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(54) Title: METHOD FOR IDENTIFYING SPECIFIC CANCER PATIENT SUBGROUPS AND NOVEL CANCER THERAPY FOR SPECIFIC CANCER PATIENT SUBGROUPS

(57) Abstract: The present invention relates to a method for predicting whether a subject having cancer will benefit from G quadruplex binding ligand treatment by determining the level of one or more subunits of the SWI/SNF complex in a biological sample. The present invention also relates to the use of a G quadruplex binding ligand in inducing cell death of cancer cells deficient in a subunit of the SWI/SNF complex. The methods, compositions and uses described herein may be used to induce cell death of cancer cells deficient in a subunit of the SWI/SNF complex. The cells may be in vitro, ex vivo or in vivo. The cancer cells deficient in a subunit of the SWI/SNF complex may be present within a subject (e.g. cancer patients carrying a SWI/SNF subunit deficient malignant tumour).

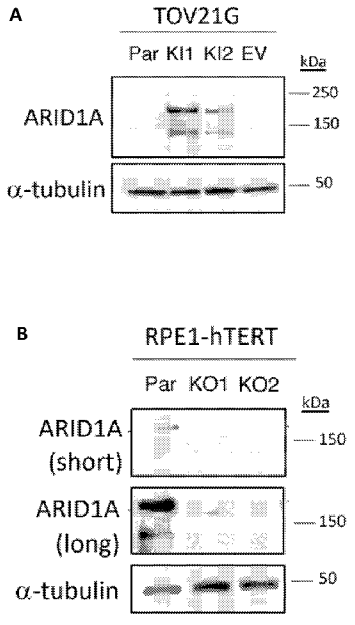

Figure 1

WO 2025/003680 A1

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METHOD FOR IDENTIFYING SPECIFIC CANCER PATIENT SUBGROUPS AND NOVEL CANCER THERAPY FOR SPECIFIC CANCER PATIENT SUBGROUPS

FIELD OF THE INVENTION

The present invention relates to a method for predicting whether a subject having cancer will benefit from G quadruplex binding ligand treatment by determining the level of one or more subunits of the SWI/SNF complex in a biological sample. The present invention also relates to the use of a G quadruplex binding ligand in inducing cell death of cancer cells deficient in a subunit of the SWI/SNF complex. The methods, compositions and uses described herein may be used to induce cell death of cancer cells deficient in a subunit of the SWI/SNF complex. The cells may be *in vitro*, *ex vivo* or *in vivo*. The cancer cells deficient in a subunit of the SWI/SNF complex may be present within a subject (e.g. cancer patients carrying a SWI/SNF subunit deficient malignant tumour).

BACKGROUND

Maintaining genome stability is vital for the accurate transmission of genetic information, and genome instability is a hallmark of cancer cells. G-quadruplex (G4) structures, with their distinctive four-stranded helical arrangement, are prevalent in regulatory regions of the human genome, including promoter elements (Spiegel, et al., 2020). The formation and stability of G4 structures appears to be tightly regulated by a repertoire of proteins that can recognise and bind them, thereby modulating biological processes, such as transcription. In addition, G4 binding proteins and associated pathways are important for their resolution (for example, (Sato, et al., 2021)). Notably, the dysregulation of G4-associated proteins has been implicated in genome instability and the pathogenesis of various diseases, including cancer. Recently, instability at genome regions with G4-structure forming potential has been found to shape the mutational burden of human cancers (Bacolla, et al., 2019) and cellular survival following exposure to G4 binding ligands depends on multiple cellular pathways, including both homologous recombination (HR) and non-homologous end joining (NHEJ) double-strand break (DSB) repair pathways (Masud, et al., 2021, Zimmer, et al., 2016, Olivieri, et al., 2020). The clinical exploration of G4 binding ligands is underway, and gaining a deeper understanding of the genetic vulnerabilities in cancer cells that makes them sensitive to G4 ligands will enhance their targeted and effective application.

The SMARCA4 (or BRG1) protein is an ATPase that forms the catalytic subunit of three highly related mammalian SWI/SNF chromatin remodelling complexes; BAF, PBAF and

ncBAF (or GBAF) (Harrod et al., 2020). SMARCA4 is frequently misregulated in cancer cells, with frequent loss of function mutations (Harrod et al., 2020).

ARID1A (also called BAF250A) is a subunit that is found only in the BAF complex. The ARID1A gene is mutated in up to 50% of ovarian clear cell carcinomas (OCCC) (Harrod et al., 2020). Identifying treatments that selectively target ARID1A deficient cells could therefore provide clinical utility. Typically, the mutation in the ARID1A gene is a loss-of-function (e.g. frameshift) mutation which results in no functional protein expression of ARID1A.

SWI/SNF is important for regulation of gene expression, and there is good evidence that this activity is important for the tumour-suppressor activity of the SWI/SNF complexes (Mittal and Roberts 2020). There is also evidence that SWI/SNF complexes function in DNA repair and replication stress response pathways (Brownlee, Meisenberg et al., 2015, Harrod et al., 2020), which are critical for preventing genome instability. Given the prevalence of SWI/SNF misregulation in cancer, it is critical to develop a complete understanding of its many activities in order to understand the cellular consequences of its loss.

BRIEF SUMMARY OF THE DISCLOSURE

SMARCA4 was reported to bind G4 sequences *in vitro* and to map to G4-containing sequences in cells (Zhang, Spiegel et al., 2021), suggesting that SMARCA4-dependent chromatin remodelling at genes containing G4 regulatory elements is important for their transcription. Here, the inventors explored the interaction between SMARCA4 and G4 structures in more depth and have identified a G4 binding motif in the C-terminal tail of SMARCA4. In the absence of SMARCA4, the inventors found that cells are vulnerable to treatment with G4 binding ligands, raising a potential therapeutic approach for patients with SMARCA4-deficient cancers. The inventors further investigated the potential impact of SMARCA4 on genome instability at G4-containing sequences. The inventors found that SMARCA4 deficient cells are more likely to have mutations or unrepaired DNA breaks at sequences with G4-forming potential when compared with the isogenic control cells. Notably, this is apparent in the absence of any cellular perturbations. These data suggest that loss of SMARCA4 removes a protective cellular mechanism for preventing G4-dependent genome instability, and this could be an important factor during the development of cancer.

Additionally, using isogenic cell lines, the inventors have also shown that ARID1A deficiency leads to cellular sensitivity to G4 binding ligands. In the absence of ARID1A, the inventors

found evidence of increased DNA damage, consistent with impaired DNA repair activity. The inventors further have shown that ARID1A is required for the efficient relocalisation of NHEJ repair proteins onto chromatin in response to treatment with G4 binding ligands. These data suggest that G4 binding ligands represent a promising new therapeutic strategy for OCCC patients with ARID1A deficiency.

Taken together, the inventors have surprisingly shown that the loss of various subunits of the SWI/SNF complex leads to sensitisation of cancer cells to treatment with G quadruplex binding ligands.

In one aspect, a method for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand is provided, the method comprising the steps of:

a) determining the level of one or more biomarker in a biological sample from the test subject, wherein the one or more biomarker is a subunit of the SWI/SNF complex;

b) comparing the level of the one or more biomarker with a threshold level or range; and

c) predicting that:

i) the test subject will respond to treatment with a G quadruplex ligand if the test subject's biological sample has a decreased level of the one or more biomarker compared to the threshold level or range; and

ii) the test subject will not respond to treatment with a G quadruplex ligand if the test subject's biological sample has an increased level of the one or more biomarker compared to the threshold level or range.

In specific embodiment, the level of the one or more biomarker in the biological sample is decreased by at least 1.5 fold, at least 2 fold, at least 2.5 fold, or at least 5 fold compared to the threshold level or range.

In one aspect, a pharmaceutical composition comprising a G quadruplex binding ligand for use in inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex in a subject is provided.

In one aspect, a method of inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex is provided, comprising subjecting the cell to a G quadruplex binding ligand.

In a specific embodiment, the method comprises inducing cell death of a cell deficient in a subunit of the SWI/SNF complex in a subject, wherein a G quadruplex binding ligand is administered to the subject.

In one aspect, a method for treating a subject having cancer is provided, the method comprising: administering to a subject a G quadruplex binding ligand, wherein the subject is identified as likely to benefit from treatment with a G quadruplex binding ligand based on having, in a biological sample, a decreased level of one or more biomarkers compared to a threshold level or range, wherein the one or more biomarkers is a subunit of the SWI/SNF complex.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the subunit of the SWI/SNF complex is selected from the group consisting of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1, or wherein the cell is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the subunit of the SWI/SNF complex is ARID1A and/or SMARCA4, or wherein the cell is deficient in ARID1A and/or SMARCA4.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the cell is a cancer cell selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lymphoma, brain, neuroblastoma, lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, cervical, breast, melanoma, bladder, endometrial, head and neck, liver, renal and prostate cancer cell, or wherein the cancer is selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lymphoma, brain, neuroblastoma, lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, cervical, breast, melanoma, bladder, endometrial, head and neck, liver, renal and prostate cancer cell.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the G quadruplex binding ligand is selected from the group consisting of: a nucleic acid, a peptide or a binding molecule, optionally wherein the binding molecule is a small molecule or an antibody.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided for treating ARID1A/and or SMARCA4 deficient cancer in a subject.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided ,wherein the subject is human.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided ,wherein the composition is for use in combination with one or more anti-cancer therapy.

In a specific embodiment, the methods of any one of the preceding aspects and embodiments are provided, wherein: a) the subject is undergoing treatment with, has been treated with, or has been prescribed treatment with, one or more anti-cancer therapy; and/or b) the method further comprises administering one or more anti-cancer therapy to the subject.

In a specific embodiment, t the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the anti-cancer therapy is selected from the group consisting of surgery, radiation therapy, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, adjuvant therapy, and immunotherapy.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the chemotherapy is a cytotoxic agent.

In a specific embodiment, the composition for use or methods of any one of the preceding aspects and embodiments are provided, wherein the cytotoxic agent is selected from the group consisting of a platinum agent, mitomycin C, a poly (ADP-ribose) polymerase (PARP) inhibitor, a radioisotope, a vinca alkaloid, a taxane, an antitumor alkylating agent, a monoclonal antibody and an antimetabolite.

In a specific embodiment, the platinum agent is carboplatin.

In one aspect, the use of one or more biomarkers selected from the group consisting of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1 is provided as a biomarker for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of them mean "including but not limited to", and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

Various aspects of the invention are described in further detail below.

DESCRIPTION OF THE FIGURES

Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1. Generation of isogenic ARID1A cell line models. A) Western blot analysis of extracts prepared from TOV21G OCCC cells (Par), and with ARID1A expression constructs (KI1 and KI2) or empty vector expression construct (EV). Blots were probed with an antibody against ARID1A, and α-tubulin was used as a loading control. B) Western blot analysis of extracts prepared from RPE1-hTERT (par) or two independently derived ARID1A knockout lines (KO1 and KO2) using an antibody against ARID1A or α-tubulin as a loading control. Short and long exposures of the ARID1A analysis are shown.

Figure 2. ARID1A deficiency sensitises cells to G4 binding ligands. A) ARID1A-deficient TOV21G cells (par or EV) and ARID1A-expressing cells (KI1 and KI2) were treated with 10 μM PDS and cell growth relative to untreated cells was measured. B) Cell lines as in A were

analysed for cell growth following exposure to CX-5461. C) ARID1A KO and parental RPE1-hTERT cells were analysed for cell growth in the presence of 1 μM PDS relative to untreated cells. D) ARID1A KO and parental HCT116 cells were analysed for cell growth in the presence of PDS relative to untreated cells. E) Survival of ARID1A KO clonal cell lines and parental RPE1-hTERT cells was measured following exposure to the indicated dose of PDS by clonogenic survival assay and survival is plotted relative to untreated cells. F) Dead cells measured by propidium iodide incorporation as a percentage of total cells were quantified in ARID1A-deficient TOV21G cells (Parental and EV) and in ARID1A-expressing TOV21G cells (KI1 and KI2) after exposure to the indicated dose of PDS. G) Dead cells measured by propidium iodide incorporation as a percentage of total cells were quantified in two ARID1A KO clonal cell lines and parental RPE1-hTERT cells as in F. H) Cell growth assay of two PBRM1 KO clonal cell lines and parental RPE1-hTERT following exposure to the indicated dose of PDS and plotted relative to untreated cells.

Figure 3. Cells lacking ARID1A, but not PBRM1, show evidence of increased DNA damage after PDS exposure. A) The number of γH2AX foci per cell was measured in ARID1A deficient TOV21G cells (Par or EV) and ARID1A-expressing cells (KI1) after treatment with 10 μM PDS for the indicated time. Foci in untreated cells was also quantitated. B) The number of γH2AX foci per cell was measured in PBRM1 knockout cell lines (KO1 and KO2) and the parental RPE1-hTERT cell line (par) as in A.

Figure 4. Non-homologous end-joining (NHEJ) DNA repair proteins fail to mobilise onto chromatin in response to PDS when ARID1A is deficient. A) Pathway analysis of the top 500 chromatin-bound proteins in PDS treated ARID1A proficient cells. Significantly enriched pathways are indicated. B) Correlation analysis of chromatin-bound proteins in PDS treated ARID1A proficient cells showing good correlation between the two ARID1A-expressing clonal cell lines KI1 and KI2. Inset indicates the chromatin enriched NHEJ proteins in the ARID1A-expressing cells after PDS treatment. C) Heatmap showing enrichment of the indicated NHEJ pathway proteins in the ARID1A-proficient and ARID1A-deficient TOV21G cell lines before and after PDS treatment. D) Western blot analysis of cellular fractions following PDS treatment in ARID1A-proficient or ARID1A-deficient cells. Fractions were analysed with antibodies against the NHEJ proteins p-DNA-PK, Ku70/80, and PAXX. An antibody against H2A was used to monitor successful chromatin fractionation.

Figure 5. Identification of a G4 binding motif in the SMARCA4 subunit of SWI/SNF chromatin remodelling complexes. A) Western blot of pull-down assays using recombinant SMARCA4 and biotinylated oligos corresponding to the G4 sequence of the indicated promoter (ss G4), a mutated version that is not capable of forming a G4 (ss mut) or the

double-stranded version (ds). G4-forming sequences from the Myc, Kit1, and Bcl2 promoter were used as indicated. The presence of SMARCA4 in the pull-down was assayed using an antibody against SMARCA4. B) Western blot of pull-down assay using nuclear extract and the indicated biotinylated oligos. Proteins associated with the oligos were analysed by Western blotting using an antibody against SMARCA4. C) Cartoon depicting the domain organisation of SMARCA4 with the sequence of the disordered C-terminal tail indicated below. The putative G4 binding motif is highlighted. D) Western blot of pull-down assays using a recombinant FLAG-tagged C-terminal SMARCA4 construct corresponding to aa1446-1647 and biotinylated oligos as in A. The presence of recombinant protein in the pull-downs was assayed by Western blotting using an antibody against the FLAG epitope. E) Sequence of the tiled peptides corresponding to the C-terminal disordered region of SMARCA4. F) Pull-down assays using fluorescently labelled peptides as in E and biotinylated oligos as in panel A. Peptides associated with the oligos were detected by fluorescence after electrophoresis on a Tricine gel.

Figure 6. SMARCA4 deficient cells are sensitive to G4 binding ligands. A) Western blot analysis of whole cell extract prepared from RPE1-hTERT parental cells or two CRISPR-Cas9 generated SMARCA4 knockout (KO) clones, C9 and H12. Tubulin is used as a loading control. B) Quantification of cell growth of SMARCA4 KO cell lines compared with parental RPE1-hTERT cells in response to treatment with the indicated dose of PDS using an SRB cell growth assay. Left panel is survival (relative cell growth) after 6 days of treatment and right panel shows survival after 9 days of treatment. n=3 biological replicates. C) SMARCA4 KO cells show increased levels of γH2AX foci following PDS treatment compared with the parental control cells. Left panels show representative immunofluorescence images stained with DAPI or the antibody against γH2AX as indicated. Right panel shows the quantification of foci after 3 or 6 hours of PDS treatment compared with untreated cells (merge). Data represent the mean +/- SEM, n=3 biological replicates. Size bar= 10μm.

Figure 7. Locations of genome instability in SMARCA4 deficient cells show a greater propensity to co-localise with G4 structures when compared with the parental control cells. A) Genomic locations of unrepaired DNA DSBs using INDUCE-seq in the RPE1-hTERT parental cell lines and two SMARCA4 KO clones showing similar distributions across indicated categories. B) Top panel shows workflow of analysis. Briefly, locations of unrepaired breaks, SNVs or INDELS (query) were intersected with G4 structures (feature). Bottom panel shows heatmap of intersection data (scale bar on right). The intersection was performed using feature maps from in silico, in vitro or in vivo approaches as indicated, and data is shown for the parental line (WT) or two SMARCA4 KO cell lines (C9 or H12) that were untreated (UT) or treated with hydroxyurea (HU).

Figure 8. ARID1A deficiency sensitises cells to G4 binding ligands. A) ARID1A-deficient TOV21G cells (par or EV) and ARID1A-expressing clonal lines (C1 and C2) were treated with 10 μM PDS and cell growth relative to untreated cells was measured using SRB assays. B) Cell death was monitored in cell lines as in A following exposure to the indicated concentration of PDS using propidium iodide incorporation as a readout of inviability. C) Cell lines as in A were monitored for cell growth following exposure to 8 μM CX-5461. D) ARID1A KO and parental RPE1-hTERT cells were analysed for cell growth in the presence of 1 μM PDS relative to untreated cells. E) Cell death was monitored in cell lines as in D following exposure to the indicated concentration of PDS using propidium iodide incorporation as a readout of inviability. F) Cell lines as in D were monitored for cell growth following exposure to CX-5461.D) ARID1A KO and parental HCT116 cells were analysed for cell growth in the presence of PDS relative to untreated cells. G) ARID1A KO and parental RPE-hTERT cells were analysed for cell growth in the presence of 5 μM PhenDC3 relative to untreated cells. H) ARID1A KO and parental cells created in the HCT116 cell line background were analysed for viability in the presence of PDS. All assays were performed in biological triplicate, and plotted using the mean +/- SD, except panel C, where n=1.

Figure 9. ARID1A deficient tumours grow more slowly when mice are treated with CX-5461. A) Schematic of *in vivo* experimental design. Nude mice were injected with TOV21G (ARID1A deficient) or TOV21.C1 or C2 (ARID1A proficient) cells. The ARID1A proficient cells failed to form tumours. B and C) Tumour volume plotted as the area under the curve (B) or over time (C) in untreated or CX-5461-treated mice.

Figure 10. CX-5461 treatment leads to apoptotic cell death, and acute treatment widens the therapeutic window for ARID1A-deficient cancer cells. A) Caspase 3/7 staining was used to monitor apoptosis in TOV21G (ARID1A deficient) and TOV21.C1 or C2 (ARID1A proficient) cells in untreated (top) or PDS-treated conditions (bottom) over 72h. B) Cell fate profiling shows that more of the ARID1A-proficient cells (C1 and C2) survive following PDS treatment. C) and D) ARID1A-deficient TOV21G cells (par or EV) and ARID1A-expressing clonal lines (C1 and C2) were treated with 10 μM PDS and cell growth relative to untreated cells was measured using SRB assays. The PDS was maintained in culture continuously (no washout) or for 4 hours (washout after 4 hours). Surviving fraction plotted in C shows a greater differential effect following acute exposure. Representative images of SRB assays are shown in D. Assays were performed in biological triplicate and plotted as the mean +/- SD.

The patent, scientific and technical literature referred to herein establish knowledge that was available to those skilled in the art at the time of filing. The entire disclosures of the issued patents, published and pending patent applications, and other publications that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of any inconsistencies, the present disclosure will prevail.

Various aspects of the invention are described in further detail below.

DETAILED DESCRIPTION

G quadruplex structures are prevalent in regulatory regions of the human genome; and instability at genome regions with G quadruplex structure forming potential has been found to shape the mutational burden of human cancers (Spiegel, et al., 2020, Bacolla, et al., 2019). SWI/SNF complexes function in DNA repair and replication stress response pathways (Brownlee, Meisenberg et al. 2015, Harrod, Lane et al. 2020), which are critical for preventing genome instability. The SMARCA4 (or BRG1) protein is an ATPase that forms the catalytic subunit of three highly related mammalian SWI/SNF complexes; BAF, PBAF and ncBAF (or GBAF) (Harrod et al., 2020). SMARCA4 was reported to bind G4 sequences *in vitro* and to map to G4-containing sequences in cells (Zhang, Spiegel et al. 2021)).

The invention is based on the surprising finding that stabilisation of G quadruplex structures using G quadruplex binding ligands strongly reduces the viability of SMARCA4 -deficient cells compared to SMARCA4 non-deficient cells.

The invention is also based on the surprising finding that stabilisation of G quadruplex structures using G quadruplex binding ligands strongly reduces the viability of ARID1A -deficient cells compared to ARID1A non-deficient cells.

Consequently, the inventors have surprisingly shown that stabilisation of G quadruplex structures using G quadruplex binding ligands strongly reduces the viability of SWI/SNF complex subunit deficient cells compared to SWI/SNF complex subunit non-deficient cells. The inventors have thus identified G quadruplex as a therapeutic target in SWI/SNF complex subunit deficient cells to specifically reduce the viability and/or kill SWI/SNF complex subunit deficient cells.

The invention therefore provides a method for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand.

The invention also provides pharmaceutical compositions for use in inducing cell death in SWI/SNF complex subunit deficient cancer cells. The invention therefore provides pharmaceutical compositions for use in inducing cell death in SMARCA4-deficient cancer cells and/or ARID1A-deficient cancer cells. The invention also provides methods for inducing cell death in such cells.

Methods for predicting the therapeutic effect to a G quadruplex binding ligand

Methods for predicting whether a test subject having cancer is likely to benefit from treatment with a G quadruplex binding ligands ARE therefore provided. The methods may comprise the steps of a) determining the level of one or more biomarker in a biological sample from the test subject, wherein the one or more biomarker is a subunit of the SWI/SNF complex; b) comparing the level of the one or more biomarker with a threshold level or range; and c) predicting that predicting that: i) the test subject will respond to treatment with a G quadruplex ligand if the test subject's biological sample has a decreased level of the one or more biomarker compared to the threshold level or range; and ii) the test subject will not respond to treatment with a G quadruplex ligand if the test subject's biological sample has an increased level of the one or more biomarker compared to the threshold level or range.

As used herein, the term "predicting" refers to the expected evolution of the treatment of a subject and refers to the prediction regarding the responsiveness of the subject to the treatment now or in the future (in order words, regarding the benefit of the treatment to the subject). It will be appreciated that a prediction might not always be accurate and/or informative. However, in the context of the invention, "predicting" requires that the prediction be accurate and/or informative regarding the responsiveness of the subject to the treatment in at least some of the a statistically significant part of subjects, for example in at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more of the subjects. It will be appreciated that the prediction may be not solely based on the levels of the one or more biomarkers, and may be made in conjunction with, for example, other clinical observations, such as symptom severity, subject's age and family history. Suitably, the prediction may provide an accurate and/or informative prediction regarding the benefit of the treatment to the subject in a statistically significant proportion of the subjects. The amount that is statistically significant can be established by a person skilled in the art by using different statistical tools, for example, but not limited to, by

determining confidence intervals, determining the significant p-value, Student's t-test or Fisher's discriminant function, non-parametric Mann-Whitney measurements, Spearman's correlation, logistic regression, linear regression, area under the ROC curve (AUC). Preferably, the confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99%. Preferably, the p-value is less than 0.1, than 0.05, than 0.01, than 0.005 or than 0.0001. In some examples, the prediction may be sufficiently accurate such that the term “predicting” can be used interchangeably with “determining” or “identifying”.

The methods are for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand. The methods may be for predicting whether a G quadruplex binding ligand treatment naïve subject will respond to G quadruplex binding ligand treatment in the future. Alternatively, the methods may be for predicting whether a subject that is currently undergoing treatment is responding to the treatment, and/or predicting whether they will respond to (ongoing) G quadruplex binding ligand treatment in the future. Based on these predictions, a decision can be made on whether G quadruplex binding ligand treatment should be started, continued, supplemented with additional treatments, or replaced, as appropriate.

The methods are for predicting whether a subject having cancer will respond to treatment with a G quadruplex ligand. Subjects that respond to the desired treatment are referred to as a “responder” herein. Subjects that do not respond to the desired treatment are referred to as a “non-responder” herein.

The term "responder" refers to a subject that shows a positive effect in response to a treatment. “G quadruplex binding ligand responder” therefore refers to a subject that shows a positive effect in response to G quadruplex binding ligand treatment. Responders and non-responders can be determined using pre-existing standard-of-care clinical (such as for example, different imaging modalities) and biochemical tests currently in use to determine whether a patient is responsive to treatment. For example, a subject having cancer (e.g. cancer patient) responsive to a treatment may have a complete response, partial response, or stable disease. Complete response, partial response and stable disease are well-known definitions in the field, as defined by the RECIST 1.1 Guidelines for Tumour Response Assessment. The skilled person is aware of the limitations of the RECIST 1.1 criteria with regards to the variability of tumour size measurements and tumoral heterogeneity both within a lesion and among different lesions in a patient. The skilled person also knows that there are limitations of RECIST 1.1 in precision cancer therapy and in particular in tumours harbouring specific genomic abnormalities treated with targeted therapy (for details see

Nishino, 2018). Furthermore, the skilled person knows that haematological malignancies are not staged by RECIST criteria but by complete blood cell counts comprising white blood cell count, red blood cell count, platelet count, haemoglobin concentration, and haematocrit concentration. In another example, the treatment responses “cure”, “complete remission/complete response”, “partial response”, “stable disease”, “disease-free survival”, “progression-free survival”, and “survival time” may be identified using the definition system described by the MSD Manual (https://www.msdmanuals.com/en-gb/professional/multimedia/table/defining-response-to-cancer-treatment). Therefore, a responder may have the treatment response “cure”, “complete remission/complete response”, “partial response”, or “stable disease”. A responder may have a longer “disease-free survival”, “progression-free survival”, and “survival time” compared to a non-responder. In yet another example, biochemical tests may include but are not limited to measuring the levels of pan-cancer biomarkers such as for example the detection of neurotrophic tyrosine receptor kinase (NTRK) gene fusions, microsatellite instability (MSI), and/or tumour mutational burden (TMB). Hence, a responder may have lower levels of the NTRK gene fusions, MSI and/or TMB burden compared to a non-responder. In even another example, biochemical tests may include measuring cancer type specific biomarkers such as for example, CA-125 (ovarian cancer) or PSA (prostate cancer). Thus, a responder may have lower levels of for example, CA-125 or PSA compared to a non-responder.

A "non-responder" refers to a subject that does not show a positive effect in response to a treatment. “G quadruplex binding ligand non-responder” therefore refers to a subject that does not show a positive effect in response to G quadruplex binding ligand treatment. A non-responder can be identified using the same criteria as for a “responder above”. For example, a subject having cancer (e.g. cancer patient) non-responsive to a treatment may have progressive disease. Progressive disease is a well-known definition in the field, as defined by the RECIST 1.1 Guidelines for Tumour Response Assessment. The skilled person is aware of the limitations of the RECIST 1.1 criteria with regards to the variability of tumour size measurements and tumoral heterogeneity both within a lesion and among different lesions in a patient. The skilled person also knows that there are limitations of RECIST 1.1 in precision cancer therapy and in particular in tumours harbouring specific genomic abnormalities treated with targeted therapy (for details see Nishino, 2018). Furthermore, the skilled person knows that haematological malignancies are not staged by RECIST criteria but by complete blood cell counts comprising white blood cell count, red blood cell count, platelet count, haemoglobin concentration, and haematocrit concentration. In another example, the treatment responses “disease-free survival”, “progression-free survival”, and “survival time” may be identified using the definition system described by the MSD Manual

(https://www.msdmanuals.com/en-gb/professional/multimedia/table/defining-response-to-cancer-treatment). Therefore, a non-responder may have a shorter “disease-free survival”, “progression-free survival”, and “survival time” compared to a responder. In yet another example, biochemical tests may include but are not limited to measuring the levels of pan-cancer biomarkers such as for example the detection of neurotrophic tyrosine receptor kinase (NTRK) gene fusions, microsatellite instability (MSI), and/or tumour mutational burden (TMB). Hence, a non-responder may have higher levels of the NTRK gene fusions, MSI and/or TMB burden compared to a responder. In even another example, biochemical tests may include measuring cancer type specific biomarkers such as for example, CA-125 (ovarian cancer) or PSA (prostate cancer). Thus, a non-responder may have higher levels of for example, CA-125 or PSA compared to a responder.

Subjects that are G quadruplex binding ligand non-responders are those whose cancer cannot be adequately treated with a G quadruplex binding ligand alone. In other words, they do not benefit from treatment with a G quadruplex binding ligand only. However, they may benefit from treatment with a G quadruplex binding ligand in combination with other cancer treatments, such as one or more cytotoxic agents. Treatment regimens comprising a G quadruplex binding ligand and another cancer treatment, such as a first cytotoxic agent (e.g. platinum agent) and/or a second cytotoxic agent (e.g. taxane) may therefore be selected for such subjects. Alternatively, treatment regimens that do not comprise a G quadruplex binding ligand but do include another cancer treatment(s), such as a first cytotoxic agent (e.g. platinum agent) and/or a second cytotoxic agent (e.g. taxane) may also be selected for such subjects. In other words, the G quadruplex binding ligand may be replaced with another treatment as a first cytotoxic agent (e.g. platinum agent) and/or a second cytotoxic agent (e.g. taxane) for treating cancer in such subjects.

Subjects that are G quadruplex binding ligand responders are those whose cancer can be effectively treated with a G quadruplex binding ligand alone. In other words, they do benefit from treatment with a G quadruplex binding ligand only. They do not require treatment with a G quadruplex binding ligand in combination with other cancer treatments per se, such as one or more cytotoxic agents. Treatment regimens comprising G quadruplex binding ligand as the only active ingredient may therefore be selected for such subjects. In the alternative, subjects that are G quadruplex binding ligand responders could benefit from a combination of G quadruplex binding ligand and other cancer treatments such as the combination of G quadruplex binding ligand with one or more cytotoxic agents, or further interventions such as surgery and/or radiation therapy. The alternative may depend on various clinical factors such as the TNM stage of the subject having cancer and/or the ECOG performance status of the

patient having cancer. The skilled person knows these concepts very well and regularly makes clinical decisions based on the TNM stage of the subject and/or the ECOG performance status.

The methods described herein comprise the step of comparing the level of the one or more biomarker (i.e. its amount per se or its activity) in the biological fluid sample (test sample) with a threshold level (or range) for the same biomarker.

As used herein, "biomarker threshold level", “threshold level”, “biomarker control level” or “control level” may also be referred to as a "cutoff value", “control value” or "threshold value". It refers to a biomarker level that can be used to distinguish between a first “condition” and a second “condition” (e.g., wherein the first condition may be individuals who are G quadruplex binding ligand responders and the second condition may be individuals who are G quadruplex binding ligand non-responders) such that a biomarker level in a sample that is above the threshold level indicates an increased likelihood of the individual having the second condition (e.g. being a G quadruplex binding ligand non-responder and thus requiring additional or alternative treatment for cancer than that provided by a G quadruplex binding ligand alone). Thus, a “threshold level" refers to an assay value (e.g., level of a biomarker), which is an approximate value that distinguishes the likelihood that a condition is present in the individual tested from the likelihood that a condition is not present in the individual tested, with a preselected specificity and/or sensitivity. Notably, the threshold may be a single value (“threshold level”) or a range of values (“threshold range”).

For example, a biomarker threshold level (or range) can represent an approximate level (or range) of a biomarker that detects affected subjects at a desired sensitivity (e.g., at least 55%, at least about 60%, at least 70%, or at least 80% or more). Thus, for example, an individual having a biomarker level that is greater than a threshold level (or range) has at least about 60% or greater likelihood of having that condition. It will be appreciated that the precise number value for threshold values (or ranges) can vary with the type of assay and reagents used to detect the biomarkers as well as the sensitivity and specificity desired from the assay. However, regardless of the assay and reagents used, the correlations between a threshold level (or range) and likelihood of a disease state (e.g., a cancer patient that is a G quadruplex binding ligand non-responder or G quadruplex binding ligand responder) will be present regardless of the assays and reagents used. Thus, as long as the test samples are assayed for the biomarker of interest using an assay platform and reagents of the same general type (e.g., protein assay) and similar sensitivity as the assay platform and reagents

used to determine the threshold level (or range) of the biomarker, the findings upon which the methods described herein are based will be preserved.

Appropriate threshold levels (or threshold ranges) can be determined using routine methods known in the art, e.g., by assaying levels of the biomarker(s) of interest in control populations, in cancer subjects known to be G quadruplex binding ligand responders, and in cancer subjects known to be G quadruplex binding ligand non-responders. Through application of statistical analysis, such methods can be used to identify a biomarker level that is present in at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 70%, at least 80%, or at least 90% or more of cancer patients that do not respond to G quadruplex binding ligand therapy and which provides a specificity of at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100%. Typically, the threshold level (or range) of the biomarker of interest may be determined by assaying the level of the biomarker in cancer patients that are known to respond to G quadruplex binding ligand therapy and assaying the level of the biomarker in cancer patients that are known to be non-responders to G quadruplex binding ligand therapy. The levels of the biomarker in such patients can be used to identify the range of biomarker levels that are observed in non-responders, which can be used to set the threshold level (or range).

The individuals used to generate the threshold level (or range) are typically of the same species, age or sex as the subject from which the test sample is obtained.

A threshold level can be single cut-off value, such as a median or mean. It can be a range of cut-off (or threshold) values, such as a confidence interval. It can be established based upon comparative groups, such as where the risk in one defined group is a fold higher, or lower, (e.g., approximately 2-fold, 4-fold, 8-fold, 16-fold or more) than the risk in another defined group. It can be a range, for example, where a population of subjects (e.g., control subjects) is divided equally (or unequally) into groups, such as a low-risk group, a medium-risk group and a high-risk group, or into quartiles, the lowest quartile being subjects with the lowest risk and the highest quartile being subjects with the highest risk, or into n-quantiles (i.e., n regularly spaced intervals) the lowest of the n-quantiles being subjects with the lowest risk and the highest of the n-quantiles being subjects with the highest risk. Moreover, the reference could be a calculated reference, most preferably the average or median, for the relative or absolute amount of a biomarker of a population of individuals comprising the subject to be investigated. How to calculate a suitable reference value, preferably, the

average or median, is well known in the art. The population of subjects referred to before shall comprise a plurality of individuals, preferably, at least 5, 10, 50, 100, 1,000 subjects.

Suitably, the level of the specific biomarker detected in a sample (e.g. a test sample, a control sample etc.) may be normalized by adjusting the measured level (amount or activity) of the biomarker using the level of a reference protein in the same sample, wherein the reference protein is not a marker itself (it is *e.g.*, a protein that is constitutively expressed). This normalization allows the comparison of the biomarker level in one sample to another sample, or between samples from different sources. This normalised level can then optionally be compared to the threshold level or range.

For example, when measuring a protein biomarker in a whole blood sample the biomarker may be expressed as an absolute concentration or, alternatively, it may be normalized against a known protein constitutively expressed in whole blood such as albumin, immunoglobulins or plasma protein concentration.

As another example, when measuring a protein biomarker in a serum sample the biomarker may be expressed as an absolute concentration or, alternatively, it may be normalized against a known protein constitutively expressed in serum.

The methods described herein include the step of predicting that the test subject will respond to treatment with a G quadruplex ligand (i.e. predicting that the test subject is a G quadruplex binding ligand responder if the test subject has a decreased level of the one or more biomarker compared to the threshold level or range. Conversely, if the test subject has an increased level of the one or more biomarker compared to the threshold level or range, the prediction is that the test subject will not respond to treatment with a G quadruplex ligand (i.e. the prediction is that the test subject is a G quadruplex binding ligand non-responder and thus requires additional or alternative treatment for cancer than that provided by a G quadruplex binding ligand alone.

The terms "decrease", "decreased" "reduced", "reduction" or 'down- regulated", “lower”, “less than” are all used herein generally to mean a decrease by a statistically significant amount. However, for avoidance of doubt, "reduced", "reduction", "decreased" or "decrease" etc means a decrease by at least 10% as compared to a reference level/control, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (i.e. absent level as compared to a

threshold value or range), or any decrease between 10-100% as compared to a threshold value or range, or at least about a 0.5-fold, or at least about a 1.0-fold, or at least about a 1.2-fold, or at least about a 1.5-fold, or at least about a 2-fold, or at least about a 3 -fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold decrease, or any decrease between 1.0-fold and 10-fold or greater as compared to a threshold value or range.

The terms "increased", "increase" or "up-regulated", “higher” etc are all used herein to generally mean an increase by a statically significant amount; for the avoidance of any doubt, the terms "increased" or "increase" means an increase of at least 10% as compared to a threshold value or range, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a threshold value or range, or at least about a 0.5-fold, or at least about a 1.0-fold, or at least about a 1.2-fold, or at least about a 1.5-fold, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5 -fold or at least about a 10-fold increase, or any increase between 1.0-fold and 10-fold or greater as compared to a threshold value or range.

The methods can further comprise selecting, and optionally administering, a treatment regimen for the subject based on the comparison of the levels of the biomarkers with the threshold level (or range). Treatment can include, for example, administration of one or more G quadruplex binding ligand, either on its own, or with additional cancer therapeutic agents, such as one or more cytotoxic agents. The treatment can therefore include a combination of a G quadruplex binding ligand and other cytotoxic agents. In other cases, the treatment may include cytotoxic agents other than a G quadruplex binding ligand (in other words, a cytotoxic agent such as a platinum agent (e.g. carboplatin), or a cytotoxic agent such as a taxane (e.g. docetaxel), without the addition of a G quadruplex binding ligand.

As an example, for test subjects that are predicted to be G quadruplex binding ligand non-responders, the method may further comprise selecting, or selecting and administering one or more cytotoxic agents. The one or more cytotoxic agents such as for example a platinum agent and/or a taxane may be selected, or selected and administered, in combination with a G quadruplex binding ligand, or without a G quadruplex binding ligand.

In general, the methods described are *in vitro* methods that are performed using a sample that has already been obtained from the subject (i.e. the sample is provided for the method,

and the steps taken to obtain the sample from the subject are not included as part of the method).

However, in some examples, the methods may include the step of providing a biological sample from a subject.

As used herein, “provide”, "obtain" or "obtaining" can be any means whereby one comes into possession of the sample by "direct" or "indirect" means. Directly obtaining a sample means performing a process (e.g., performing a physical method such as extraction) to obtain the sample. Indirectly obtaining a sample refers to receiving the sample from another party or source (e.g., a third party laboratory that directly acquired the sample).

As used herein, the terms "biological sample", “test sample”, "sample" and variations thereof refer to a sample obtained or derived from a subject. For the purposes described herein, the sample is, or comprises solid tissue samples such as a biopsy specimen, cells, tissue cultures, cells derived from tissue cultures and the progeny thereof. The samples being tested in the methods described herein are also referred to as “test samples”.

For the purposes described herein, the sample may be, or may comprise solid tissue samples such as a biopsy specimen, cells, tissue cultures, cells derived from tissue cultures and the progeny thereof.

In the alternative, the sample may be, or may comprise, a biological fluid (also referred to herein as a bodily fluid) sample. As used herein, the term “biological fluid sample” encompasses a blood sample. The term biological fluid sample also encompasses other bodily fluids such as a urine sample or a saliva sample.

The methods provided herein may therefore comprise providing a biological fluid sample (for example a blood sample, such as a serum or plasma sample) from a subject.

A solid tissue sample can be biopsy specimen, cells, tissue cultures, cells derived from tissue cultures and the progeny thereof. The sample may be collected by fine needle aspiration, scraping or washing a cavity to collects cells or tissue therefrom. The sample may be of a tumour such as, for example, solid and hematopoietic tumours as well as of neighbouring healthy tissue. The sample may be of a healthy tissue, diseased tissue or tissue suspected of being diseased tissue. The sample may be a smear of individual cells or a tissue section.

In a preferred example, the biological sample is a solid tissue sample.

A blood sample may be a whole blood sample, or a processed blood sample/ a blood fraction e.g. serum, plasma etc. Methods for obtaining biological fluid samples (e.g. whole blood, serum, plasma, urine, saliva etc) from a subject are well known in the art. For example, methods for obtaining blood samples from a subject are well known and include established techniques used in phlebotomy. The obtained blood samples may be further processed using standard techniques to obtain e.g. a serum sample, or a plasma sample. Advantageously, methods for obtaining biological fluid samples from a subject are typically low-invasive or non-invasive.

A whole blood sample is defined as a blood sample drawn from the human body and from which (substantially) no constituents (such as platelets or plasma) have been removed. In other words, the relative ratio of constituents in a whole blood sample is substantially the same as a blood in the body. In this context, “substantially the same” allows for a very small change in the relative ratio of the constituents of whole blood e.g. a change of up to 5%, up to 4%, up to 3%, up to 2%, up to 1% etc. Whole blood contains both the cell and fluid portions of blood. A whole blood sample may therefore also be defined as a blood sample with (substantially) all of its cellular components in plasma, wherein the cellular components (i.e. at least comprising the requisite white blood cells, red blood cells, platelets of blood) are intact.

Where the biological sample is a blood sample, the blood sample can be obtained from fresh blood or stored blood (e.g., in a blood bank). The biological sample can be a blood sample expressly obtained for the methods described herein or a blood sample obtained for another purpose which can be subsampled for the methods described herein. Cell free biological fluid samples include serum and plasma.

In a preferred example, the biological fluid sample is serum or plasma.

Samples can be manipulated after or during procurement, such as, by treatment with reagents (e.g., anti-coagulants), dilution, and/or enrichment for certain components for an analyte (s) to be assayed. Samples can be pre-treated as necessary by dilution in an appropriate buffer solution, concentrated if desired, or fractionated by any number of methods including but not limited to ultracentrifugation, fractionation by fast performance liquid chromatography (FPLC), precipitation, preservation, and fixation. Any of a number of

standard aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH can be used. In general, after isolation, samples (such as blood samples) are stored at - 80 °C until assaying. In the alternative, samples such as paraformaldehyde-fixed paraffin-embedded (FFPE) tissue blocks and sections thereof on, for example, microscopy slides are stored at room temperature. Therefore, the sample may be embedded in a matrix such as an FFPE block or fixated in a (buffered) paraformaldehyde solution.

Methods for analysing (and optionally isolating, enriching for or extracting) protein biomarkers from blood, plasma, serum etc samples have been described previously.

A biomarker is an organic biomolecule (e.g. a protein, polypeptide, peptide, isomeric form thereof, immunologically detectable fragment thereof, corresponding nucleic acid molecule (e.g. mRNA, cDNA etc)) which is differentially present in a sample taken from a subject having a disease (e.g. having a SWI/SNF subunit deficient cancer) as compared with a subject not having the disease (e.g. not having a SWI/SNF subunit deficient cancer). A biomarker is differentially present if the mean or median level of the biomarker in the different groups is calculated to be statistically significant. Common tests for statistical significance include, among others, t-test (e.g., student t-test), ANOVA, Kruskal-Wallis, Wilcoxon, Mann- Whitney, Receiver Operating Characteristic (ROC curve), accuracy and odds ratio. Biomarkers, alone or in combination, provide measures of relative risk that a subject belongs to one phenotypic status or another. Therefore, they are useful as markers for disease (diagnostics), therapeutic effectiveness of a drug and drug toxicity.

The methods described herein help predict whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand. Therefore, the methods also may help determining treatment and/or stratifying subjects based on detection of decreased levels of one or more biomarkers, wherein the one or more biomarker is a subunit of the SWI/SNF complex.

Typically, the biomarker (i.e. the subunit of the SWI/SNF complex) referred to herein is measured at the protein level. Details of each of the biomarkers is provided below.

The level of biomarker present in the biological fluid sample may be determined by e.g. assaying the amount of protein biomarker present in the sample. Assays for measuring the amount of a specified protein are well known in the art and include direct or indirect measures.

The level of protein biomarker in a sample may also be determined by determining the level of protein biomarker activity in a sample. Accordingly, protein “level” encompasses both the amount of protein per se, or its level of activity.

By way of example, the level of a protein biomarker in a biological fluid sample can be determined (e.g., measured) by any suitable methods and materials known in the art, including, for example, a process selected from the group consisting of mass spectrometry, immunoassays, enzymatic assays, spectrophotometry, colorimetry, fluorometry, bacterial assays, protein microarrays, compound separation techniques, or other known techniques for determining the presence and/or quantity of an analyte such as immunohistochemistry or immunofluorescence. Examples of relevant techniques include enzyme linked immunosorbent assays (ELISAs), immunoprecipitation, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), Western blot analysis, and Lateral Flow (using e.g. Lateral Flow Devices (LFDs) utilizing a membrane bound antibody specific to the protein biomarker). Preferably, the level of a protein biomarker in a biological fluid sample is measured by ELISA or lateral flow. Preferably, the level of a protein biomarker in a solid tissue sample is measured by immunohistochemistry or immunofluorescence.

The methods described herein help predict whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand. Therefore, the methods also may help determining treatment and/or stratifying subjects based on detection of decreased levels of one or more biomarkers, where the one or more biomarkers is a variant, variant gene or gene variant that encodes a subunit of the SWI/SNF complex as defined elsewhere herein. In some examples, the determination of one or more biomarkers is across the whole genome of a subject’s tumour such as by whole genome sequencing. In some examples, the determination of one or more biomarkers may be more targeted, for example by sequencing parts of a subject’s tumour genome. For example, selected genes may be sequenced in a targeted panel containing only genes SWI/SNF complex subunits described here.

As used herein, the terms “variant”, “variant gene” and "gene variant" refer to any change in nucleotide sequence relative to the native or wild type sequences. These terms are used interchangeably with “mutant”, “mutant gene” and “gene mutation”. Examples include, but are not limited to, single nucleotide polymorphisms (SNPs), deletions, inversions, splice variants, frameshift variants, nonsense variants or haplotypes.

As is described herein, the invention is particularly relevant to cancer and cancer cells that are deficient in a SWI/SNF subunit (also referred to as being deficient in a subunit of the SWI/SNF complex herein). The methods provided herein provide a prediction on whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand based on an analysis of whether the cancer has a decreased level of a subunit of the SWI/SNF complex (i.e. whether the cancer is deficient in a SWI/SNI complex).

The phrases “deficient in a SWI/SNF subunit” and “deficient in a subunit of the SWI/SNF complex” are used interchangeably herein.

In some examples, “deficient in a SWI/SNF subunit” means that there is a deleterious variant of the SWI/SNF subunit, yielding a decreased level of the one or more biomarker. In some examples deficient in a SWI/SNF subunit means a deleterious variant or mutation is present. "Deleterious mutation" and “deleterious variant” refer to variants or mutations that compromise or alter the normal function of a gene product for example by decreasing or increasing activity of the gene product or alters expression of the gene product in the subject for example by decreasing or increasing expression of the gene product. In some examples, “deficient in a SWI/SNF subunit” may be a loss of function variant or mutation. The term “loss of function mutation” refers to a mutation that results in a gene product no longer being able to perform its normal function or its normal level of activity, in whole or in part. Loss of function mutations are also referred to as inactivating mutations and typically result in the gene product having less or no function, i.e., being partially or wholly inactivated (e.g., a non-functional protein has less than 50%, 40%, 30%, 20%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or less activity than its native or wild-type counterpart).

In some examples, “deficient in a SWI/SNF subunit” means that there is a likely deleterious variant of the SWI/SNF subunit. Therefore, in some examples, the “deficient in a SWI/SNF subunit” is a predicted deleterious mutant. Determination of whether “deficient in a SWI/SWF subunit” is likely to be deleterious or is predicted to be deleterious may be done using any suitable variant annotation tool. For example using, SnpEff, Combined Annotation Dependent Depletion (CADD), ANNOVAR, AnnTools, NGS-SNP, Sequence Variant Analyzer (SVA), SeattleSeq Annotation Server, Variant (VARIANT), Variant Effect Predictor (VEP) or combinations thereof. In some examples, the germline variant is predicted to be likely to be deleterious as determined by Variant Effect Predictor (VEP) see “Variant Effect Predictor,” Genome Biology 17, p. 122, doi: 10.1186/s13059-016-0974-4 each of which is hereby incorporated by reference.

In some examples, “deficient in a SWI/SNF subunit” means that there is a predicted or likely loss of function variant of the SWI/SNF subunit. Determination of predicted or likely loss of function variants may be done using any suitable annotation and/or prediction tools such as loss-of-function transcript effect estimator (LOFTEE) (available via https://github.com/konradjk/loftee).

In some examples, “deficient in a SWI/SNF subunit” means that there is a protein-truncating variant of the SWI/SNF subunit or mutation. For example a protein-truncating loss of function and/or deleterious or predicted loss of function and/or deleterious mutation. Protein-truncating variants are genetic variants that are predicted to or do shorten the coding sequence of a gene, through for example a stop-gain mutation. Protein-truncating variants are sometimes categorized under the umbrella term frameshift or truncating variants (FTVs), which includes both protein-truncating variants and DNA variants caused by frameshift mutation.

In some examples, “deficient in a SWI/SNF subunit” means that is a nonsense variant, frameshift variant or splice site variant. Nonsense mutation or variant refers to a mutation in which a sense codon that corresponds to one of the twenty amino acids specified by the genetic code is changed to a chain-terminating codon (i.e. stop codon). Frameshift mutation or variant refers to a mutation caused by the addition or deletion of a base pair or base pairs in the DNA of a gene resulting in the translation of the genetic code in an unnatural reading frame from the position of the mutation to the end of the gene. Splice site variant or mutation refers to a genetic alteration in the DNA sequence of a gene that occurs at the boundary of an exon and an intron (splice site). This change can disrupt RNA splicing resulting in the loss of exons or the inclusion of introns and an altered protein-coding sequence.

In some examples, “deficient in a SWI/SNF subunit” means that the variation or mutation occurs in the first 95% of the protein encoded by the variant gene. For example a protein-truncating loss of function and/or deleterious or predicted loss of function and/or deleterious mutation occurring in the first 95% of a protein encoded by the gene.

In some examples, “deficient in a SWI/SNF subunit” means that there may be a missense variant. A missense mutation or variant lead to a change in a single base pair that causes the substitution of an amino acid for a different amino acid in the resulting protein, in particular, a non-conservative amino acid substitution.

Pharmaceutical Compositions

A pharmaceutical composition comprising a G quadruplex binding ligand for use in inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex in a subject is therefore provided. Also provided is a pharmaceutical composition comprising a G quadruplex binding ligand for use in inducing cell death of a cancer cell deficient in SMARCA4 and/or ARID1A in a subject.

Also provided is a method of inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex, the method comprising subjecting the cell to a G quadruplex binding ligand. Also provided is a method of inducing cell death of a cancer cell deficient in SMARCA4 and/or ARID1A, the method comprising subjecting the cell to a G quadruplex binding ligand.

The methods described herein may be performed *in vitro, ex vivo,* or *in vivo*.

The pharmaceutical compositions and methods described herein cell death of a cancer cell deficient in a subunit of the SWI/SNF complex. The pharmaceutical compositions and methods described herein induce cell death in a cancer cell deficient in SMARCA4 and/or ARID1A.

As used herein, “a cell deficient in a subunit of the SWI/SNF complex” (also referred to as “the cell” herein) refers to a cell characterized by a lack, or a deficiency of a functional subunit of the SWI/SNF complex. Due to the nature of the SWI/SNF complex, a lack, or a deficiency of a functional subunit such as SMARCA4 can lead to reduced or abrogated activity of the SWI/SNF complex in its entirety. In other words, a cell deficient in a subunit of the SWI/SNF complex has a reduced or abrogated ability to remodel chromatin *inter alia*.

Cells deficient in a subunit of the SWI/SNF complex may therefore be readily identified by a reduced or abrogated ability to remodel chromatin. Alternatively, SWI/SNF subunit complex deficiency can be determined by analysis of the SWI/SNF subunit complex expression level using, for example Western blotting, ELISA, immunofluorescence, immunohistochemistry, mass spectrometry, RT-PCR, nucleic acid hybridisation, RNA-sequencing (RNA-seq), or karyotypic analysis. The terms “deficient in SWI/SNF complex”, “deficient in a SWI/SNF subunit complex”, “SWI/SNF complex deficient”, “SWI/SNF subunit complex deficient” and equivalents are used interchangeably herein.

As used herein, “SWI/SNF complex” refers to any SWI/SNF complex such as for example BRG1/BRM-associated factor (BAF), non-canonical BAF (ncBAF) and any SWI/SNF complex which will be discovered in future. In one example, “SWI/SNF complex” refers to BRG1/BRM-associated factor (BAF), and non-canonical BAF (ncBAF). In another example, “SWI/SNF complex” refers to BRG1/BRM-associated factor (BAF), non-canonical BAF (ncBAF) and any SWI/SNF complex which will be discovered in future and any SWI/SNF complex which will be discovered in future. The terms “SWI/SNF complex” and “SWI/SNF” are used interchangeably herein.

As used herein, “subunit of a SWI/SNF complex” refers to any subunit of a SWI/SNF complex. Therefore, a subunit of the SWI/SNF complex can be any one of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2 as well as combinations thereof. In one example, a subunit of the SWI/SNF complex can be any one of ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2 as well as combinations thereof.

As used herein, “a cell deficient in SMARCA4” (also referred to as “the cell” herein) refers to a cell characterized by a lack of, or a deficiency in, a functional SMARCA4 domain due to for example one or mutations as defined elsewhere herein. SMARCA4 deficiency can be defined by analysis of SMARCA4 expression level using, for example Western blotting, ELISA, immunofluorescence, immunohistochemistry, mass spectrometry, RT-PCR, nucleic acid hybridisation, RNA-sequencing (RNA-seq), or karyotypic analysis. The terms “deficient in SMARCA4”, and “SMARCA4 deficient” and equivalents are used interchangeably herein.

As used herein, “a cell deficient in ARID1A” (also referred to as “the cell” herein) refers to a cell characterised by a lack of, or a deficiency in, a functional ARID1A domain. ARID1A deficiency can be defined by analysis of ARID1A expression level using, for example Western blotting, ELISA, immunofluorescence, immunohistochemistry, mass spectrometry, RT-PCR, nucleic acid hybridisation, RNA-sequencing (RNA-seq), or karyotypic analysis. The terms “deficient in ARID1A”, and “ARID1A deficient” and equivalents are used interchangeably herein.

Generally, SWI/SNF-deficiency arises from a mutation or mutations in one or more SWI/SNF-associated genes, such as but not limited to the genes encoding ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA,

BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2 , SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In one example, SWI/SNF-deficiency arises from a mutation or mutations in one or more SWI/SNF-associated genes, such as the genes encoding ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2 , SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

Methods of identifying a cell (or cells) deficient in a subunit of the SWI/SNF complex are well known in the art. The SWI/SNF subunit status of a cell can be determined by, for example, sequence analysis of SWI/SNF subunit genes. Suitable sequencing techniques are readily identifiable to a person skilled in the art and may include whole genome sequencing. Whole genome sequencing may be conducted on cells and/or tumour samples. The SWI/SNF subunit status of a cell can also be determined by, for example, a SWI/SNF complex subunit specific CGH classifier or determining the epigenetic status of a SWI/SNF complex subunit such as the methylation status of for example SMARCA4 or ARID1A. Expression levels may be determined, for example, by Western blot, ELISA, RT-PCR, nucleic acid hybridisation, RNA-sequencing (RNA-seq), mass spectrometry or karyotypic analysis.

In some examples, the cell deficient in a subunit of the SWI/SNF complex is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, and SS18L1.

In a particular example, the cell deficient in a subunit of the SWI/SNF complex is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, and SS18L1.

In a particular example, the cell deficient in a subunit of the SWI/SNF complex is deficient in ARID1A and/or SMARCA4.

In another example, the cell deficient in a subunit of the SWI/SNF complex is deficient in ARID1A.

In yet another example, the cell deficient in a subunit of the SWI/SNF complex is deficient in SMARCA4.

ARID1A and SMARCA4 are both known subunits of the SWI/SNF complex. As discussed elsewhere herein, one of the functions of the SWI/SNF complex appears to be a genome maintenance factor. The association of *ARID1A* and *SMRACA4* mutations with human cancers is well-characterized in the art. Methods to identify *ARID1A* and or *SMARCA4* mutations, e.g. mutations in either of the *ARID1A* and *SMARCA4* genes, are commonly known in the art and described elsewhere herein.

As described herein, a cell deficient in one or more proteins may be deficient in one or more proteins through either absence of the protein, a reduction in amount of the protein (compared to non-deficient cells), or dysfunction of the protein, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of mutation or polymorphism in a gene encoding a regulatory factor. Accordingly, as used herein, a cell deficient in one or more proteins is a cell wherein expression and/or activity of the one or more proteins or a nucleic acid (DNA or RNA) encoding the one or more proteins is reduced, abolished or dysfunctional relative to a control cell (e.g. a non-deficient cell). A cell deficient in one or more proteins may, for example, be heterozygous or homozygous for mutations or polymorphisms in the nucleic acid encoding the protein, or its regulatory elements, which reduce expression or activity.

As would be appreciated by the skilled person, a cell deficient in one or more proteins may possess a level (i.e. an amount) or activity of the protein which is less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the normal level of the active protein (e.g. in a non-deficient cell). A deficient cell includes a null cell which contains no active protein or substantially no active protein i.e. the activity of the protein, is abolished or substantially abolished in a null cell.

In some examples, the expression and/or activity of the one or more proteins may be reduced by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or at least 1000-fold. In some examples, the expression and/or activity of the one or more proteins may be reduced by about 2-fold to about 500-fold.

By way of non-limiting example, a cell deficient in ARID1A may be deficient in ARID1A through either absence of the protein, a reduction in amount of the protein (compared to non-deficient cells), or dysfunction of the protein, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of mutation or polymorphism in a

gene encoding a regulatory factor. Accordingly, a cell deficient in ARID1A is a cell wherein expression and/or activity of ARID1A or a nucleic acid (DNA or RNA) encoding ARID1A is reduced, abolished or dysfunctional relative to a control (e.g. the normal level of the active protein (e.g. in a non-deficient cell)). A cell deficient in the ARID1A protein may, for example, be heterozygous or homozygous for mutations or polymorphisms in the nucleic acid encoding the protein, or its regulatory elements, which reduce expression or activity. Accordingly, as would be appreciated by the skilled person, a cell deficient in ARID1A may possess a level (i.e. an amount) or activity of the protein which is less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the normal level of the active protein (e.g. in a non-deficient cell).

In some examples, the expression and/or activity of ARID1A may be reduced by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or at least 1000-fold. In some examples, the expression and/or activity of ARID1A may be reduced by about 2-fold to about 500-fold.

In another non-limiting example, a cell deficient in the SMARCA4 may be deficient in SMARCA4 through either absence of the protein, a reduction in amount of the protein (compared to non-deficient cells), or dysfunction of the protein, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of mutation or polymorphism in a gene encoding a regulatory factor. Accordingly, a cell deficient in SMARCA4 is a cell wherein expression and/or activity of SMARCA4 or a nucleic acid (DNA or RNA) encoding SMARCA4 is reduced, abolished or dysfunctional relative to a control (e.g. the normal level of the active protein (e.g. in a non-deficient cell)). A cell deficient in the SMARCA4 protein may, for example, be heterozygous or homozygous for mutations or polymorphisms in the nucleic acid encoding the protein, or its regulatory elements, which reduce expression or activity. Accordingly, as would be appreciated by the skilled person, a cell deficient in SMARCA4 may possess a level (i.e. an amount) or activity of the protein which is less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the normal level of the active protein (e.g. in a non-deficient cell).

In some examples, the expression and/or activity of SMARCA4 may be reduced by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, or at least 1000-fold. In some examples, the expression and/or activity of SMARCA4 may be reduced by about 2-fold to about 500-fold.

The expression status of the one or more proteins can be determined, for example, by measuring the level of mRNA and/or protein using methods known in the art, such as but not limited to, Northern blot, quantitative PCR, nucleic acid microarray technologies, Western blot, ELISA or ELISPOT, antibodies microarrays, RNA-sequencing (RNA-seq), mass spectrometry or immunohistochemistry.

The activity level of one or more proteins can be determined using routine methods known in the art (e.g. measuring enzyme activity etc).

The pharmaceutical compositions and methods described herein induce cell death of a cancer cell deficient in a subunit of the SWI/SNF complex.

The cell may be *in vitro*, *ex vivo* or *in vivo*.

In a particular example, the cell is a cancer cell. The cancer cell may be *in vitro*, *ex vivo* or *in vivo*. For example, it may be part of an *in vivo* cancer (or tumour). The compositions and methods described herein may therefore be used to induce cell death of SWI/SNF complex-subunit deficient cancer cells (and thus may be used to treat SWI/SNF complex subunit-deficient cancers or tumours).

Cancer cells in general are characterised by abnormal proliferation relative to normal or healthy cells and typically form clusters or tumours in an individual having a cancer condition. As would be clear to a person skilled in the art, a cancer may comprise one or more cancer cells. Accordingly, an individual having a cancer condition may comprise one or more cancer cells. Methods of identifying cancer and cancer cell(s) are well known in the art.

It is well known that SWI/SNF complexes are commonly mutated in human cancers at and their exact role as potential tumour suppressors or oncogenes has to be further elucidated in the development of cancer. Examples of cancers known to have mutations in genes of the SWI/SNF complex (and are, thus, considered as SWI/SNF complex subunit deficient cancers herein) include, but are not limited to, ovarian cancer, breast cancer, prostate cancer, non-Hodgkin's lymphoma, colon cancer, lipoma, uterine leiomyoma, basal cell skin carcinoma, squamous cell skin carcinoma, osteosarcoma, acute myelogenous leukemia (AML), and other cancers (See, e.g. ,Mittal and Roberts, 2020).

In some examples, the compositions and methods described herein may be used to induce cell death of one or more SWI/SNF complex subunit deficient cancer cells selected from the

group consisting of: adrenocortical, rhabdoid, sarcoma, lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, cervical, breast, melanoma, bladder, endometrial, head and neck, liver and prostate cancer cell. By way of a non-limiting example, an ovarian cancer cell is a cancer cell obtained from an ovarian cancer (and thus may be used to treat these SWI/SNF-deficient cancers or tumours).

In a specific example, the compositions and methods described herein may be used to induce cell death of one or more SMARCA4 -deficient cancer cells selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lung, non-small cell lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, ovarian clear cell, cervical, breast, melanoma, bladder, endometrial, head and neck, renal, liver and prostate cancer cell.

In a specific example, the compositions and methods described herein may be used to induce cell death of one or more ARID1A -deficient cancer cells selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lung, non-small cell lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, ovarian clear cell, cervical, breast, melanoma, bladder, endometrial, head and neck, renal, liver and prostate cancer cell.

As would be appreciated by the person skilled in the art, the SWI/SNF subunit deficient cancer cell may be deficient in one or more proteins selected from the group consisting of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, and SS18L1.

In a particular example, the SWI/SNF subunit deficient cancer cell is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, and SS18L1.

In an example, the SWI/SNF subunit deficient cancer cell is deficient in SMARCA4 and/or ARID1A.

In another example, the SWI/SNF subunit deficient cancer cell is deficient in SMARCA4.

In yet another example, the SWI/SNF subunit deficient cancer cell is deficient in ARID1A.

The invention is based on the surprising finding that treatment with a G quadruplex binding ligand is toxic to cells deficient in a subunit of the SWI/SNF complex. The pharmaceutical compositions and methods provided herein therefore specifically induce cell death of a cancer cell deficient in a subunit of the SWI/SNF complex subunit by subjecting the cell to a G quadruplex binding ligand.

As used herein, “binding ligands” refers to any agent that inhibits, reduces, slows, halts, blocks, suppresses, abolishes and/or prevents the activity, and/or formation of a target structure in a cell. A binding ligand may reduce, slow, halt, block, suppress, abolish and/or prevent the activity , and/or formation of a target structure in a cell relative to a cell subjected to a control (or a cell that is not subjected to the binding ligand). As would be clear to a person skilled in the art, a binding ligand may function at the level of the target structure. The term “binding ligand” as used herein may be used to refer to a G quadruplex binding ligand.

In some examples, "bind", “reduce”, "block", "suppress", "prevent" etc means that the activity being blocked, reduced, suppressed, or prevented is reduced by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% as compared to the activity of a control (e.g., activity in the absence of the binding ligand). In some examples, "block", “reduce”, "suppress", "prevent" etc means that the formation of the target of the binding ligand, for example the G quadruplex binding ligand is reduced by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% as compared to a control (e.g., the expression in the absence of the binding ligand).

G quadruplex structures are secondary structures formed in nucleic acids by guanine rich sequences and are therefore, found at guanine rich motifs in a cell. Therefore, G quadruplex structures can be found at for example, promoters and telomeres which are guanine rich. G quadruplex structures are helical in shape and comprise so called guanine tetrads forming one or more strands. Guanine tetrads are a planar structure formed by four guanine cases and associated by Hoogsten hydrogen bonding. G quadruplex structures are formed by two or more guanine tetrads. The G quadruplex structure is further stabilised by the presence of a cation, preferably potassium. Said cation sits in a central channel between each pair of tetrads

Preferably, the G quadruplex structure is a DNA G quadruplex structure.

In some examples, the pharmaceutical compositions and methods provided herein specifically induce cell death of a cancer cell deficient in a subunit of the SWI/SNF complex by subjecting the cell to a G quadruplex binding ligand.

As used herein, a "G quadruplex binding ligand" refers to any agent that stabilises the G quadruplex structure at guanine rich motifs in a cell, for example, promoters and telomeres and thereby inhibits, reduces, slows, halts, blocks, supresses, abolishes and/or prevents the activity and/or expression of genes in a cell. Accordingly, as would be clear to a person skilled in the art, there are G quadruplex binding ligand which are "pan binders" and therefore, bind any G quadruplex structure.

It is also clear to the skilled person that there are also G quadruplex binding ligands which selectively bind to G quadruplex binding ligands of target genes of interest. Exemplary G quadruplex binding ligand binding to target genes of interest are for example phenanthrolines binding to G quadruplex structures of *c-KIT* and *c-MYC*.

Accordingly, G quadruplex binding ligand can be an agent (e.g. an inhibitory nucleic acid, a binding molecule (e.g. a small molecule or an antibody), or peptides. A person of skill in the art will be able to readily identify suitable G quadruplex binding ligands using known methods in the art.

Preferably, the G quadruplex binding ligand is a "pan binder". As used herein, a "pan binder" binds to any G quadruplex structure in a cell of interest. The G quadruplex binding ligand can be for example any of CX-3543, CX-5461, pyridostatin, pyridodicarboxamide (PDC), Tmpyp4, CMO3, NDI1, PDI1,3, pyrvinium pamoate, WZZ02, MTR106, telomestatin or combinations thereof.

In one example, the G quadruplex binding ligand is CX-3543.

In another example, the G quadruplex binding ligand is CX-5461.

In yet another example, the G quadruplex binding ligand is pyridostatin.

In another non-limiting example, the G quadruplex binding ligand can be any G quadruplex binding ligand found on the publicly available database G4DLB2.2 (http://www.g4ldb.com).

The skilled person is able to retrieve and identify a suitable G quadruplex binding ligand in said database.

In some examples, a G quadruplex binding ligand is a molecule that reduces or prevents expression of target genes such as *c-KIT, c-MYC, k-RAS, STAT3, BCL2* .

As described herein, the G quadruplex binding ligand may be a targeted binding ligand. As used herein, "targeted binding ligand" refers to G quadruplex binding ligand that directly targets the target gene, transcript or protein. Thereby, for example oncogene transcription is abolished. In the alternative, telomere dysfunction could be averted. Therefore, the targeted binding ligand directly inhibits the protein, an transcript and/or gene, thereby reducing the associated protein activity and/or expression. Non-limiting examples of targets are *c-KIT, c-MYC, k-RAS, STAT3, BCL2*. Another non-limiting example are telomeres.

Accordingly, in some examples, the G quadruplex binding ligand directly targets c-KIT, c-MYC, k-RAS, STAT3, and/or BCL2 gene, RNA transcript or protein. For example, the G quadruplex binding ligand may directly bind to the c-KIT, c-MYC, k-RAS, STAT3, and/or BCL2 gene, RNA transcript or protein.

Any suitable G quadruplex binding ligand may be used. In a particular example, the G quadruplex binding ligand may be selected from the group consisting of: an inhibitory nucleic acid, a binding molecule (e.g. a small molecule or an antibody (including functional fragments thereof)) and a peptide. Suitable examples of, binding molecules (e.g. small molecules, or antibodies (including functional fragments thereof)) or peptides would be readily identifiable to a person of skill in the art.

In some examples, the G quadruplex binding ligand is a binding molecule. Preferably, the binding molecule binds to G quadruplex structures and inhibits their activity (for example it inhibits one or more of the activities for G quadruplex described elsewhere herein). In a non-limiting example, the binding molecule may bind to any G quadruplex structure which is also known in the art as "pan binder". In another example, the binding molecule binds to telomeres. In yet another example, the binding molecule binds to the G quadruplex structure of a specific target but not limited to *c-KIT, c-MYC, k-RAS, STAT3,* and *BCL2* and thereby inhibits, reduces, slows, halts, blocks, suppresses, abolishes and/or prevents for example, oncogene transcription. In even another example, the binding molecule binds to the G quadruplex structure of telomeres and thereby inhibits, reduces, slows, halts, blocks, suppresses, abolishes and/or prevents for example, telomere dysfunction.

An example of a binding molecule is a small molecule. Binding molecules also include antibodies as well as non-immunoglobulin binding agents, such as phage display-derived peptide binders, and antibody mimics, e.g., affibodies, tetranectins (CTLDs), adnectins (monobodies), anticalins, DARPins (ankyrins), avimers, iMabs, microbodies, peptide aptamers, Kunitz domains, aptamers and affilins. The term "antibody" includes, for example, both naturally occurring and non-naturally occurring antibodies, polyclonal and monoclonal antibodies, chimeric antibodies and wholly synthetic antibodies and fragments thereof, such as, for example, the Fab', F(ab')2, Fv or Fab fragments, or other antigen recognizing immunoglobulin fragments. Antibodies which bind a particular epitope can be generated by methods known in the art. For example, polyclonal antibodies can be made by the conventional method of immunizing a mammal (e.g., rabbits, mice, rats, sheep, goats). Polyclonal antibodies are then contained in the sera of the immunized animals and can be isolated using standard procedures (e.g., affinity chromatography, immunoprecipitation, size exclusion chromatography, and ion exchange chromatography). Monoclonal antibodies can be made by the conventional method of immunization of a mammal, followed by isolation of plasma B cells producing the monoclonal antibodies of interest and fusion with a myeloma cell (see, e.g., Mishell, et al., 1980). Screening for recognition of the epitope can be performed using standard immunoassay methods including ELISA techniques, radioimmunoassays, immunofluorescence, immunohistochemistry, and Western blotting (Ausubel, et al., 1992). *In vitro* methods of antibody selection, such as antibody phage display, may also be used to generate antibodies (see, e.g., Schirrmann et al. 2011). Preferably, a nuclear localisation signal is added to the antibody in order to increase localization to the nucleus.

In one example, the G quadruplex binding ligand is G quadruplex antibody.

The pharmaceutical compositions and methods described herein reduce the viability of a cell deficient in at least one subunit of the SWI/SNF complex by subjecting the cell to a G quadruplex binding ligand. The reduction in cell viability is due to the synthetic lethal relationship between HR-deficiency and loss of EXO1 or BLM. Synthetic lethality is a term which was first used in genetics and defines that loss of gene A or gene B as a single event is well-tolerated, but loss of both genes simultaneously is severely toxic. Synthetic lethality can also be observed between two drug treatments and between a genetic deficiency and a drug treatment targeting A or B. Hence, a synthetic lethality as used herein is the deficiency of one subunit of a SWI/SNF complex and a G quadruplex binding ligand.

As described herein, subjecting the cell deficient in at least one subunit of the SWI/SNF complex to a G quadruplex binding ligand may include exposing and/or introducing the cell to a G quadruplex binding ligand under conditions suitable to reduce the viability of the cell. Appropriate conditions are readily identifiable to a person of skill in the art using routine experimentation.

In some examples, the cell may be subjected to the G quadruplex binding ligand *in vitro* or *ex vivo*.

In some examples, the cell may be subjected to the G quadruplex binding ligand *in vivo*.

Methods for determining cell death are well known in the art. For example cell viability may be assessed by Trypan blue exclusion assay or other similar means. Other similar means include the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay and examination of cell colony formation post extraction.

As detailed elsewhere herein, the methods described herein may be performed *in vivo*. In this context, the method may comprise inducing cell death of a cell deficient in a subunit of the SWI/SNF complex in a subject by administering a G quadruplex binding ligand to the subject. Accordingly, in some examples, the cell deficient in a subunit of the SWI/SNF complex may be in a subject.

The pharmaceutical composition or method may therefore be for treating SWI/SNF complex subunit deficient cancer (or malignant tumour) in a subject, wherein the SWI/SNF complex subunit deficient cancer (or malignant tumour) is treated by inducing cell death of SWI/SNF subunit deficient cells (cancer cells) within the subject. Accordingly, any reference to "inducing cell death of a SWI/SNF subunit deficient cell" herein may also be for the purpose of inducing cell death of a SWI/SNF subunit-deficient cancer cell within a subject suffering from or afflicted with a SWI/SNF subunit deficient cancer.

The pharmaceutical composition or method may alternatively be for treating a SWI/SNF complex subunit deficient benign tumour in a subject.

As used herein, the term "subject" is intended to include humans and animals. Typically, a subject refers to a human or animal that, within their body, has a cell deficient in homologous recombination. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In some

examples, subjects include companion animals, e.g. dogs, cats, rabbits, and rats. In some examples, subjects include livestock, e.g., cows, pigs, sheep, goats, and rabbits. In some examples, subjects include thoroughbred or show animals, e.g. horses, pigs, cows, and rabbits.

As discussed elsewhere herein, in some examples, the cell may be a cancer cell. Accordingly, the subject may be suffering from or afflicted with a cancer or any disorder involving, directly or indirectly, a cancer, comprising one or more cells deficient in a subunit of the SWI/SNF complex. Accordingly, in some examples, the subject may be suffering from or afflicted with a SWI/SNF deficient cancer.

In a particular example, the SWI/SNF subunit deficient cancer may be a cancer deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In another particular example, the SWI/SNF subunit deficient cancer may be a cancer deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

In some examples, the SWI/SNF deficient cancer may be a SMARCA4 and/or ARID1A deficient cancer. In a particular example, the SWI/SNF deficient cancer may be a SMARCA4 deficient cancer deficient cancer. In a particular example, the SWI/SNF deficient cancer may be an ARID1A deficient cancer deficient cancer.

The subject may be symptomatic (e.g., the subject presents symptoms associated with having a cell deficient in a subunit of the SWI/SNF complex (e.g. cancer)), or the subject may be asymptomatic (e.g., the subject does not present symptoms associated with having a cell deficient in a subunit of the SWI/SNF complex (e.g. cancer)).

In some examples, the subject is a human. For example, the subject may be a human having, at risk of having, or potentially capable of having a cancer deficient in a SWI/SNF complex subunit (e.g. a SMARCA4 and/or ARID1A deficient cancer).

In some examples, the subject has been identified as having a SWI/SNF complex subunit deficient cancer. In some examples, the subject has been identified as having a SWI/SNF complex subunit deficient cancer deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In some examples, the subject has been identified as having a SWI/SNF complex subunit deficient cancer deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In some examples, the subject has been identified as having a cancer deficient in SMARCA4 and/or ARID1A. In another example the subject has been identified as having a cancer deficient in SMARCA4. In yet another example the subject has been identified as having a cancer deficient in ARID1A. Methods of identifying such a cancer are discussed elsewhere herein.

The subject may be referred to herein as a patient i.e. a subject in need of treatment. A subject in need of treatment is a subject identified as having a SWI/SNF subunit deficient cancer, i.e., the subject has been diagnosed by a physician as having a SWI/SNF subunit deficient cancer. As discussed elsewhere herein, methods of identifying cancer cells and methods of identifying cells deficient in a subunit of the SWI/SNF complex are well known in the art. The subject may have received treatment for the condition, disorder or symptom. Alternatively, the subject has not been treated prior to the treatment described herein. The terms “subject”, “individual”, and “patient” are used herein interchangeably.

As used herein, the terms “treat”, “treating” and "treatment" are taken to include an intervention performed with the intention of preventing the development or altering the pathology of a condition, disorder or symptom (e.g. a hyperproliferative disease or condition, such as cancer). Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted condition, disorder or symptom. In other words, terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of e.g. cancer. “Treatment” therefore encompasses a reduction, slowing or inhibition of the amount or concentration of malignant cells, for example as measured in a sample obtained from the subject, of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% when compared to the amount or concentration of malignant cells before treatment. Methods of measuring the amount or concentration of malignant cells include, for

example, qRT-PCR, and quantification of hyperproliferative specific biomarkers in a sample obtained from the subject. Methods of measuring the amount or concentration of malignant cells also include, for example, histo-pathologic examination of tumour (e.g. malignant tumour) material and quantification of tumour biomarkers (not limited to hyperproliferative biomarkers).

In some examples, treatment may be administered after one or more signs or symptoms of the disease have developed or have been observed. In other examples, treatment may be administered in the absence of signs or symptoms of the disease. For example, treatment may be administered to a susceptible subject prior to the onset of symptoms (e.g., in light of a history of symptoms). Treatment may also be continued after symptoms have resolved, for example, to delay and/or prevent recurrence.

As discussed elsewhere herein, the cell deficient in a subunit of the SWI/SNF complex may be a cancer cell (in a subject). Accordingly, the pharmaceutical compositions or methods described herein may be for treating SWI/SNF complex subunit deficient cancer in a subject, optionally wherein the cancer is a SMARCA4 and/or ARID1A deficient cancer. Examples of cancers known to have mutations in SWI/SNF complex subunit genes (and are, thus, SWI/SNF complex subunit deficient cancers) are provided elsewhere herein.

In some examples, the pharmaceutical composition or method may be for treating SWI/SNF complex subunit deficient cancer in a subject wherein the cancer is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In some examples, the pharmaceutical composition or method may be for treating SWI/SNF complex subunit deficient cancer in a subject wherein the cancer is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

In some examples, the pharmaceutical composition or method may be for treating SWI/SNF complex subunit deficient cancer in a subject wherein the cancer is SMARCA4 deficient cancer. In some examples, the pharmaceutical composition or method may be for treating SWI/SNF complex deficient cancer in a subject wherein the cancer is ARID1A deficient cancer.

As used herein, a “SWI/SNF subunit complex deficient cancer” refers to a cancer characterized by a lack of a functional, or a deficiency in a SWI/SNF subunit complex. In other words, a cancer deficient in a SWI/SNF complex is characterised by a reduced or abrogated ability to remodel chromatin. Accordingly, a SWI/SNF subunit complex deficient cancer comprises one or more cells (e.g. one or more cancer cells) deficient in a subunit of the SWI/SNF complex (cancer, cancer cells and cells deficient in SWI/SNF complex are described elsewhere herein). Generally, SWI/SNF subunit complex deficiency arises from a mutation or mutations in one or more SWI/SNF-associated genes, such as but not limited to ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1. In some examples, the SWI/SNF subunit complex deficiency arises from a mutation or mutations in one or more SWI/SNF-associated genes, such as ARID1A, SMARCA4, ARID1B, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD9, DPF1, DPF2, DPF3, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

Accordingly, in some examples, the subject has been identified as having a SWI/SNF subunit complex deficient cancer. Methods of identifying a cancer deficient in a SWI/SNF complex are well known in the art. See for example the methods described elsewhere herein for identifying cancer cells and cells deficient a SWI/SNF complex.

The term a “ARID1A deficient cancer” as used herein refers to cancer comprising one or more cells (e.g. one or more cancer cells) deficient in ARID1A. Cancer, cancer cells and cells deficient in ARID1A are described elsewhere herein. In some examples, the cancer cell deficient in ARID1A may be deficient in ARID1A through either absence of the protein, a reduction in amount of the protein (compared to non-deficient cells), or dysfunction of the protein, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of mutation or polymorphism in a gene encoding a regulatory factor. For example, a cancer cell deficient in ARID1A may be a cell wherein expression and/or activity of ARID1A or a nucleic acid (DNA or RNA) encoding ARID1A is reduced, abolished or dysfunctional relative to a control cell (e.g. a non-deficient cell). A cancer cell deficient in ARID1A may, for example, be heterozygous or homozygous for mutations or polymorphisms in the nucleic acid encoding the protein, or its regulatory elements, which reduce expression or activity. In some examples, a ARID1A deficient cancer may be characterized by a ARID1A deficient phenotype. Accordingly, as would be appreciated by the skilled person, a cancer cell deficient in ARID1A may possess a level (i.e. an amount) or activity of the protein

which is less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the normal level of the active protein (e.g. in a non-deficient cell). Methods of identifying an ARID1A deficient cancer are well known in the art.

As used herein, a "SMARCA4 deficient cancer" refers to cancer comprising one or more cells (e.g. one or more cancer cells) deficient in SMARCA4. Cancer, cancer cells and cells deficient in SMARCA4 are described elsewhere herein. For example, the cancer cell deficient in SMARCA4 may be deficient in SMARCA4 through either absence of the protein, a reduction in amount of the protein (compared to non-deficient cells), or dysfunction of the protein, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of mutation or polymorphism in a gene encoding a regulatory factor. For example, a cancer cell deficient in SMARCA4 may be a cell wherein expression and/or activity of SMARCA4 or a nucleic acid (DNA or RNA) encoding SMARCA4 is reduced, abolished or dysfunctional relative to a control cell (e.g. a non-deficient cell). A cancer cell deficient in SMARCA4 may, for example, be heterozygous or homozygous for mutations or polymorphisms in the nucleic acid encoding the protein, or its regulatory elements, which reduce expression or activity. In some examples, a SMARCA4 deficient cancer may be characterized by a SMARCA4 deficient phenotype. In some examples, SMARCA41 activity may be reduced or abolished in the cancer cells. Accordingly, as would be appreciated by the skilled person, a cancer cell deficient in SMARCA4 may possess a level (i.e. an amount) or activity of the protein which is less than 50%, less than 40%, less than 30%, less than 20% or less than 10% of the normal level of the active protein (e.g. in a non-deficient cell). Methods of identifying a SMARCA4 deficient cancer are well known in the art.

In some examples, the SWI/SNF complex subunit deficient cancer is a lung cancer, colon cancer, pancreatic cancer, gastric cancer, ovarian cancer, cervical cancer, breast cancer, melanoma, bladder cancer, endometrial cancer, head and neck cancer, liver or prostate cancer.

In one example, the SWI/SNF complex subunit deficient cancer is lung cancer, colon cancer, pancreatic cancer, gastric cancer, gall bladder cancer, ovarian cancer, bladder cancer, endometrial cancer, brain cancer, lymphoma, neuroblastoma cancer.

In some examples, the ARID1A deficient cancer is ovarian cancer, colon cancer, endometrial cancer, gastric cancer, pancreatic cancer, gall bladder cancer, bladder cancer and neuroblastoma cancer.

In one example, the ovarian cancer deficient in ARID1A is a ovarian clear cell carcinoma (OCCC).

In one example, the colon cancer deficient in ARID1A is colorectal cancer.

In one example, the bladder cancer deficient in ARID1A is transitional cell carcinoma of the bladder.

In some examples, the SMARCA4 deficient cancer is lung cancer, brain cancer or lymphoma.

In one example, the lung cancer deficient in SMARCA4 is non-small-cell lung cancer (NSCLC).

In one example, the brain cancer deficient in SMARCA4 is medulloblastoma.

In one example, the lymphoma deficient in SMARCA4 is Burkitt's lymphoma.

In some examples, the SWI/SNF complex subunit deficient cancer is breast cancer. Breast cancer includes, but is not limited to, lobular carcinoma in situ (LCIS), a ductal carcinoma in situ (DCIS), an invasive ductal carcinoma (IDC), inflammatory breast cancer, Paget disease of the nipple, Phyllodes tumour, Angiosarcoma, adenoid cystic carcinoma, low- grade adenosquamous carcinoma, medullary carcinoma, mucinous carcinoma, papillary carcinoma, tubular carcinoma, metaplastic carcinoma, micropapillary carcinoma, mixed carcinoma, or another breast cancer, including but not limited to triple negative, HER positive, oestrogen receptor positive, progesterone receptor positive, HER and estrogenic receptor positive, HER and progesterone receptor positive, oestrogen and progesterone receptor positive, and HER and estrogenic and progesterone receptor positive.

In some examples, the SWI/SNF complex subunit deficient cancer is breast cancer. Breast cancer includes, but is not limited to, lobular carcinoma in situ (LCIS), a ductal carcinoma in situ (DCIS), an invasive ductal carcinoma (IDC), inflammatory breast cancer, Paget disease of the nipple, Phyllodes tumour, Angiosarcoma, adenoid cystic carcinoma, low- grade adenosquamous carcinoma, medullary carcinoma, mucinous carcinoma, papillary carcinoma, tubular carcinoma, metaplastic carcinoma, micropapillary carcinoma, mixed carcinoma, or another breast cancer, including but not limited to triple negative, HER positive, oestrogen receptor positive, progesterone receptor positive, HER and oestrogen

receptor positive, HER and progesterone receptor positive, oestrogen and progesterone receptor positive, and HER and oestrogen and progesterone receptor positive.

In some examples, the SWI/SNF complex subunit deficient cancer is ovarian cancer. Ovarian cancer includes, but is not limited to, ovarian clear cell carcinomas (OCCC), epithelial ovarian carcinomas (EOC), maturing teratomas, dysgerminomas, endodermal sinus tumours, granulosa-theca tumours, Sertoli-Leydig cell tumours, and primary peritoneal carcinoma.

Additional examples of cancers that may be deficient in a subunit of the SWI/SNF complex include, but are not limited to, lung cancer (e.g. , bronchogenic carcinoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), adenocarcinoma of the lung); kidney cancer (e.g., nephroblastoma, a.k.a. Wilms' tumour, renal cell carcinoma); acoustic neuroma; acute myeloid leukaemia; adenocarcinoma; adrenal gland cancer; anal cancer; angiosarcoma (e.g., lymphangio sarcoma, lymphangioendotheliosarcoma, hemangio sarcoma); appendix cancer; benign monoclonal gammopathy; biliary cancer (e.g. , cholangiocarcinoma); bladder cancer; breast cancer (e.g., adenocarcinoma of the breast, papillary carcinoma of the breast, mammary cancer, medullary carcinoma of the breast); brain cancer (e.g. , meningioma, glioblastomas, glioma (e.g., astrocytoma, oligodendroglioma), medulloblastoma); bronchus cancer; carcinoid tumour; cervical cancer (e.g. , cervical adenocarcinoma); choriocarcinoma; chordoma; craniopharyngioma; colorectal cancer (e.g., colon cancer, rectal cancer, colorectal adenocarcinoma); connective tissue cancer; epithelial carcinoma; ependymoma; endothelio sarcoma (e.g., Kaposi' s sarcoma, multiple idiopathic haemorrhagic sarcoma); endometrial cancer (e.g. , uterine cancer, uterine sarcoma); oesophageal cancer (e.g., adenocarcinoma of the oesophagus, Barrett's adenocarcinoma); Ewing's sarcoma; ocular cancer (e.g., intraocular melanoma, retinoblastoma); familiar hypereosinophilia; gall bladder cancer; gastric cancer (e.g., stomach adenocarcinoma); gastrointestinal stromal tumour (GIST); germ cell cancer; head and neck cancer (e.g. , head and neck squamous cell carcinoma, oral cancer (e.g., oral squamous cell carcinoma), throat cancer (e.g., laryngeal cancer, pharyngeal cancer, nasopharyngeal cancer, oropharyngeal cancer)); heavy chain disease (e.g. , alpha chain disease, gamma chain disease, mu chain disease; hemangioblastoma; hypopharynx cancer; inflammatory myofibroblastic tumours; immunocytic amyloidosis; liver cancer (e.g., hepatocellular cancer (HCC), malignant hepatoma); leiomyosarcoma (LMS); mastocytosis (e.g., systemic mastocytosis); muscle cancer; myelodysplastic syndrome (MDS); mesothelioma; myeloproliferative disorder (MPD) (e.g., polycythemia vera (PV), essential thrombocytosis (ET), agnogenic myeloid metaplasia (AMM) a.k.a. myelofibrosis (MF),

chronic idiopathic myelofibrosis, chronic myelocytic leukaemia (CML), chronic neutrophilic leukaemia (CNL), hypereosinophilic syndrome (HES)); neuroblastoma; neurofibroma (e.g. , neurofibromatosis (NF) type 1 or type 2, schwannomatosis); neuroendocrine cancer (e.g., gastroenteropancreatic neuroendoctrine tumour (GEP-NET), carcinoid tumour); osteosarcoma (e.g., bone cancer); ovarian cancer (e.g., cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma); papillary adenocarcinoma; pancreatic cancer (e.g., pancreatic adenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), Islet cell tumours); penile cancer (e.g., Paget' s disease of the penis and scrotum); pinealoma; primitive neuroectodermal tumour (PNT); plasma cell neoplasia; paraneoplastic syndromes; intraepithelial neoplasms; prostate cancer (e.g. , prostate adenocarcinoma); rectal cancer; rhabdomyosarcoma; salivary gland cancer; skin cancer (e.g., squamous cell carcinoma (SCC), keratoacanthoma (KA), melanoma, basal cell carcinoma (BCC)); small bowel cancer (e.g., appendix cancer); soft tissue sarcoma (e.g., malignant fibrous histiocytoma (MFH), liposarcoma, malignant peripheral nerve sheath tumour (MPNST), chondrosarcoma, fibrosarcoma, myxosarcoma); sebaceous gland carcinoma; small intestine cancer; sweat gland carcinoma; synovioma; testicular cancer (e.g., seminoma, testicular embryonal carcinoma); thyroid cancer (e.g., papillary carcinoma of the thyroid, papillary thyroid carcinoma (PTC), medullary thyroid cancer); urethral cancer; uveal melanoma; vaginal cancer; and vulvar cancer (e.g., Paget' s disease of the vulva).

In some examples, the subject is undergoing treatment with, has been treated with, or has been prescribed treatment with, one or more anti-cancer therapy.

In some examples, the method further comprises administering one or more anti-cancer therapy to the subject.

In some examples, the subject is undergoing treatment with, has been treated with, or has been prescribed treatment with, one or more anti-cancer therapy; and/or the method further comprises administering one or more anti-cancer therapy to the subject.

Accordingly, in some examples, one or more of G quadruplex binding ligand, an anti-cancer therapy or a pharmaceutical composition may be provided in a form which is suitable for sequential (consecutive), separate and/or simultaneous (concurrent) administration to the subject, in any order. For example, a G quadruplex binding ligand (e.g. as part of a pharmaceutical composition) may be provided in a form that is suitable for sequential, separate and/or simultaneous administration with an anticancer therapy (or vice versa). In some examples, a G quadruplex binding ligand (e.g. as part of a pharmaceutical

composition) may be administered to the subject at the same time or before an anti-cancer therapy is administered. In cases where they are administered simultaneously, the G quadruplex binding ligand (e.g. as part of a pharmaceutical composition) and anti-cancer therapy may be administered as separate compositions that are administered at the same time, or may be administered as a combined composition that includes both. The same applies for the optional addition of a further anti-cancer therapy (i.e. it may be provided in a form which is suitable for sequential (consecutive), separate and/or simultaneous (concurrent) administration with the 6 quadruplex binding ligand (e.g. as part of a pharmaceutical composition) and/or the anti-cancer therapy, in any order, as described in detail above).

As used herein, "anti-cancer therapy" refers to any agent, composition or medical technique (e.g., surgery, radiation treatment, etc.) useful for the treatment of cancer. For example, an anti-cancer agent can be a small molecule, antibody, peptide or antisense compound. Examples of antisense compounds include, but are not limited to interfering RNAs (e.g., dsRNA, siRNA, shRNA, miRNA, and amiRNA) and antisense oligonucleotides (ASO).

In some examples, the anti-cancer therapy is selected from the group consisting of surgery, radiation therapy, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, adjuvant therapy, and immunotherapy.

As used herein, the phrase “chemotherapeutic agent” refers to (but is not limited to) compounds that are used in chemotherapy for the treatment of proliferative disorders such as cancer. For the avoidance of doubt, reference to a chemotherapeutic agent herein refers to an additional agent to the G quadruplex binding ligand. Accordingly, where G quadruplex binding ligand functions as a chemotherapeutic agent, and the claims or description refers to an G quadruplex binding ligand and a chemotherapeutic agent, an additional chemotherapeutic agent is intended, in addition to the G quadruplex binding ligand.

Several chemotherapeutic agents are known, some of which are clinically approved or awaiting approval as cancer therapies. Suitable examples include nucleoside analogues, topoisomerase inhibitors, platinum complexes, microtubule-targeting drugs and combinations thereof.

In some examples, the chemotherapy is a cytotoxic agent. As used herein a “cytotoxic agent” refers to any substance that kills cells, including cancer cells e.g. the cytotoxic agent may stop cancer cells from dividing and growing and may cause tumours to shrink in size. In

some examples, the chemotherapy comprises administering to the subject a cytotoxic agent in an amount effective to treat the SWI/SNF complex subunit deficient cancer.

In some examples, the cytotoxic agent is selected from the group consisting of a platinum agent, mitomycin C, a poly (ADP-ribose) polymerase (PARP) inhibitor, a radioisotope, a vinca alkaloid, a taxane an antitumour alkylating agent, a monoclonal antibody and an antimetabolite.

Examples of platinum agents include, but are not limited to cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, Nedaplatin, Triplatin, and Lipoplatin. Examples of antitumour alkylating agents include, but are not limited to nitrogen mustards, cyclophosphamide, mechlorethamine or mustine (HN2), uramustine or uracil mustard, melphalan, chlorambucil, ifosfamide, bendamustine, nitrosoureas, carmustine, lomustine, streptozocin, alkyl sulfonates, busulfan, thiotepa, procarbazine, altretamine, triazenes, dacarbazine, mitozolomide, and temozolomide. Examples of anti-cancer monoclonal antibodies include, but are not limited to necitumumab, dinutuximab, nivolumab, blinatumomab, pembrolizumab, ramucirumab, obinutuzumab, adotrastuzumab emtansine, pertuzumab, brentuximab, ipilimumab, ofatumumab, catumaxomab, bevacizumab, cetuximab, tositumomab-1131, ibritumomab tiuxetan, alemtuzumab, gemtuzumab ozogamicin, trastuzumab, and rituximab. Examples of vinca alkaloids include, but are not limited to vinblastine, vincristine, vindesine, vinorelbine, desoxyvincaminol, vincaminol, vinburnine, vincamajine, vineridine, vinburnine, and vinpocetine. Examples of taxanes include, but are not limited to docetaxel, paclitaxel and cabazitaxel. Examples of antimetabolites include, but are not limited to fluorouracil, cladribine, capecitabine, mercaptopurine, pemetrexed, fludarabine, gemcitabine, hydroxyurea, methotrexate, nelarbine, clofarabine, cytarabine, decitabine, pralatrexate, floxuridine, and thioguanine. In some embodiments, the anti-cancer therapy is an immunotherapy, such as, but not limited to, cellular immunotherapy, antibody therapy or cytokine therapy. Examples of cellular immunotherapy include, but is not limited to, dendritic cell therapy and Sipuleucel-T. Examples of antibody therapy include, but is not limited to Alemtuzumab, Ipilimumab, Nivolumab, Ofatumumab, Pembrolizumab, and Rituximab. Examples of cytokine therapy include, but is not limited to, interferons (for example, IFNa, IENβ, IENγ, IENλ) and interleukins. In some embodiments, the immunotherapy comprises one or more immune checkpoint inhibitors. Examples of immune checkpoint proteins include, but are not limited to, CTLA-4 and its ligands CD80 and CD86, PD- 1 with its ligands PD-L1 and PD-L2, and 4- IBB.

Additional examples of anti-cancer therapies include, but are not limited to, abiraterone acetate (e.g., ZYTIGA), ABVD, ABVE, ABVE-PC, AC, AC-T, ADE, ado- trastuzumab emtansine (e.g., KADCYLA), afatinib dimaleate (e.g., GILOTRIF), aldesleukin (e.g., PROLEUKIN), alemtuzumab (e.g., CAMPATH), anastrozole (e.g., ARIMIDEX), arsenic trioxide (e.g., TRISENOX), asparaginase erwinia chrysanthemi (e.g., ERWINAZE), axitinib (e.g., INLYTA), azacitidine (e.g., MYLOSAR, VIDAZA), BEACOPP, belinostat (e.g., BELEODAQ), bendamustine hydrochloride (e.g., TREANDA), BEP, bevacizumab (e.g., AVASTIN), bicalutamide (e.g., CASODEX), bleomycin (e.g., BLENOXANE), blinatumomab (e.g., BLINCYTO), bortezomib (e.g., VELCADE), bosutinib (e.g., BOSULIF), brentuximab vedotin (e.g., ADCETRIS), busulfan (e.g., BUSULFEX, MYLERAN), cabazitaxel (e.g., JEVTANA), cabozantinib- s-malate (e.g., COMETRIQ), CAF, capecitabine (e.g., XELODA), CAPOX, carboplatin (e.g., PARAPLAT, PARAPLATIN), carboplatin-taxol, carfilzomib (e.g., KYPROLIS), carmustine (e.g., BECENUM, BICNU, CARMUBRIS), carmustine implant (e.g., GLIADEL WAFER, GLIADEL), ceritinib (e.g., ZYKADIA), cetuximab (e.g., ERBITUX), chlorambucil (e.g., AMBOCHLORIN, AMBOCLORIN, LEUKERAN, LINFOLIZIN), chlorambucil-prednisone, CHOP, cisplatin (e.g., PLATINOL, PLATINOL-AQ), clofarabine (e.g., CLOFAREX, CLOLAR), CMF, COPP, COPP- ABV, crizotinib (e.g., XALKORI), CVP, cyclophosphamide (e.g., CLAFEN, CYTOXAN, NEOSAR), cytarabine (e.g., CYTOSAR-U, TARABINE PFS), dabrafenib (e.g., TAFINLAR), dacarbazine (e.g., DTIC-DOME), dactinomycin (e.g., COSMEGEN), dasatinib (e.g., SPRYCEL), daunorubicin hydrochloride (e.g., CERUBIDINE), decitabine (e.g., DACOGEN), degarelix, denileukin diftitox (e.g., ONTAK), denosumab (e.g., PROLIA, XGEVA), Dinutuximab (e.g., UNITUXIN), docetaxel (e.g., TAXOTERE), doxorubicin hydrochloride (e.g., ADRIAMYCIN PFS, ADRIAMYCIN RDF), doxorubicin hydrochloride liposome (e.g., DOXIL, DOX-SL, EVACET, LIPODOX), enzalutamide (e.g., XTANDI), epirubicin hydrochloride (e.g., ELLENCE), EPOCH, erlotinib hydrochloride (e.g., TARCEVA), etoposide (e.g., TOPOSAR, VEPESID), etoposide phosphate (e.g., ETOPOPHOS), everolimus (e.g., AFINITOR DISPERZ, AFINITOR), exemestane (e.g., AROMASIN), FEC, fludarabine phosphate (e.g., FLUDARA), fluorouracil (e.g., ADRUCIL, EFUDEX, FLUOROPLEX), FOLFIRI , FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, FU-LV, fulvestrant (e.g., FASLODEX), gefitinib (e.g., IRESSA), gemcitabine hydrochloride (e.g., GEMZAR), gemcitabine-cisplatin, gemcitabine-oxaliplatin, goserelin acetate (e.g., ZOLADEX), Hyper-CVAD, ibritumomab tiuxetan (e.g., ZEVALIN), ibrutinib (e.g., IMBRUVICA), ICE, idelalisib (e.g., ZYDELIG), ifosfamide (e.g., CYFOS, IFEX, IFOSFAMIDUM), imatinib mesylate (e.g., GLEEVEC), imiquimod (e.g., ALDARA), ipilimumab (e.g., YERVOY), irinotecan hydrochloride (e.g., CAMPTOSAR), ixabepilone (e.g., IXEMPRA), lanreotide acetate (e.g., SOMATULINE DEPOT), lapatinib ditosylate (e.g., TYKERB), lenalidomide (e.g., REVLIMID), lenvatinib (e.g., LENVIMA),

letrozole (e.g., FEMARA), leucovorin calcium (e.g., WELLCOVORIN), leuprolide acetate (e.g., LUPRON DEPOT, LUPRON DEPOT-3 MONTH, LUPRON DEPOT-4 MONTH, LUPRON DEPOT-PED, LUPRON, VIADUR), liposomal cytarabine (e.g., DEPOCYT), lomustine (e.g., CEENU), mechlorethamine hydrochloride (e.g., MUSTARGEN), megestrol acetate (e.g., MEGACE), mercaptopurine (e.g., PURINETHOL, PURIXAN), methotrexate (e.g., ABITREXATE, FOLEX PFS, FOLEX, METHOTREXATE LPF, MEXATE, MEXATE-AQ), mitomycin c (e.g., MITOZYTREX, MUTAMYCIN), mitoxantrone hydrochloride, MOPP, nelarabine (e.g., ARRANON), nilotinib (e.g., TASIGNA), nivolumab (e.g., OPDIVO), obinutuzumab (e.g., GAZYVA), OEPA, ofatumumab (e.g., ARZERRA), OFF, olaparib (e.g., LYNPARZA), omacetaxine mepesuccinate (e.g., SYNRIBO), OPPA, oxaliplatin (e.g., ELOXATIN), paclitaxel (e.g., TAXOL), paclitaxel albumin-stabilized nanoparticle formulation (e.g., ABRAXANE), PAD, palbociclib (e.g., IBRANCE), pamidronate disodium (e.g., AREDIA), panitumumab (e.g., VECTIBIX), panobinostat (e.g., FARYDAK), pazopanib hydrochloride (e.g., VOTRIENT), pegaspargase (e.g., ONCASPAR), peginterferon alfa-2b (e.g., PEG-INTRON), peginterferon alfa-2b (e.g., SYLATRON), pembrolizumab (e.g., KEYTRUDA), pemetrexed disodium (e.g., ALIMTA), pertuzumab (e.g., PERJETA), plerixafor (e.g., MOZOBIL), pomalidomide (e.g., POMALYST), ponatinib hydrochloride (e.g., ICLUSIG), pralatrexate (e.g., FOLOTYN), prednisone, procarbazine hydrochloride (e.g., MATULANE), radium 223 dichloride (e.g., XOFIGO), raloxifene hydrochloride (e.g., EVISTA, KEOXIFENE), ramucirumab (e.g., CYRAMZA), R-CHOP, recombinant HPV bivalent vaccine (e.g., CERVARIX), recombinant human papillomavirus (e.g., HPV) nonavalent vaccine (e.g., GARDASIL 9), recombinant human papillomavirus (e.g., HPV) quadrivalent vaccine (e.g., GARDASIL), recombinant interferon alfa-2b (e.g., INTRON A), regorafenib (e.g., STIVARGA), rituximab (e.g., RITUXAN), romidepsin (e.g., ISTODAX), ruxolitinib phosphate (e.g., JAKAFI), siltuximab (e.g., SYLVANT), sipuleucel-t (e.g., PROVENGE), sorafenib tosylate (e.g., NEXAVAR), STANFORD V, sunitinib malate (e.g., SUTENT), TAC, tamoxifen citrate (e.g., NOLVADEX, NOVALDEX), temozolomide (e.g., METHAZOLASTONE, TEMODAR), temsirolimus (e.g., TORISEL), thalidomide (e.g., SYNOVIR, THALOMID), thiotepa, topotecan hydrochloride (e.g., HYCAMTIN), toremifene (e.g., FARESTON), tositumomab and iodine 1 131 tositumomab (e.g., BEXXAR), TPF, trametinib (e.g., MEKINIST), trastuzumab (e.g., HERCEPTIN), VAMP, vandetanib (e.g., CAPRELSA), VEIP, vemurafenib (e.g., ZELBORAF), vinblastine sulfate (e.g., VELBAN, VELSAR), vincristine sulfate (e.g., VINCASAR PFS), vincristine sulfate liposome (e.g., MARQIBO), vinorelbine tartrate (e.g., NAVELBINE), vismodegib (e.g., ERIVEDGE), vorinostat (e.g., ZOLINZA), XELIRI, XELOX, ziv-aflibercept (e.g., ZALTRAP), zoledronic acid (e.g., ZOMETA), or a combination thereof.

In certain examples, the anti-cancer therapy is selected from the group consisting of epigenetic or transcriptional modulators (e.g. , DNA methyltransferase inhibitors, histone deacetylase inhibitors (HDAC inhibitors), lysine methyltransferase inhibitors), antimitotic drugs (e.g. , taxanes and vinca alkaloids), hormone receptor modulators (e.g., oestrogen receptor modulators and androgen receptor modulators), cell signalling pathway inhibitors, modulators of protein stability (e.g. , proteasome inhibitors), Hsp90 inhibitors, glucocorticoids, all-trans retinoic acids, and other agents that promote differentiation.

In some examples, the cytotoxic agent is a platinum agent.

In some examples, the platinum agent is carboplatin.

In some examples, the cytotoxic agent is a taxane.

In some examples, the taxane is docetaxel.

In some examples, the cytotoxic agent is a platinum agent and a taxane.

In some examples, the platinum agent is carboplatin and the taxane is docetaxel.

In some examples, an G4 quadruplex binding agent can be administered in combination with an anti-cancer therapy including, but not limited to, surgery, radiation therapy, transplantation (e.g., stem cell transplantation, bone marrow transplantation), immunotherapy, and chemotherapy.

In some examples, the SWI/SNF complex subunit deficient cancer is resistant to conventional first line therapy, including, for example, first line chemotherapy (such as nucleoside analogues, topoisomerase inhibitors, platinum-based drugs, microtubule-targeting drugs, anthracyclines, and combinations thereof) and/or irradiation.

In some examples, the SWI/SNF complex subunit deficient cancer is resistant to treatment with a PARP inhibitor alone and/or is resistant to conventional first line therapy. PARP is an enzyme that plays a critical role in DNA repair. Consequently, PARP inhibition has been put forward as a potential strategy to treat human cancers. Several small molecule inhibitors of PARP activity have been developed and brought forward into clinical development. Some have shown growth inhibitory activity in a small but distinct number of human cancer cell

lines and patient tumours that lack specific DNA repair mechanisms either through inherited mutations and/or non-inherited silencing of genes.

As used herein, the term "PARP" includes at least PARP1 and PARP2.

Agents that inhibit the activity of PARP or reduce the expression level of PARP have been tested in cancer therapy. Such inhibitors are collectively referred to herein as "PARP inhibitors (PARPi)". Inhibitors are defined elsewhere herein and apply equally here. Examples of PARPi include, but are not limited to, iniparib (BSI 201), talazoparib (BMN-673), olaparib (AZD-2281, TOPARP-A), rucaparib (AG014699, PF-01367338), veliparib (ABT-888), CEP 9722, MK 4827, BGB-290 and 3-aminobenzamide, 4-amino-l,8-napthalimide, benzamide, BGP-15, BYK204165, 3,4- Dihydro-5-[4-(1 -piperidinyl)butoxyl] - 1 (2H)-isoquinolinone, DR2313, 1,5- Isoquinolinediol, MC2050, ME0328, PJ-34 hydrochloride hydrate, and UPF-1069.

As used herein, a cancer that is resistant to a PARP inhibitor (i.e. resistant to treatment with a PARP inhibitor alone) means that the cancer does not respond to such inhibitor, for example as evidenced by continued proliferation and/or increasing tumour growth and burden. In some examples, the cancer may have initially responded to treatment with such inhibitor (referred to herein as a previously administered therapy) but may have grown resistant after a time. In some examples, the cancer may have never responded to treatment with such inhibitor at all. Cancers resistant to PARP inhibitors can be identified using methods known in the art (see, e.g., WO 2014205105, US 8729048). Examples of cancers resistant to PARP-inhibitors include, but are not limited to, breast cancer, ovarian cancer, lung cancer, bladder cancer, liver cancer, head and neck cancer, pancreatic cancer, gastrointestinal cancer, and colorectal cancer.

The binding ligands described herein may be part of a pharmaceutical composition. As used herein, “pharmaceutical composition” refers to a composition comprising one or more compounds that is formulated for administration to a subject. Pharmaceutical compositions typically comprise one or more active ingredients (e.g. in this case a G quadruplex binding ligand) and one or more pharmaceutically acceptable materials. The pharmaceutical compositions described herein may therefore comprise a G quadruplex binding ligand and one or more other components. For example, the pharmaceutical composition may comprise a G quadruplex binding ligand and a pharmaceutically acceptable excipient, diluent and/or carrier. Pharmaceutical compositions may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary

immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents or compounds.

In some examples, the SWI/SNF complex subunit deficient cancer is resistant to treatment with a PARP inhibitor alone and/or is resistant to conventional first line therapy.

The G quadruplex binding ligands described herein may be part of a pharmaceutical composition. As used herein, “pharmaceutical composition” refers to a composition comprising one or more compounds that is formulated for administration to a subject. Pharmaceutical compositions typically comprise one or more active ingredients (e.g. in this case a G quadruplex binding ligand) and one or more pharmaceutically acceptable materials. The pharmaceutical compositions described herein may therefore comprise a G quadruplex binding ligand and one or more other components. For example, the pharmaceutical composition may comprise a G quadruplex binding ligand and a pharmaceutically acceptable excipient, diluent and/or carrier. Pharmaceutical compositions may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents or compounds.

As used herein, "pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected compound (e.g. binding ligand) without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

In some examples, the pharmaceutical composition comprises a pharmaceutically acceptable diluent. Diluents are diluting agents. Pharmaceutically acceptable diluents are well known in the art. A suitable diluent is therefore easily identifiable by one of ordinary skill in the art.

In some examples, the pharmaceutical composition comprises a pharmaceutically acceptable excipient. Excipients are natural or synthetic substances formulated alongside an active ingredient (e.g. the vaccine, binding ligand, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate)), included for the purpose of bulking-up the formulation or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating drug absorption or solubility. Excipients can also be useful in the manufacturing process, to aid in the handling of the

active substance concerned such as by facilitating powder flowability or non-stick properties, in addition to aiding in vitro stability such as prevention of denaturation over the expected shelf life. Pharmaceutically acceptable excipients are well known in the art. A suitable excipient is therefore easily identifiable by one of ordinary skill in the art. By way of example, suitable pharmaceutically acceptable excipients include water, saline, aqueous dextrose, glycerol, ethanol, and the like.

In some examples, the pharmaceutical composition may comprise a G quadruplex binding ligand and a pharmaceutically acceptable carrier. Carriers are non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the formulation. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. Pharmaceutically acceptable carriers are well known in the art. A suitable carrier is therefore easily identifiable by one of ordinary skill in the art.

The pharmaceutical composition may be administered using any suitable method and dosage form, as described in detail below.

The terms "administer," "administering," or "administration" refers to implanting, absorbing, ingesting, injecting, inhaling, or otherwise introducing a compound described herein (e.g. a G quadruplex binding ligand, an anti-cancer therapy or a pharmaceutical composition of the invention), or a composition thereof, in or on a subject. The compounds described herein can be administered by any suitable route (e.g. any conventional route) including enteral (e.g., oral, for example in tablet form), parenteral, intravenous, intramuscular, intracerebral, intravascular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, intracavity, transdermal, intradermal, rectal, intravaginal, percutaneous, intratracheal, intralesional, epidural, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, buccal, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol, and/or as an injection, and/or by infusion, and/or by gradual infusion over time. Specifically, contemplated routes are oral administration, intravenous administration (e.g., systemic intravenous injection), regional administration via blood and/or lymph supply, and/or direct administration to an affected site. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the agent (e.g., its stability in the environment of the gastrointestinal tract), and/or the condition of the subject (e.g., whether the subject is able to tolerate oral administration).

The compounds described herein may therefore be in a form suitable for the appropriate mode of administration. For example, suitable forms for oral administration include a tablet or capsule; suitable forms for nasal administration or administration by inhalation include a powder or solution; suitable forms for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) include a sterile solution, suspension or emulsion; suitable forms for topical administration include a patch, an ointment or cream; and suitable forms for rectal administration include a suppository. Alternatively, the route of administration may be by injection (e.g. i.v.), or infusion.

The compounds described herein (e.g. a G quadruplex binding ligand, an anti-cancer therapy or a pharmaceutical composition of the invention) may be administered as an acute dose and/or chronic dose to the subject. As used herein, the term “acute dose” refers to the short-term exposure of a compound to a subject. For example, providing the subject with a compound for a short period.

Surprisingly, as shown in the examples, the inventors identified that the selective sensitivity of ARID1A deficient cells to PDS (G quadruplex binding ligand) treatment is widened when cells are exposed to an acute dose of PDS instead of a chronic dose of PDS.

Suitably, the G quadruplex binding ligand is administered to the subject as an acute dose. Suitably, the acute dose is for at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 8 hours, at least about 10 hours, or at least about 11 hours. Suitably, the acute dose is for up to about 12 hours. Preferably, the G quadruplex binding ligand is administered to the subject for at least about 4 hours.

As used herein, the term “chronic dose” refers to the long-term exposure of a compound to a subject. For example, providing the subject with a compound continuously for a long period. Suitably, the G quadruplex binding ligand is administered to the subject as a chronic dose. Suitably, the chronic dose is for at least about 12 hours, at least about 24 hours, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 10 days, at least about 12 days, at least about 14 days, at least about 28 days, at least about 2 months, at least about 6 months, or at least about 12 months. Preferably, the G quadruplex binding ligand is administered to the subject for at least about 6 days.

Preferably, the compounds described herein (e.g. a G quadruplex binding ligand, an anti-cancer therapy or a pharmaceutical composition of the invention) are provided at an effective dose. The actual dose used will depend on a number of parameters (e.g. if it is for *in vivo* or *ex vivo* use).

The G quadruplex binding ligand, anti-cancer therapy or pharmaceutical composition of the invention (as appropriate) may advantageously be presented in unit dosage form. Dosage forms (also called unit doses) are pharmaceutical drug products in the form in which they are marketed for use, with a specific mixture of active ingredients and inactive components (excipients), in a particular configuration (such as a capsule shell, for example), and apportioned into a particular dose. Depending on the route of administration, dosage forms include liquid, solid, and semisolid dosage forms. Common dosage forms include pills, tablet, capsule, drinks or syrups.

Where the administration of separate formulations of two components (e.g. two or more of a G quadruplex binding ligand or an anti-cancer therapy (as appropriate)) is sequential or separate, the delay in administering the second formulation should not be such as to lose the beneficial effect of the combination therapy.

Preferably, the combination(s) described herein will provide a benefit to the treatment of a disease (e.g. a homologous recombination deficient cancer) in a subject in need thereof. For example, and as described elsewhere herein, a G quadruplex binding ligand (or a pharmaceutical composition of the invention) may be used in combination with one or more anti-cancer therapies. For example, wherein the anti-cancer therapy is selected from the group consisting of surgery, radiation therapy, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, adjuvant therapy, and immunotherapy.

In particular examples, a G quadruplex binding ligand (or a pharmaceutical composition described herein) may be used in combination with any agent selected from the group consisting of a platinum agent, mitomycin C, a poly (ADP-ribose) polymerase (PARP) inhibitor, a radioisotope, a vinca alkaloid, a taxane, an antitumour alkylating agent, a monoclonal antibody and an antimetabolite.

In some examples, the combination may have an additive or synergistic effect on the treatment of a disease in a subject in need thereof. A combination treatment is defined as affording an “additive effect” “synergistic effect” or a “synergistic treatment” if the effect is therapeutically superior, as measured by, for example, the extent of the response, the

response rate, the time to disease progression or the survival period, to that achievable on dosing one or other of the components of the combination treatment at its conventional dose. For example, the effect of the combination treatment is additive if the effect is therapeutically superior to the effect achievable with G quadruplex binding ligand. For example, the effect of the combination treatment may be synergistic if the effect of the combination treatment supersedes the effect of the individual treatments added together. Further, the effect of the combination is beneficial (e.g. additive or synergistic) if a beneficial effect is obtained in a group of subjects that does not respond (or responds poorly) to any members of the combination alone. In addition, the effect of the combination treatment is defined as affording a benefit (e.g. additive or synergistic effect) if one of the components is dosed at its conventional dose and the other component is dosed at a reduced dose and the therapeutic effect, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, is equivalent to or better than that achievable on dosing conventional amounts of either one of the components of the combination treatment.

In some examples, the combination(s) described herein may reduce side effects. Side effects may be reduced as lower doses of the individual compounds described herein (e.g. an G quadruplex binding ligand, a pharmaceutical composition described herein or an anti-cancer therapy) may be used in a combination therapy in comparison to in a single treatment therapy (e.g. treatment with a G quadruplex binding ligand, a pharmaceutical composition described herein or an anti-cancer therapy alone) which often requires higher doses.

The compounds described herein (e.g. a G quadruplex binding ligand, any anti-cancer therapy or the pharmaceutical composition described herein) are for administration in an “effective amount”. An “effective amount” (or “therapeutically effective amount”) is an amount that alone, or together with further doses, produces the desired (therapeutic) response (e.g. reducing the viability of a cell deficient in homologous recombination). The (therapeutically) effective amount to be used will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the subject. A suitable dosage for a given subject can be determined by an attending physician, taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Accordingly, in one example, a suitable dose (of e.g. a G quadruplex binding ligand and/or a pharmaceutical composition as described herein) is selected based on the body weight of the subject. The dosages and schedules may be varied according to the particular disease state and the overall condition of the patient. For example, it may be

necessary or desirable to reduce the doses of the components of the combination treatment in order to reduce toxicity. Suitable doses may also be determined for subgroups of subjects, e.g. based on their heredity and/or pharmacogenetic profile(s).

Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods.

The compounds described herein (e.g. a G quadruplex binding ligand and/or a pharmaceutical composition described herein) are therefore administered to a subject in an effective amount to produce the desired response.

An example of a desired response is the induction of cell death in a SWI/SNF complex subunit deficient cell (e.g. *in vitro, ex vivo* or *in vivo*, for example in a subject comprising one or more cells deficient in a subunit of the SWI/SNF complex and/or in a subject with a SWI/SNF complex subunit deficient cancer). Another example of a desired response is reversing, alleviating, delaying the onset of, or inhibiting the progress of a SWI/SNF complex subunit deficient cancer (e.g. a SWI/SNF complex subunit deficient cancer deficient in ARID1A and/or SMARCA4) in a subject. Specific examples of the therapeutic response in the context of e.g. a cancer patient (including patients with a SWI/SNF complex subunit deficient cancer) include a reduction in the tumour to lower risk levels, a reduction in the severity of tumour, and increase in survival rate. Methods for measuring these responses are well known.

The invention therefore provides pharmaceutical compositions for use in inducing cell death in SWI/SNF complex subunit deficient cancer cells. The invention also provides methods for inducing cell death in such cells.

A pharmaceutical composition comprising G quadruplex binding ligand for use in inducing cell death of a cancer cell deficient in a SWI/SNF complex subunit in a subject is therefore provided.

Also provided is a method of inducing cell death of a cancer cell deficient in a SWI/SNF subunit complex, the method comprising subjecting said cell to a G quadruplex binding ligand. The methods described herein may be performed *in vitro, ex vivo,* or *in vivo*.

The pharmaceutical compositions and methods described herein induce cell death of a cancer cell deficient in a subunit of the SWI/SNF complex.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, Dictionary of Microbiology and Molecular Biology, 2d Ed., John Wiley and Sons, NY (1994); and Hale and Marham, The Harper Collins Dictionary of Biology, Harper Perennial, NY (1991) provide those of skill in the art with a general dictionary of many of the terms used in the invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole. Also, as used herein, the singular terms "a", "an," and "the" include the plural reference unless the context clearly indicates otherwise. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.

Aspects of the invention are demonstrated by the following non-limiting examples.

EXAMPLES

EXAMPLE 1: Specific killing of ARID1A-deficient cancer cells by G quadruplex binding ligands

Materials and Methods

Cell culture

TOV21G (ATCC) and derived cell lines were grown in Medium 199 in combination with MCDB105 supplemented with sodium bicarbonate and Fetal Bovine Serum (FBS, Gibco) at 37°C in a humidified incubator with 5% CO_2. RPE1-hTERT (ATCC) and derived cell lines were grown in DMEM/F12 supplemented with 10% Fetal Bovine Serum (FBS, Gibco), 1% penicillin/streptomycin (Gibco), 3.5% sodium bicarbonate (Gibco), and 1% glutamax (Gibco) at 37°C in a humidified incubator with 5% CO_2. HCT116-derived cell lines (parental and ARID1A KO; Horizon Discovery) were grown in McCoys 5A (Modified) Medium (Glico, 26600023) supplemented with 10% Fetal Bovine Serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO_2.

Generation of ARID1A expressing TOV21G cells

pLenti-puro-ARID1A (Addgene #39478) and an empty vector control, pLenti-puro (Addgene #39481) plasmids were introduced into the ARID1A-deficient OCCC cell line TOV21G. Plasmids were prepared using the Qiagen Plasmid Plus kit as per manufacturer's protocol and stored at -20°C. Viral particles containing EV or ARID1A were prepared and used to infect the TOV21G cells. After selection, cells were single cell sorted and screened by Western blotting and immunofluorescence for ARID1A expression.

Generation of ARID1A knockout clones in RPE1-hTERT

Generation of *ARID1A* KOs in an RPE1 background was carried out using CRISPR-Cas9-mediated genome engineering. Guide RNA (gRNA) primers were designed, re-suspended in H_2O at 100μM concentration (Sigma-Aldrich), annealed and cloned into px458 (Addgene #48138). 3 gRNAs were cloned using the following primers:

ARID1A_sg1_FW: 5'-CACCGAACCCATACTCGCAGCAACA

ARID1A_sg1_RV: 5'-AAACTGTTGCTGCGAGTATGGGTTC

ARID1A_sg2_FW: 5'-CACCGATGGTCATCGGGTACCGCTG

ARID1A_sg2_RV: 5'-AAACCAGCGGTACCCGATGACCATC

ARID1A_sg_3_FW: 5'-CACCGCGGTACCCGATGACCATGC

ARID1A_sg3_RV: 5'-AAACGCATGGTCATCGGGTACCGC

RPE1 cells were transfected with one of the three gRNA constructs using Lipofectamine 3000 as per manufacturer's protocol. After 24 hours, cells were single cell sorted based on GFP expression on a BD ARIA II flow cytometer (BD Biosciences). Clones were expanded and screened for ARID1A expression. Genomic DNA was extracted from *ARID1A* KOs and parental RPE1 cells using the Genomic DNA extraction kit (Eurogentec) and the targeted region was expanded by PCR using the following primers:

ARID1A_gDNA_FW1: 5'-GTTCTGAGGCGGGTCAGTT

ARID1A_gDNA_RV1: 5'-GAGCACACATGGAACGGTGC

Following PCR cleanup (QIAGEN), amplified DNA was subjected to Sanger sequencing of the targeted area (Genewiz) to confirm gene disruption.

Sulforhodamine B (cell growth) assay

Cells were seeded in 96-well plates at the appropriate densities. 24 hours later, drugs were added, and cells were allowed to grow for 6 days. After 6 days, cells were fixed by adding 10% TCA to the medium and plates were kept at 4°C for additional 24 hours. Plates were then washed 5 x with water and left to dry. Once dried, cells were stained with 0.0057% SRB in 1% acetic acid, incubated for 1 hour RT and then washed with 1% acetic acid and left to dry overnight. For cell mass quantification, cells were resuspended in Sulforhodamine B (SRB), incubated for 10 minutes in a shaker, washed and then measured under the wavelength of 492 nm.

Clonogenic survival assay

RPE1-hTERT and KO cell lines were seeded in triplicate in 6cm^2 plates (600 cells/dish). After 24h, cells were treated with indicated concentrations of Pyridostatin (Sigma-Aldrich). Cells were grown for 10 days to enable colony formation, and were then fixed and stained with methylene blue (1% methylene blue, 70% methanol) for 1 hour at room temperature. Colonies were counted using a manual colony counter (ThermoFisher Scientific).

Cell death analysis

Cells were seeded at the appropriate densities in 96-well plates. The next day cells were treated with different concentrations of PDS (0, 5, 10, 15μM) and incubated for 4-5 days. Propidium iodide (PI), which doesn't stain live cells, was used as a readout of dead cells and Hoechst staining was used to identify total cell number. Stained cells were analysed on a Celigo imaging cytometer, and data are presented as percent PI-positive (dead) relative to Hoechst-positive (total).

γH2AX foci analysis

Cells were seeded in coverslips and after 24 h were treated with 10mM PDS for 1, 3 or 6h followed by fixation with 4% PFA. After washing with 1x PBS, cells were permeabilized with 0.5% Triton in PBS for 10 minutes then washed with 1x PBS. Coverslips were incubated with blocking solution (1% BSA in PBS) for 30 minutes RT. Primary antibodies were prepared in 1% BSA/PBS and incubated in the coverslips, overnight, at 4°C. Following washes in 1x PBS, cells were incubated in fluorescent secondary antibodies protected from light for 1 hour at room temperature. Coverslips were mounted onto poly-L-lysine glass slides with Vectashield mounting medium and sealed with nail varnish. The cells were imaged with an Advanced Spinning Disc Confocal Microscope and analysed with Slidebook 6 software and ImageJ. All the images were obtained with 63x oil lens with z-stacks (1.02μm intervals). The exposure times for each condition was kept constant. γH2AX primary antibody was Cell

Signalling 20E3 and the secondary antibody was Alexa flour 488 Anti-rabbit (Thermo Fisher Scientific). DNA Hoechst fluorescent stain (Thermo Fisher Scientific) was used at 10µg/ml.

Proteomic analysis

Chromatin fractions of the TOV21G cell line panel were prepared and analysed by mass spectroscopy as in (Feng, et al, Genes and Development 36, 790-806 (2022)).

Protein extract preparation and Western blot analysis

For whole cell analysis, proteins were extracted by boiling cell pellets in sample buffer (0.35 M Tris pH 6.8, 0.1 g/ml sodium dodecyl sulfate, 93 mg/ml dithiothreitol, 30% glycerol, 50 µg/ml bromophenol blue), resolved by SDS-PAGE, then electroblotted onto Amersham™ Protan™ 0.45 mM Nitrocelluose Blotting Membranes (Cytiva). Following blocking in 5% dried skimmed milk dissolved in TBST (50 mM Tris pH 7.6, 150 mM NaCl, 0.1% Tween-20), membranes were incubated with primary antibodies overnight at 4°C. ARID1A/BAF250 (D2A8U) Rabbit mAb (Cell Signalling Technology) was used in 1:500 dilution, and α-tubulin (DM1A) mouse mAb (Abcam) was used in 1:10,000 dilution. Secondary antibodies polyclonal goat Anti-rabbit immunoglobulins/HRP (Dako) and polyclonal rabbit Anti-mouse immunoglobulins/HRP (Dako) were used for ARID1A and tubulin respectively in 1:10,000 dilutions. Membranes were then washed three times in TBST and incubated for at least 1 hr with appropriate horseradish-peroxidase-conjugated secondary antibodies (Invitrogen). After washing in TBST, bound secondary antibodies were detected using Luminata™ Forte Western HRP Substrate (Merck Millipore) in an iBright 750 imaging system (Invitrogen).

For isolation of cellular fractions, cells were incubated in NIB-250 + 0.3% NP-40 buffer then nuclear fraction was pellet and cytoplasmic fraction was collected. Nuclear fraction was washed with NIB-250 buffer, pelleted again and chromatin fraction was extracted by incubation in hypotonic buffer. Chromatin was pelleted and the nucleoplasmic fraction in the supernatant was collected. The chromatin pellet for SDS-PAGE was resuspended in the appropriate volume of 4x LSB (Laemmli sample buffer), sonicated with the Bioruptor Pico – 2 cycles of 30 seconds each, with a 30 second gap in between, then boiled and analysed by Western blotting as above.

Results

Generation of isogenic ARID1A cell lines

In order to gain deeper insights into ARID1A biology, the inventors set out to create cell lines with genetic modulation of ARID1A (Table 1).

Table 1: Table of cell lines used in this study of ARID1A. Derived cell lines indicates the number of clonal cell lines in each parental cell line background. EV, empty vector; KI, knock in, KO, knockout.

Parental Cell Line	Origin	ARID1A Status	Derived Cell Lines
TOV21G	Human OCCC	c.1650dup/c.2272del	EV, KI1 (C1), and KI2 (C2)
RPE1-hTERT	hTERT immortalised human retinal pigment epithelial cells	WT	KO1 and KO2
HCT116	Human colorectal carcinoma	WT	KO1

Because of the prevalence of ARID1A mutations in ovarian clear cell carcinoma (OCCC), the inventors began by re-expressing ARID1A in the TOV21G cell line, which is an OCCC derived line that has a loss of function mutation in ARID1A leading to lack of protein expression. The inventors generated two independently derived clonal cell lines in which were introduced an ARID1A expression construct, referred to here as KI1 and KI2. ARID1A expression was apparent by Western blot analysis (Fig. 1A), with expression levels higher in KI2 than KI1. As an additional control, the inventors introduced the empty expression construct into the TOV21G cell line (EV).

The inventors also generated CRISPR-Cas9 knockout (KO) mutations of ARID1A in the non-cancerous immortalised epithelial cell line RPE1-hTERT (Table 1, Fig. 1B). These were screened by Western blotting and validated by Sanger sequencing. Finally, the inventors obtained ARID1A KO and parental control cells generated in the colorectal HCT116 cell line (Table 1).

<u>ARID1A deficient cells show selective sensitivity to G4 binding ligands</u>

Treatment of cells with the G4 binding ligand pyridostatin (PDS) leads to impaired growth (Harrod, et al., 2020). To test whether the effect is modulated by the presence of ARID1A, the inventors treated their panel of TOV21G cell lines with PDS and monitored growth over time. Consistent with previous reports, the inventors found that PDS impairs growth of all cell lines. Importantly, however, the inventors found that the cells with ARID1A expression (KI1 and KI2) survives better than the isogenic parental and EV cells lacking ARID1A expression (Fig. 2A). A derivative of PDS, CX-5461, has recently entered clinical trials (9). When this compound was tested in viability assays, the inventors found that the cell lines expressing ARID1A also have improved growth rate in the presence of CX-5461 compared with the cells lacking ARID1A (Fig. 2B).

The inventors then tested the survival of the ARID1A KO cell lines relative to their isogenic parental controls in response to PDS exposure. The inventors found that the ARID1A KO clones in both the RPE1-hTERT and the HCT116 cell line backgrounds show decreased cell growth following treatment with PDS (Fig. 2C,D), consistent with a protective effect of ARID1A expression. The inventors used two orthogonal approaches to look at viability to ensure these results were robust. First, the inventors performed clonogenic survival assays on the RPE1-hTERT derived cells and found that there is a significant decrease in colony formation in the ARID1A KO clones when cells are exposed to PDS when compared with the parental control cells (Fig. 2E).

Next, the inventors used an assay to directly monitor cell death. The inventors did this using propidium iodide staining, which is a healthy membrane-impermeable nuclear dye that selectively stains dead cells. Using this assay, the inventors found that the ARID1A deficient cells have increased levels of cell death in a dose-dependent manner following exposure to PDS when compared with the ARID1A proficient cells. This was apparent in all three cell line models tested (Fig. 2F-H). Together, these data indicate that ARID1A loss leads to increased sensitivity to G4 binding ligands.

ARID1A is found specifically in the BAF complex, but not in the other two highly related SWI/SNF complexes PBAF or GBAF. To see whether this phenotype is shared by other SWI/SNF complexes, the inventors used cell lines with CRISPR-Cas9 mediated KO of PBRM1 (Rodriguez, et al., 2012), which is found specifically in the PBAF complex. These were tested in cell growth assays alongside the isogenic parental RPE1-hTERT cell line. In contrast to the inventors' data with ARID1A KO, the inventors found no reproducible or significant sensitivity to exposure to PDS (Fig. 2H), suggesting that not all SWI/SNF complexes are involved in the cellular response to G4 binding ligands.

The inventors looked to see if there is evidence of increased DNA damage in the ARID1A deficient cells following PDS exposure by analysing γH2AX foci. This histone variant is rapidly phosphorylated in response to DNA damage forming visible foci, and this provides a sensitive readout of DNA damage. The inventors tested γH2AX foci formation in the TOV21G cell lines and found, consistent with previous reports, that foci numbers increase over time after exposure to PDS (Fig. 3A). While the difference is modest, the number of foci in the cells with ARID1A re-expression is reproducibly lower than the parental and EV controls cells with no ARID1A expression (Fig. 3A), suggesting that there are more DNA DSBs after PDS treatment when ARID1A is deficient. In contrast and consistent with the survival data, the inventors saw no consistent increase in γH2AX foci in the PBRM1 KO cells following PDS treatment (Fig. 3B). These data suggest that the increase in cell death when

ARID1A is deficient could be a consequence of increased persistent or unrepaired DNA DSBs, and support a role for BAF, but not PBAF, in mediating responses to G4 binding ligands.

ARID1A deficient cells fail to recruit non-homologous end joining (NHEJ) proteins to chromatin after PDS exposure

To understand what pathways were altered in the absence of ARID1A, the inventors analysed the proteins associated with the chromatin fraction in the TOV21G cell line panel before and after PDS treatment by mass spectroscopy. The inventors identified the proteins that were recruited to chromatin in the ARID1A proficient cells upon PDS treatment and focused on those that failed to do so in the ARID1A deficient cells. When pathway analysis was performed on this group of proteins, the inventors found that the NHEJ pathway was a top hit (Fig. 4A). There was very good concordance between the two independently derived ARID1A re-expressing clones with NHEJ proteins associating with chromatin post-PDS in both (Fig. 4B). In contrast, these proteins were not evident in the chromatin fraction of PDS-treated ARID1A deficient parental or EV cells (Fig. 4C).

To investigate this using an orthogonal approach, the inventors performed Western blot analysis of cytoplasmic, nucleoplasmic, and chromatin-bound fractions prepared from the TOV21G cell line panel before and after PDS treatment. The inventors found that there is a detectable increase in chromatin-bound NHEJ proteins after PDS treatment in the two clones with ARID1A re-expression, but not in the parental and EV controls (Fig. 4D). Together, these data suggest that ARID1A is important for recruiting NHEJ repair proteins to chromatin after PDS treatment.

Discussion

OCCC is a cancer of unmet need with poor prognosis and limited treatment options. ARID1A deficiency is frequent, and this provides an opportunity to identify therapeutic approaches that target vulnerabilities associated with ARID1A loss. Here, the inventors used an OCCC cell line model to identify G4 binding ligands as a selective sensitivity in ARID1A deficient cells, and the inventors' data is consistent with a defect in DNA DSB repair using NHEJ. ARID1A was previously shown to recruit NHEJ proteins to laser irradiation induced DNA DSBs (Watanabe, et al., 2014), suggesting that the same mechanism is used in response to G4 ligand induced DNA DSBs. Whether this recruitment of NHEJ proteins to chromatin is through a direct interaction or a result of chromatin remodelling to allow NHEJ binding is still unclear.

G4 binding ligands have generated significant interest as a therapeutic approach for cancer cells. A full understanding of the molecular mechanisms that dictate sensitivity to G4 binding ligands will require further investigation, but there is evidence that cells with impaired DNA DSB repair pathways are vulnerable to G4 binding ligands (Masud, et al., 2021, Zimmer, et al., 2016, Olivieri, et al., 2020). The PDS derivative CX-5461 is now in phase I clinical trials (Hilton, et al., 2022), and it is important to identify patients who will respond well to these. The inventor's data suggest that OCCC patients with loss of function mutations in ARID1A are good candidates for CX-5461 treatment and therefore, for all clinically suitable G quadruplex binding ligands.

Interestingly, the inventors found similar sensitivity to PDS in non-OCCC derived cell lines when ARID1A is deficient. This suggests that sensitivity to G4 binding ligands could be a general feature of ARID1A deficient cells and that the clinical utility could extend beyond OCCC.

EXAMPLE 2: Specific killing of SMARCA4 -deficient cancer cells by G quadruplex binding ligands

Materials and Methods

Oligonucleotide preparation for G4 binding assays

For the G4 binding assays, 3'-biotinylated oligonucleotides corresponding to G4-containing elements of the c-MYC, KIT1, or BCL2 promoters were used as described by (7). The oligonucleotides were prepared at 0.02 μM in 10 mM Tris HCl pH 7.4 containing 100 mM KCl. G-quadruplex folding (or dsDNA formation) was promoted by heating the oligos at 95 °C for 5 minutes followed by gradual cooling at a rate of -0.3 °C per minute to 10 °C. Oligos were transferred to ice and stored at -20 °C.

G4 ss Myc	5' TGA GGG TGG GTA GGG TGG GTA ATT TTT 3'
ss mut Myc	5' TGA GTG TGT GTA GTG TGT GTA ATT TTT 3'
ds Myc	5' TTA CCC ACC CTA CCC ACC CTC A 3'
G4 ss Kit1	5' AGG GAG GGC GCT GGG AGG AGG GTT TTT 3'
ss mut Kit1	5' AGT GAG TGC GCT GTG AGG AGT GTT TTT 3'
ds Kit1	5' CCC TCC TCC CAG CGC CCT CCC T 3'
G4 ss BCL2	5' GGG CGC GGG AGG AAT TGG GCG GGT TTT T 3'

ss mut BCL2 5' GTG CGC GTG AGG AAT TGT GCG TGT TTT T 3'

G4 pull-down assay

For these experiments, Streptavidin MagneSphere Paramagnetic Particles (Promega, Z5482) were used as described by (7) with a few modifications. In summary, 0.05 mL of Streptavidin MagneSphere slurry for sample was washed twice with 0.3 mL pull-down buffer (25 mM HEPES pH 7.5, 120 mM KCl, 1 mM $MgCl_2$, 0.01 mM $ZnCl_2$, 20% v/v glycerol, 0.1% Igepal C-630, and protease and phosphatase inhibitor cocktails (Roche, cOmplete Protease Inhibitor Cocktail 04693116001 and PhosSTOP 4906837001) plus the addition of 3% bovine serum albumin (BSA, Sigma Aldrich A9647) and 0.2 g/L salmon sperm DNA (Invitrogen, 15632011). The streptavidin magnespheres were resuspended in 0.3 mL of pull-down buffer plus BSA and salmon sperm DNA, and 0.02 mL of the folded biotinylated oligonucleotides were added and incubated by shaking at room temperature for 30-60 minutes. Beads were washed twice with pull-down buffer plus BSA and salmon sperm DNA to remove the unbound biotinylated oligonucleotides and resuspended in 0.3 mL of pull-down buffer plus BSA and salmon sperm DNA. Depending on the experiment, 40 ng of recombinant human full length SMARCA4 (Abcam, ab82237), 500 ng of C-terminus SMARCA4, 100 ng of labelled SMARCA4 peptide or 75-100 ng of nuclear protein extracts were added to the biotinylated oligonucleotides bound to the streptavidin magnespheres and incubated by rotation at 4 °C overnight. Beads were washed three times with pull-down buffer and resuspended in LDS sample buffer (Life technologies, NP0007) containing DTT before being boiled and electrophoresed on a polyacrylamide gel (glycine-SDS-PAGE or tricine gel (Invitrogen Novex 16%, Tricine, Mini Protein Gels, EC66952BOX)). Full length SMARCA4, C-terminus SMARCA4 or the peptides were detected using anti-SMARCA4 (SantaCruz, sc-17798), anti-FLAG (Sigma-Aldrich, F3165) or by fluorescent label, respectively. For the western blots, Immobilon Forte Western HRP substrate (WBLUF0500, Millipore) was used. Images were captured using iBright 750 (Life Technologies).

Expression and purification of SMARCA4 C-terminus

The expression plasmid for the SMARCA4 C-terminus (Brg1 Del4 pET28C) was a gift from Paul Herring (Addgene plasmid # 122118). The SMARCA4 fragment is labelled with a 6x His tag and a FLAG tag. Expression was induced with 0.2 mM IPTG at 20 °C for 18 hours and the cell pellet was frozen and thawed before being resuspended in lysis buffer (PBS + 0.2 mg/mL lysozyme (Sigma-Aldrich, L2879)) and frozen again. The sample was thawed and sonicated on ice for 30 seconds and 30% amplitude (Cole Palmer Ultrasonic Processor GEX 600). After centrifugation, the supernatant was incubated with the beads from a nickel column (HisPur™ Ni-NTA Spin Purification Kit, Thermo Scientific 88227) containing 10 mM

imidazole by rotation at room temperature for 90 minutes. Beads were transferred back to the column and washed three times with 1xPBS plus 25 mM imidazole and bound proteins were eluted using 1xPBS plus 250 mM imidazole.

SMARCA4 peptide labelling

SMARCA4 peptides (sequences in Fig. 1E) were synthetized by GenScript USA Inc. and resuspended in 1xPBS to get a concentration of 2 mg/mL. Peptides were labelled using Alexa Fluor™ 488 Protein Labeling Kit (Invitrogen, A10235) according to the manufacturer's protocol, except that 0.125 mL of peptide was mixed with 0.0225 mL of 1M $NaHCO_3$ and 0.1 mL of Alexa Fluor 488 dye (from a 0.5 mL PBS reconstituted solution) and incubated at room temperature by 1000 rpm shaking in a thermomixer for 1 hour.

Nuclear extract preparation

RPE1-hTERT cells were collected, resuspended to $30x10^6$ cells/mL in low salt buffer (20 mM HEPES, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EDTA, 1 mM DTT and protease and phosphatase cocktails), and incubated on ice for 15 minutes. This was followed by the addition of 0.05 mL of 10% NP-40 per 1 mL of cell suspension, and samples were vortexed for 1 min. After centrifugation at 900 g and 4 °C for 10 min, the nuclear pellet was washed once with low salt buffer and resuspended to $12x10^7$ nuclei/mL in high salt buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 3 mM $MgCl_2$, 0.2 mM EDTA, 0.5% NP-40 and 1 mM DTT and protease and phosphatase cocktails) and sonicated in a Diagenode Bioruptor Pico (10 cycles, 30 seconds on and 30 seconds off at 4 °C). The samples were centrifuged at 16000 g and 4 °C for 10 minutes and the supernatant was collected. Protein concentration was measured by using Bradford reagent.

Generation of SMARCA4 knockout cell lines

RPE1-hTERT cells (ATCC) were grown in DMEM/F-12 medium (Sigma-Aldrich, D6421) supplemented with glutamax (Gibco, 35050038), sodium bicarbonate (Gibco, 25080060), penicillin/streptomycin (Sigma-Aldrich, P4333) and 10% fetal bovine serum (Sigma-Aldrich, F7524). Six single guide RNA (sgRNA) were cloned in the lentiviral vector lentiCRISPRv2.

5'-CACCGCGCGAGGCTTCCCCTCGTT 3' (sgSMARCA4_ex1_1.1)

5'-AAACAACGAGGGGAAGCCTCGCGC 3' (sgSMARCA4_ex1_1.2)

5'-CACCGCGCCGGGAAGTCGACGGCGC 3' (sgSMARCA4_ex1_2.1)

5'-AAACGCGCCGTCGACTTCCCGGCGC 3' (sgSMARCA4_ex1_2.2)

5'-CACCGCCGGCGAGCGCGCGCGCAGC 3' (sgSMARCA4_ex1_3.1)

5'-AAACGCTGCGCGCGCGCTCGCCGGC 3' (sgSMARCA4_ex1_3.2)

5'-CACCGTGGCCGAGGAGTTCCGCCCA 3' (sgSMARCA4_ex2_4.1)

5'-AAACTGGGCGGAACTCCTCGGCCAC 3' (sgSMARCA4_ex2_4.2)

5'-CACCGGCTCCGCCCACAGCATGAT 3' (sgSMARCA4_ex2_5.1)

5'-AAACATCATGCTGTGGGCGGAGCC 3' (sgSMARCA4_ex2_5.2)

5'-CACCGTTGTCCTGAGGGTACCCTCC 3' (sgSMARCA4_ex2_6.1)

5'-AAACGGAGGGTACCCTCAGGACAAC 3' (sgSMARCA4_ex2_6.2)

RPE1-hTERT cells were infected with sgRNA containing lentiviral particles, single cell sorted, and screened by Western blotting. Clonal cell lines lacking SMARCA4 expression were validated by Sanger sequencing.

Sulforhodamine B (cell growth) assay

Cells were seeded in 96-well plates at the appropriate densities. 24 hours later, drugs were added, and cells were allowed to grow for 6 days. After 6 days, cells were fixed by adding 10% TCA to the medium and plates were kept at 4°C for additional 24 hours. Plates were then washed 5 x with water and left to dry. Once dried, cells were stained with 0.0057% SRB in 1% acetic acid, incubated for 1 hour RT and then washed with 1% acetic acid and left to dry overnight. For cell mass quantification, cells were resuspended in Sulforhodamine B (SRB), incubated for 10 minutes in a shaker, washed and then measured under the wavelength of 492 nm. Data was plotted as percent survival (cell growth after PDS treatment relative to cell growth in untreated conditions), n=3 biological replicates presented as the mean +/- SD.

Whole genome sequencing and INDUCE-seq

For WGS, cell line preparation, library construction, and data processing were performed as in (17). Briefly, parental RPE1-hTERT and two SMARCA4 KO clonal cell lines were grown for one month in the presence or absence of 0.1 mM hydroxyurea. Following single cell sorting, clonal cell lines (6 per condition) were expanded and processed for sequencing (Illumina; Novogen). Reads were mapped to human reference genome (GRCh37/hg19) using BWA-MEM. Mapped reads were quality filtered with SAMtools and a custom script, and then converted into BED files. Genome blacklist regions were excluded and SNVs and INDELs were called and intersected with G4 containing locations.

For INDUCE-seq, library construction and data processing were performed as in (16). Briefly, 1X105 cells were seeded to each well of a 96-well plate pre-coated with Poly-L-lysine and fixed in 4% PFA for 10min at room temperature. Cells were subjected to the two-step permeabilisation as described in the protocol and washed three times in 1xCutSmart® Buffer (NEB, B7204S). The DNA double strand ends were end repaired with Quick Blunting Kit (NEB) and A-tailed with NEBNext® dATailing Module (NEB) according to manufacturer's protocol. A-tailed ends were ligated to the modified P5 adapter and excessive P5 adapter was washed away after ligation. Genomic DNA was extracted with Genomic DNA Clean & Concentrator™-10 (Zymo), sonicated to 300-500bp fragments, and size selected to exclude fragments smaller than 150 bp. Fragmented and size-selected DNA was then end-repaired and ligated to P7 adapter with NEBNext® Ultra™ II Ligation Module (NEB) according to the manufacturer's protocol. The ligated sequencing libraries were size selected to remove fragments smaller than 200bp and residual P7 adapter. Samples were pooled and concentrated before sequencing on an Illumina NextSeq 500 platform with 75bp single-ended reads. For the data processing pipeline, FASTQ files were demultiplexed and remove adapter sequence with Trim Galore. Reads were mapped to human reference genome (GRCh37/hg19) using BWA-MEM. Mapped reads were quality filtered with SAMtools and a custom script, and then converted into BED files. Genome blacklist regions were excluded for the further analysis. The breakend position was recorded as the first 5' nucleotide relative to the strand orientation and optical read duplicates were removed with a custom script. Breakend positions were then intersected with G4 containing locations.

Results

SMARCA4 binds to G quadruplex sequences through a motif in the C-terminal tail

To understand more about the interaction between SMARCA4 and G4 structures, the inventors first performed pull-down assays using recombinant full-length SMARCA4. The inventors used oligos as in (Zhang, et al., 2021), which correspond to the Myc promoter single-stranded G4-forming sequence, a mutated version that is incapable of forming G4s, and the Myc G4 paired to its complementary sequence. Consistent with the previous report (Zhang, et al., 2021), the inventors found that SMARCA4 preferentially binds to the G4-containing sequence (Fig. 5A). Similarly, the inventors tested G4 forming sequences and controls (mutated, dsDNA) from the Kit1 or Bcl2 promoters and found preferential SMARCA4 binding as reported (Fig. 5A). To determine whether SMARCA4 can bind to G4 structures in the context of the SWI/SNF complex, the inventors performed pull-down assays using nuclear extracts. Western blot analysis using an antibody against SMARCA4 showed a preferential association with the G4-containing oligos (Fig. 5B).

Arginine (R) and glycine (G) rich sequences have been shown to facilitate interactions with G4 structures, and an RGG domain comprising a series of RGG repeats separated by other amino acid residues is often found in G4 binding proteins (Brazda, et al., 2018). The inventors therefore analysed the SMARCA4 protein sequence to identify putative G4 binding regions. While there are no canonical RGG domains, the inventors found a motif that is enriched in arginine and glycine residues in the C-terminal tail of the protein (Fig. 5C).

The inventors therefore expressed and purified a FLAG-tagged SMARCA4 construct corresponding to amino acids 1146 through 1647. This construct comprises the bromodomain and the C-terminal tail of SMARCA4 containing the putative G4 binding motif (Fig. 5D). Similar to the full-length protein, the inventors found that this construct preferentially interacts with G4 containing oligos relative to the controls (Fig. 5D). To further define the interacting region, the inventors created a series of tiled peptides (P1-P4) across the putative G4 binding motif (Fig. 5E). Peptides P1 and P4, which do not contain the intact G4 binding motif, show no substantial binding to any of the oligos. In contrast, peptide P2, which does contain this motif, interacts with the oligos under these conditions and preferentially binds to the G4-containing sequences (Fig. 5F), suggesting that the motif the inventors identified is important for G4 binding. However, both P2 and P3 contained the G4 binding motif, but P3 binding to oligos in the pull-down assay is not detectable (Fig. 5F). This suggests that the upstream amino acid residues present in P2, while insufficient on their own, also contribute to G4 binding. Together, these data identify a motif in the C-terminal tail of SMARCA4 that has G4 binding ability.

Cells lacking SMARCA4 are sensitive to G4 binding ligands

G4s can be stabilised by compounds that interact with these structures, such as pyridostatin (PDS; (Kosiol, et al., 2021)). When treated with PDS, cells show increased levels of transcription- and replication-dependent DNA double strand breaks (DSBs) and growth arrest (Rodriguez, et al., 2012). Cells with defects in DSB repair pathways are particularly sensitive to PDS treatment (for example, (Zimmer, et al., 2016)), and this has been developed in the clinic for targeting BRCA-deficient cancers (Xu, et al., 2017, Hilton, et al., 2022).

To see whether the absence of SMARCA4 impacted on cellular sensitivity to G4 binding ligands, the inventors first created two independent CRISPR-Cas9 mediated SMARCA4 knockout (KO) clonal cell lines in the RPE1-hTERT background. These were screened by Western blotting and validated by Sanger sequencing (Fig. 6A). The inventors then performed survival assays and found that the SMARCA4 KO cells were more sensitive to

treatment with PDS than the isogenic parental cells (Fig. 6B). The inventors then monitored phosphorylation of H2AX (to form γH2AX) as a readout of DNA DSBs and found that levels were modestly but significantly increased in the SMARCA4 KO cells following PDS treatment (Fig. 6C). These data indicate that there is more DNA damage and reduced viability in SMARCA4-deficient cells when G4 binding ligands are present.

Genome instability events in SMARCA4 deficient cells exhibit an increased propensity to co-localise with G4 structures

G4 structures have been shown to act as an impediment to replication, leading to the generation of DNA breaks and genome instability (Rodriguez, et al., 2012, Lemmens, et al., 2015), and SMARCA4 deficient cells have been shown to display signs of replication stress (for example, (Kurashima, et al., 2020)). This raises the interesting possibility that SMARCA4 is important for preventing genome instability at G4 sequences.

To test this, the inventors first mapped the locations of unrepaired DNA DSBs in our SMARCA4 KO clones and the isogenic parental control. The inventors did this using INDUCE-seq (Dobbs, et al., 2020), which avoids the use of amplification during library preparation, thus allowing the detection of low frequency endogenous breaks. In addition to mapping breaks in untreated cells, the inventors also mapped breaks in cells following treatment with hydroxyurea (HU) for 16 hours to induce mild replication stress. The distribution of DSBs across genomic regions was not changed in the SMARCA4 KO cells relative to the parental cells (Fig. 7A).

The inventors wanted to compare the location of the DNA breaks to G4 structures. The inventors used two methods for G4 identification. First, the inventors made use of *in silico* prediction tools that identify sequences in the genome that have the propensity to form G4 structures. However, not all sequences with G4 forming potential form G4s in cells. The inventors therefore also made use of datasets where G4 structures have been mapped, either through G4-seq or using an antibody against G4 structures in ChIP-seq or Cut&Run assays. The inventors intersected the presence of unrepaired DNA breaks in our INDUCE-seq data with these datasets and found that there is an increased propensity for the DSBs to be located at a G4 forming sequence in the KO cells relative to the parental cells (Fig. 7B). While the absolute numbers were different, the relative difference was consistent across the analyses. Moreover, the presence of HU didn't change the overall patterns. These data show that in the absence of SMARCA4, unrepaired DNA breaks are more likely to be located at a G4 forming sequence, suggesting that SMARCA4 plays a protective role at G4 structures.

To take this analysis further, the inventors performed whole genome sequencing on SMARCA4 KO cells and the parental control under untreated and HU-treated conditions. The inventors mapped the location of single nucleotide variations (SNVs) or insertion/deletion (INDEL) mutational events and intersected them with the G4 datasets as above. Here, the inventors found that there is an increased propensity for SNVs in SMARCA4 KO cells to be located at G4 forming sequences, although in this case, the patterns were less pronounced for the untreated cells and more obvious following HU-induced replication stress (Fig. 7B). In contrast, the inventors didn't find a clear pattern of INDEL association with G4 forming sequences, regardless of HU treatment (Fig. 7B). Together, these data suggest that SMARCA4 plays a protective role at G4 forming sequences.

Discussion

SMARCA4 and the SWI/SNF chromatin remodelling complexes are critical regulators of gene expression in cells. The ability of SMARCA4 to bind G4 sequences in the regulatory elements of genes contributes to this function. Here, the inventors defined the motif in SMARCA4 that mediates this interaction. Interestingly, this motif is not conserved in the orthologous SMARCA2 protein, raising the possibility that SMARCA2 does not share the ability to bind G4 sequences.

The inventors also found that SMARCA4 plays a protective role and prevents mutations and DNA breaks at G4 sequences. This could play a role during cancer progression. SMARCA4 loss of function mutations are frequent and often occur early during tumourigenesis, which means that the evolution of cancer following SMARCA4 loss is likely to have increased instability around G4 forming sequences. It will be of interest to further explore the genomic landscape of SMARCA4 deficient cancers.

Finally, the inventors found that the absence of SMARCA4 leads to selective sensitivity to the G4 binding ligand PDS. The inventors also found that PDS treatment leads to increased levels of γH2AX formation in SMARCA4 deficient cells, which indicates increased DNA breaks. PDS binding to G4s stabilises the structures, which creates impediments to replication and transcription machinery and leads to DNA breaks (Rodriguez, et al., 2012). One possible interpretation of the inventors' data is that when present, SMARCA4 binds and protects G4s from PDS, such that the ligand is incapable of stabilising the structure and creating a DNA DSB. Alternatively, the presence of SMARCA4-containing complexes at PDS-stabilised G4 structures could help to repair the DSBs as they arise. In support of this latter possibility, SWI/SNF complexes have been shown to promote DNA DSB repair (Harrod, et al., 2020).

In either case, the absence of SMARCA4 creates a vulnerability to treatment with G4 stabilising ligands. Since these are now being used in the clinic (Hilton, et al., 2022), this presents an exciting new therapeutic approach for patients with SMARCA4-deficient tumours.

EXAMPLE 3:

Materials and Methods

Cell culture

TOV21G (ATCC) and derived cell lines were grown in Medium 199 in combination with MCDB105 supplemented with sodium bicarbonate and Fetal Bovine Serum (FBS, Gibco) at 37oC in a humidified incubator with 5% CO2. RPE1-hTERT (ATCC) and derived cell lines were grown in DMEM/F12 supplemented with 10% Fetal Bovine Serum (FBS, Gibco), 1% penicillin/streptomycin (Gibco), 3.5% sodium bicarbonate (Gibco), and 1% glutamax (Gibco) at 37oC in a humidified incubator with 5% CO2. HCT116-derived cell lines (parental and ARID1A KO; Horizon Discovery) were grown in McCoys 5A (Modified) Medium (Glico, 26600023) supplemented with 10% Fetal Bovine Serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco) at 37oC in a humidified incubator with 5% CO2.

Sulforhodamine B (cell growth) assay

Cells were seeded in 96-well plates at the appropriate densities. 24 hours later, drugs were added, and either washed out after 4h or not. Then cells were allowed to grow for 6 days. After 6 days, cells were fixed by adding 10% TCA to the medium and plates were kept at 4°C for additional 24 hours. Plates were then washed 5 x with water and left to dry. Once dried, cells were stained with 0.0057% SRB in 1% acetic acid, incubated for 1 hour RT and then washed with 1% acetic acid and left to dry overnight. For cell mass quantification, cells were resuspended in Sulforhodamine B (SRB), incubated for 10 minutes in a shaker, washed and then measured under the wavelength of 492nm.

In vivo studies

5 x 10^6 TOV21G or TOV.C1 or TOV.C2 cells were resuspended in Matrigel and injected subcutaneously into nude mice (Charles River Laboratories). After 30 days growth, mice were treated three times weekly (changing to twice weekly following weight loss) with 75mg/kg CX-5461 or vehicle alone (50mM NaH_2PO_4) by oral gavage until tumours reached 15mm size or until mice had greater than acceptable weight loss.

Cell fate profiling

Cells were seeded in 96 well plates and monitored using Caspase 3/7 staining or by imaging using an IncuCyte (Sartorius). Images were collected every 20 minutes and used to monitor proliferation of the population. Individual cells were manually tracked using exported images, and the time and stage of cell death (interphase or mitosis) were recorded.

Results

Further evidence that ARID1A deficient cells show selective sensitivity to G4 binding ligands

To test whether ARID1A deficiency leads to impaired survival in the presence of G4 ligands, cells were treated with 10 μM pyridostatin (PDS) and growth was monitored. The inventors find that the ARID1A deficient TOV21G and TOV.EV cell lines show impaired growth when compared with cells where ARID1A expression was re-introduced (TOV.C1 and TOV.C2; Figure 8A). Consistent results were obtained when varying doses of PDS between 5 and 15 μM were tested (Figure 8B). In addition, cells showed the same pattern of sensitivity when a different G4 ligand, CX-5461, was tested (Figure 8C).

The inventors then tested ARID1A knockout (KO) cell lines generated in the RPE1 epithelial cell line and found similar responses to PDS (Figures 8D and 8E), CX-5461 (Figure 8F) and an additional G4 ligand, PhenDC3 (Figure 8G). Finally, the inventors tested an ARID1A isogenic cell line created in the HCT116 cell line background and found that, consistent with the other cell line models, ARID1A deficiency leads to decreased growth when compared with the parental control cell line (Figure 8H).

ARID1A-deficient tumours show trend towards response following treatment with CX-5461

To understand whether treatment with G4 ligands holds clinical value, the inventors investigated the response to the clinically approved G4 ligand CX-5461 in mouse models. The inventors did this using the ARID1A-deficient ovarian cancer line TOV21G. In addition, the TOV21G cell lines with ARID1A re-expression (C1 and C2) were tested in parallel. The inventors found that ARID1A re-expression resulted in a failure to establish tumours in mice (Figure 9A), consistent with the role of ARID1A as a tumour suppressor.

Mice bearing tumours established from TOV21G cells were treated with vehicle or CX-5461. Mice were culled when tumours reached 15mm in size or when they displayed greater than acceptable weight loss. As a result, only 4 of 11 mice in the vehicle group and 5 of 11 mice in the CX-5461 group were viable at the end of the study. There was a clear trend towards reduced tumour volume in the CX-5461 treated cohort compared with those treated with vehicle alone (Figures 9B and 9C).

Treatment with G4 ligands leads to apoptosis, and acute treatment results in increased survival specifically in ARID1A proficient cells

To understand how PDS influences cell growth, the inventors monitored cells for the production of Caspase 3/7 as a readout of apoptosis. In untreated cells, the inventors detected a low level of apoptotic cells in the ARID1A deficient TOV21G cell line, which was not apparent in the two ARID1A re-expressing cell lines (TOV.C1 and TOV.C2; Figure 10A top panel). When these cell lines were treated over the course of 72 hours with 10 μM PDS, the inventors found that the Caspase 3/7 signal was highest in the ARID1A-deficient cells at late time points (Figure 10A bottom panel).

The inventors next performed live cell imaging to track the fate of cells over time in untreated or PDS-treated conditions over 72 hours. In untreated conditions, the inventors find that there is a higher percentage of cells that undergoes cell death (either in mitosis or interphase) in the ARID1A deficient TOV21G cell line when compared with the two ARID1A re-expressing clones (Figure 10B). The difference is much greater following PDS treatment, with the majority of TOV21G cells undergoing cell death, whereas almost half of the cell population remains viable in both ARID1A re-expressing cell lines (Figure 10B).

To determine whether the response to PDS is influenced by the treatment window, the inventors compared the cellular response to acute versus chronic exposure to PDS. In this assay, cells were either treated with PDS continuously for 6 days (no washout) or for four hours, followed by 6 days in fresh media (washout after 4 hours). The inventors found that there was no difference in the surviving fraction of the ARID1A deficient cells (TOV21G or TOV.EV; Figures 10C and 10D), suggesting that the commitment to apoptosis was made early in response to PDS exposure. In contrast, the two ARID1A re-expressing cell lines

(TOV.C1 and TOV.C2) showed increased survival following acute PDS exposure when compared with chronic exposure (Figures 10C and 10D).

Discussion

These data demonstrate that ARID1A modulates the response to G4 stabilising ligands, including CX-5461, which is already in clinical use. ARID1A is frequently deficient in ovarian clear cell carcinoma, which is a cancer with poor prognosis and limited treatment options. Using an ARID1A-deficient ovarian cell line model, the inventors show that there is a trend towards response to CX-5461 treatment.

Prompted by the inventors finding that the ovarian cell line models were undergoing apoptosis in response to PDS treatment, the inventors tested whether acute treatment would lead to different survival outcomes. Notably, the inventors found that the selective sensitivity of ARID1A deficient cells to pyridostatin treatment is widened when cells are exposed to acute PDS treatment instead of chronic exposure. This difference is the result of increased survival of ARID1A proficient cells, and suggests that short exposure times could spare normal healthy tissue, while still leading to substantial toxicity in ARID1A deficient cancer cells.

Table 2: List of sequences disclosed in this application

SEQ ID NO:	Sequence (5' → 3')	Name of the sequence
1	CACCGAACCCATACTCGCAGCAACA	ARID1A_sg1_FW
2	AAACTGTTGCTGCGAGTATGGGTTC	ARID1A_sg1_RV
3	CACCGATGGTCATCGGGTACCGCTG	ARID1A_sg2_FW
4	AAACCAGCGGTACCCGATGACCATC	ARID1A_sg2_RV
5	CACCGCGGTACCCGATGACCATGC	ARID1A_sg_3_FW
6	AAACGCATGGTCATCGGGTACCGC	ARID1A_sg3_RV
7	GTTCTGAGGCGGGTCAGTT	ARID1A_gDNA_FW1
8	GAGCACACATGGAACGGTGC	ARID1A_gDNA_RV1
9	TGAGGGTGGGTAGGGTGGGTAATTTTT	G4 ss Myc
10	TGAGTGTGTGTAGTGTGTGTAATTTTT	ss mut Myc
11	TTACCCACCCTACCCACCCTCA	ds Myc
12	AGGGAGGGCGCTGGGAGGAGGGTTTTT	G4 ss Kit1
13	AGTGAGTGCGCTGTGAGGAGTGTTTTT	ss mut Kit1
14	CCCTCCTCCCAGCGCCCTCCCT	ds Kit1
15	GGGCGCGGGAGGAATTGGGCGGGTTTTT	G4 ss BCL2
16	GTGCGCGTGAGGAATTGTGCGTGTTTTT	ss mut BCL2
17	CACCGCGCGAGGCTTCCCCTCGTT	sgSMARCA4_ex1_1.1
18	AAACAACGAGGGGAAGCCTCGCGC	sgSMARCA4_ex1_1.2
19	CACCGCGCCGGGAAGTCGACGGCGC	sgSMARCA4_ex1_2.1
20	AAACGCGCCGTCGACTTCCCGGCGC	sgSMARCA4_ex1_2.2
21	CACCGCCGGCGAGCGCGCGCGCAGC	sgSMARCA4_ex1_3.1
22	AAACGCTGCGCGCGCGCTCGCCGGC	sgSMARCA4_ex1_3.2
23	CACCGTGGCCGAGGAGTTCCGCCCA	sgSMARCA4_ex2_4.1
24	AAACTGGGCGGAACTCCTCGGCCAC	sgSMARCA4_ex2_4.2
25	CACCGGCTCCGCCCACAGCATGAT	sgSMARCA4_ex2_5.1
26	AAACATCATGCTGTGGGCGGAGCC	sgSMARCA4_ex2_5.2
27	CACCGTTGTCCTGAGGGTACCCTCC	sgSMARCA4_ex2_6.1
28	AAACGGAGGGTACCCTCAGGACAAC	sgSMARCA4_ex2_6.2

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CLAIMS

1. A method for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand, the method comprising the steps of:
a) determining the level of one or more biomarker in a biological sample from the test subject, wherein the one or more biomarker is a subunit of the SWI/SNF complex;
b) comparing the level of the one or more biomarker with a threshold level or range; and
c) predicting that:
i) the test subject will respond to treatment with a G quadruplex ligand if the test subject's biological sample has a decreased level of the one or more biomarker compared to the threshold level or range; and
ii) the test subject will not respond to treatment with a G quadruplex ligand if the test subject's biological sample has an increased level of the one or more biomarker compared to the threshold level or range.

2. The method of claim 1, wherein the level of the one or more biomarker in the biological sample is decreased by at least 1.5 fold, at least 2 fold, at least 2.5 fold, or at least 5 fold compared to the threshold level or range.

3. A pharmaceutical composition comprising a G quadruplex binding ligand for use in inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex in a subject.

4. A method of inducing cell death of a cancer cell deficient in a subunit of the SWI/SNF complex, comprising subjecting the cell to a G quadruplex binding ligand.

5. The method of claim 4, wherein the method comprises inducing cell death of a cell deficient in a subunit of the SWI/SNF complex in a subject, wherein a G quadruplex binding ligand is administered to the subject.

6. The method of claim 5, wherein the G quadruplex binding ligand is administered to the subject as an acute dose.

7. The method of claim 6, wherein the acute dose is at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least

about 6 hours, at least about 8 hours, at least about 10 hours, at least about 11 hours, or up to about 12 hours.

8. The method of claims 6 or 7, wherein the acute dose is at least about 4 hours.

9. A method for treating a subject having cancer, the method comprising: administering to a subject a G quadruplex binding ligand, wherein the subject is identified as likely to benefit from treatment with a G quadruplex binding ligand based on having, in a biological sample, a decreased level of one or more biomarkers compared to a threshold level or range, wherein the one or more biomarkers is a subunit of the SWI/SNF complex.

10. The composition for use, or methods of any preceding claim, wherein the subunit of the SWI/SNF complex is selected from the group consisting of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1, or wherein the cell is deficient in one or more proteins selected from the group consisting of: ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1.

11. The composition for use, or methods of any preceding claim, wherein the subunit of the SWI/SNF complex is ARID1A and/or SMARCA4, or wherein the cell is deficient in ARID1A and/or SMARCA4.

12. The composition for use, or methods of any preceding claim, wherein the cell is a cancer cell selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lymphoma, brain, neuroblastoma, lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, cervical, breast, melanoma, bladder, endometrial, head and neck, liver, renal and prostate cancer cell, or wherein the cancer is selected from the group consisting of: adrenocortical, rhabdoid, sarcoma, lymphoma, brain, neuroblastoma, lung, colon, pancreatic, gall bladder, gastric, oesophageal, mesothelioma, ovarian, cervical, breast, melanoma, bladder, endometrial, head and neck, liver, renal and prostate cancer cell.

13. The composition for use, or methods of any preceding claim, wherein the G quadruplex binding ligand is selected from the group consisting of: a nucleic acid, a peptide or a binding molecule, optionally wherein the binding molecule is a small molecule or an antibody.

14. The composition for use of any preceding claim or method of claims 4 to 13, for treating ARID1A/and or SMARCA4 deficient cancer in a subject.

15. The composition for use of any preceding claim, or methods of any preceding claim, wherein the subject is human.

16. The composition for use of any preceding claim, or method of any one of claims 4 to 15, wherein the composition is for use in combination with one or more anti-cancer therapy.

17. The method of any one of claims 4 to 16, wherein:
a) the subject is undergoing treatment with, has been treated with, or has been prescribed treatment with, one or more anti-cancer therapy; and/or
b) the method further comprises administering one or more anti-cancer therapy to the subject.

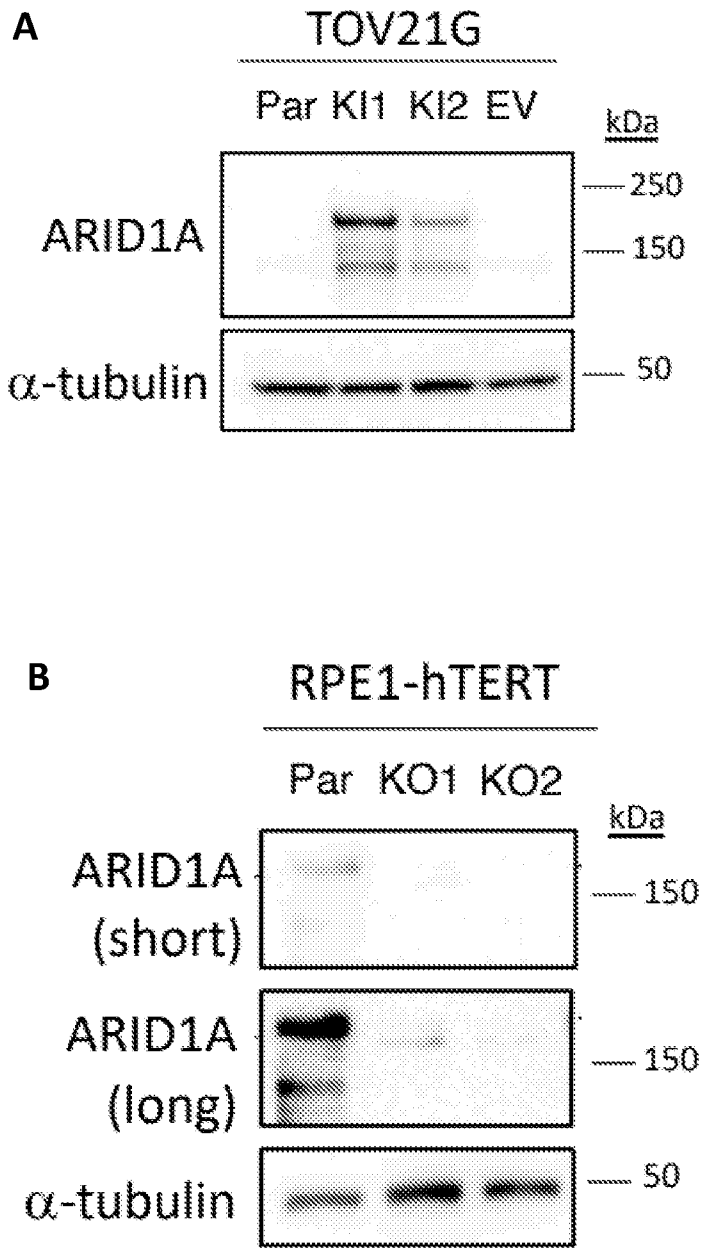
18. The composition for use according to claim 16, or method of claim 17, wherein the anti-cancer therapy is selected from the group consisting of surgery, radiation therapy, chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, adjuvant therapy, and immunotherapy.

19. The composition for use, or method, of claim 18, wherein the chemotherapy is a cytotoxic agent.

20. The composition for use, or method, of claim 19, wherein the cytotoxic agent is selected from the group consisting of a platinum agent, mitomycin C, a poly (ADP-ribose) polymerase (PARP) inhibitor, a radioisotope, a vinca alkaloid, a taxane, an antitumor alkylating agent, a monoclonal antibody and an antimetabolite.

21. The composition for use, or method, of claim 20, wherein the platinum agent is carboplatin.

22. Use of one or more biomarkers selected from the group consisting of ARID1A, SMARCA4, ACTB, ACTL6A, ACTL6B, ARID1B, ARID2, BCL7A, BCL7B, BCL7C, BICRA, BICRL, BRD7, BRD9, DPF1, DPF2, DPF3, PBRM1, SMARCA2, SMARCB1, SMARCC1, SMARCC2, SMARCD1, SMARCD2, SMARCD3, SMARCE1, SS18, SS18L1 as a biomarker for predicting whether a subject having cancer is likely to benefit from treatment with a G quadruplex binding ligand.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TOV21G
Par KI1 KI2 EV
kDa
ARID1A
250
150
α-tubulin
50
B
RPE1-hTERT
Par KO1 KO2
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ARID1A (short)
150
ARID1A (long)
150
α-tubulin
50

Figure 1

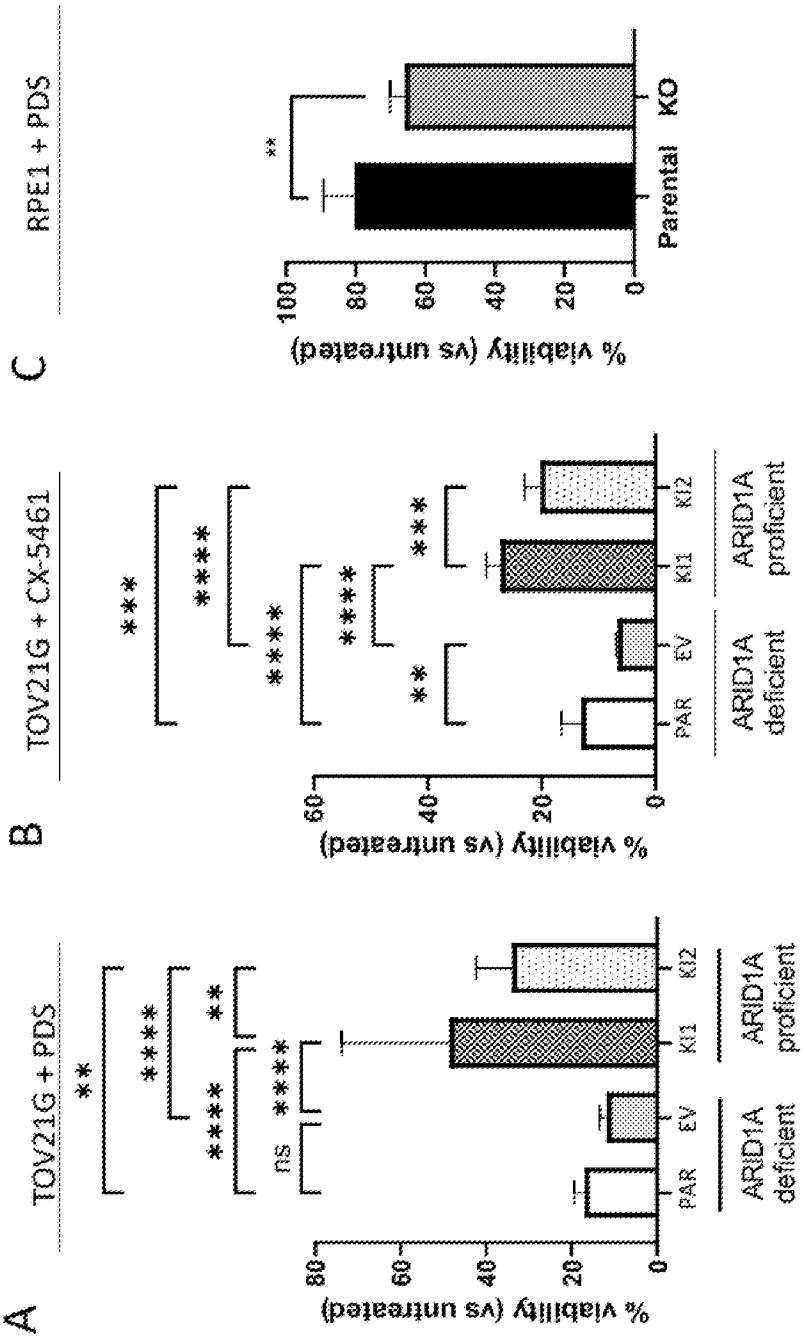
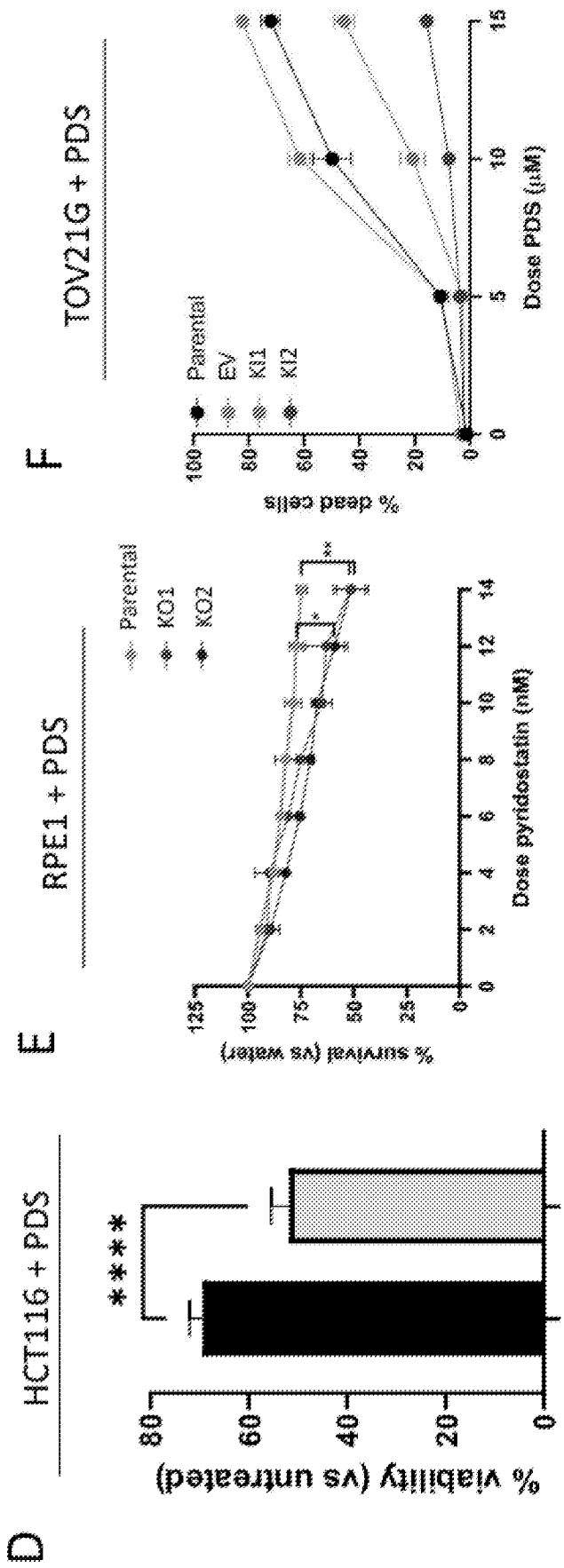

A
TOV21G + PDS
% viability (vs untreated)
ARID1A deficient
ARID1A proficient
PAR
EV
KI1
KI2
B
TOV21G + CX-5461
% viability (vs untreated)
C
RPE1 + PDS
% viability (vs untreated)
Parental
KO

Figure 2



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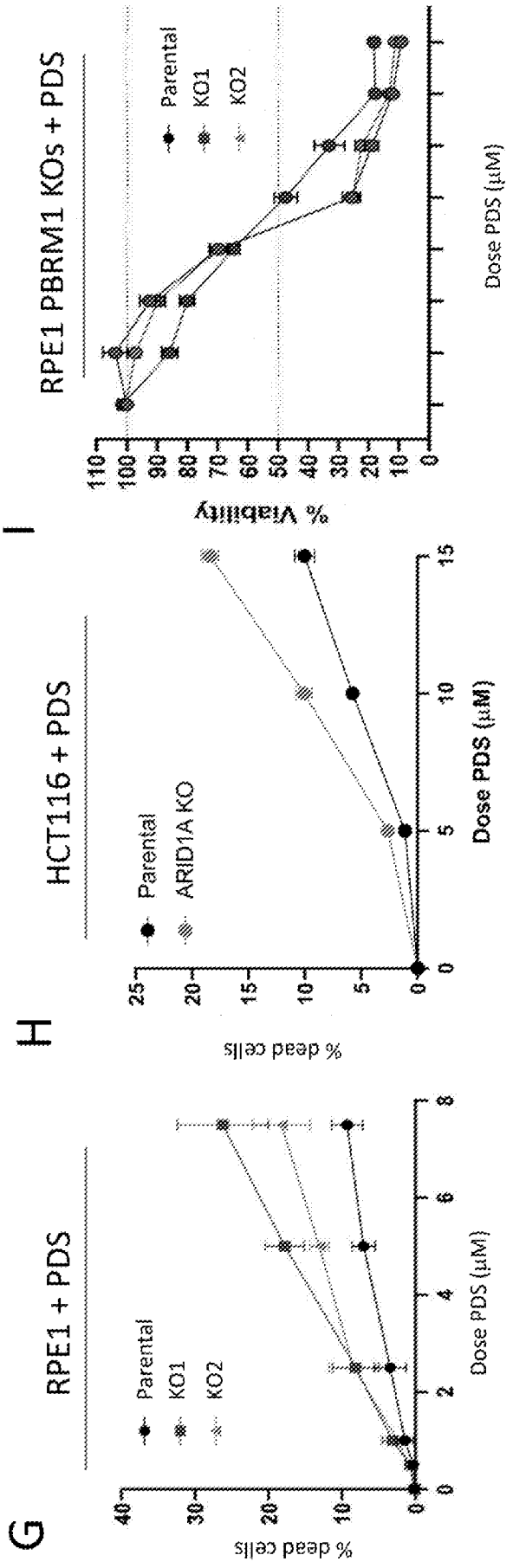


Figure 2 continued

A

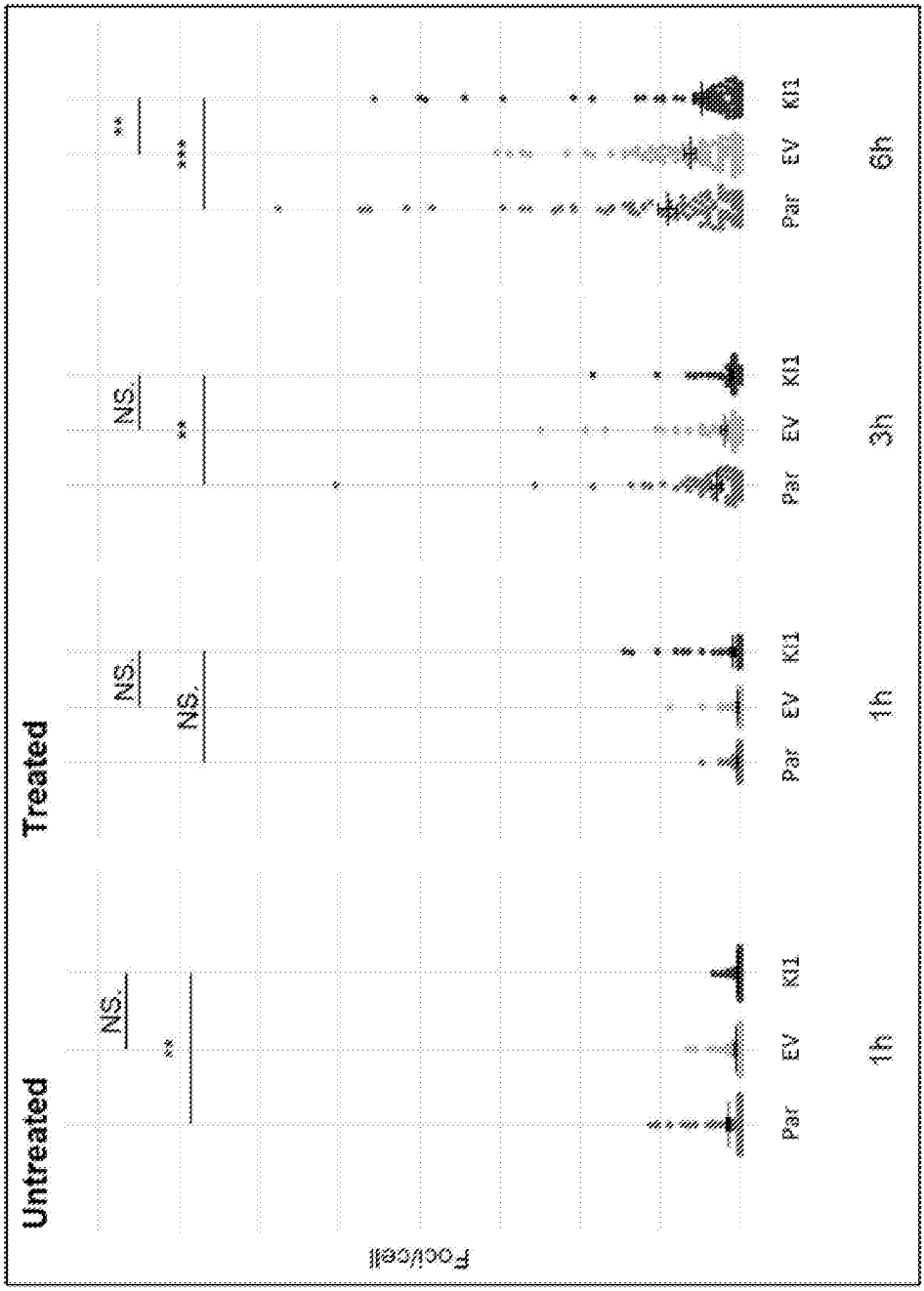
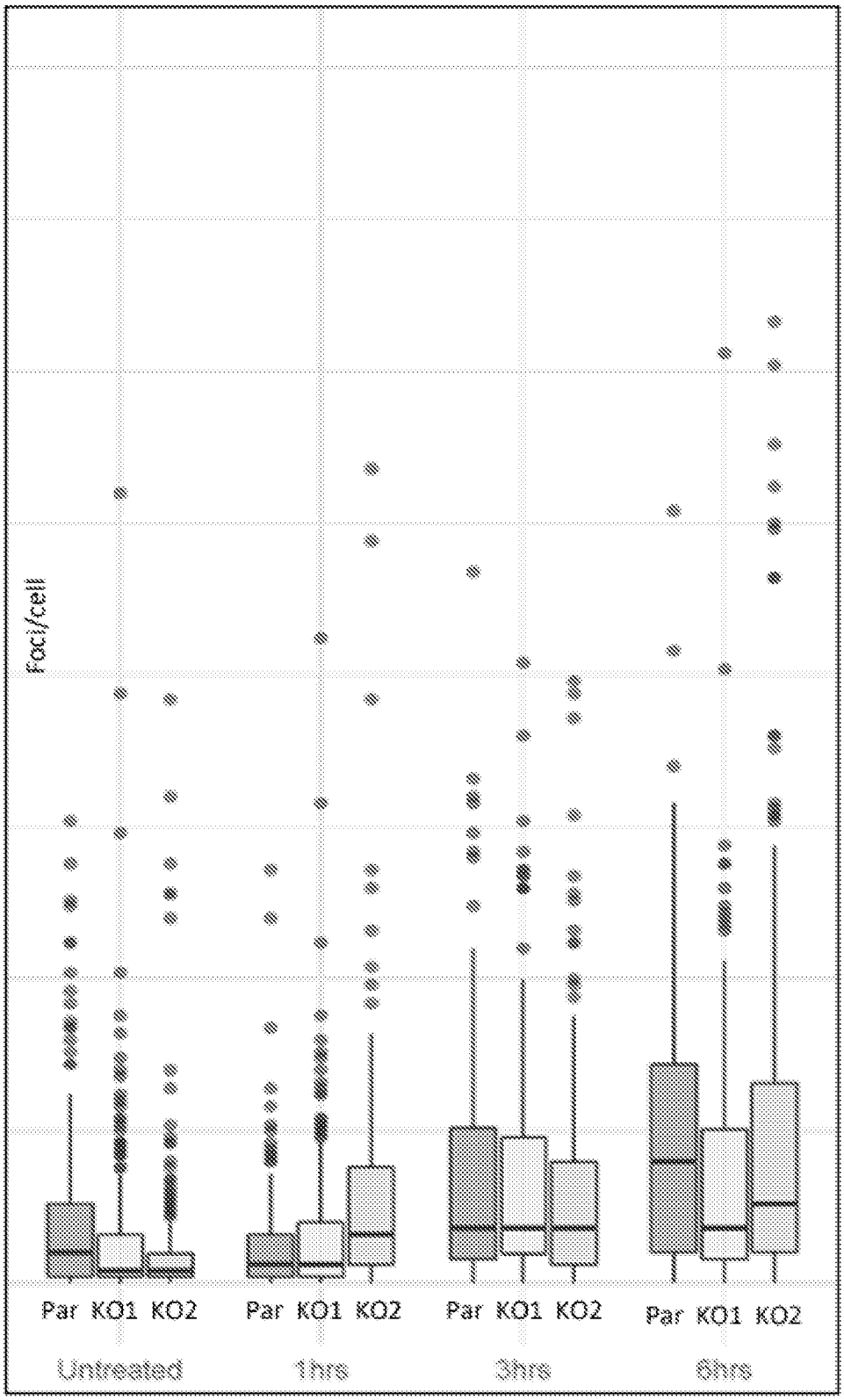


Figure 3

B



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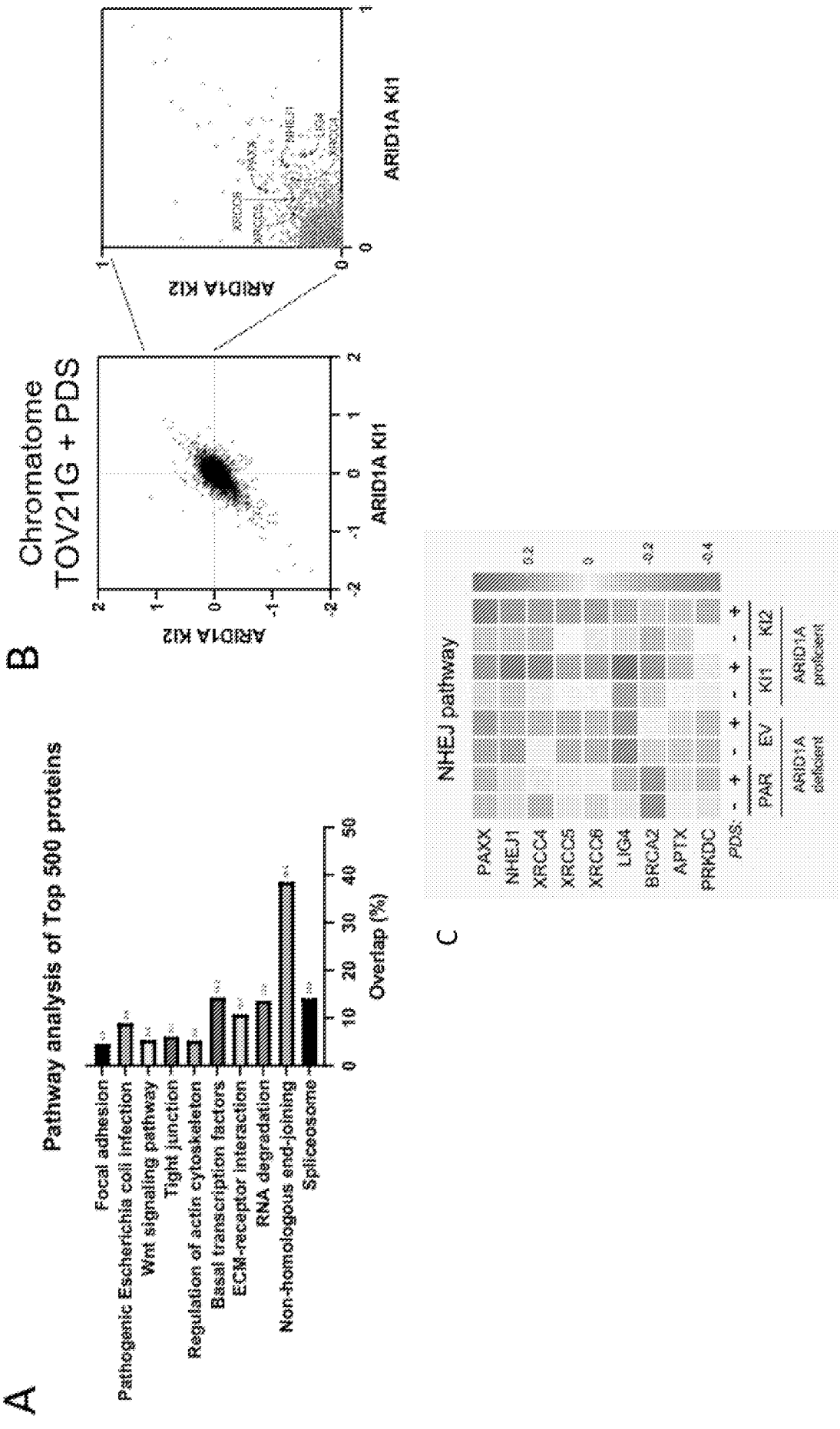

A
Pathway analysis of Top 500 proteins
Focal adhesion
Pathogenic Escherichia coli infection
Wnt signaling pathway
Tight junction
Regulation of actin cytoskeleton
Basal transcription factors
ECM-receptor interaction
RNA degradation
Non-homologous end-joining
Spliceosome
0 10 20 30 40 50
Overlap (%)
B
Chromatome
TOV21G + PDS
ARID1A KI2
ARID1A KI1
ARID1A KI2
ARID1A KI1
C
NHEJ pathway
PAXX
NHEJ1
XRCC4
XRCC5
XRCC6
LIG4
BRCA2
APTX
PRKDC
0.2
0
-0.2
-0.4
PDS - + - + - + - +
PAR EV KI1 KI2
ARID1A deficient
ARID1A proficient

Figure 4

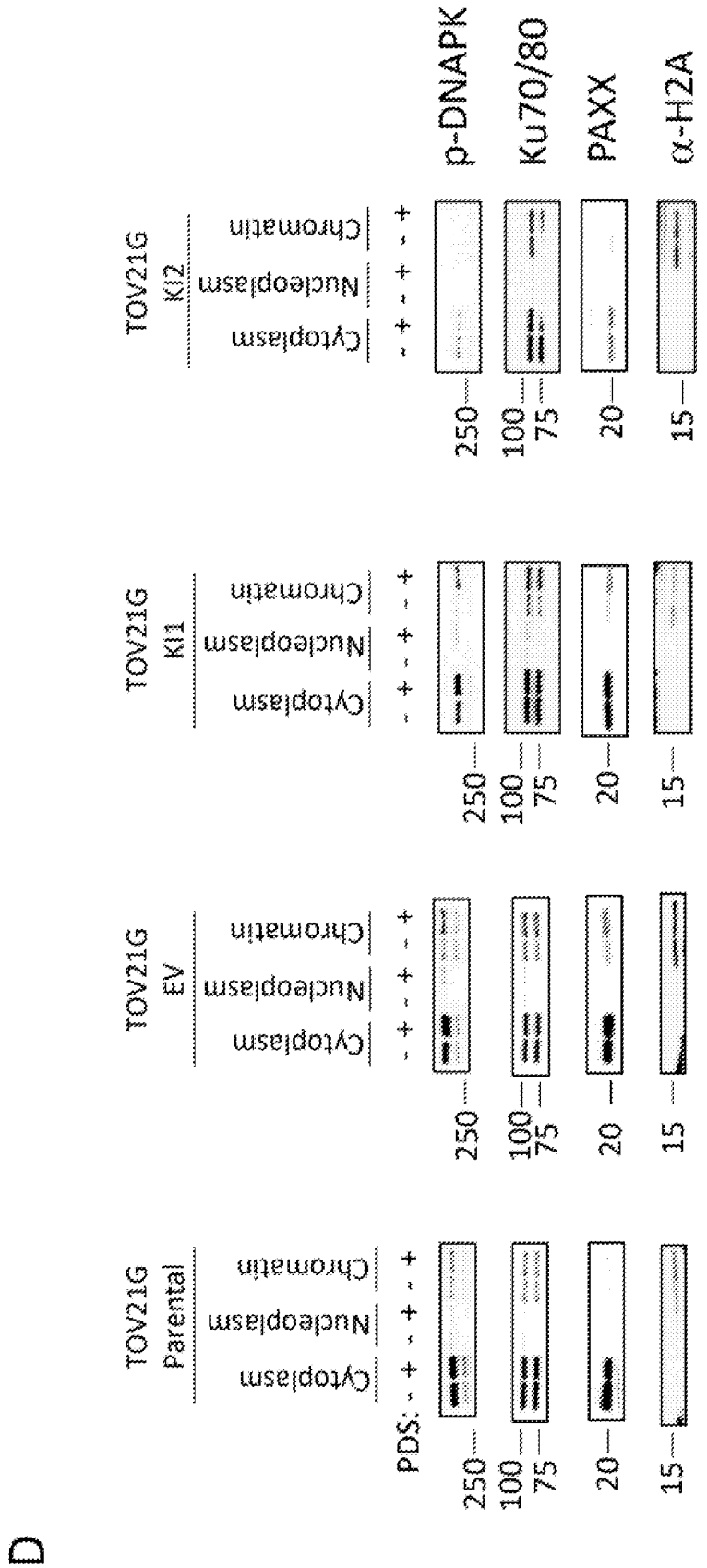


Figure 4 continued

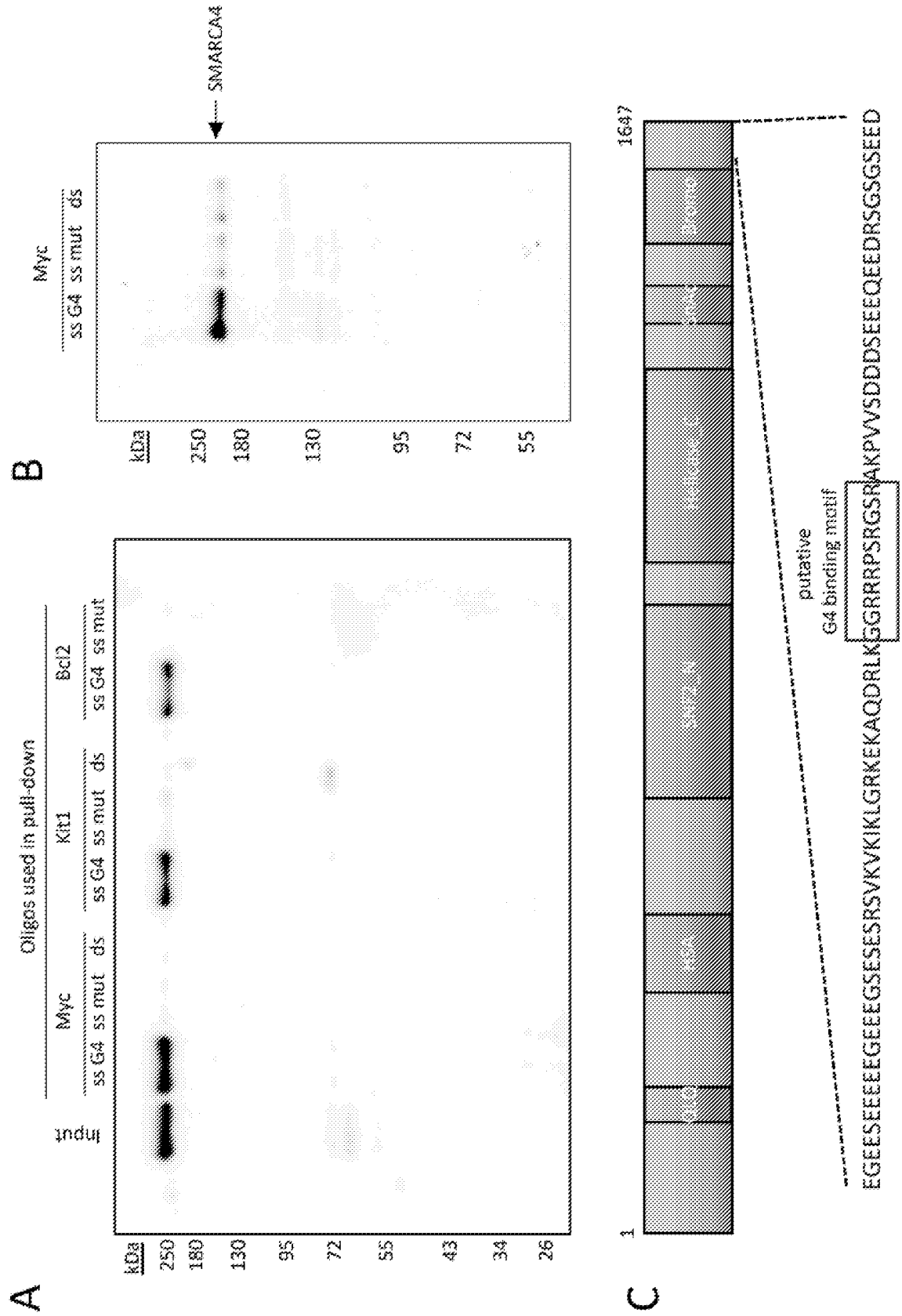
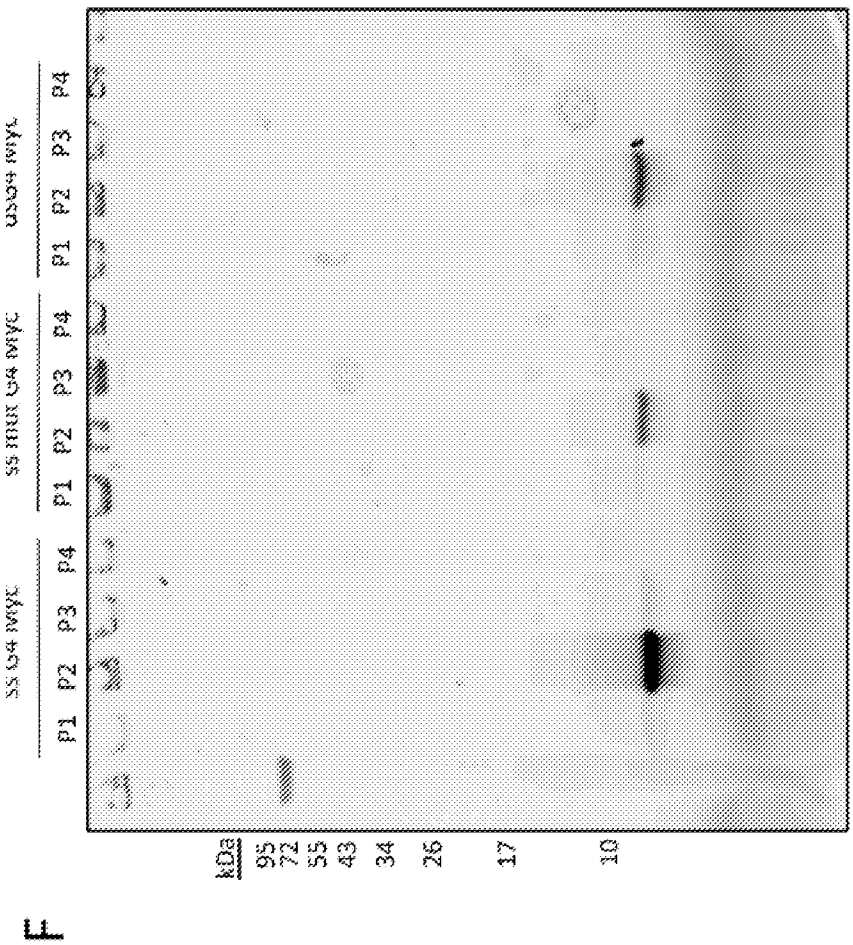

A
Oligos used in pull-down
Input
Myc
ss G4
ss mut
ds
Kit1
ss G4
ss mut
ds
Bcl2
ss G4
ss mut
kDa
250
180
130
95
72
55
43
34
26
B
Myc
ss G4
ss mut
ds
SMARCA4
kDa
250
180
130
95
72
55
C
1
1647
putative
G4 binding motif
EGEESEEEEEGEEEGSESESRSVKVKIKLGRKEKAQDRLKGGRRRPSRGSRAKPVVSDDSEEEQEEDRSGSGSEED

Figure 5



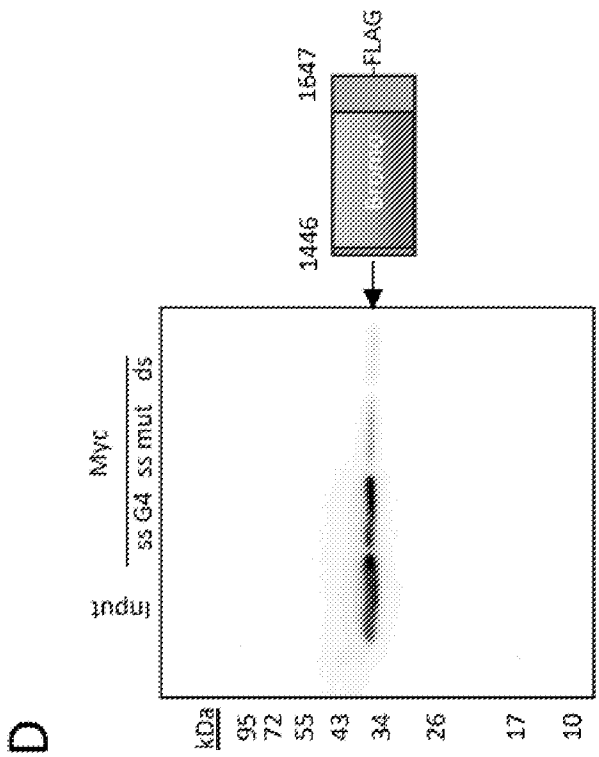


Figure 5 continued

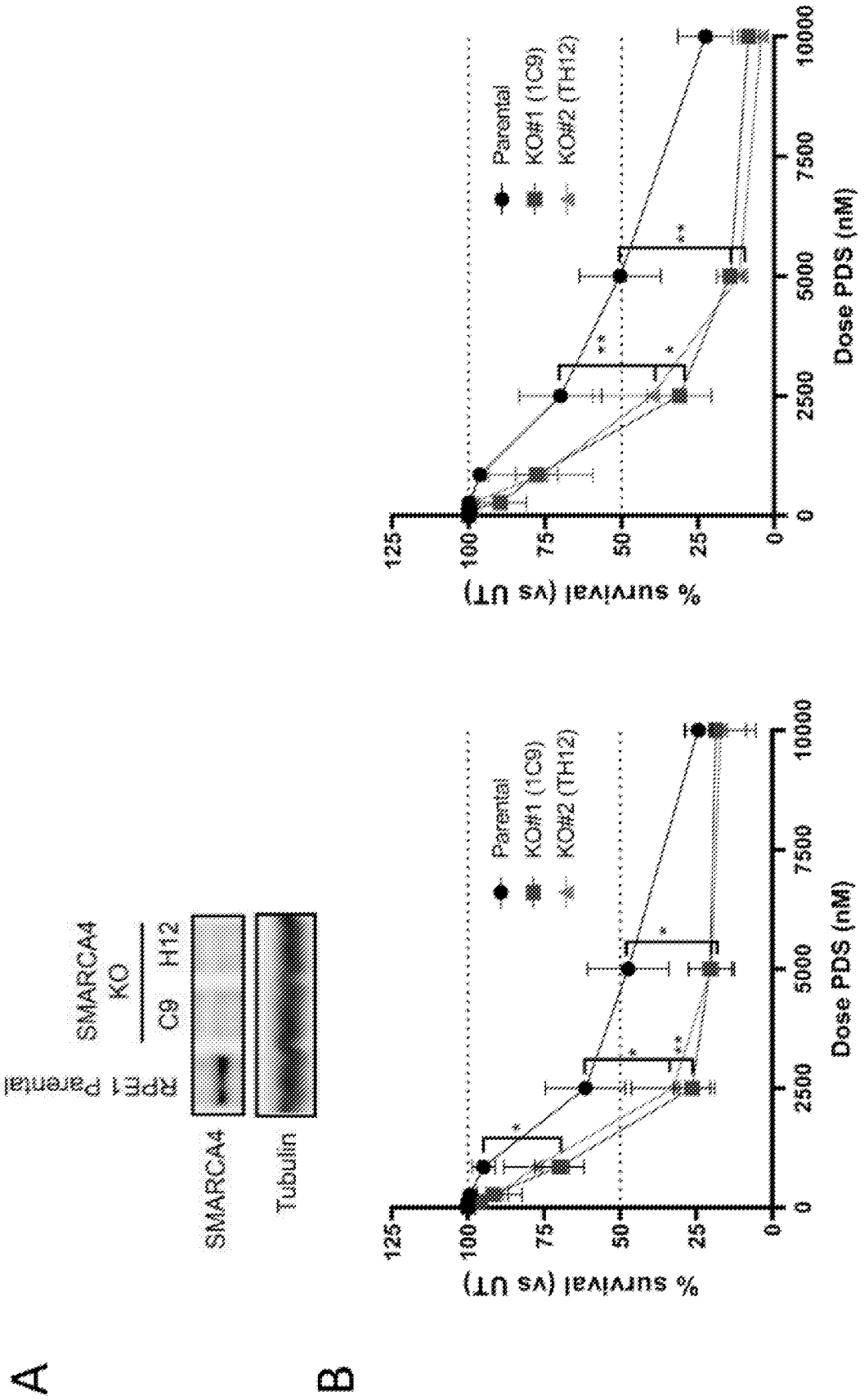

A
RPE1 Parental
SMARCA4 KO
C9
H12
SMARCA4
Tubulin
B
% survival (vs UT)
Dose PDS (nM)
Parental
KO#1 (1C9)
KO#2 (TH12)

Figure 6

C

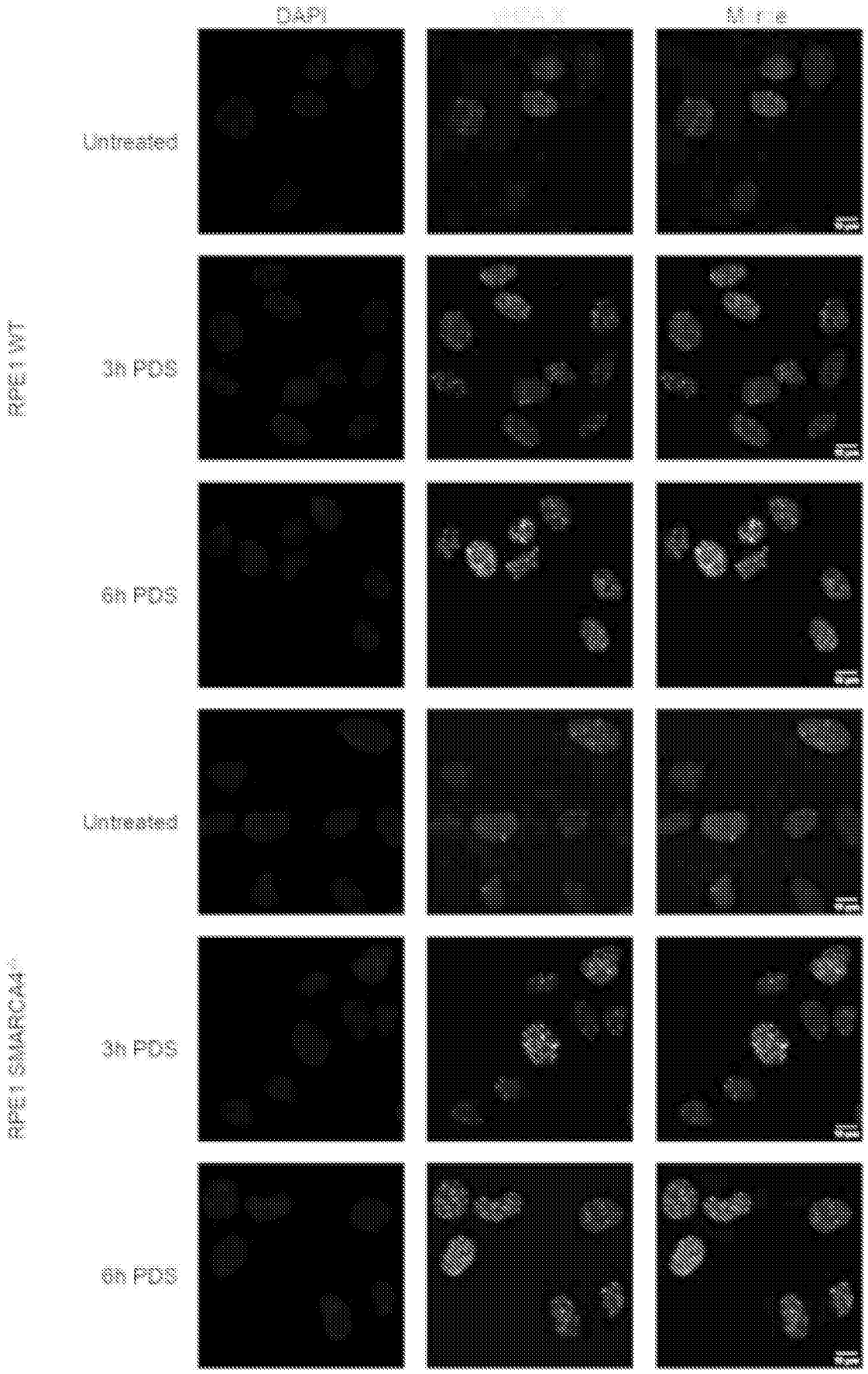


Figure 6 continued

C

continued

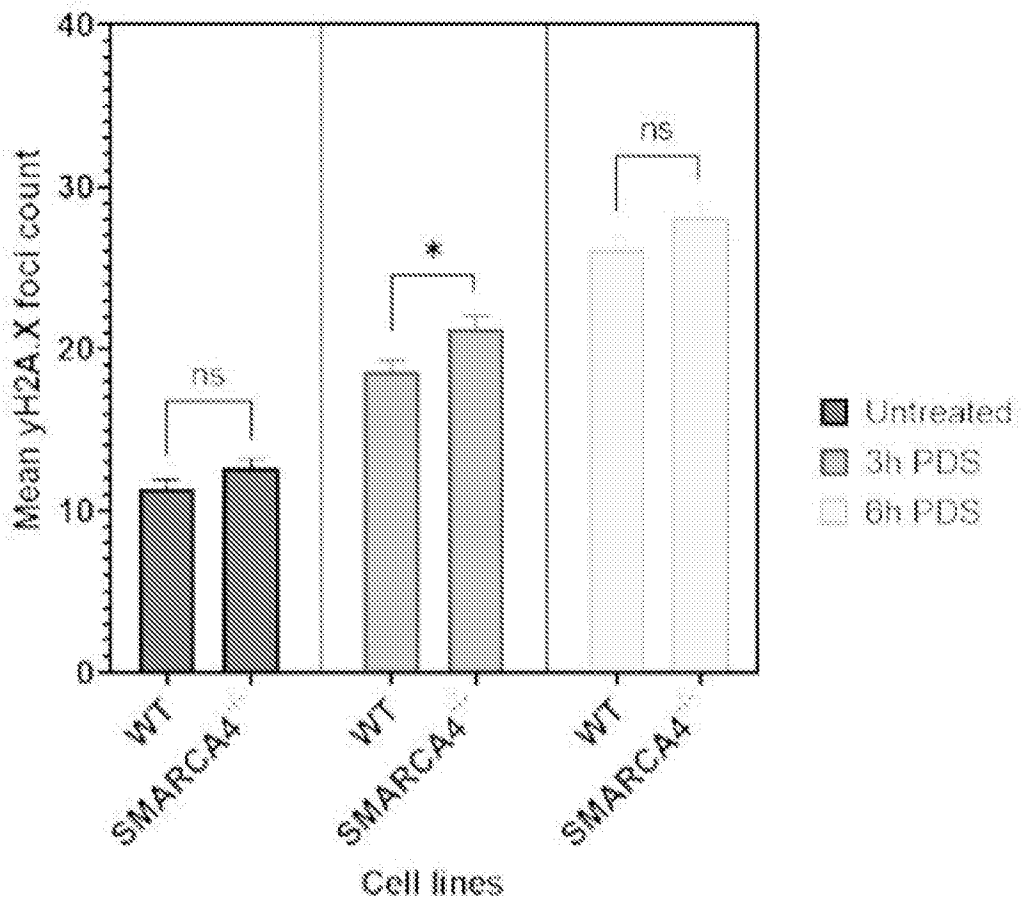


Figure 6 continued

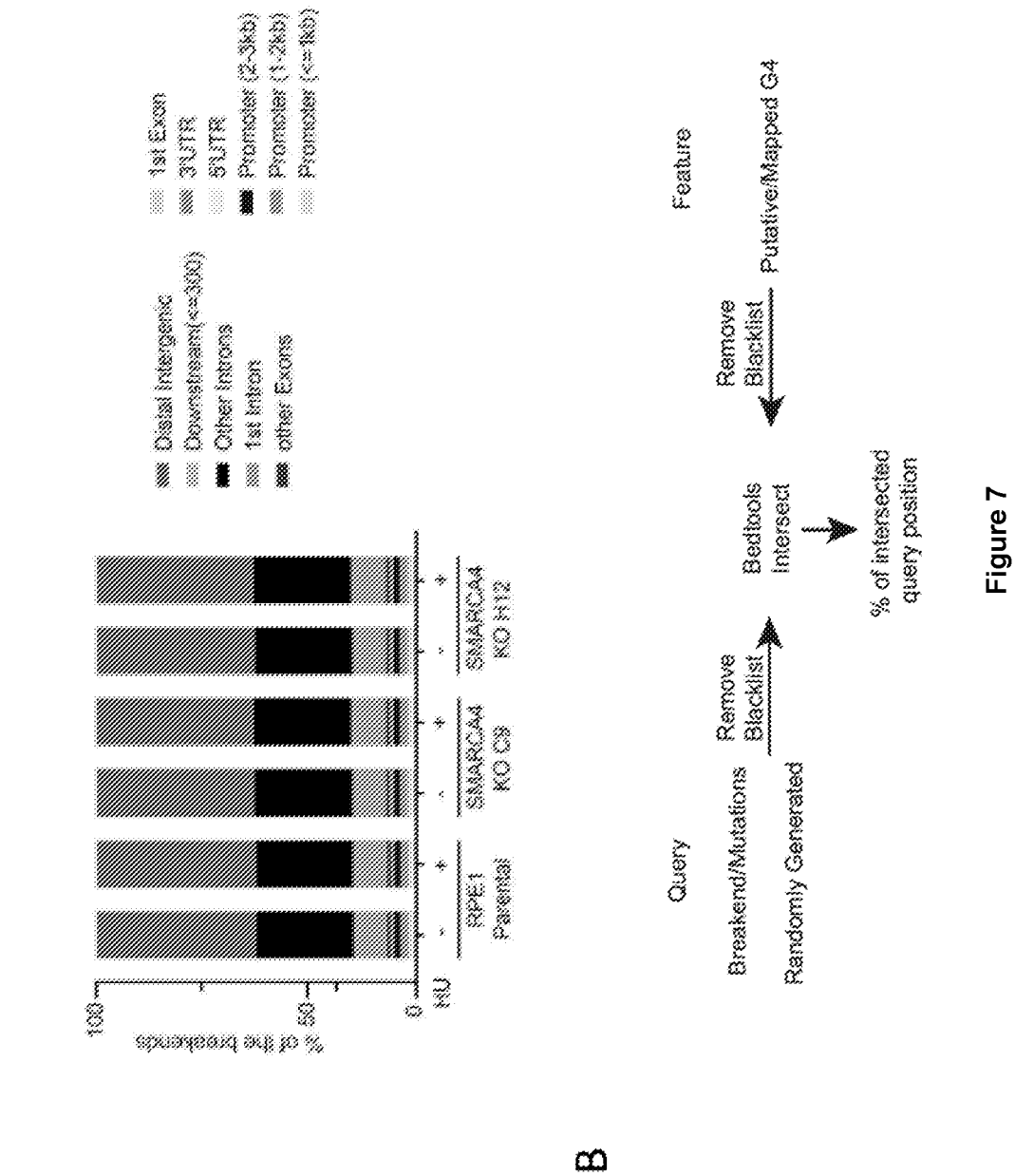

A
% of the breakends
100
50
0
KU
RPE1 Parental
SMARCA4 KO C9
SMARCA4 KO H12
Distal Intergenic
Downstream(<=300)
Other Introns
1st Intron
other Exons
1st Exon
3'UTR
5'UTR
Promoter (2-3kb)
Promoter (1-2kb)
Promoter (<=1kb)
B
Query
Breakend/Mutations
Randomly Generated
Remove Blacklist
Bedtools Intersect
% of intersected query position
Feature
Remove Blacklist
Putative/Mapped G4

Figure 7

B

continued

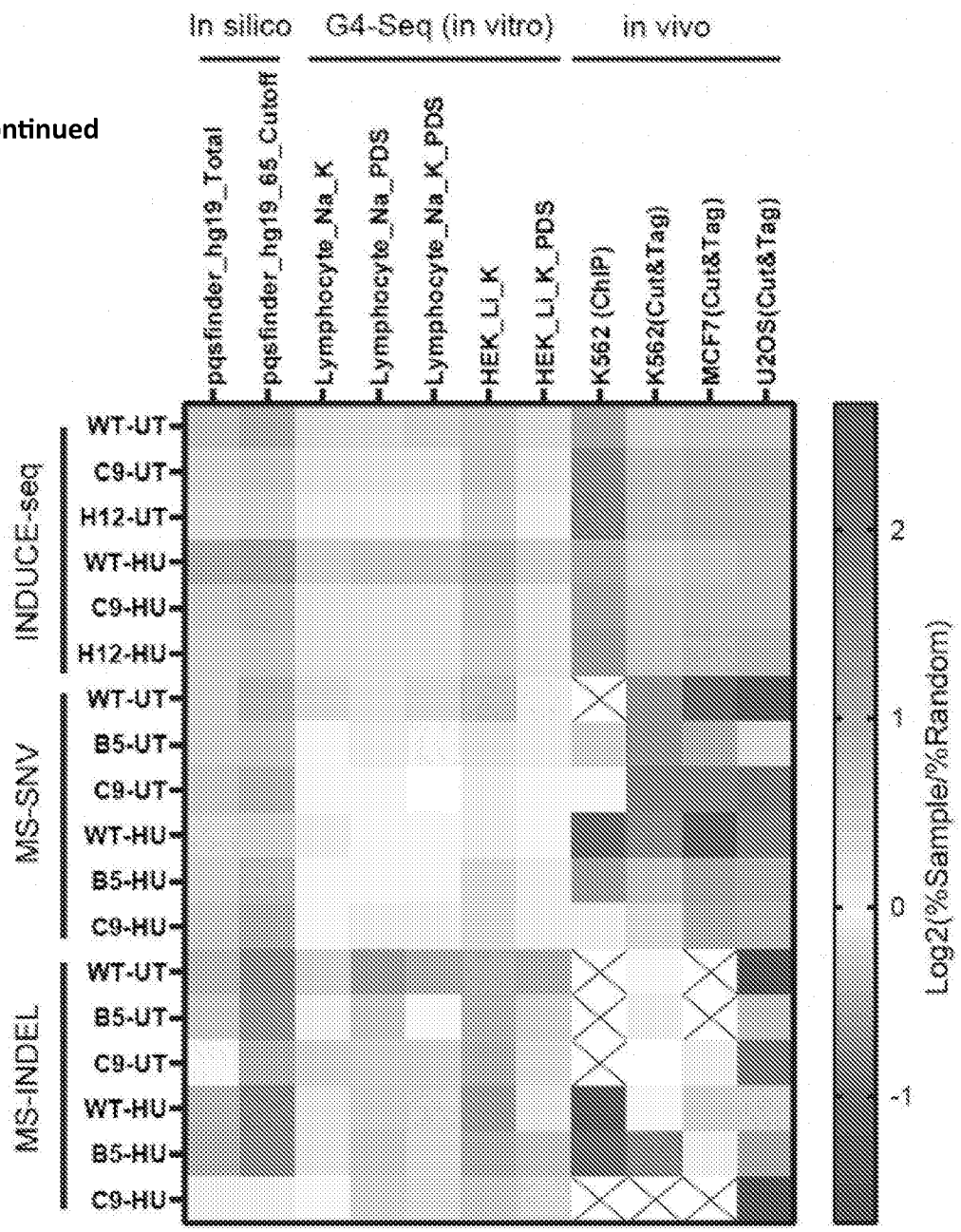


Figure 7 continued

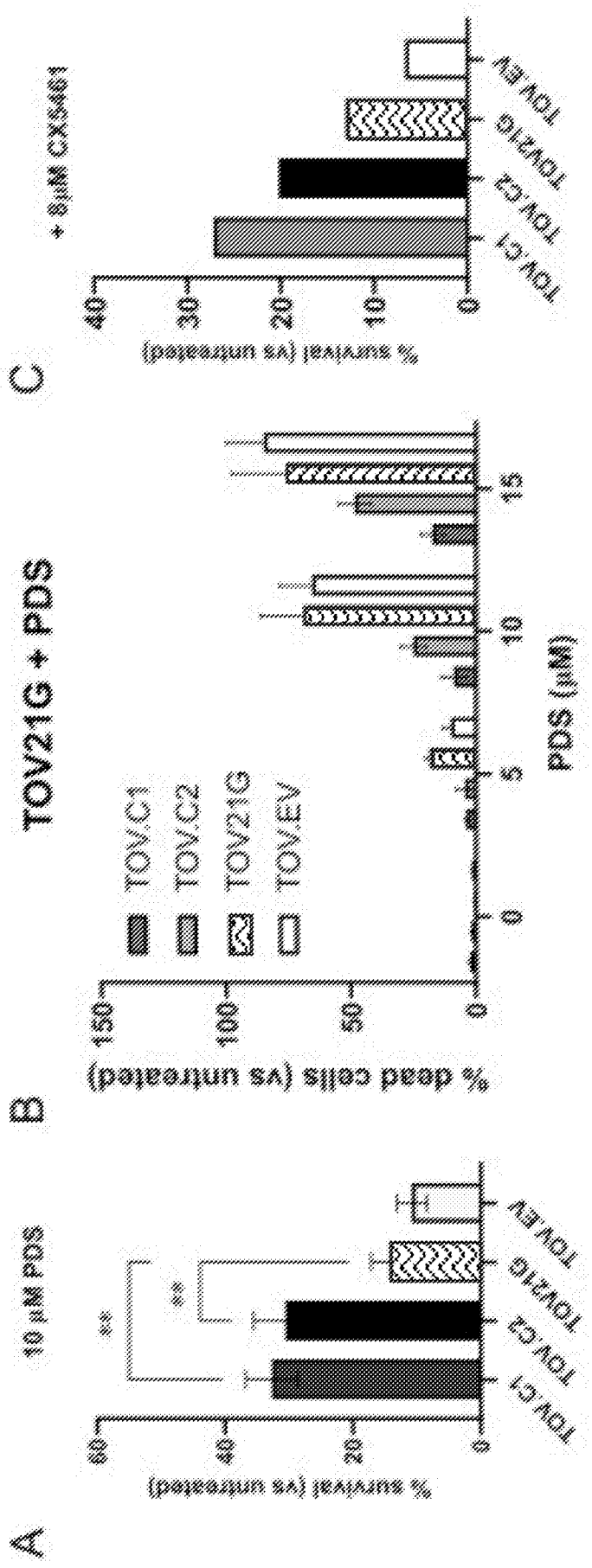

A
10 μM PDS
% survival (vs untreated)
60
40
20
0
**
**
TOV.C1
TOV.C2
TOV21G
TOV.EV
B
TOV21G + PDS
% dead cells (vs untreated)
150
100
50
0
TOV.C1
TOV.C2
TOV21G
TOV.EV
0
5
10
15
PDS (μM)
C
+ 8 μM CX5461
% survival (vs untreated)
40
30
20
10
0
TOV.C1
TOV.C2
TOV21G
TOV.EV

Figure 8

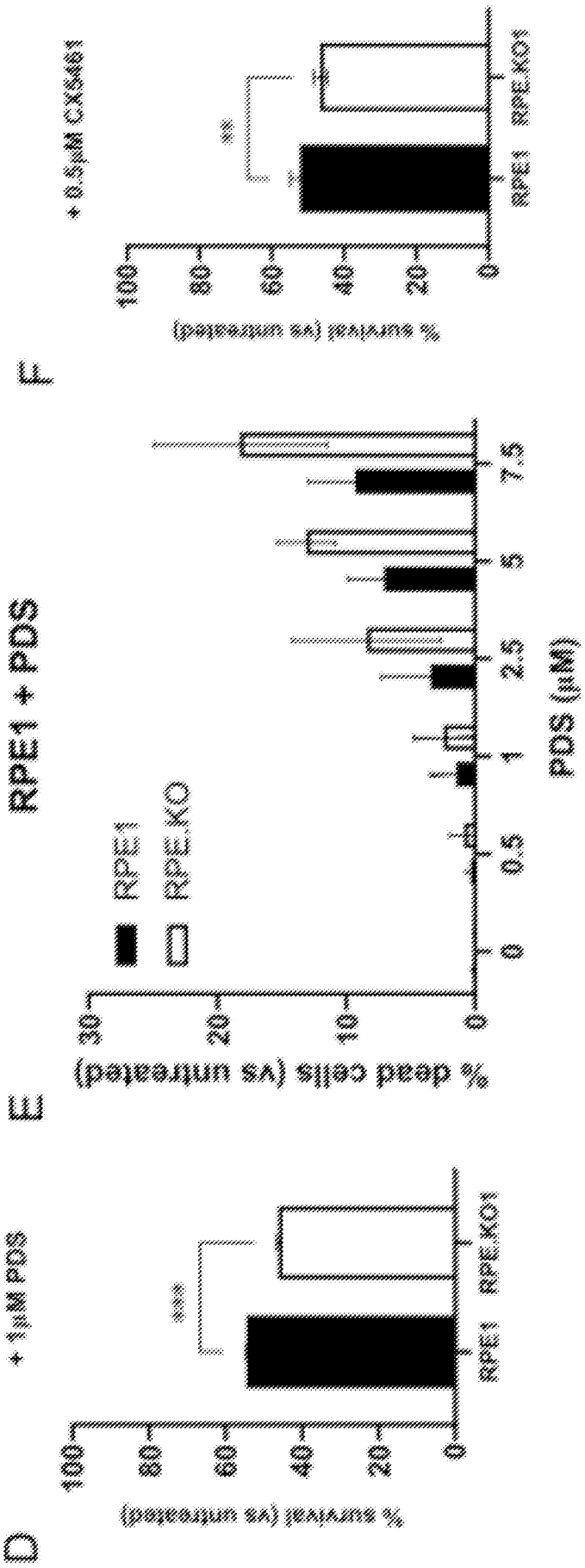
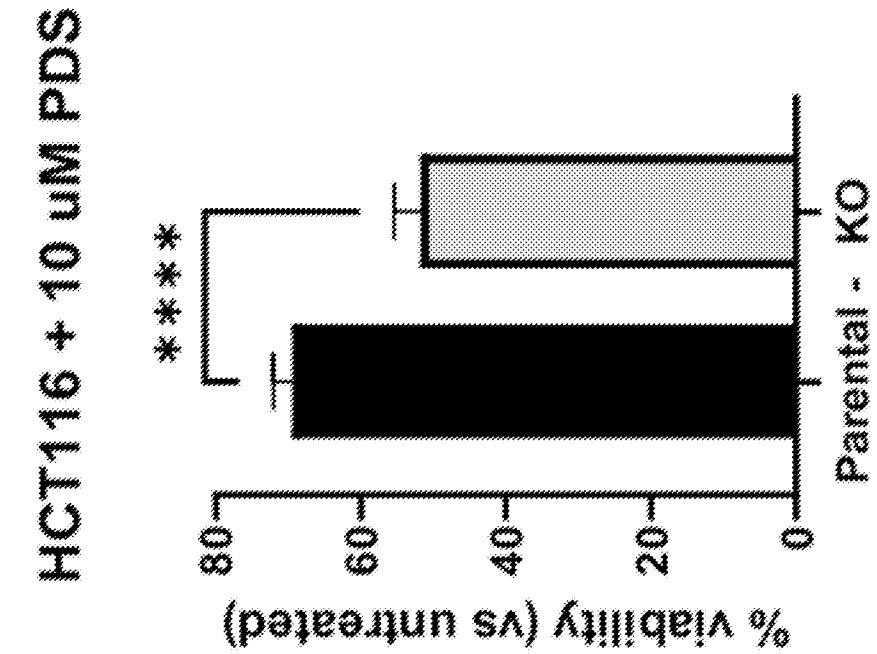


Figure 8 continued



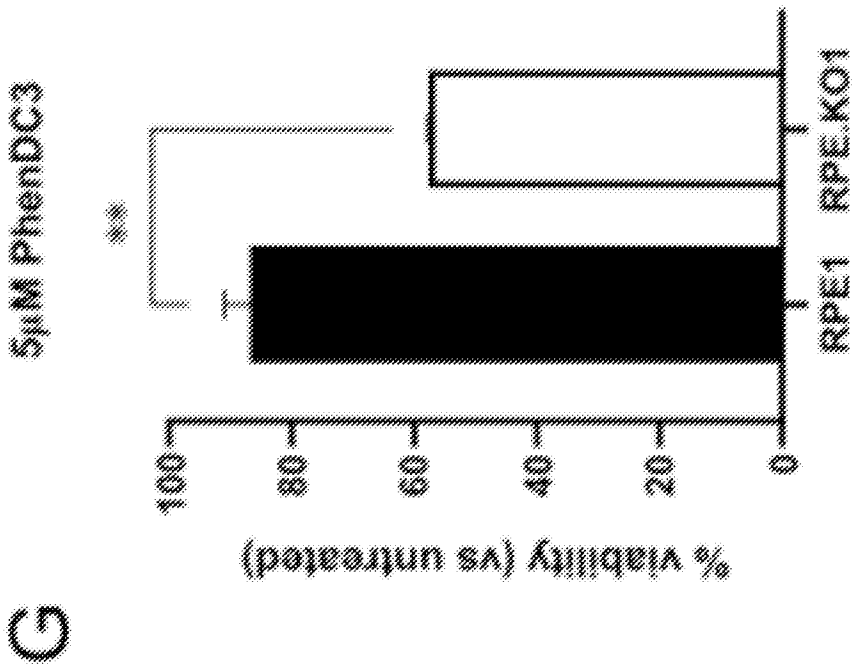


Figure 8 continued

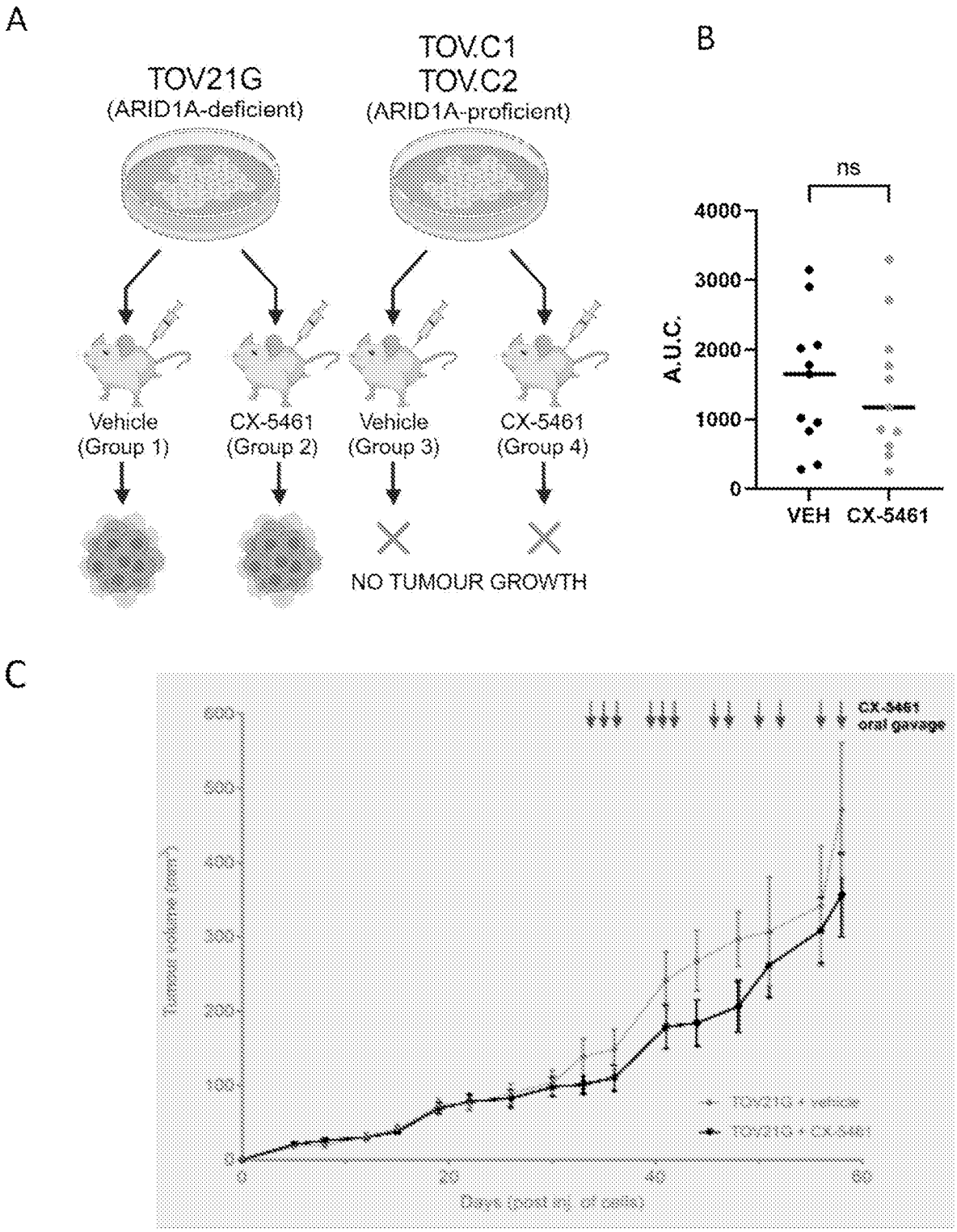
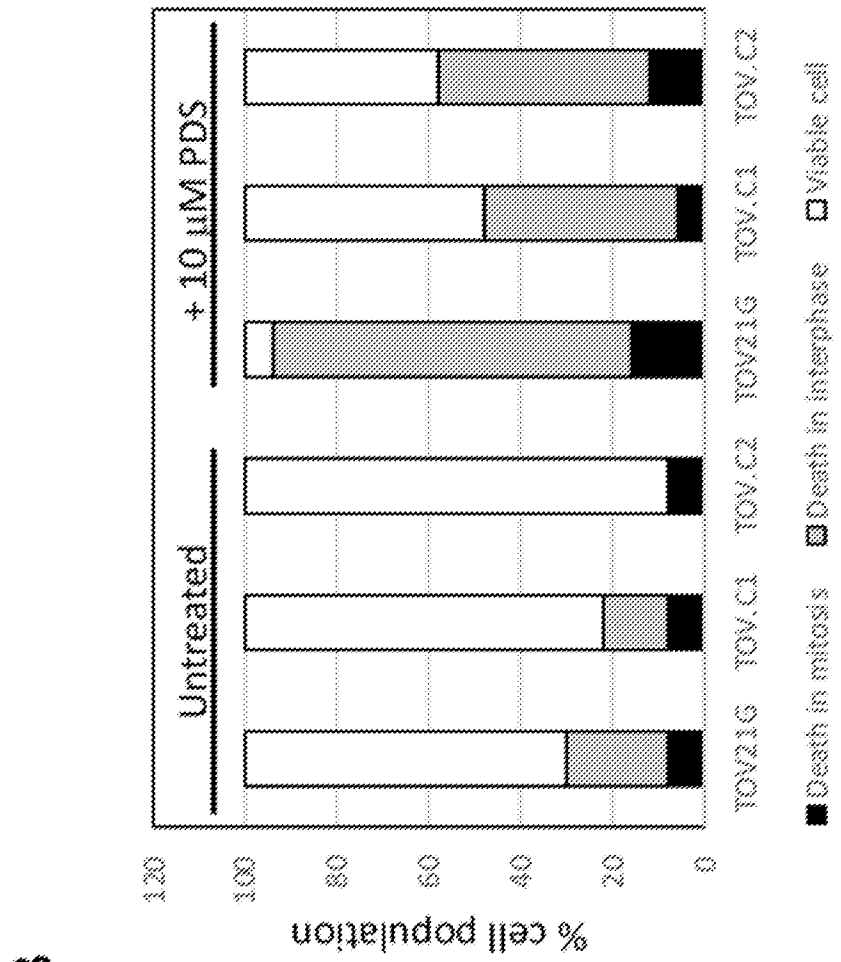

A
TOV21G
(ARID1A-deficient)
TOV.C1
TOV.C2
(ARID1A-proficient)
Vehicle
(Group 1)
CX-5461
(Group 2)
Vehicle
(Group 3)
CX-5461
(Group 4)
NO TUMOUR GROWTH
B
ns
A.U.C.
4000
3000
2000
1000
0
VEH
CX-5461
C
CX-5461
oral gavage
Days (post inj. of cells)

Figure 9


B
Untreated
+ 10 uM PDS
TOV21G
TOV.C1
TOV.C2
TOV21G
TOV.C1
TOV.C2
Death in mitosis
Death in interphase
Viable cell
% cell population
120
100
80
60
40
20
0

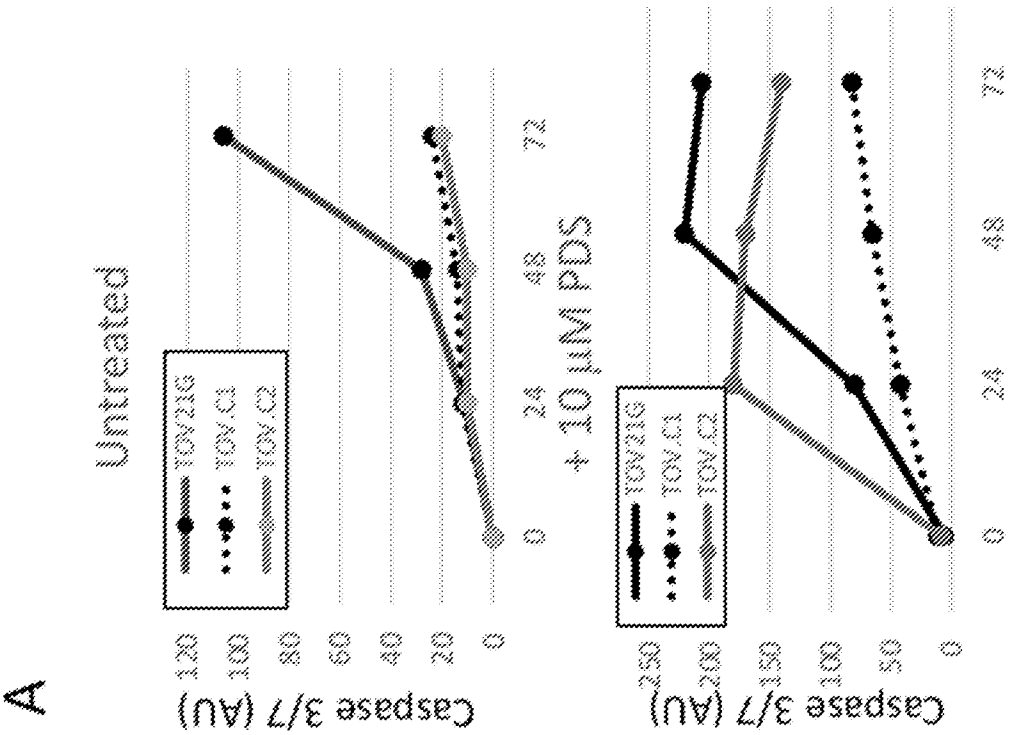

A
Untreated
+ 10 μM PDS
TOV21G
TOV.C1
TOV.C2
Caspase 3/7 (AU)
120
100
80
60
40
20
0
250
200
150
100
50
0
24
48
72

Figure 10

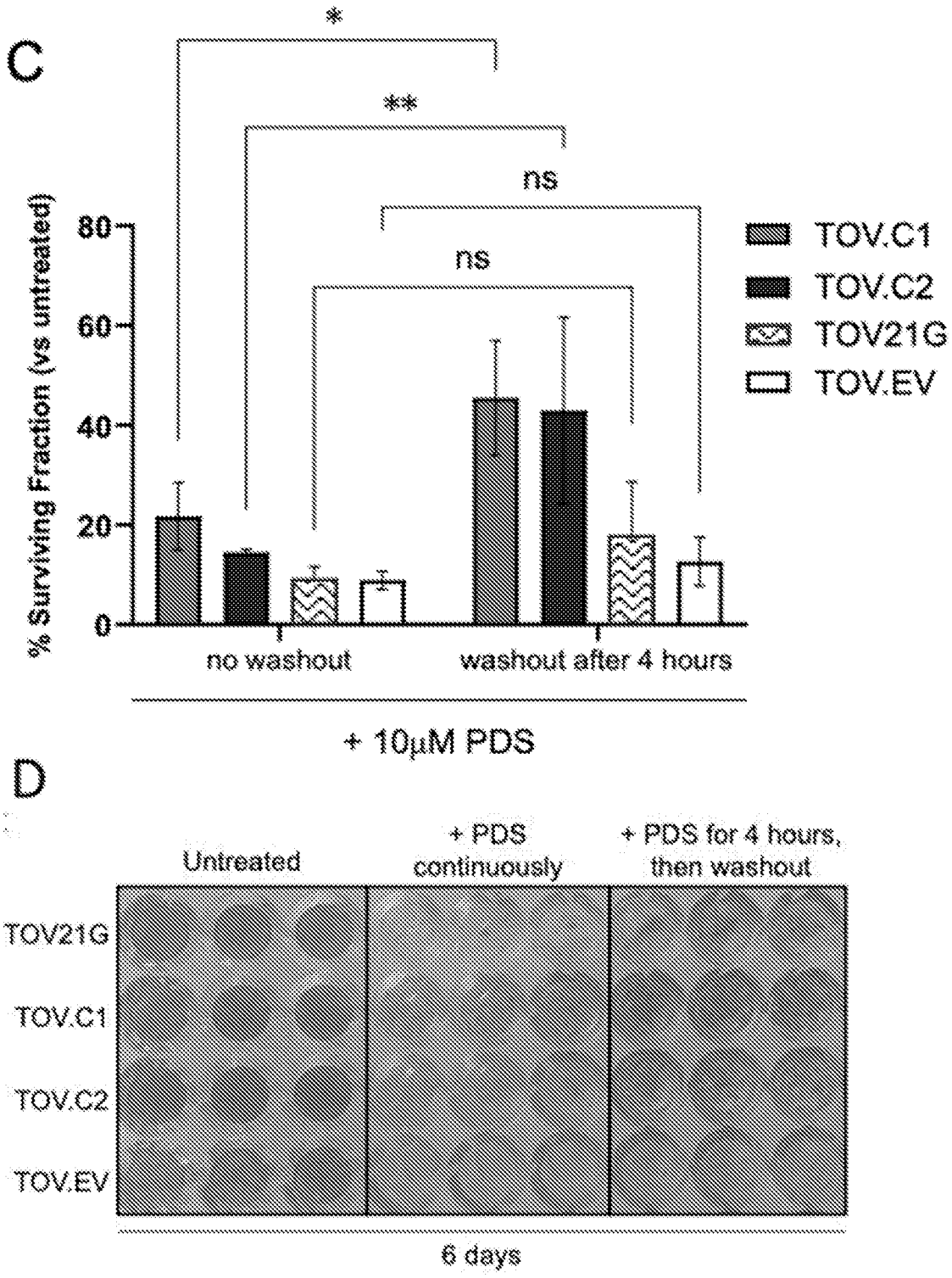


Figure 10 continued

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051658

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574 A61K45/06
ADD.

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

B. FIELDS SEARCHED

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

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, COMPENDEX, EMBASE, FSTA, INSPEC, 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	US 2022/062294 A1 (LIU HSHIOU-TING [US] ET AL) 3 March 2022 (2022-03-03) paragraphs [0063], [0129], [0131], [0136], [0206] - [0209]	1-22
A	ZHANG XIAOYUN ET AL: "Chemical profiling of DNA G-quadruplex-interacting proteins in live cells", NATURE CHEMISTRY, NATURE PUBLISHING GROUP UK, LONDON, vol. 13, no. 7, 28 June 2021 (2021-06-28), pages 626-633, XP037495107, ISSN: 1755-4330, DOI: 10.1038/S41557-021-00736-9 [retrieved on 2021-06-28] the whole document	1-22

-/--

[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
27 September 2024	23/10/2024

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	Jacques, Patrice

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051658

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZIMMER JUTTA ET AL: "Targeting BRCA1 and BRCA2 Deficiencies with G-Quadruplex-Interacting Compounds", MOLECULAR CELL, ELSEVIER, AMSTERDAM, NL, vol. 61, no. 3, 31 December 2015 (2015-12-31), pages 449-460, XP029408447, ISSN: 1097-2765, DOI: 10.1016/J.MOLCEL.2015.12.004 the whole document	1-22
A	SILLAPAPONGWARAKORN SITTICHAI ET AL: "Molecular docking based screening of triterpenoids as potential G-quadruplex stabilizing ligands with anti-cancer activity", BIOINFORMATION, vol. 13, no. 09, 30 September 2017 (2017-09-30), pages 284-292, XP093209517, Singapore ISSN: 0973-8894, DOI: 10.6026/97320630013284 the whole document	1-22
A	YAN TING ET AL: "Characterization of G-quadruplex formation in the ARID1A promoter", INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, ELSEVIER BV, NL, vol. 147, 23 January 2020 (2020-01-23), pages 750-761, XP086073826, ISSN: 0141-8130, DOI: 10.1016/J.IJBIOMAC.2020.01.210 [retrieved on 2020-01-23] the whole document	1-22
A	MITTAL PRIYA ET AL: "The SWI/SNF complex in cancer - biology, biomarkers and therapy", NATURE REVIEWS CLINICAL ONCOLOGY, NATURE, NY, US, vol. 17, no. 7, 17 April 2020 (2020-04-17) , pages 435-448, XP037175958, ISSN: 1759-4774, DOI: 10.1038/S41571-020-0357-3 [retrieved on 2020-04-17] the whole document	1-22

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051658

C(Continuation). **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOSIOL NILS ET AL: "G-quadruplexes: a promising target for cancer therapy", MOLECULAR CANCER, vol. 20, no. 1, 25 February 2021 (2021-02-25), XP055870284, DOI: 10.1186/s12943-021-01328-4 the whole document -----	1-22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2024/051658

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2024/051658

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2022062294 A1	03-03-2022	TW 202227090 A	16-07-2022
		US 2022062294 A1	03-03-2022
		WO 2022051491 A1	10-03-2022