferation is due to cell cycle arrest or apoptosis. In a further embodiment a reduction in catalytic activity of USP11 is measured *in vitro* by a reduction in the deubiquitylating activity of USP11.

Inhibition of catalytic activity of USP11 may be measured using *in vitro* techniques as described in Burkhart RA. *et al.*, Mol Cancer Res. 2013 Aug;11(8):901-11, such methods are incorporated herein by reference.

Test agents may be small molecules, peptides, polypeptides, polynucleotides, oligonucleotides, inhibitory RNA (RNAi) molecules, antibodies or libraries thereof.

According to a further aspect of the invention there is provided a method of determining the responsiveness of a subject having a cancer to a USP11 inhibitor, the method comprising determining whether the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, wherein the presence of said DDR deficiency or resistance to platinum-based chemotherapy indicates that the subject is responsive to a USP11 inhibitor.

The inventors have demonstrated that deficiencies in one or more of DNA damage response proteins BRCA2, ATR and ATM in selective killing of cells where USP11 is silenced either alone or in combination with chemotherapeutic agents. Therefore the absence of functional BRCA2, ATR and ATM proteins represent useful biomarkers that can be used to select or screen for patients that would benefit from USP11 inhibitor treatment. Screening of cancer patients suitable for USP11 inhibitor treatment has the advantage that treatment in patients who have an absence of deficiencies in the DNA damage response proteins described above is avoided, thus ensuring relevant patient populations are targeted.

In one embodiment the responsiveness of a subject to a USP11 inhibitor is determined by the absence of one or more biomarkers selected from BRCA2, ATR and ATM functional proteins. In another embodiment the responsiveness of a subject to a USP11 inhibitor is determined by the presence of one or more amino acid or nucleic acid mutations in the sequence of BRCA2, ATR or ATM. The responsiveness of a subject to a USP11 inhibitor may be determined by measuring the

levels of BRCA2, ATR or ATM mRNA, wherein abnormally low levels indicate that the subject is responsive to a USP11 inhibitor.

According to a further aspect, there is provided the use of the presence of a mutated *BRCA2* gene, mutated BRCA2 protein, mutated *ATR* gene, mutated ATR protein, mutated *ATM* gene or mutated ATM protein in a sample of cancer cells as a biomarker for determining the sensitivity of said cancer with treatment with a USP11 inhibitor.

The sample of cancer cells may be taken according to any standard practice, including biopsy.

In an embodiment, the biomarker can determine the sensitivity of said cancer to treatment with a USP11 inhibitor alone or in combination with a further anti-tumour therapeutic agent. In one embodiment the method further comprises identifying the responsiveness of a cancer cell to a USP11 inhibitor, wherein the presence of said deficiency indicates that the cancer cell is responsive to a USP11 inhibitor.

In certain embodiments the cancer cell has defective ATM, ATR, BRCA2 or a combination thereof. The defect may be in the *ATM, ATR or BRCA2* gene, expression of the *ATM, ATR or BRCA2* gene or in the ATM, ATR or BRCA2 protein, such as post-translational modifications. Thus, the defective ATM, ATR or BRCA2 may be due to mutations in, the absence of, or defective expression of the gene encoding ATM, ATR or BRCA2. Defective ATM, ATR or BRCA2 may be mutated, absent or deficient. It has been found that defective ATM, ATR or BRCA2 is a useful biomarker which is predictive of the sensitivity of cancer cells to treatment with a USP11 inhibitor. In particular, it has been found that the presence of a mutated *ATM*, *ATR* or *BRCA2* gene, deficient or absent ATM, ATR or BRCA2 protein correlates well with increased cell killing resulting from treatment with a USP11 inhibitor.

According to an aspect of the invention there is provided a method of determining the responsiveness of a subject having a cancer to a USP11 inhibitor, the method comprising determining whether the cancer comprises cells with defective ATM, ATR or BRCA2, wherein the presence of said defect indicates that the subject is responsive to a USP11 inhibitor. If the defect is present, the subject may be treated with a USP11 inhibitor.

The defect can be any defect in the *ATM*, *ATR* or *BRCA2* gene, the ATM, ATR or BRCA2 protein or the expression of the latter, such as those discussed above. Determining whether a cancer cell has a mutation in the nucleotides encoding ATM, ATR or BRCA2 can be performed in any suitable way.

The BRCA2 gene is approximately 10,200 base pairs in length comprising 26 coding exons. The wild-type gene encodes a protein comprised of 3418 amino acids. Various mutations in BRCA2 have been associated with cancer. These include copy number abnormalities, deletion or duplication mutants, nonsense and frameshift mutations that prematurely truncate the protein, missense mutations and non-coding intervening sequence (IVS) mutations, deletions and duplications of entire or multiple exons, large genomic rearrangements, in frame deletions/duplications and sequence mutations, such as insertions, deletions or substitutions of nucleotides. Examples of polymorphisms, deletions and insertions known to be associated with an increased risk of cancer are c.5946delT, c.8363G>A, c.8754+1G>A, c.3860delA, c.7806-2A>G, c.8357_8538delAG, c.3170_3174del5, c.5857G>T, c2808_2811del4, c.6629_6630delAA, c.9026_9030del5, c.9310_9311delAA, c.5146_5149del4, c.156_157insAlu, c.516+1G>A, c.6275_6276delTT, c8904delC, c.5351dupA, c.6275_6276delTT, c.1813dupA, c.4478del4, c.9098dupA, c.7913_7917del5, c.8357_8538del2, c.9098dupA, c.5946delT, c.8755delG, c.6373delA, c.1310_1013del4, c.6486_6489del4, c.3847_3848delGT, c.4528delG, c.2808del4, c.3847delGT, c.7480C>T, c.8327T>G, c.9118-2A>G, c.9117+1G>A, c771_775del5, c.6275_6726delTT and c.1929delG (BRCA2 is numbered according to Genbank U43746 reference sequence). Any of these variations can be detected.

All mutations are named according to Human Genome Variation Society recommendations – http://www.hgvs.org/rec.html

The mutation in ATR may be in any part of the nucleotide sequence for ATR, including non-coding or coding parts of the gene. The mutation may cause no ATR to be translated or allow for translation of a truncated or shortened version of ATR. Alternatively, the mutation in ATR may be a mutation of one or more nucleotides, insertions, deletions or replacements. A missense mutation resulting in a substituted amino acid residue c.6431A>G (p.Gln2144Arg) may be associated with oropharyngeal-cancer lesion (NCBI RefSeq accession number NM_001184.3). Other substituted amino acid residues associated with cancer are p.Glu2537Gln, p.Glu2438Lys, p.Ala1488Pro and p.Ala2002Gly. (Numbered according to GenBank accession number: U76308.1)

The mutation in ATM may be in any part of the nucleotide sequence for ATM, including non-coding or coding parts of the gene. The mutation may cause no ATM to be translated or allow for translation of a truncated or shortened version of ATM. Alternatively, the mutation in ATM may be a mutation of one or more nucleotides, insertions, deletions or replacements. Human ATM is published as Genbank Accession Number: U82828.1.

Any of the mutations discussed previously or known in the art in ATM, ATR or BRCA2 can be a biomarker according to the present invention.

Determining whether a cancer cell has defective ATM, ATR or BRCA2 can be performed by any technical means. Said means may be on the basis of analysis for genetic changes, for example by examining DNA for nucleotide mutations, nucleotide additions or nucleotide deletions in the ATM, ATR or BRCA2 gene. Additionally, DNA can be examined for larger-scale changes affecting the ATM, ATR or BRCA2 gene, including inversions, translations, translocations, loss of heterozygosity, deletions, and/or amplification. Alternatively, the cells can be examined for epigenetic changes, such as DNA methylation or chromatin restructuring, which may alter the expression of the ATM, ATR or BRCA2 gene. Further means to determine whether a cancer cell has defective ATM, ATR or BRCA2 on the basis of RNA or mRNA expression from the ATM, ATR or BRCA2 gene. A further alternative means is on the basis of mutations in the polypeptide sequence compared to the wild-type sequence or modification of the protein, such as the presence or absence of post-translational modifications, i.e. glycosylation, phosphorylation or methylation. Such changes may be detected by standard techniques, including the use of antibodies specific for the mutation or post-translational modification. Alternatively, defective ATM, ATR or BRCA2 can be detected on the basis of ATM, ATR or BRCA2 protein expression, which can be established by standard techniques, such as Western blotting. It is possible to monitor blood samples from a subject for circulating DNA, mRNA or cells containing defective ATM, ATR or BRCA2 using any of the techniques outlined herein. Immunohistochemistry may be used to visualise aberrant gene products.

For example, nucleotide mutations may be detected by any DNA sequencing, i.e. using Real-Time PCR based assays, primer-extension assays, Sanger sequencing assays, or quantitative parallel pyrosequencing methods.

According to an aspect of the invention, there is provided a method of selecting subjects having a cancer for treatment with a USP11 inhibitor, the method comprising determining whether the

cancer comprises cells with defective ATM, ATR or BRCA2 and selecting said subjects showing defective ATM, ATR or BRCA2 for treatment. Said method may involve determination of whether the cancer cell has defective ATM, ATR or BRCA2 as defined above. Following selection, the patient may be treated with a USP11 inhibitor.

The method may be used to aid selection of an appropriate dose of USP11 inhibitor, in order to optimise treatment for each subject. The method may be used to determine the effectiveness of treatment with a USP11 inhibitor. For example, the method may be used to quantify the amount or proportion of cells in a sample of the cancer cells that include a defective ATM, ATR or BRCA2. A decrease or increase in this amount/proportion may indicate that the treatment with USP11 is effective.

In another embodiment the responsiveness of a subject to a USP11 inhibitor is determined by the presence of cancer cells resistant to platinum-based chemotherapy. Resistance is detected by the persistence or recurrence of tumour growth and /or the presence of metastasis following previous treatment with platinum-based chemotherapy. Alternatively the responsiveness of a subject is determined by an *in vitro* assay measuring survival of the tumour cells when exposed to platinum-based chemotherapy agents. Such methods are known to the skilled person in the art and the determination of whether a patient has a cancer resistant to platinum-based treatment is a routine assessment carried out by clinicians. Patients having cancer cells that respond to platinum-based chemotherapy for less than six months may be defined as having a cancer resistant to platinum-based treatment.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising an agent for use in the in the treatment of cancer combined with any pharmaceutically acceptable carrier, adjuvant or vehicle wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways. The agent is a USP11 agent as hereinbefore defined.

The term “pharmaceutical composition” in the context of this invention means a composition comprising an active agent and additionally one or more pharmaceutically acceptable excipients.

Suitable pharmaceutically acceptable excipients are known to those skilled in the art and generally include an acceptable composition, material, carrier, diluent or vehicle suitable for administering a USP11 inhibitor of the invention to an animal.

The pharmaceutical composition may further comprise one or more anti-tumour therapeutic agent. Such agents are as previously defined.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising an agent of USP11 and an anti-tumour therapeutic agent combined with any pharmaceutically acceptable carrier, adjuvant or vehicle for use in treating cancer.

In one embodiment the animal is a mammal. In another embodiment the mammal is human.

The pharmaceutical compositions of the invention may be administered in any effective manner suitable effective for targeting cancer cells, for example by oral, intravenous, intramuscular, intranasal or topical methods. The pharmaceutical composition may be administered directly at the site of the tumour, for example during a surgical procedure or by intravenous methods.

Dosages may be varied depending on the requirements of the patient, the severity of the condition being treated and the characteristics of the active ingredient being employed. Determination of the effective dose is within the remit of the skilled person, without undue burden. Suitable dosage forms for administration to mammals, including humans are typically in the range of up to 100mg/kg body weight, or may be 0.1mg/kg, 10mg/kg, 20mg/kg, 30mg/kg for example.

According to a further aspect of the invention there is provided a method of treating a subject having a cancer comprising cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, the method comprising administering to the subject a therapeutically effective amount of a USP11 inhibitor.

According to a further aspect of the invention there is provided a method of treating a subject having a cancer, the method comprising administering to the subject a therapeutically effective amount of a USP11 inhibitor and an anti-tumour therapeutic agent.

USP11 inhibitors for any aspect of the invention may be selected from small molecules, peptides, oligonucleotides, inhibitory RNA (RNAi) molecules and antibodies. In one embodiment a USP11 inhibitor is a small molecule compound.

The USP11 inhibitor may be administered prior to, in combination with or subsequent to the administration of an additional anti-tumour therapeutic agent, for example chemotherapeutic drugs or inhibitors of other DNA damage response proteins. In one embodiment the additional anti-tumour therapeutic agent is a PARP (poly ADP ribose polymerase) inhibitor. In another embodiment PARP inhibitors may be selected from one or more of iniparib (BSI 201), olaparib (AZD-2281), rucaparib (AG014699, PF-01367338) and veliparib (ABT-888), MK-4827, CEP-9722, E7016(GPI-21016), LT-673, MP-124, NMS-P118. The additional anti-tumour agent may be an ATR inhibitor, for example ADZ6738 or VE-822. The additional anti-tumour therapeutic agent may be an ATM inhibitor, such as 2-Morpholin-4-yl-6-thianthren-1-yl-pyran-4-one (KU 55933), (2R,6S-rel)-2,6-Dimethyl-N-[5-[6-(4--morpholinyl)-4-oxo-4H-pyran-2-yl]-9H-thioxanthen--2-yl-4-morpholineacetamide (KU 60019) or KU59403. The additional anti-tumour therapeutic agent may be a BRCA2 inhibitor, such as siRNA targeting BRCA2. In one embodiment, the anti-tumour therapeutic agent may be an inhibitor of ATR, ATM or BRCA2.

In a further embodiment the additional anti-tumour agent is a chemotherapeutic agent. Chemotherapeutic agents may be selected from olaparib, mitomycin C, cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin, triplatin, ionizing radiation (IR), bleomycin, camptothecin, irinotecan, topotecan, temozolomide, taxanes, 5-fluoropyrimidines, gemcitabine, etoposide and doxorubicin.

Embodiments described in one aspect of the invention may also be combined in other aspects of the invention.

The invention is described further in the following non-limiting examples.

Materials and Methods

Measuring selective killing of platinum-resistant tumour cells using CellTiter Glo

6µl of 1µM control or USP11-specific siRNAs were diluted in 80µl OptiMEM serum-free medium (Invitrogen) containing 0.932µl Lipofectamine RNAiMAX (Invitrogen) (for A2780 and A2780CIS cells) or 1.4µl Lipofectamine RNAiMAX (for PEO1 and PEO4 cells). Transfection mixtures were then incubated at room temperature for 90min. 20µl of each transfection complex was spotted into 4 wells of a 96 well plate. Cells were then trypsinised, counted and diluted to 17400 cells/ml (A2780), 34800 cells/ml (A2780CIS), or 78300 cells/ml (PEO1 and PEO4) in RPMI + 10% FBS + 2mM Sodium Pyruvate. 120µl of each cell suspension was added to 2 wells of each transfection complex. Cells

were incubated under normal growth conditions (37°C and 5% CO_2) for a further 5 days (A2780 and A2780CIS) or 6 days (PEO1 and PEO4). 50µl of reconstituted Cell Titer Glo reagent (Promega) was added to the wells. The plates were placed on a plate shaker for 4 minutes and luminescence was read after 10 more minutes using the Clariostar plate reader.

Measuring selective sensitisation of platinum-resistant tumour cells using CellTiter Glo

6µl of 1µM control or USP11-specific siRNAs were diluted in 80µl OptiMEM serum-free medium (Invitrogen) containing 1.4µl Lipofectamine RNAiMAX (Invitrogen). Transfection mixtures were then incubated at room temperature for 90min. 20µl of each transfection complex was spotted into 4 wells of a 96 well plate. PEO1 and PEO4 cells were then trypsinised, counted and diluted to 78300 cells/ml in RPMI + 10% FBS + 2mM Sodium Pyruvate. 115µl of each cell suspension was added to 2 wells of each transfection complex. Cells were incubated under normal growth conditions (37°C and 5% CO_2) overnight. The cells were then treated with a final concentration of 5µM carboplatin or vehicle only. The plates were placed back in the incubator and left for a further 5 days. 50µl of reconstituted Cell Titer Glo reagent (Promega) was added to the wells. The plates were placed on a plate shaker for 4 minutes and luminescence was read after 10 more minutes using the Clariostar plate reader.

Measuring synthetic lethality by colony forming assay (CFA)

15.0µl of 1µM control or USP11-specific siRNAs were diluted in 0.5ml OptiMEM serum-free medium (Invitrogen) containing 3.5µM Lipofectamine RNAiMAX (Invitrogen) then incubated at room temperature for 10-20min in the well of a 6 well plate. 2.5 x 10^5 cells in 2.5ml medium was added to the transfection mixture then incubated overnight at 37°C, 5% CO_2. Control and matched DDR-deficient cells (Tebu-bio) were then trypsinised, counted and diluted to 400 cells per ml and 2.0ml added to the wells of a 6 well tissue culture plate then incubated overnight. The cells were then treated with DNA damaging agents by adding 222µl 10 x drug solution to the appropriate wells. The plates were returned to the incubator for 1-2 weeks until the colonies have formed to ≥50 cells per colony. The medium was then removed from the wells and replaced with 1ml Giemsa stain (Sigma-Aldrich) for 10-20min at room temperature. The stain was removed and the plates washed 4 times in tap water. The plates were dried overnight, scanned and the colonies counted using ImageJ. The data was expressed as percentage of colonies in the control wells.

Measuring synthetic lethality by monitoring cytotoxicity using the IncuCyte

Control and DDR-deficient cells (Tebu-bio) were then trypsinised, counted and diluted to 5.5 x 10^3 cells per ml and 180µl added to the wells of a 96 well tissue culture plate then incubated overnight. The cells were then treated with USP11 inhibitor by adding 20.0µl 10 times concentrated drug solution to the appropriate wells. The plates were then placed in the IncuCyte FLR (Essen Biosciences) and monitored every 4 hours using the confluence algorithm for 1-2 weeks. The object count algorithm was then run to detect the cells that have taken up the YOYO1 dye (Invitrogen), representing dead or dying cells.

Measuring chemosensitisation by Sulforhodamine B (SRB) assay

18.0µl of 1µM control or USP11-specific siRNAs were diluted in 0.3ml OptiMEM serum-free medium (Invitrogen) containing 3.5µM Lipofectamine RNAiMAX (Invitrogen) then incubated at room temperature for 10-20min. HCT116, HeLa and Cal51 cells were then trypsinised, counted and diluted in DMEM + 10% FBS + Glut only at concentrations of 1.8 x 10^5, 4.0 x 10^5 or 3.3 x 10^5 cells per ml respectively. 1.5ml of each cell suspension was added to the transfection mix in a 6 well plate. Complexes and cells were mixed thoroughly before the plate was returned to the incubator (37°C and 5% CO_2) overnight. Transfected HCT116, HeLa and Cal51 cells were trypsinised and re-seeded into 96 well plates at 450, 2500 and 1600 cells per well respectively in 180µl medium before incubating for a further 24hours. The cells were then treated with serial dilutions of various chemotherapeutic agents by diluting to 10 x final concentration and adding 20µl solution per well. The plate was then returned to the incubator and left for a further 4 days. The medium was then removed from the plate and 100µl of 10% trichloracetic acid (TCA) (Sigma-Aldrich) was added to each well for 30 min at 4°C. The TCA was removed and the plate washed 4 x in tap water. The plate was then stained with 100µL/well 0.4% SRB (sulphorhodamine B – Sigma-Aldrich) in 1% acetic acid for 15 minutes at room temp. Excess stain was removed by washing 4 x with 1% acetic acid before allowing plate to air dry. The stained precipitate was then re-solubilised with 100µL/well 10 mM Tris Buffer pH 9.5, and absorbance determined at 560nm using the Pherastar plate reader. The data was expressed as percentage of absorbance in the control wells.

Expression and purification of USP11

The USP11 construct was PCR amplified and cloned into a pFLAG-CMV-6a vector (Sigma-Aldrich) with an N-terminal FLAG tag. HEK293T cells were transfected with FLAG-USP11 using TransIT-LT1 transfection reagent (Mirus) according to the manufacturer's instructions. Cells were harvested 40 hours after transfection. Cells were washed once with PBS and scraped in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 3 mM EDTA, 0.5% NP40, 10% glycerol, 5 mM beta-mercaptoethanol, protease

inhibitors (complete mini, Roche) and phosphatase inhibitors (PhosSTOP mini, Roche). Lysates were incubated for 30 min on ice and centrifuged at 1200 rpm for 10 min at 4° C. Soluble supernatant was added to FLAG affinity resin (EZview Rad ANTI-FLAG M2 affinity gel, Sigma-Aldrich) equilibrated in low salt buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 5 mM beta-mercaptoethanol) and incubated at 4° C for 3 hours rotating. The resin was spun at 2000 rpm for 2 min and the supernatant was removed. The resin was washed two times with low salt buffer and one time with high salt buffer (20 mM Tris, pH 7.5, 500 mM NaCl, 0.5 mM EDTA, 5 mM beta-mercaptoethanol, protease inhibitors (complete mini, Roche) and phosphatase inhibitors (PhosSTOP mini, Roche). To elute the bound USP11, elution buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 10% glycerol, 0.5% NP40, 5 mM beta-mercaptoethanol, 0.15 mg/ml 3X FLAG peptide (Sigma-Aldrich)) was added to the resin and incubated at 4° C for 2.5 hours rotating. The resin was centrifuged at 4000 rpm for 30 seconds, and the supernatant containing purified FLAG-USP11 was removed and stored at -80° C.

USP11 biochemical assay

Reactions were performed in duplicate in black 384 well plates (small volume, Greiner 784076) in a final reaction volume of 21 µl. USP11 was diluted in reaction buffer (20 mM Tris, pH 7.5, 100 mM NaCl, 0.05% Tween 20, 0.5 mg/ml BSA, 5 mM - beta mercaptoethanol) to the equivalent of 0, 0.01, 0.05, 0.1, 0.5, and 1 µl/well. Buffer was optimised for optimal temperature, pH, reducing agent, salts, time of incubation, and detergent. Reactions were initiated by the addition of 50 nM of TAMRA labelled peptide linked to ubiquitin via an iso-peptide bond as fluorescence polarisation substrate. Reactions were incubated at room temperature and read every 2 min for 120 min. Readings were performed on a Pherastar Plus (BMG Labtech), λ Excitation 540 nm; λ Emission 590 nm.

Ubiquitin-VME activity probe assay

U2OS cells were plated in 6-well dishes. The following day, cells were treated with DMSO or the indicated concentrations of inhibitor for 1 hour at 37°C. Cells were washed with PBS and lysed in 75 µl of lysis buffer [50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% NP-40, 0.5% CHAPS, 5 mM MgCl2, 5 mM beta-mercaptoethanol, protease inhibitor tablet (Roche) and phosphatase inhibitor tablet (Roche)]. Cells were scraped in lysis buffer and incubated on ice for 30 min. Lysates were centrifuge at 8000 rpm for 5 min at 4°C and the supernatant transferred to a new tube. Protein concentration was determined by using Coomassie Plus Protein Assay Reagent (Thermo Scientific) with BSA as a standard according to the manufacturer's recommendation. Lysates (15 µg) were diluted in lysis

buffer and incubated in the absence or presence of ubiquitin-VME (150 ng) in a final assay volume of 20 µl. Reactions were incubated for 60 min at room temperature and terminated by the addition of SDS-loading buffer and boiled for 5 min. Protein were separated by SDS-PAGE and transferred to nitrocellulose. Western blotting was performed with anti-USP11 antibodies (Bethyl MMS-A301-613A, 1:4000 dilution) and detected using a GE LAS4010 imaging system.

Table 1: USP siRNA sequences:

Name	Sequence	SEQ ID NO:
USP11_3:	AAGGUCGAAGUGUACCCAGUA	2
USP11_4:	CCCAUUGAACGCAAGGUCAUA	3
USP11_5:	CUGCGUCGGGUACGUGAUGAA	4
USP11_6:	ACCGAUUCUAUUGGCCUAGUA	5
USP11_10:	GGAAAGAACAGGUCGUGUC	6

Table 2: USP11 amino acid sequence (1 letter code – SEQ ID NO 1):

Human USP11 amino acid sequence (NP_004642.2)
mavaprlfgg lcfrfrdqnp evavegrlpi shscvgcrre rtamatvaan paaaaaavaa aaavtedrep qheelpglds qwrqienges grerplrage swflvekhwy kqweayvqgg dqdsstfpgc innatlfqde inwrlkeglv egedyvllpa aawhylvswy glehgqppie rkvielpniq kvevypvell lvrhndlgks htvqfshtds iglvlrtare rflvepqedt rlwaknsegs ldrlydthit vldaaletgq liimetrkkd gtwpsaqlhv mnnnmseede dfkgqpgicg ltnlgntcfm nsalqclsnv pqlteyflnn cyleelnfrn plgmkgeiae ayadlvkqaw sghhrsivph vfknkvghfa sqflgyqqhd sqellsflld glhedlnrvk kkeyvelcda agrpdqevaq eawqnhkrrn dsvivdtfhg lfkstlvcpd cgnvsvtfdp fcylsvplpi shkrvlevff ipmdprrkpe qhrlvvpkkg kisdlcvals khtgisperm mvadvfshrf yklyqleepl ssildrddif vyevsgriea iegsredivv pvylrertpa rdynnsyygl mlfghpllvs vprdrftweg lynvlmyrls ryvtkpnsdd eddgdekedd eedkddvpgp stggslrdpe peqagpssgv tnrcpflldn clgtsqwppr rrrkqlftlq tvnsngtsdr ttspeevhaq pyiaidwepe mkkryydeve aegyvkhdcv gyvmkkapvr lqecielftt vetlekenpw ycpsckqhql atkkldlwml peiliihlkr fsytkfsrek ldtlvefpir dldfsefviq pqnesnpely kydliavsnh yggmrdghyt tfacnkdsgq whyfddnsvs pvnenqiesk aayvlfyqrq dvarrllspa gssgapaspa cssppssefm dvn

Examples

Example 1: Purification of USP11 from mammalian cells

The method used for expression and purification of USP11 from human cells is detailed above. Purified proteins were separated by SDS-PAGE and visualised by protein staining (Figure 1).

Example 2: USP11 assay for high throughput screening of compounds using an isopeptide-linked substrate.

The proteolytic activity (deubiquitiylating activity) of purified FLAG-USP11 was measured in the presence of TAMRA (Tetramethyl-6-Carboxyrhodamine) labelled peptide linked to ubiquitin via an iso-peptide bond using fluorescence polarisation (Figure 2).

Example 3: Cells transfected with siUSP11 are hypersensitive to carboplatin treatment.

Carboplatin is a known chemotherapeutic drug which causes DNA cross linking resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to carboplatin is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by carboplatin treatment. It was observed that cells treated with carboplatin were more sensitive to USP11 depletion (Figure 3a, 3b and 3c) indicating that USP11 inhibitors are useful in the treatment of tumours where carboplatin treatment is at least partially effective.

Example 4: Cells transfected with siUSP11 are hypersensitive to camptothecin treatment.

Camptothecin is a known chemotherapeutic drug which inhibits the DNA Topoisomerase I enzyme, generating DNA damage resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to camptothecin is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by camptothecin treatment. It was observed that cells treated with camptothecin were more sensitive to USP11 depletion (Figure 4a and 4b) indicating that USP11 inhibitors are useful in the treatment of tumours where camptothecin treatment is at least partially effective.

Example 5: Cells transfected with siUSP11 are hypersensitive to temozolomide treatment.

Temozolomide is a known chemotherapeutic drug and alkylating agent that generates DNA damage resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to temozolomide is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by temozolomide treatment. It was observed that cells treated with temozolomide were more sensitive to USP11 depletion (Figure 5) indicating that USP11 inhibitors are useful in the treatment of tumours where temozolomide treatment is at least partially effective.

Example 6: Cells transfected with siUSP11 are hypersensitive to etoposide treatment. Etoposide is a known chemotherapeutic drug that inhibits the DNA Topoisomerase II enzyme generating DNA damage resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to etoposide is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by etoposide treatment. It was observed that cells treated with etoposide were more sensitive to USP11 depletion (Figure 6) indicating that USP11 inhibitors are useful in the treatment of tumours where etoposide treatment is at least partially effective.

Example 7: Cells transfected with siUSP11 are hypersensitive to Mitomycin C (MMC) treatment. MMC is a known chemotherapeutic drug and DNA cross-linking agent that generates DNA damage resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to MMC is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by MMC treatment. It was observed that cells treated with MMC were more sensitive to USP11 depletion (Figure 7) indicating that USP11 inhibitors are useful in the treatment of tumours where MMC treatment is at least partially effective.

Example 8: Cells transfected with siUSP11 are hypersensitive to bleomycin treatment. Bleomycin is a known chemotherapeutic drug and radiomimetic that generates DNA damage resulting in apoptosis and cell death. In addition to the characteristic side effects associated with chemotherapy, resistance to bleomycin is commonly observed resulting in recurrence of tumours following treatment.

Cells were transfected with siUSP11 followed by bleomycin treatment. It was observed that cells treated with bleomycin were more sensitive to USP11 depletion (Figure 8) indicating that USP11

inhibitors are useful in the treatment of tumours where bleomycin treatment is at least partially effective.

Example 9: Synthetic lethal interaction between USP11 and ATM, ATR and BRCA2 deficient cells

Isogenic ATM, ATR and BRCA2 deficient and wild type control cells were obtained from TeBu bio and the effect of USP11-specific siRNA measured according to the colony forming assay (CFA) method described above.

Selective killing was observed indicating that ATM, ATR and BRCA2 deficient cells are sensitive to USP11 inhibition (Figure 9).

Example 10: USP11 assay for measuring on-target activity of USP11 inhibitors in cells using an activity probe

U2OS osteosarcoma cells were incubated with DMSO or various concentrations of USP11 inhibitor as indicated. Cells were washed and lysed. Lysates were incubated in the absence or presence of an ubiquitin-VME probe. Proteins were separated by SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane. USP11 activity was determined via measurement of a shifted (active, Ub-USP11) relative to non-shifted (inactive, USP11) form of USP11 as indicated by Western blotting with an anti-USP11 antibody (Figure 10).

Example 11: DDR-deficient cells are hypersensitive to USP11 inhibition

Isogenic ATM, ATR and BRCA2 deficient and wild type control cells were obtained from TeBu bio and the effect of USP11 small molecule inhibitors measured according to the IncuCyte growth assay (method described above).

Selective killing was observed indicating that ATM, ATR and BRCA2 deficient cells are sensitive to USP11 inhibition (Figure 11).

Example 12: Platinum-resistant ovarian cancer cells are preferentially sensitised to carboplatin as compared to matched platinum-sensitive ovarian cancer cells

Matched ovarian cancer cells derived pre- and post-relapse from the same individual receiving platinum based therapy were transfected with USP11-targetting siRNAs followed by carboplatin treatment. It was observed that the USP11 siRNAs more efficiently killed the drug-resistant cells (PE04) compared to the drug-sensitive cells (PE01) (Figure 12). This indicates that USP11 inhibitors can reverse tumour resistance to platinum-based therapies and that they are useful in the treatment of tumours where platinum treatment is ineffective.

Example 13: Platinum-resistant endometrioid cancer cells are preferentially killed as compared to matched platinum-sensitive endometrioid cancer cells

Matched *in vitro*-derived platinum-sensitive (A2780) and platinum-resistant (CIS) endometrioid cancer cells were transfected with USP11-targetting siRNAs.

It was observed that the USP11 siRNAs more efficiently killed the drug-resistant cells compared to the drug-sensitive cells (Figure 13). This indicates that USP11 inhibitors can selectively kill tumours that are resistant to platinum-based therapies and that they are useful in the treatment of tumours where platinum treatment is ineffective.

Example 14: USP11 depletion selectively kills platinum-resistant ovarian cancer cells as compared to matched platinum-sensitive ovarian cancer cells

Matched ovarian cancer cells derived pre- and post-relapse from the same individual receiving platinum based therapy were transfected with USP11-targetting siRNAs. It was observed that the USP11 siRNAs more efficiently killed the drug-resistant cells (PE04) compared to the drug-sensitive cells (PE01) (Figure 16). This indicates that USP11 inhibitors can selectively kill tumours that are resistant to platinum-based therapies and that they are useful in the treatment of tumours where platinum treatment is ineffective.

Example 15: Platinum resistant A2789cis cells are significantly more sensitive to USP11 inhibition that platinum sensitive A2789 cells.

USP11 inhibitors lead to selective killing in platinum resistant cells (CIS) in preference to platinum-sensitive cells (A2780) (Figure 14). This further supports the data shown in Example 13 and indicates that USP11 inhibitors can reverse tumour resistance to platinum-based therapies and that they are useful in the treatment of tumours where platinum treatment is ineffective.

Example 16: BRCA2-deficient cells are hypersensitive to USP11 inhibition

USP11 inhibitors selectively prevent proliferation of BRCA2 deficient cells (Figure 15).

The effect of USP11 small molecule inhibitors on isogenic BRCA2 deficient and wild type control cells was measured according to the colony forming assay (method described above).

Selective killing was observed indicating BRCA2 deficient cells are sensitive to USP11 inhibition (Figure 15).

Example 17: Platinum resistant A2789cis cells are significantly more sensitive to USP11 inhibition that platinum sensitive A2789 cells.

Matched in vitro-derived platinum-sensitive (A2780) and platinum-resistant (CIS) endometrioid cancer cells were treated with USP11 inhibitor, this inhibitor is different to the one used in Examples 15 and 16.

It was observed that the USP11 inhibitors led to selective killing in platinum resistant cells in preference to platinum-sensitive cells (Figure 17). This further supports the data shown in Example 13 and indicates that USP11 inhibitors can be effective in the treatment of tumours where platinum treatment is ineffective.

Example 18: BRCA2-deficient cells are hypersensitive to USP11 inhibition

Isogenic BRCA2 deficient and wild-type control cells (TeBu bio) were treated with USP11 inhibitor. It was observed that the USP11 inhibitors selectively killed BRCA2-deficient cells compared to the control (Figure 18). These inhibitors are different to the one used in Examples 15 and 16.

This further supports the data shown in examples 9 and 11 and indicates that USP11 inhibitors can be effective in the treatment of tumours where BRCA2 is defective.

Claims

1. A USP11 inhibitor for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

2. Use according to claim 1 wherein one or more DNA damage (DDR) pathways is selected from base excision repair, single strand break repair, mismatch repair, nucleotide excision repair, transcription-coupled repair, non-homologous end joining repair, translesion synthesis, and double strand break repair processes.

3. Use according to claim 2 wherein one or more DNA damage (DDR) pathways is selected from homologous recombinational repair, DNA interstrand cross-link repair, base excision repair and double strand break (DSB) signalling.

4. Use according to any previous claim wherein deficiencies in one or more DDR pathways are due to mutations in, the absence of or defective expression of a gene encoding proteins selected from one or more of BRCA2, ATM and ATR.

5. Use according to any previous claim wherein cells deficient in one or more DNA damage (DDR) pathways are hyper-dependent on USP11 associated pathways for survival.

6. Use according to any preceding claim wherein cells deficient in one or more DDR pathways have increased sensitivity to DNA damaging agents.

7. Use according to any preceding claim wherein the cancer comprises cells resistant to carboplatin, cisplatin, oxaliplatin, picoplatin or satriplatin.

8. Use according to any previous claim wherein the USP11 inhibitor causes a reduction in functional activity of USP11.

9. Use according to any previous claim wherein the USP11 inhibitor is selected from a polypeptide, polynucleotide, antibody, peptide, small molecule, an inhibitory (RNAi) molecule or any other suitable chemical.

10. Use according to claim 9 wherein the USP11 inhibitor is an inhibitory (RNAi) molecule or a small molecule compound.

11. Use according to claims 9 or 10 wherein the USP11 inhibitor is selective for USP11.

12. Use according to any previous claim wherein cancer is selected from breast, ovarian, prostate, lung, kidney, gastric, colorectal, testicular, head and neck, pancreas, brain, melanoma, bone, oesophageal, bladder, cervix, endometrial or other cancers of tissue organs and cancers of the blood cells such as lymphomas and leukaemia.

13. Use according to any preceding claim wherein the USP11 inhibitor is combined with an anti-tumour therapeutic agent.

14. Use according to claim 14 wherein said anti-tumour therapeutic agent is selected from a chemotherapeutic agent, a ATM inhibitor, a ATR inhibitor, a BRCA2 inhibitor, a PARP inhibitor or ionising radiation.

15. A method of screening for agents of USP11 suitable for use in the treatment of cancer, wherein the cancer comprises cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy, comprising the steps of:

a) obtaining purified or recombinant USP11
b) contacting the isolated USP11 with one or more test agents
c) selecting those agents that demonstrate a reduction in USP11 activity.

16. A method according to claim 15 wherein a reduction in activity of USP11 is measured *in vitro* by a reduction in cell proliferation of cells deficient in at least one DDR pathway.

17. A method according to claim 16 wherein a reduction in catalytic activity of USP11 is measured *in vitro* by a reduction in the deubiquitylating activity of USP11.

18. A method of determining the responsiveness of a subject having a cancer to a USP11 inhibitor, the method comprising determining whether the cancer comprises cells deficient in one or more DNA damage response (DDR) pathway and / or cells resistant to platinum-based

chemotherapy, wherein the presence of said DDR deficiency or resistance to platinum-based chemotherapy indicates that the subject is responsive to a USP11 inhibitor.

19. A method according to claim 18 wherein the responsiveness of a subject to a USP11 inhibitor is determined by the absence or reduction in one or more biomarkers selected from BRCA2, ATM and ATR protein or mRNA, or by the presence of one or more amino acid or nucleic acid mutations in the sequence of BRCA2, ATM or ATR or by the presence of cancer cells resistant to platinum-based chemotherapy.

20. A use of the presence of a mutated *BRCA2* gene, mutated BRCA2 protein, mutated *ATR* gene, mutated ATR protein, mutated *ATM* gene or mutated ATM protein in a sample of cancer cells as a biomarker for determining the sensitivity of said cancer with treatment with a USP11 inhibitor.

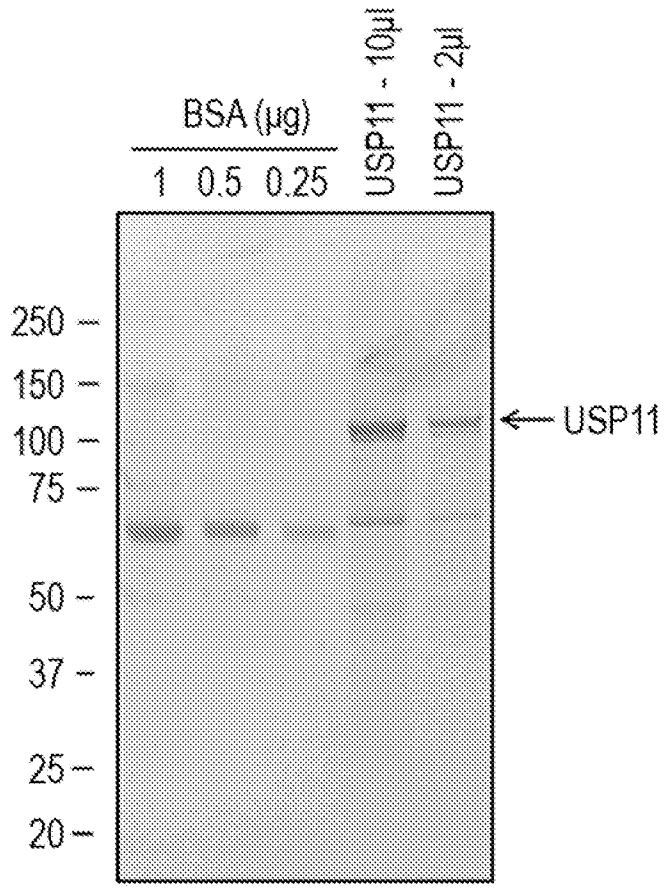
21. A pharmaceutical composition comprising an agent of USP11 for use in the in the treatment of cancer combined with any pharmaceutically acceptable carrier, adjuvant or vehicle wherein the cancer comprises cells deficient in one or more DNA damage (DDR) pathways and / or cells resistant to platinum-based chemotherapy.

22. A method of treating a subject having a cancer comprising cells deficient in one or more DNA damage response (DDR) pathways and / or cells resistant to platinum-based chemotherapy the method comprising administering to the subject a therapeutically effective amount of a USP11 inhibitor.

23. A method according to claim 22 wherein the USP11 inhibitor is administered prior to, in combination with or subsequent to the administration of an additional anti-tumour therapeutic agent.

24. A method according to claim 23 wherein the anti-tumour agent is a chemotherapeutic agent, ionizing radiation or a poly ADP ribose polymerase inhibitor.

25. A method use according to claim 23 or claim 24 wherein the anti-tumour agent is an ATM inhibitor, an ATR inhibitor and/or a BRCA2 inhibitor.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USP11 - 2µl
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150
100
75
50
37
25
20
USP11

Fig. 1

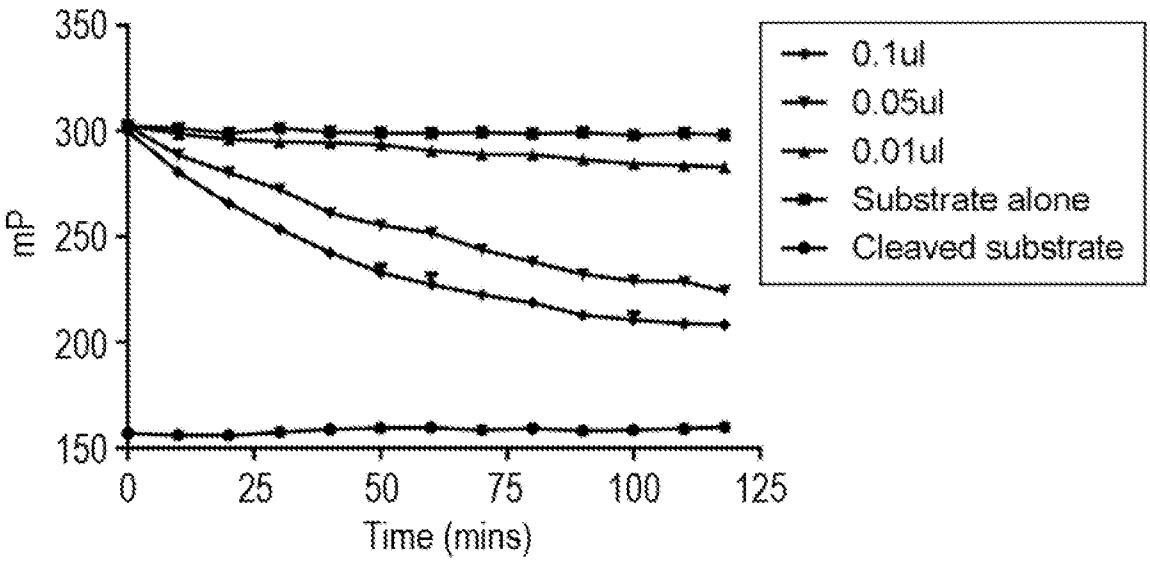
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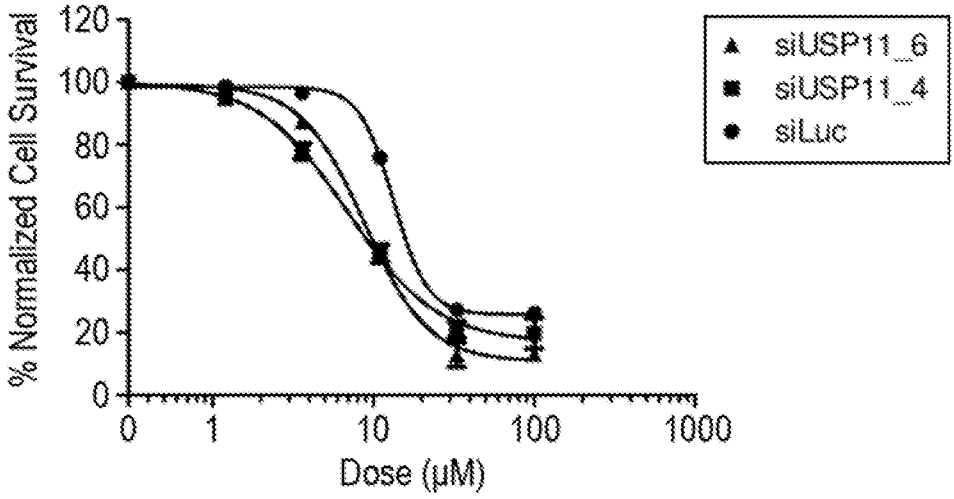
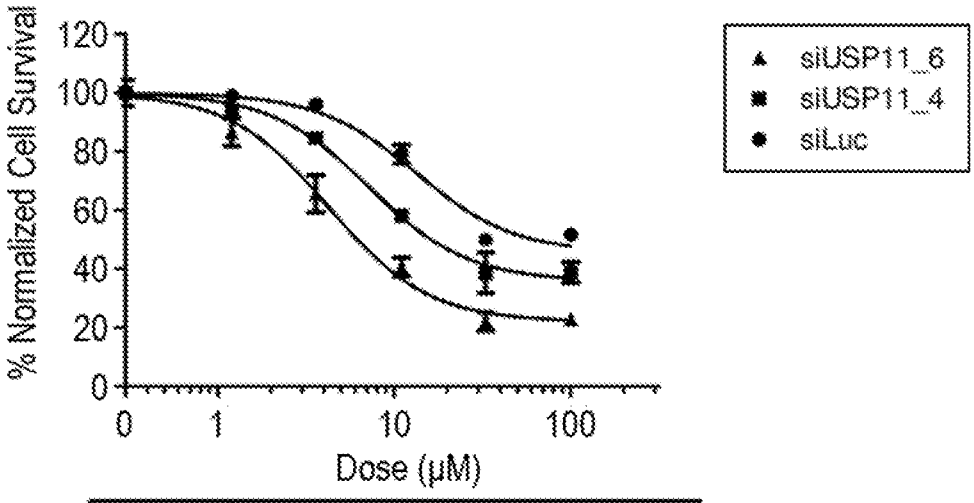
Fig. 2



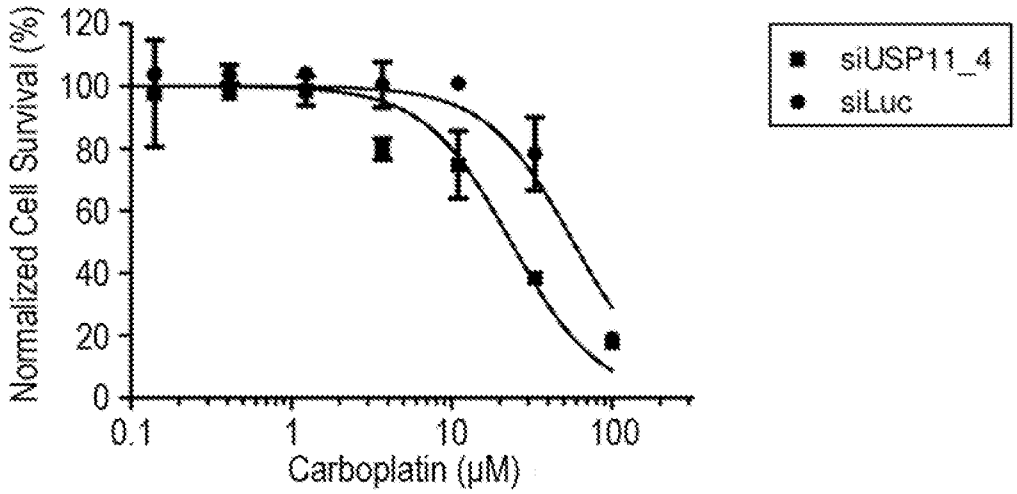
	siLuc	siUSP11_4	siUSP11_6
IC50	13.33	6.934	8.803

Fig. 3(a)



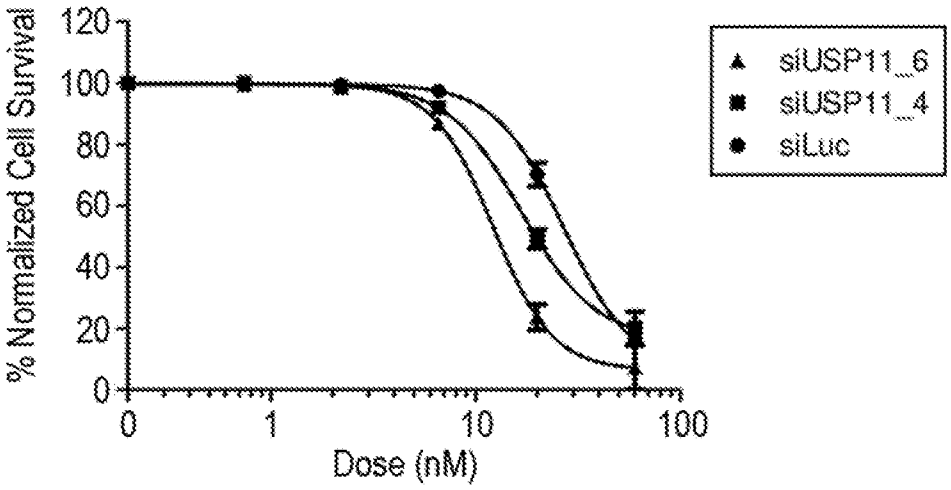
	siLuc	siUSP11_4	siUSP11_6
IC50	13.38	7.234	4.271

Fig. 3(b)



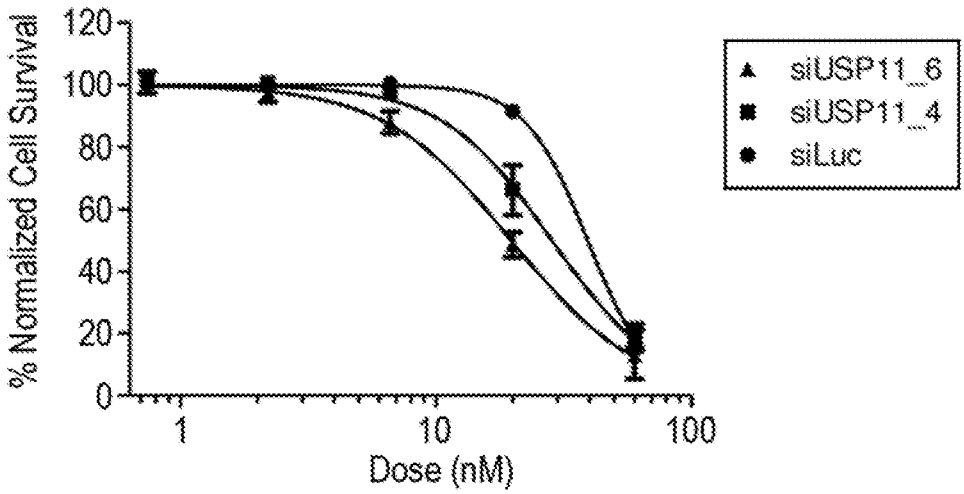
	siLuc	siUSP11_4
IC50	57.29	23.44

Fig. 3(c)



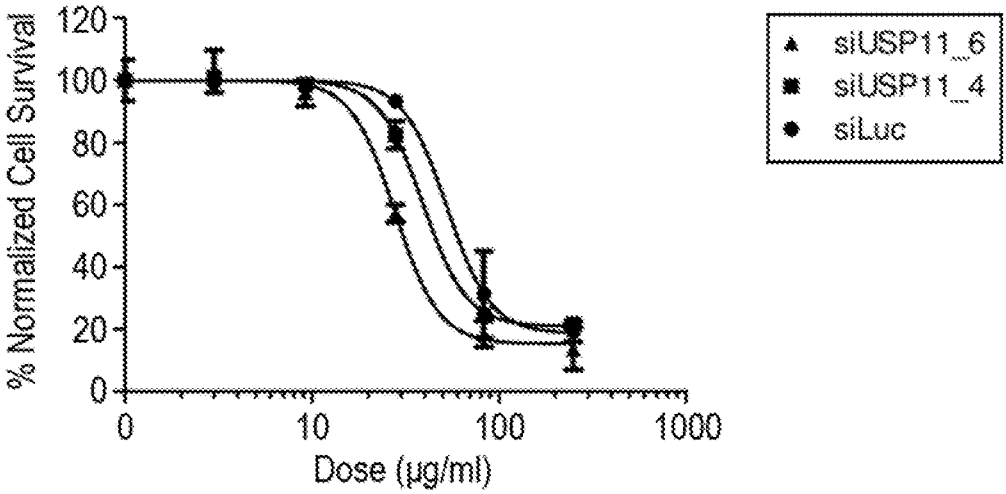
	siLuc	siUSP11_4	siUSP11_6
IC50	27.05	16.71	12.18

Fig. 4(a)



	siLuc	siUSP11_4	siUSP11_6
IC50	39.31	28.50	19.74

Fig. 4(b)



	siLuc	siUSP11_4	siUSP11_6
IC50	52.64	39.13	27.82

Fig. 5

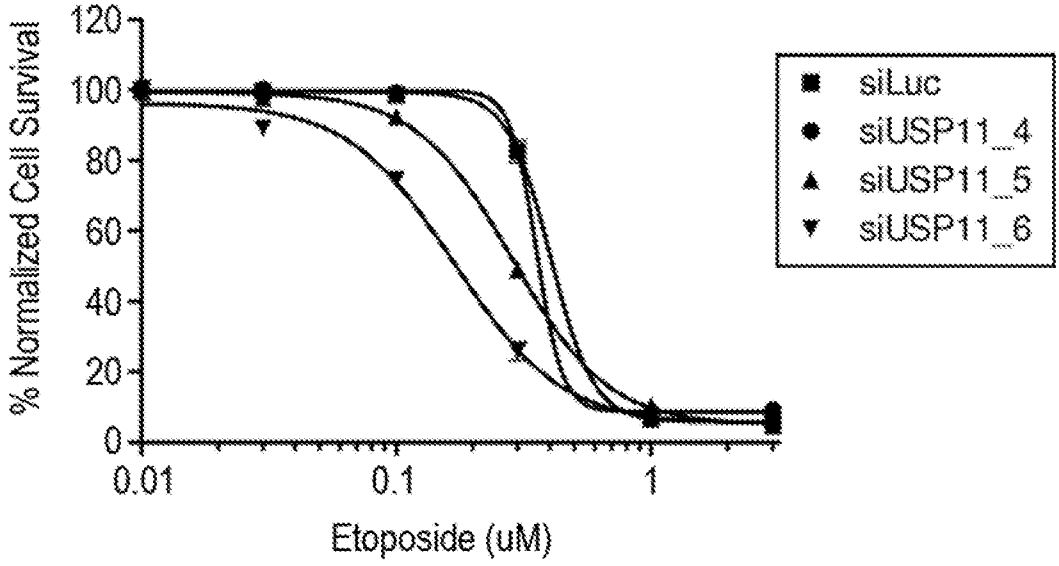
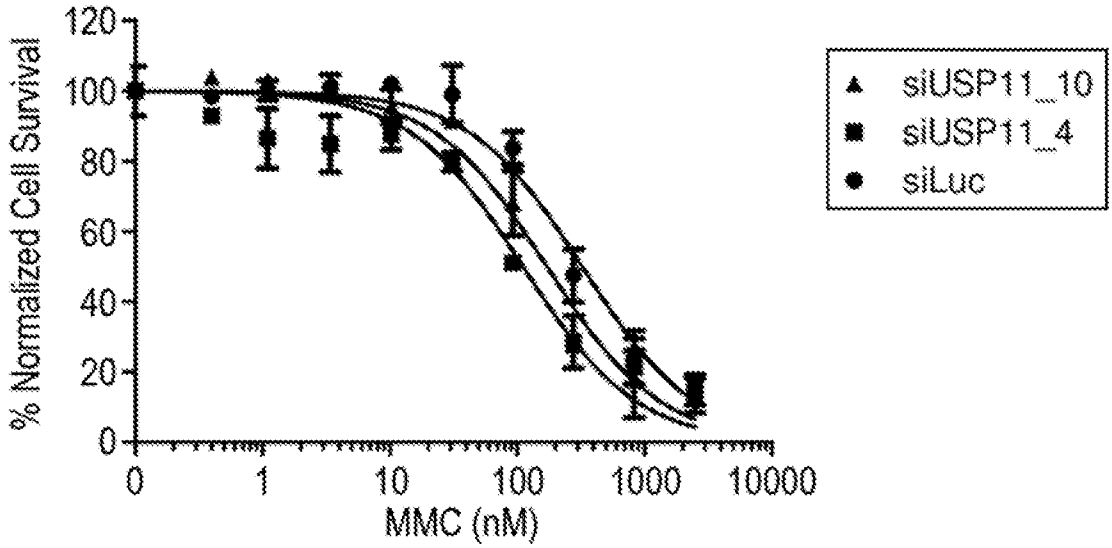
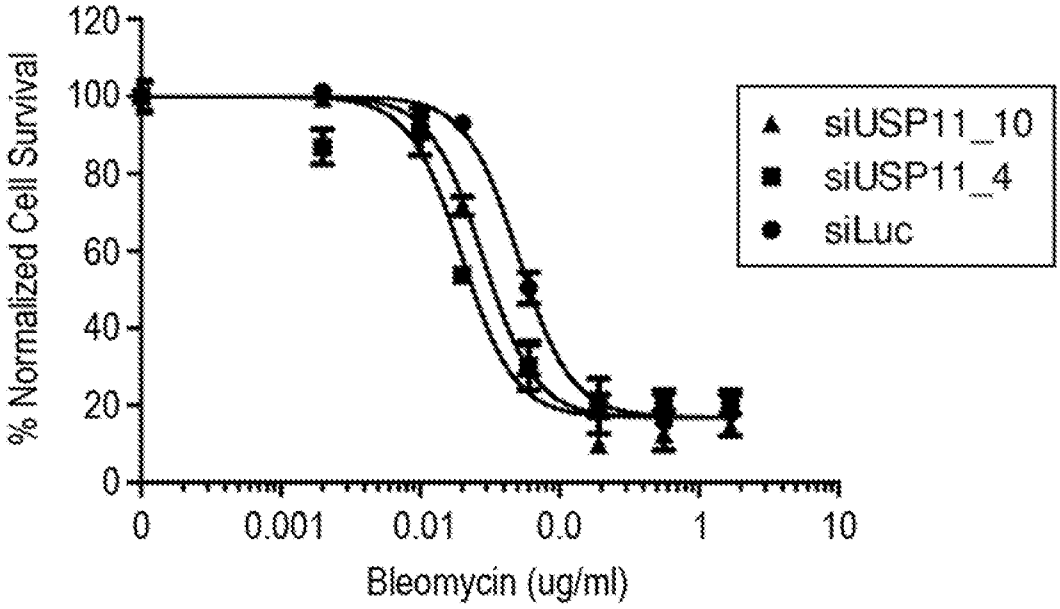


Fig. 6



	siLuc	siUSP11_4	siUSP11_10
IC50	324.5	111.4	172.1

Fig. 7



	siLuc	siUSP11_4	siUSP11_10
IC50	0.05080	0.01996	0.02808

Fig. 8

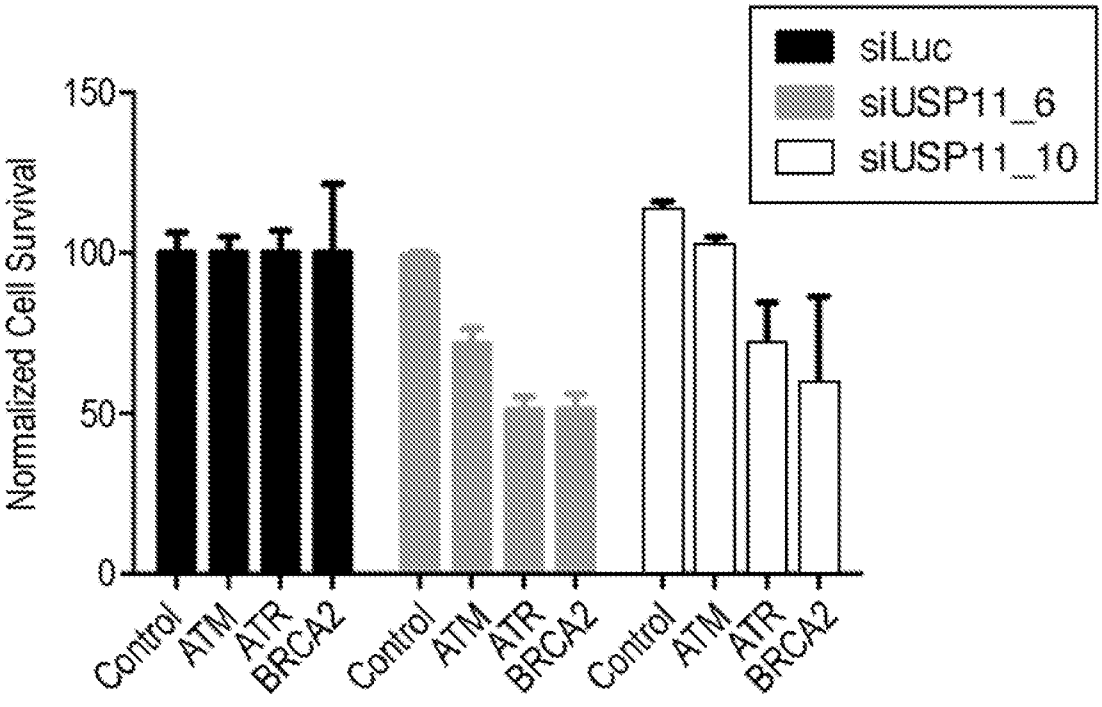


Fig. 9

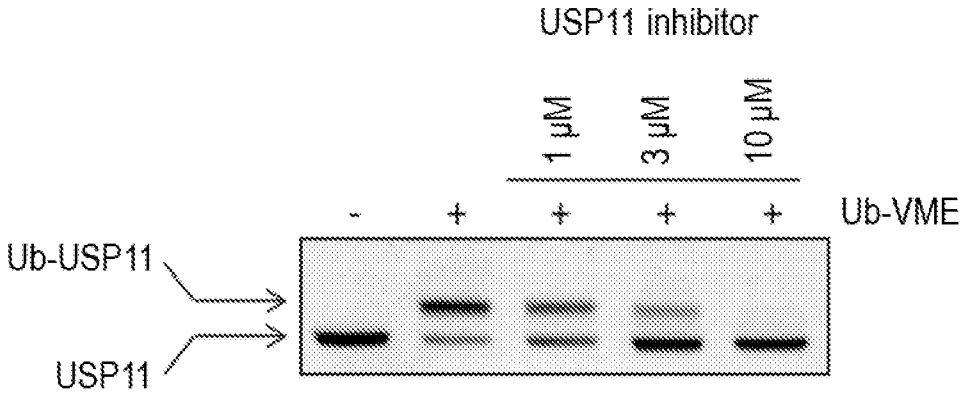
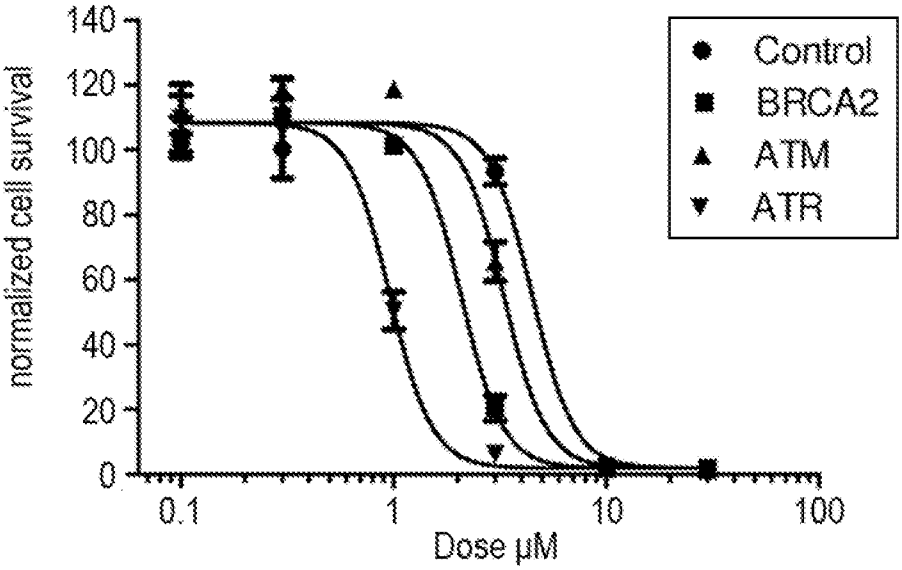


Fig. 10



	Control	BRCA2	ATM	ATR
EC50	4.490	2.078	3.296	0.9621

Fig. 11

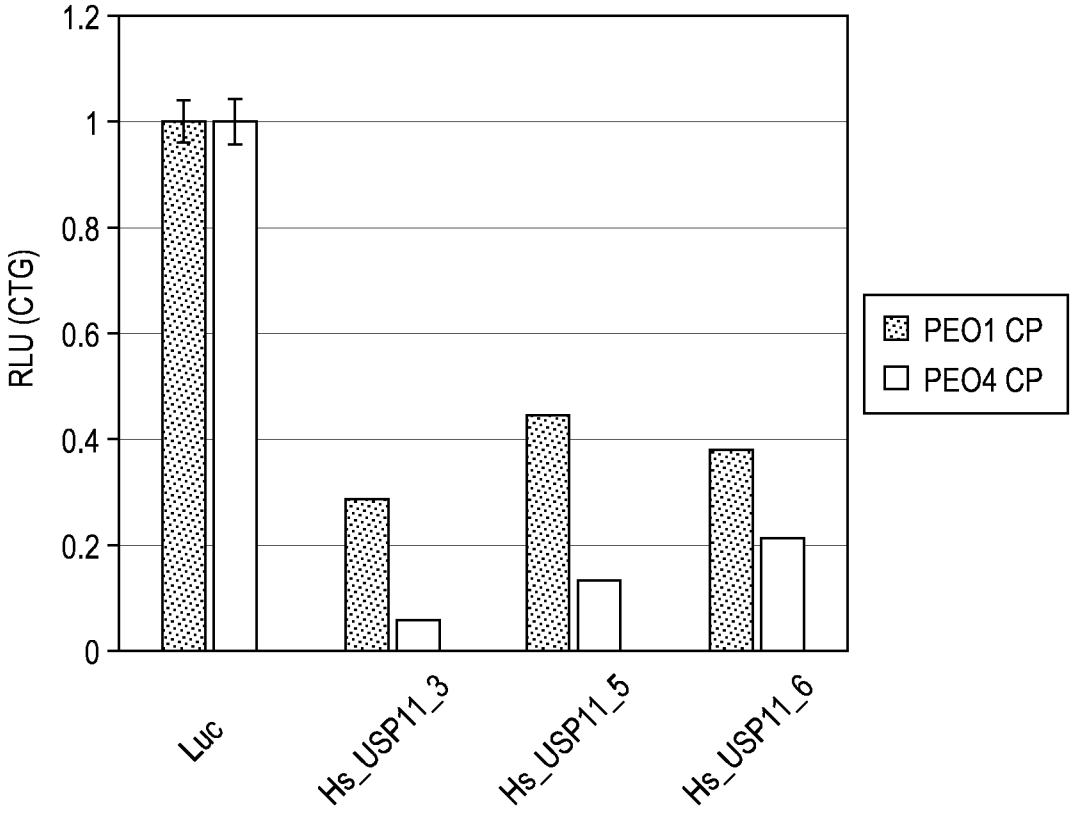

1.2
1
0.8
0.6
0.4
0.2
0
RLU (CTG)
PEO1 CP
PEO4 CP
Luc
Hs_USP11_3
Hs_USP11_5
Hs_USP11_6

Fig. 12

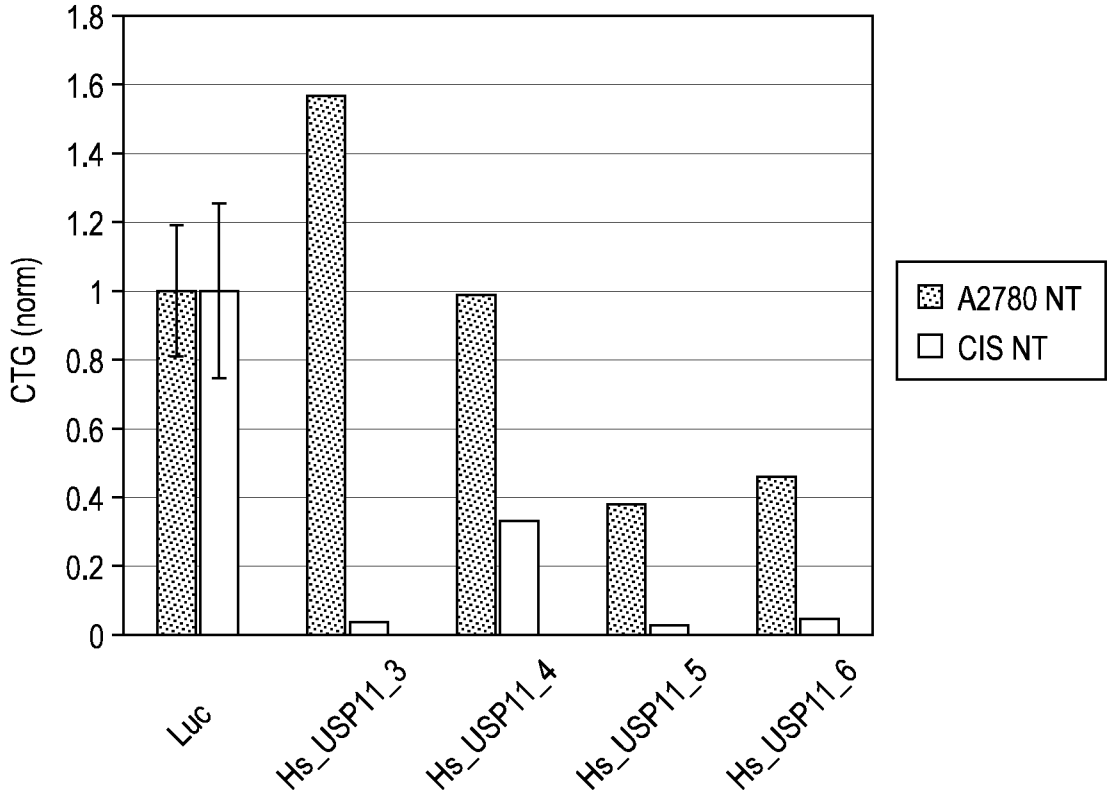
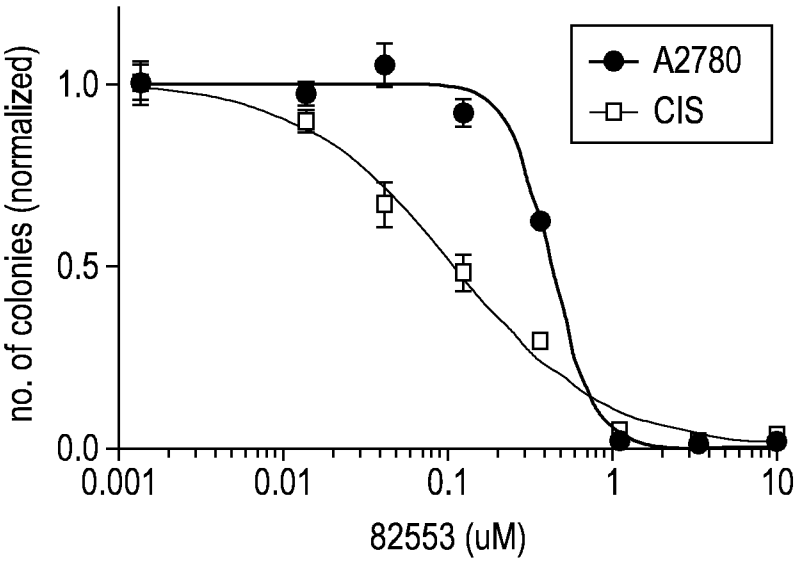
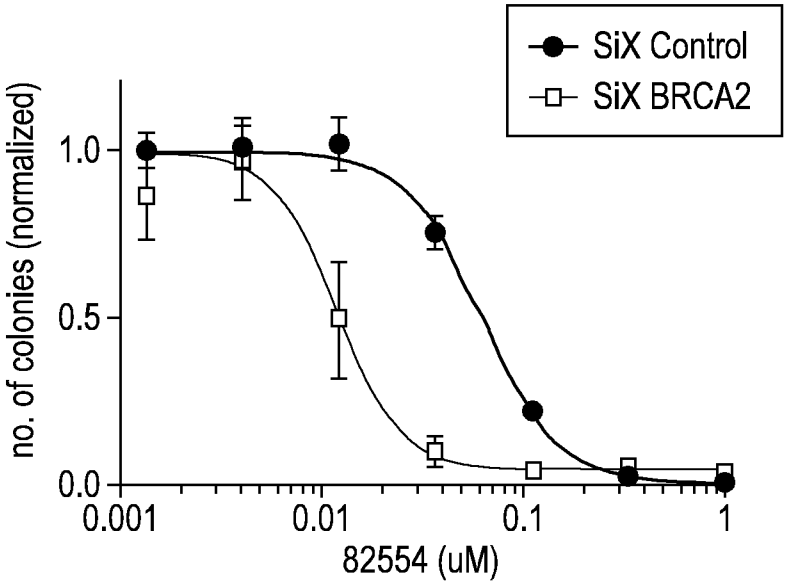

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CTG (norm)
A2780 NT
CIS NT
Luc
Hs_USP11_3
Hs_USP11_4
Hs_USP11_5
Hs_USP11_6

Fig. 13



	A2780	CIS
IC50	0.4279	0.1098

Fig. 14



	SiX Control	SiX BRCA2
IC50	0.06204	0.01191

Fig. 15

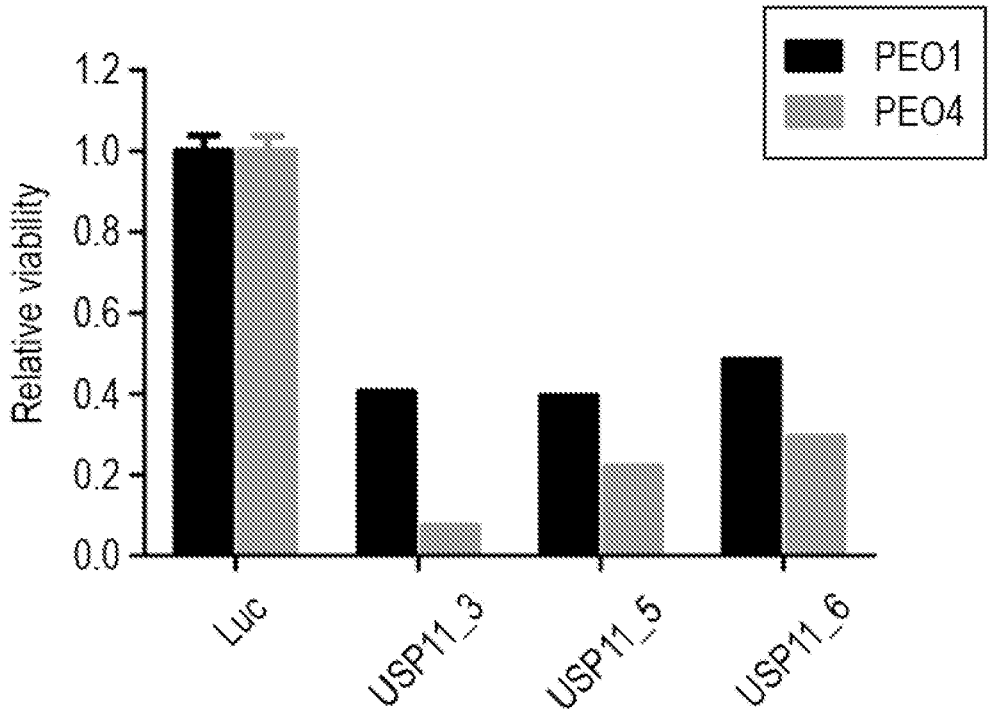
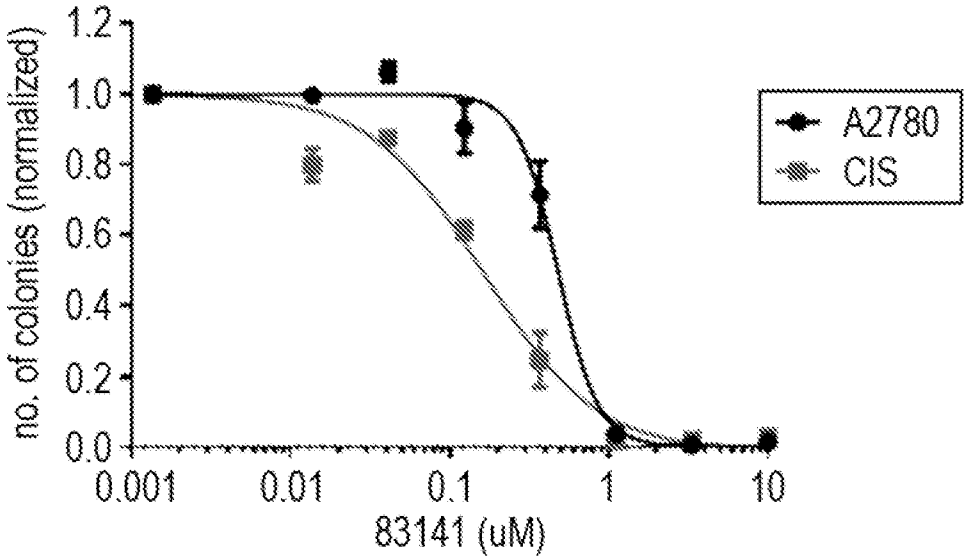
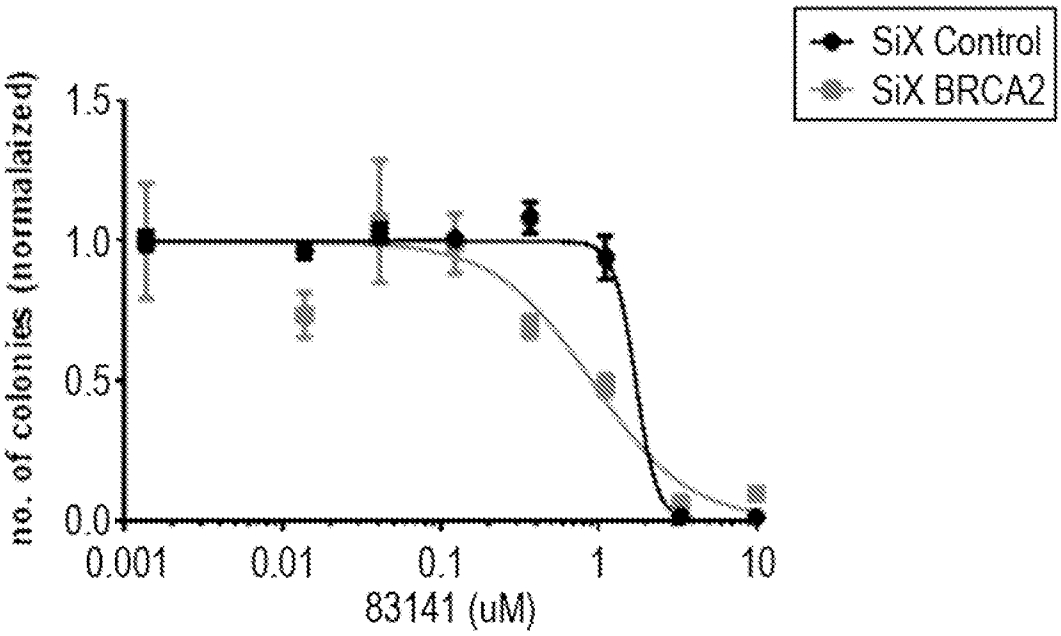

PEO1
PEO4
Relative viability
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Luc
USP11_3
USP11_5
USP11_6

Fig. 16



	A2780	CIS
IC50	0.4781	0.1633

Fig. 17



	SiX Control	SiX BRCA2
IC50	1.722	0.9084

Fig. 18

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/050326

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N9/16 A61K31/713
ADD. G01N33/573 A61K38/46 A61P35/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K G01N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R. A. BURKHART ET AL: "Mitoxantrone Targets Human Ubiquitin-Specific Peptidase 11 (USP11) and Is a Potent Inhibitor of Pancreatic Cancer Cell Survival", MOLECULAR CANCER RESEARCH, vol. 11, no. 8, 21 May 2013 (2013-05-21), pages 901-911, XP055182825, ISSN: 1541-7786, DOI: 10.1158/1541-7786.MCR-12-0699 cited in the application	1-6, 8-17, 21-25
Y	the whole document	1-25
	----- -/--	

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2015	22/04/2015

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/050326

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WILTSHIRE TIMOTHY D ET AL: "Sensitivity to poly(ADP-ribose) polymerase (PARP) inhibition identifies ubiquitin-specific peptidase 11 (USP11) as a regulator of DNA double-strand break repair", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 285, no. 19, 7 May 2010 (2010-05-07), pages 14565-14571, XP002613116, ISSN: 1083-351X, DOI: 10.1074/JBC.M110.104745 cited in the application the whole document	1-25
A	WO 2008/066624 A2 (DANA FARBER CANCER INST INC [US]; DNAR INC [US]; UNIV OREGON HEALTH &) 5 June 2008 (2008-06-05) the whole document	1-25
A	WO 2012/071684 A1 (SHANGHAI DE NOVO PHARMATECH CO LTD [CN]; GAO DAXIN [CN]) 7 June 2012 (2012-06-07) the whole document	1-25
A	WO 2013/151638 A1 (INDUS PHARMACEUTICALS INC [US]; CHATURVEDI PRAVIN R; MANIVASAKAM PALAN) 10 October 2013 (2013-10-10) the whole document	1-25
A	A. R. SCHOENFELD ET AL: "BRCA2 Is Ubiquitinated In Vivo and Interacts with USP11, a Deubiquitinating Enzyme That Exhibits Prosurvival Function in the Cellular Response to DNA Damage", MOLECULAR AND CELLULAR BIOLOGY, vol. 24, no. 17, 16 August 2004 (2004-08-16), pages 7444-7455, XP055182826, ISSN: 0270-7306, DOI: 10.1128/MCB.24.17.7444-7455.2004 cited in the application the whole document	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2015/050326

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		JP 2013544261 A	12-12-2013
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		WO 2013151638 A1	10-10-2013