

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

26 March 2020 (26.03.2020)



(10) International Publication Number

WO 2020/061391 A1

(51) International Patent Classification:

C07K 14/00 (2006.01) *C12Q 1/68* (2018.01)

A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2019/052046

(22) International Filing Date:

20 September 2019 (20.09.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/734,088 20 September 2018 (20.09.2018) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: METHODS FOR INHIBITING TUMOR CELLS USING INHIBITORS OF FOXO3A ANTAGONISTS

(57) Abstract: The present disclosure provides for methods and compositions for inhibiting tumor cell growth or increasing tumor cell death. The tumor cell is contacted with an effective amount of an inhibitor of a FOXO3A antagonist, such as an inhibitor of ZNF274. An inhibitor of a FOXO3A antagonist, such as an inhibitor of ZNF274 can be used to modulate the subcellular localization of FOXO3A, or to regulate the activity/expression of FOXO3A in a cell.

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METHODS FOR INHIBITING TUMOR CELLS USING INHIBITORS OF FOXO3A ANTAGONISTS

5 CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/734,088 filed on September 20, 2018, which is incorporated herein by reference in its entirety.

GOVERNMENT LICENSE RIGHTS

10 This invention was made with government support under HG003008 and AG047344
awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to methods and compositions for inhibiting tumor cell growth and increasing tumor cell death. In particular, the present invention relates to the use of an inhibitor of a FOXO3A antagonist, such as an inhibitor of zinc finger protein 274 (ZNF274), in inhibiting tumor cell growth or increasing tumor cell death.

BACKGROUND OF THE INVENTION

Forkhead box O3, also known as FOXO3 or FOXO3A, is a member of the FOXO subfamily of forkhead transcription factors that mediate a variety of cellular processes including apoptosis, proliferation, cell cycle progression, DNA damage and tumorigenesis. It also responds to several cellular stresses such as UV irradiation and oxidative stress. FOXO3A is implicated in a variety of diseases, particularly in malignancy of breast, liver, colon, prostate, bladder, and nasopharyngeal cancers. Known as a tumor suppressor, FOXO3A is frequently inactivated in cancer cell lines by mutation of the FOXO3A gene or cytoplasmic sequestration of the FOXO3A protein. Overexpression of FOXO3A is shown to inhibit the proliferation, tumorigenic potential, and invasiveness of cancer cells, while silencing of FOXO3A results in marked attenuation in protection against tumorigenesis. The role of FOXO3A in both normal physiology as well as in cancer development have presented a great challenge to formulating an effective therapeutic strategy for cancer (Liu et al., Critical role of FOXO3a in carcinogenesis, *Molecular Cancer*,

2018, 17:104). FOXO3A also plays an important role in longevity. Activation of FOXO3A induces a stress response that can be generally beneficial to the cell and extend life span. Depending on the circumstances, the cell can focus on stress response and damage repair – activated by FoxO3A – or on growth and development.

5 Reduced insulin/IGF-1-like signaling (IIS) greatly extends the lifespan of many organisms, including the nematode *C. elegans*. This effect is almost entirely dependent on activation of the FOXO transcription factor DAF-16 (Kenyon et al., 1993, A *C. elegans* mutant that lives twice as long as wild type, *Nature* 366, 461–464; Lin et al., 1997, daf-16: An HNF-3/ forkhead family member that can function to double the life-span of *Caenorhabditis elegans*,
10 *Science* 278, 1319–1322; Ogg et al., 1997, The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389, 994–999). The IIS pathway is conserved, with increased longevity requiring the DAF-16 ortholog dFOXO in *Drosophila* and FOXO3A in mammals (Kenyon, 2005, The plasticity of aging: insights from long-lived mutants. *Cell* 120, 449–460). Under normal conditions of nutrient availability and
15 growth, AKT-dependent phosphorylation of specific amino acid residues causes DAF-16 to be retained in the cytosol and thus be transcriptionally inactive. Upon reduced insulin pathway signaling, AKT-dependent phosphorylation of DAF-16 decreases, promoting DAF-16 nuclear translocation, which leads to both upregulation and downregulation of large sets of genes (Murphy et al., 2003, Genes that act downstream of DAF-16 to influence the lifespan of
20 *Caenorhabditis elegans*. *Nature* 424, 277–283).

 In a previous study in *C. elegans*, we discovered that the transcriptional activator PQM-1 is an antagonist of DAF-16, the ortholog of FOXO3A (Tepper et al., PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity, *Cell*, 2013, 154(3): pp. 676-690). Loss of PGM-1 suppresses daf-2 longevity and further slows development,
25 and the aging process is associated with progressive loss of nuclear PGM-1.

 A better understanding of the FOXO3A pathway would facilitate identification of new or improved methods for treating cancer.

SUMMARY

The present disclosure provides for a method for inhibiting growth, or increasing cell death, of a tumor cell. The method may comprise contacting the tumor cell with an effective amount of an inhibitor of ZNF274. The contacting may be *in vitro* or *in vivo*.

The present disclosure provides for a method for inhibiting growth, or increasing cell death, of a tumor cell. The method may comprise contacting the tumor cell with an effective amount of an inhibitor of a FOXO3A antagonist. The contacting may be *in vitro* or *in vivo*.

The present disclosure also provides for a method of modulating subcellular localization, and/or regulating activity/expression of, FOXO3A in a cell. The method may comprise contacting the cell with an inhibitor of ZNF274.

The present disclosure provides for a method of modulating subcellular localization, and/or regulating activity/expression of, FOXO3A in a cell. The method may comprise contacting the cell with an inhibitor of a FOXO3A antagonist.

Also encompassed by the present disclosure is a method of treating cancer in a subject, comprising administering to the subject an effective amount of an inhibitor of ZNF274.

Further encompassed by the present disclosure is a method of treating cancer in a subject, comprising administering to the subject an effective amount of an inhibitor of a FOXO3A antagonist.

The FOXO3A antagonist may be ZNF274, ERR α , NR3C1, GATAD1, ZKSCAN1, PGC-1 α , HDAC8, or combinations thereof.

The inhibitor may be a small molecule, a polynucleotide (such as a small interfering RNA (siRNA), a shRNA, an antisense polynucleotide, and a ribozyme), or an antibody or antigen-binding portion thereof.

The inhibitor may comprise a CRISPR/Cas system (e.g., a CRISPR/Cas9 system). For example, the inhibitor may comprise Cas9, dCas9, and/or a dCas9 fusion protein. In certain embodiments, the inhibitor may comprise Cas12a/Cpf1, CasX, C2c1/2/3, mutants thereof, or variants thereof.

The tumor cell may be a breast cancer cell, a liver cancer cell, a colon cancer cell, a prostate cancer cell, a bladder cancer cell, or a nasopharyngeal cancer cell. The tumor cell may be a cell of any cancer described herein.

The present method may further comprise contacting the tumor cell with a cytotoxic agent.

The present method may further comprise administering to the subject a cytotoxic agent.

The present method may further comprise treating the subject with radiation.

5 The present method may further comprise administering to the subject a chemotherapeutic agent.

Non-limiting examples of cytotoxic agents or chemotherapeutic agents include an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and an endoplasmic reticulum stress inducing agent.

10 The cancer may be breast cancer, liver cancer, colon cancer, prostate cancer, bladder cancer, or nasopharyngeal cancer. The cancer may be any cancer described herein.

The administration of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and the cytotoxic agent may result in a synergistic increase in apoptosis of cancer cells. The administration of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and
15 the cytotoxic agent may result in a synergistic reduction in tumor volume.

The inhibitor of ZNF274 and the cytotoxic agent (or chemotherapeutic agent) may be administered simultaneously, sequentially or separately.

The inhibitor of ZNF274 and radiation may be administered simultaneously, sequentially or separately.

20 The inhibitor of a FOXO3A antagonist radiation may be administered simultaneously, sequentially or separately.

Non-limiting examples of chemotherapeutic agents or cytotoxic agents include a DNA alkylating agent, a topoisomerase inhibitor, an endoplasmic reticulum stress inducing agent, a platinum compound, an antimetabolite, an enzyme inhibitor, a receptor antagonist, and a
25 therapeutic antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B. Knockdown of ZNF274 by siRNAs leads to nuclear translocation of FOXO3A.

To reduce the level of ZNF274, a pool of three stealth siRNAs targeting ZNF274, or a negative control containing a pool of three non-targeting siRNAs, were reverse transfected into HepG2 cells. As a positive control for nuclear translocation, a PI3K inhibitor (LY2940020) was separately added to drive FOXO3A into the nucleus, and the ratios of nuclear/cytoplasmic fluorescence intensity were determined and compared for each sample. Figure 1A: The PI3K inhibitor-treated cells exhibited a higher ratio of nuclear : cytoplasmic FOXO3A compared to DMSO-treated cells ($p=0.0002$, t-test). Figure 1B: Cells treated with ZNF274 siRNAs had a higher nuclear : cytoplasmic FOXO3A ratio than the control siRNAs ($p<0.0001$, t-test).

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides for methods and compositions for inhibiting tumor cell growth and/or increasing tumor cell death (e.g., via apoptosis). The tumor cell may be contacted with an effective amount of an inhibitor of zinc finger protein 274 (ZNF274) *in vitro* or *in vivo*. The tumor cell may be contacted with an effective amount of an inhibitor of a FOXO3A antagonist *in vitro* or *in vivo*. Examples of FOXO3A antagonists also include ERR α (encoded by the *ESRRA* gene), NR3C1, GATAD1, ZKSCAN1, PGC-1 α (encoded by the *PPARGC1A* gene), and HDAC8. The present compositions and methods may be used to inhibit the growth of a cell that is resistant to a chemotherapeutic agent.

An inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) can be used to modulate the subcellular localization of FOXO3A, or to regulate the activity/expression of FOXO3A in a cell. In one embodiment, an inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) induces/increases nuclear translocation of FOXO3A, and/or increases the activity of FOXO3A.

The present disclosure also provides for methods and compositions for treating cancer. A subject having cancer may be administered an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274). The inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) may be administered alone or in combination with other treatment methods including radiation, chemotherapy, and surgery.

The pharmaceutical compositions of the present invention may be administered before, during or after radiation. The pharmaceutical compositions may be administered before, during or after the administration of a chemotherapeutic agent or a cytotoxic agent. The routes of administration of the pharmaceutical compositions include oral, intravenous, subcutaneous, intramuscular, inhalation, or intranasal administration. Additionally, specifically targeted delivery of ZNF274 inhibitor (nucleic acid, peptide, or small molecule) could be delivered by targeted liposome, nanoparticle or other suitable means.

An inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) may be used to increase fertility. The method may comprise administering to a subject an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274).

Modulating the expression/activity of a FOXO3A antagonist (e.g., ZNF274) may also be utilized to increase stress resistance in a cell or a subject. For example, an inhibitor of a

FOXO3A antagonist (e.g., a ZNF274 inhibitor) may be used to increase stress resistance in a cell or a subject. An inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) may be used to increase the life span of, or to slow the aging process in, a subject. The method may comprise contacting the cell with, or administering to a subject, an inhibitor of a FOXO3A antagonist (e.g.,
5 an inhibitor of ZNF274).

The activity and/or expression levels of a FOXO3A antagonist (e.g., ZNF274) or its alleles, can be used as biomarkers for cancer progression/development or cancer treatment.

The amount and/or activity of a FOXO3A antagonist (e.g., ZNF274) may be downregulated by RNA interference or RNAi (such as small interfering RNAs or siRNAs, small
10 hairpin RNAs or shRNAs, microRNAs or miRNAs, a double-stranded RNA (dsRNA), etc.), antisense molecules, and/or ribozymes targeting the DNA or mRNA encoding the FOXO3A antagonist (e.g., ZNF274). The amount and/or activity of the FOXO3A antagonist (e.g., ZNF274) may be downregulated by gene knockout. The amount and/or activity of ZNF274 may be downregulated by the cluster regularly interspaced short palindromic repeat-associated
15 nuclease (CRISPR) technology.

The amount and/or activity of a FOXO3A antagonist (e.g., ZNF274) may also be modulated by introducing polypeptides (e.g., antibodies) or small molecules which inhibit gene expression, protein level or activity of the FOXO3A antagonist (e.g., ZNF274).

Agents that bind to or modulate, such as down-regulating the amount, activity or
20 expression of a FOXO3A antagonist (e.g., ZNF274), may be administered to a target cell or may contact the cell. Such an agent may be administered in an amount effective to down-regulate the expression, level and/or activity of the FOXO3A antagonist (e.g., ZNF274), or by activating or down-regulating a second signal which controls the FOXO3A antagonist (e.g., ZNF274) expression, activity or amount.

25 The present disclosure provides for a method of treating a disease such as cancer, comprising the step of delivering to a subject a therapeutically effective amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274).

The present pharmaceutical composition may comprise, or consist essentially of (or consist of), an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274).

30 The present method for treating cancer may comprise the step of administering to a subject having cancer an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a

cytotoxic agent (or a chemotherapeutic agent). The combination of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a cytotoxic agent (or a chemotherapeutic agent) may produce a synergistic effect on the cancer compared to the effect of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone or the effect of the cytotoxic agent (or a chemotherapeutic agent) alone.

The present invention provides for a pharmaceutical composition comprising a first amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a second amount of a cytotoxic agent (or a chemotherapeutic agent). The combination of the first amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and the second amount of a cytotoxic agent (or a chemotherapeutic agent) may produce a synergistic effect on cancer compared to the effect of the first amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) alone or the effect of the second amount of a cytotoxic agent (or a chemotherapeutic agent) alone.

The present methods may be used *in vitro* or *in vivo* (e.g., in a subject having cancer).

Methods and compositions of the present invention can be used for prophylaxis as well as treating a disease such as cancer.

For prophylaxis, the present composition may be administered to a subject in order to prevent the onset of one or more symptoms of a disease such as cancer. In one embodiment, the subject is asymptomatic. A prophylactically effective amount of the agent or composition is administered to such a subject. A prophylactically effective amount is an amount which prevents the onset of one or more symptoms of the disease such as cancer.

The present compositions may be used *in vitro* or administered to a subject. The administration may be topical, intravenous, intranasal, or any other suitable route as described herein.

The present methods may utilize adeno-associated virus (AAV) mediated genome engineering. Wirth et al. Gene. 2013 Aug 10;525(2):162-9). Additionally, delivery vehicles such as nanoparticle- and lipid-based mRNA or protein delivery systems can be used. Further examples of alternative delivery vehicles include lentiviral vectors, lipid-based delivery system, gene gun, hydrodynamic, electroporation or nucleofection microinjection, and biolistics. Various gene delivery methods are discussed in detail by Nayerossadat et al. (Adv Biomed Res. 2012; 1: 27) and Ibraheem et al. (Int J Pharm. 2014 Jan 1;459(1-2):70-83).

The present methods may use nanoparticle-based siRNA delivery systems. Lee et al., Recent Developments in Nanoparticle-Based siRNA Delivery for Cancer Therapy, BioMed Research International, Volume 2013, Article ID 782041. The nanoparticle-formulated siRNA delivery systems may be based on polymers or liposomes. Nanoparticles conjugated to the cell-specific targeting ligand for effective siRNA delivery can increase the chance of binding the tumor surface receptor. The nanoparticles may be coated with PEG (polyethylene glycol) which can reduce uptake by the reticuloendothelial system (RES), resulting in enhanced circulatory half-life. Various nanoparticle-based delivery systems such as cationic lipids, polymers, dendrimers, and inorganic nanoparticles may be used in the present methods to provide effective and efficient siRNA delivery *in vitro* or *in vivo*.

The inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) may be administered in a local rather than systemic manner, for example, via injection of directly into the desired target site, e.g., in a depot or sustained release formulation. The composition may be administered in a targeted drug delivery system, for example, in liposomes or nanoparticles coated with tissue-specific or cell-specific antibodies. The liposomes or nanoparticles will be targeted to and taken up selectively by the desired tissue or cells. A summary of various delivery methods and techniques of siRNA administration in ongoing clinical trials is provided in Zuckerman and Davis 2015; Nature Rev. Drug Discovery, Vol. 14: 843-856, 2015. In some embodiments, the level of the FOXO3A antagonist (e.g., ZNF274) is decreased in a target cell. The expression of the FOXO3A antagonist (e.g., ZNF274) may be specifically decreased only in the desired target cell (i.e., those cells which are predisposed to the condition, or exhibiting the disease already), and not substantially in other non-diseased cells. In these methods, expression of the FOXO3A antagonist (e.g., ZNF274) may not be substantially reduced in other cells, i.e., cells which are not desired target cells. Thus, in such embodiments, the level of the FOXO3A antagonist (e.g., ZNF274), remains substantially the same or similar in non-target cells in the course of or following treatment.

The vectors comprising the present nucleic acid may be delivered into host cells by a suitable method. Methods of delivering the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) to cells may include transfection of nucleic acids or polynucleotides (e.g., using reagents such as liposomes or nanoparticles); electroporation, delivery of protein, e.g., by mechanical deformation (see, e.g., Sharei et al. *Proc. Natl. Acad. Sci. USA* (2013) 110(6): 2082-

2087); or viral transduction. Exemplary viral vectors include, but are not limited to, recombinant retroviruses, alphavirus-based vectors, and adeno-associated virus (AAV) vectors. In some embodiments, the vectors are retroviruses. In some embodiments, the vectors are lentiviruses. In some embodiments, the vectors are adeno-associated viruses.

5 Vectors of the present disclosure can comprise any of a number of promoters known to the art, wherein the promoter is constitutive, regulatable or inducible, cell type-specific, tissue-specific, or species specific. In addition to the sequence sufficient to direct transcription, a promoter sequence of the invention can also include sequences of other regulatory elements that are involved in modulating transcription (e.g., enhancers, kozak sequences and introns). Many promoter/regulatory sequences useful for driving constitutive expression of a gene are available
10 in the art and include, but are not limited to, for example, CMV (cytomegalovirus promoter), EF1a (human elongation factor 1 alpha promoter), SV40 (simian vacuolating virus 40 promoter), PGK (mammalian phosphoglycerate kinase promoter), Ubc (human ubiquitin C promoter), human beta-actin promoter, rodent beta-actin promoter, CBh (chicken beta-actin promoter),
15 CAG (hybrid promoter contains CMV enhancer, chicken beta actin promoter, and rabbit beta-globin splice acceptor), TRE (Tetracycline response element promoter), H1 (human polymerase III RNA promoter), U6 (human U6 small nuclear promoter), and the like. Moreover, inducible and tissue specific expression of an RNA, transmembrane proteins, or other proteins can be accomplished by placing the nucleic acid encoding such a molecule under the control of an
20 inducible or tissue specific promoter/regulatory sequence. Examples of tissue specific or inducible promoter/regulatory sequences which are useful for this purpose include, but are not limited to, the rhodopsin promoter, the MMTV LTR inducible promoter, the SV40 late enhancer/promoter, synapsin 1 promoter, ET hepatocyte promoter, GS glutamine synthase promoter and many others. Various commercially available ubiquitous as well as tissue-specific
25 promoters can be found at <http://www.invivogen.com/prom-a-list> and <https://www.addgene.org/>. In addition, promoters which are well known in the art can be induced in response to inducing agents such as metals, glucocorticoids, tetracycline, hormones, and the like, are also contemplated for use with the present methods and compositions. Thus, the present disclosure includes the use of any promoter/regulatory sequence known in the art that is capable of driving
30 expression of the desired protein operably linked thereto.

Vectors according to the present disclosure can be transformed, transfected or otherwise introduced into a wide variety of host cells. Transfection refers to the taking up of a vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, lipofectamine, calcium phosphate co-precipitation, electroporation, DEAE-dextran treatment, microinjection, viral transduction, and other methods known in the art. Transduction refers to entry of a virus into the cell and expression (e.g., transcription and/or translation) of sequences delivered by the viral vector genome. In the case of a recombinant vector, “transduction” generally refers to entry of the recombinant viral vector into the cell and expression of a nucleic acid of interest delivered by the vector genome.

The administration regimen may depend on several factors, including the serum or tissue turnover rate of the therapeutic composition, the level of symptoms, and the accessibility of the target cells in the biological matrix. The administration regimen can deliver sufficient therapeutic composition to effect improvement in the target disease state, while simultaneously minimizing undesired side effects.

In accordance with the present disclosure, there may be numerous tools and techniques within the skill of the art, such as those commonly used in molecular immunology, cellular immunology, pharmacology, and microbiology (see, e.g., Sambrook et al. (2001) *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.; Ausubel et al. eds. (2005) *Current Protocols in Molecular Biology*. John Wiley and Sons, Inc.: Hoboken, N.J.; Bonifacino et al. eds. (2005) *Current Protocols in Cell Biology*. John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al. eds. (2005) *Current Protocols in Immunology*, John Wiley and Sons, Inc.: Hoboken, N.J.; Coico et al. eds. (2005) *Current Protocols in Microbiology*, John Wiley and Sons, Inc.: Hoboken, N.J.; Coligan et al. eds. (2005) *Current Protocols in Protein Science*, John Wiley and Sons, Inc.: Hoboken, N.J.; and Enna et al. eds. (2005) *Current Protocols in Pharmacology*, John Wiley and Sons, Inc.: Hoboken, N.J.)

FOXO3A antagonists

Examples of FOXO3A antagonists include ZNF274, ERR α (encoded by the *ESRRA* gene), NR3C1, GATAD1, ZKSCAN1, PGC-1 α (encoded by the *PPARGC1A* gene), and HDAC8.

Zinc finger protein 274, or ZNF274, is a protein that in humans is encoded by the ZNF274 gene (Gene ID: 10782). This gene encodes a zinc finger protein containing five C2H2-type zinc finger domains, one or two Kruppel-associated box A (KRAB A) domains, and a leucine-rich domain. The encoded protein has been suggested to be a transcriptional repressor. It localizes predominantly to the nucleolus.

The NCBI Reference Sequence (RefSeq) accession numbers for human ZNF274 mRNA may include NM_001278734, NM_001278735, NM_016324, NM_016325 and NM_133502. The NCBI RefSeq accession numbers for human ZNF274 protein may include NP_001265663, NP_057408, NP_057409, and NP_598009. The NCBI RefSeq accession numbers for murine ZNF274 mRNA may include NM_178364.

There may be a number of different isoforms for each of these proteins/polypeptides discussed in this disclosure, provided herein are the general accession numbers, NCBI Reference Sequence (RefSeq) accession numbers, GenBank accession numbers, and/or UniProt numbers to provide relevant sequences. The proteins/polypeptides may also comprise other sequences.

Alternatively spliced transcript variants encoding different isoforms for ZNF274 include, but are not limited to, ZNF274d, ZNF274b, ZNF274a, and ZNF274c. These variants utilize alternative polyadenylation signals. Any isoform of ZNF274 may be inhibited by the present inhibitors. The term “ZNF274” or “*ZNF274*” is meant to include the DNA, RNA, mRNA, cDNA, recombinant DNA or RNA, or the protein arising from the gene. As used herein, ZNF274 can refer to the gene or the protein encoded by the gene, as appropriate in the specific context utilized. Additionally, in certain contexts, ZNF274 may refer to the human gene or protein, or to the mouse gene or protein, as appropriate in the specific context.

Estrogen-related receptor alpha (ERR α), also known as NR3B1 (nuclear receptor subfamily 3, group B, member 1), is a nuclear receptor that in humans is encoded by the ESRRA (Estrogen Related Receptor Alpha) gene. The NCBI Reference Sequence (RefSeq) accession numbers for human ERR α mRNA may include NM_001282450, NM_001282451, and NM_004451. The NCBI RefSeq accession numbers for human ERR α protein may include NP_001269379, NP_001269380, and NP_004442.

The glucocorticoid receptor (GR, or GCR) also known as NR3C1 (nuclear receptor subfamily 3, group C, member 1) is the receptor to which cortisol and other glucocorticoids bind. The NCBI Reference Sequence (RefSeq) accession numbers for human NR3C1 mRNA may

include NM_000176, NM_001018074, NM_001018075, NM_001018076, and NM_001018077. The NCBI RefSeq accession numbers for human NR3C1 protein may include NP_000167, NP_001018084, NP_001018085, NP_001018086, and NP_001018087. The NCBI RefSeq accession numbers for murine NR3C1 mRNA may include NM_008173, NM_001361209, NM_001361210, NM_001361211, and NM_001361212.

GATA zinc finger domain containing 1 (GATAD1) contains a zinc finger at the N-terminus, and is thought to bind to a histone modification site that regulates gene expression. The NCBI Reference Sequence (RefSeq) accession numbers for human GATAD1 mRNA may include NM_021167. The NCBI RefSeq accession numbers for human GATAD1 protein may include NP_066990.

Zinc finger protein with KRAB and SCAN domains 1 (ZKSCAN1) is a protein that in humans is encoded by the *ZKSCAN1* gene. The NCBI Reference Sequence (RefSeq) accession numbers for human ZKSCAN1 mRNA may include NM_001287054, NM_001287055, NM_003439, NM_001346579 and NM_001346580. The NCBI RefSeq accession numbers for human ZKSCAN1 protein may include NP_001273983, NP_001273984, NP_001333508, NP_001333509, and NP_001333510. The NCBI RefSeq accession numbers for murine ZKSCAN1 mRNA may include NM_029869 and NM_133906. The NCBI RefSeq accession numbers for murine ZKSCAN1 protein may include NP_084145 and NP_598667.

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) is a protein that in humans is encoded by the *PPARGC1A* gene. PPARGC1A is also known as human accelerated region 20 (HAR20). The NCBI Reference Sequence (RefSeq) accession numbers for human PGC-1 α mRNA may include NM_013261, NM_001330751, NM_001330752, and NM_001330753. The NCBI RefSeq accession numbers for human PGC-1 α protein may include NP_001317680, NP_001317681, NP_001317682, NP_037393 and NP_001341754. The NCBI RefSeq accession numbers for murine PGC-1 α mRNA may include NM_008904. The NCBI RefSeq accession numbers for murine PGC-1 α protein may include NP_032930.

Histone deacetylase 8 (HDAC8) is an enzyme that in humans is encoded by the *HDAC8* gene. The NCBI Reference Sequence (RefSeq) accession numbers for human HDAC8 mRNA may include NM_001166418, NM_001166419, NM_001166420, NM_001166422 and NM_001166448. The NCBI RefSeq accession numbers for human HDAC8 protein may include NP_001159890, NP_001159891, NP_001159892, NP_001159894 and NP_001159920. The

NCBI RefSeq accession numbers for murine HDAC8 mRNA may include NM_027382 and NM_001313742. The NCBI RefSeq accession numbers for murine HDAC8 protein may include NP_001300671 and NP_081658.

5 **Inhibitors of FOXO3A antagonists**

The present inhibitors may target the wild-type or mutant FOXO3A antagonist (e.g., ZNF274).

As used herein, the term "inhibitor" refers to agents capable of down-regulating or otherwise decreasing or suppressing the amount/level and/or activity of a FOXO3A antagonist
10 (e.g., ZNF274).

The mechanism of inhibition may be at the genetic level (e.g., interference with or inhibit expression, transcription or translation, etc.) or at the protein level (e.g., binding, competition, etc.).

A wide variety of suitable inhibitors may be employed, guided by art-recognized criteria such as efficacy, toxicity, stability, specificity, half-life, etc.

15

Inhibitory Nucleic Acids

The inhibitor used in the present methods and compositions may be a polynucleotide that reduces the expression/amount/activity of the FOXO3A antagonist (e.g., ZNF274). Thus, the method may involve administering an effective amount of a polynucleotide that specifically
20 targets a nucleotide sequence(s) encoding the FOXO3A antagonist (e.g., ZNF274). The polynucleotides reduce expression of the FOXO3A antagonist (e.g., ZNF274), to yield reduced levels of the gene product (the translated polypeptide).

For example, a nucleic acid molecule complementary to at least a portion of a human FOXO3A antagonist-encoding nucleic acid can be used to inhibit the gene expression. Short
25 RNA molecules for inhibiting gene expression include, but are not limited to, small interfering RNA (siRNA), short hairpin RNAs (shRNAs), small temporal RNAs (stRNAs), and micro-RNAs (miRNAs). Small interfering RNAs silence genes through an mRNA degradation pathway, while stRNAs and miRNAs are approximately 21 or 22 nt RNAs that are processed from endogenously encoded hairpin-structured precursors, and function to silence genes via
30 translational repression. See, e.g., McManus et al., RNA, 8(6):842-50 (2002); Morris et al., Science, 305(5688):1289-92 (2004); He and Hannon, Nat Rev Genet. 5(7):522-31 (2004).

The target sequence of the polynucleotides (e.g., siRNA, antisense oligonucleotides, and ribozymes) of the disclosure may be any location within the gene or transcript of the FOXO3A antagonist (e.g., ZNF274).

Other aspects of the invention include vectors (e.g., viral vectors, expression cassettes, plasmids) comprising or encoding polynucleotides of the inhibitory nucleic acids (e.g., siRNA, antisense nucleic acids, and ribozymes), and cells genetically modified with polynucleotides or vectors of the present disclosure.

According to the methods of the subject invention, recombinant cells can be administered to a patient, wherein the recombinant cells have been genetically modified to express a nucleotide sequence encoding an inhibitory polypeptide.

RNA Interference

RNA interference, or RNAi, is a form of post-transcriptional gene silencing (PTGS), including the introduction of a double-stranded RNA into cells (reviewed in Fire, A. Trends Genet 15:358-363 (1999); Sharp, P. Genes Dev 13:139-141 (1999); Hunter, C. Curr Biol 9:R440-R442 (1999); Baulcombe, D. Curr Biol 9:R599-R601 (1999); Vaucheret et al. Plant J 16: 651-659 (1998)).

SiRNAs (small interfering RNAs) or small-hairpin RNA (shRNA) may be used as an inhibitor of the FOXO3A antagonist (e.g., ZNF274) to reduce the level of the FOXO3A antagonist (e.g., ZNF274).

SiRNAs may have 16-30 nucleotides, e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides. The siRNAs may have fewer than 16 or more than 30 nucleotides. The polynucleotides of the invention include both unmodified siRNAs and modified siRNAs such as siRNA derivatives etc.

SiRNAs can be delivered into cells *in vitro* or *in vivo* by methods known in the art, including cationic liposome transfection and electroporation. SiRNAs and shRNA molecules can be delivered to cells using viruses or DNA vectors.

Software programs for predicting siRNA sequences to inhibit the expression of a target protein are commercially available. One program, siDESIGN from Dharmacon, Inc. (Lafayette, Colo.), permits predicting siRNAs for any nucleic acid sequence, and is available on the internet at dharmacon.com. Programs for designing siRNAs are also available from others, including

Genscript (available on the internet at genscript.com/ssl-bin/app/rnai) and, to academic and non-profit researchers, from the Whitehead Institute for Biomedical Research found on the worldwide web at "jura.wi.mit.edu/pubint/http://iona.wi.mit.edu/siRNAext/."

RNA precursors such as short hairpin RNAs (shRNAs) can also be encoded by all or a part
5 of the nucleic acid sequence encoding the FOXO3A antagonist.

Any suitable viral knockdown system could be utilized for decreasing the mRNA levels of the FOXO3A antagonist, including AAV, lentiviral vectors, or other suitable vectors.

Gene therapy may be employed to modulate the expression of the FOXO3A antagonist (e.g., ZNF274) by the target cells. For example, a polynucleotide encoding an siRNA targeting the
10 FOXO3A antagonist, or a portion of this, may be engineered for expression in a replication defective retroviral vector. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding the siRNA such that the packaging cell now produces infectious viral particles containing the sequence of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and
15 regulating expression of the the FOXO3A antagonist (e.g., ZNF274) polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

20 Antisense Polynucleotides

The inhibitor of the FOXO3A antagonist (e.g., ZNF274) may be an antisense nucleic acid sequence that is complementary to a target region within the mRNA of the FOXO3A antagonist (e.g., ZNF274). The antisense polynucleotide may bind to the target region and inhibit
translation. The antisense oligonucleotide may be DNA or RNA. The antisense oligonucleotide
25 may comprise synthetic analogs of ribo-deoxynucleotides. Thus, the antisense oligonucleotide inhibits expression of the FOXO3A antagonist (e.g., ZNF274).

An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

The antisense nucleic acid molecules of the present disclosure may be administered to a
30 subject or a cell, or generated *in situ* such that they hybridize with or bind to the mRNA of the FOXO3A antagonist (e.g., ZNF274). Alternatively, antisense nucleic acid molecules can be

modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using viruses or DNA vectors.

Ribozyme

The inhibitor of the FOXO3A antagonist (e.g., ZNF274) may be a ribozyme that inhibits expression of the gene encoding the FOXO3A antagonist.

Ribozymes can be chemically synthesized and structurally modified to increase their stability and catalytic activity using methods known in the art. Ribozyme encoding nucleotide sequences can be introduced into host cells through gene-delivery mechanisms known in the art.

Endonucleases

The FOXO3A antagonist (e.g., ZNF274) may be inhibited by using a sequence-specific endonuclease that target the gene encoding the FOXO3A antagonist (e.g., ZNF274). Thus, the inhibitor of a FOXO3A antagonist may comprise an endonuclease.

Non-limiting examples of the endonucleases include a zinc finger nuclease (ZFN), a ZFN dimer, a ZFNickase, a transcription activator-like effector nuclease (TALEN), or an RNA-guided DNA endonuclease (e.g., CRISPR/Cas). Meganucleases are endonucleases characterized by their capacity to recognize and cut large DNA sequences (12 base pairs or greater). Any suitable meganuclease may be used in the present methods to create double-strand breaks in the host genome, including endonucleases in the LAGLIDADG and PI-Sce family.

An example of sequence-specific endonucleases includes RNA-guided DNA nucleases, e.g., the CRISPR/Cas system (Geurts et al., Science 325, 433 (2009); Mashimo et al., PLoS ONE 5, e8870 (2010); Carbery et al., Genetics 186, 451-459 (2010); Tesson et al., Nat. Biotech. 29, 695-696 (2011). Wiedenheft et al. Nature 482,331-338 (2012); Jinek et al. Science 337,816-821 (2012); Mali et al. Science 339,823-826 (2013); Cong et al. Science 339,819-823 (2013)).

The sequence-specific endonuclease of the methods and compositions described herein can be engineered, chimeric, or isolated from an organism. Endonucleases can be engineered to recognize a specific DNA sequence, by, e.g., mutagenesis (Seligman et al. (2002) Mutations

altering the cleavage specificity of a homing endonuclease, *Nucleic Acids Research* 30: 3870–3879). Combinatorial assembly is a method where protein subunits form different enzymes can be associated or fused (Arnould et al. (2006) Engineering of large numbers of highly specific homing endonucleases that induce recombination to novel DNA targets, *Journal of Molecular Biology* 355: 443–458). These two approaches, mutagenesis and combinatorial assembly, may be combined to produce an engineered endonuclease with desired DNA recognition sequence.

The sequence-specific nuclease can be introduced into the cell in the form of a protein or in the form of a nucleic acid encoding the sequence-specific nuclease, such as an mRNA or a cDNA.

In one embodiment, the inhibitor of the FOXO3A antagonist (e.g., ZNF274) comprises a nuclease, including endonucleases and exonucleases. Some nucleases are specific to either single-stranded or double-stranded nucleic acid sequences. Some enzymes have both exonuclease and endonuclease properties. In addition, some enzymes are able to digest both DNA and RNA sequences.

One example of a sequence-specific nuclease system that can be used with the methods and compositions described herein includes the CRISPR system (Wiedenheft, B. et al. *Nature* 482, 331-338 (2012); Jinek, M. et al. *Science* 337, 816-821 (2012); Mali, P. et al. *Science* 339, 823-826 (2013); Cong, L. et al. *Science* 339, 819-823 (2013)). The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system exploits RNA-guided DNA-binding and sequence-specific cleavage of target DNA. The guide RNA/Cas combination confers site specificity to the nuclease. A single guide RNA (sgRNA) can bind to a target genomic DNA sequence, e.g., upstream of a genomic PAM (protospacer adjacent motifs) site (e.g., NGG). The Cas (CRISPR-associated) protein binds to the sgRNA and the target DNA to which the sgRNA binds and introduces a double-strand break in a defined location. Cas9 harbors two independent nuclease domains homologous to HNH and RuvC endonucleases, and by mutating either of the two domains, the Cas9 protein can be converted to a nickase that introduces single-strand breaks (Cong, L. et al. *Science* 339, 819-823 (2013)). It is specifically contemplated that the methods and compositions of the present disclosure can be used with the single- or double-strand-inducing version of Cas9, as well as with other RNA-guided DNA nucleases, such as other bacterial Cas9-like systems. The sequence-specific nuclease of the present methods and compositions described herein can be engineered, chimeric, or isolated from an organism. The

nuclease can be introduced into the cell in form of a DNA, mRNA and protein. The applications of the CRISPR/Cas system to inhibiting or downregulating a FOXO3A antagonist (e.g., ZNF274) can be easily adapted.

In one embodiment, the methods of the present disclosure comprise using one, two or more
5 sgRNAs to knockout, knockdown, or suppress/inhibit the expression of, a FOXO3A antagonist (e.g., ZNF274).

In one embodiment, the inhibitor is a site-specific nuclease. In another embodiment, the site-specific nuclease may be a Cas-family nuclease. In a more specific embodiment, the Cas
nuclease may be a Cas9 nuclease.

10 In one embodiment, Cas protein may be a functional derivative of a naturally occurring Cas protein.

The inhibitor of a FOXO3A antagonist may comprise a genetic engineering system such as a genome editing system. As used herein, “genome editing” refers to a method of modifying the genome, including any protein-coding or non-coding nucleotide sequence, of an organism to
15 knockout or decrease the expression of a target gene. In general, genome editing methods involve use of an endonuclease that is capable of cleaving the nucleic acid of the genome, for example at a targeted nucleotide sequence. Double-stranded breaks in the genome may be repaired introducing mutations, and/or exogenous nucleic acid may be inserted into the targeted site.

20 For the CRISPR/Cas systems, wildtype or mutant Cas enzyme may be used. In some embodiments, the nucleotide sequence encoding the Cas9 enzyme is modified to alter the activity of the protein. The mutant Cas enzyme may lack the ability to cleave one or both strands of a target polynucleotide containing a target sequence. Cas9 harbors two independent nuclease domains homologous to HNH and RuvC endonucleases, and by mutating either of the two
25 domains, the Cas9 protein can be converted to a nickase that introduces single-strand breaks (Cong, L. et al. Science 339, 819-823 (2013)). For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, D10A, H840A, N854A, N863A, and
30 combinations thereof. In some embodiments, a Cas9 nickase may be used in combination with

guide RNA(s), e.g., two guide RNAs, which target respectively sense and antisense strands of the DNA target.

As described herein, the term "base editor" refers to a protein that edits a nucleotide base. "Edit" refers to the conversion of one nucleobase to another (e.g., A to G, A to C, A to T, C to T, C to G, C to A, G to A, G to C, G to T, T to A, T to C, T to G). In some embodiments, a nucleobase editor is a macromolecule or macromolecular complex that results primarily (e.g., more than 80%, more than 85%, more than 90%, more than 95%, more than 99%, more than 99.9%, or 100%) in the conversion of a nucleobase in a nucleic acid sequence into another nucleobase (i.e., a transition or transversion) using a combination of 1) a nucleotide-, nucleoside-, or nucleobase-modifying enzyme and 2) a nucleic acid binding protein that can be programmed to bind to a specific nucleic acid sequence. A base editor may be a fusion protein comprising: (i) a DNA binding domain; and (ii) a deaminase domain. In one embodiment, the base editor is a fusion polypeptide comprising: a) an RNA-guided endonuclease; and b) a cytidine deaminase. See, e.g., Komor et al. (2016) Nature 533:420.

In some embodiments, the base editor comprises a DNA binding domain (e.g., a DNA binding domain such as a dCas9 or nCas9) that directs it to a target sequence. In some embodiments, the nucleobase editor comprises a nucleobase modifying enzyme fused to a DNA binding domain (e.g., a dCas9 or nCas9). nCas9, a point mutant (D10A) of wild-type Cas9 nuclease, has a nick endonuclease activity. A "nucleobase modifying enzyme" is an enzyme that can modify a nucleobase and convert one nucleobase to another (e.g., a deaminase such as a cytosine deaminase or an adenosine deaminase). In some embodiments, the nucleobase editor may target cytosine (C) bases in a nucleic acid sequence and convert the C to thymine (T) base. In some embodiments, the C to T editing is carried out by a deaminase, e.g., a cytosine deaminase. Base editors that can carry out other types of base conversions (e.g., adenosine (A) to guanine (G), C to G) are also contemplated.

In some embodiments, the DNA binding protein domain comprises the DNA binding domain of a zinc finger nuclease (ZFN) or a transcription activator-like effector domain (TALE). In some embodiments, the DNA binding protein domain may be programmed by a guide nucleotide sequence. In some embodiments, the DNA binding protein is a nuclease inactive Cas9, or dCas9. A dCas9 as used herein, encompasses a Cas9 that is completely inactive in its nuclease activity, or partially inactive in its nuclease activity (e.g., a Cas9 nickase). In some

embodiments, the DNA binding protein is a nuclease inactive Cpf1. In some embodiments, the DNA binding protein is a nuclease inactive Argonaute. In some embodiments, the base editor comprises a Cas9 (e.g., dCas9 and nCas9), CasX, CasY, Cpf1, C2c1, C2c2, C2c3, or Argonaute protein. For example, the base editor may be a cytosine deaminase-dCas9 fusion protein, a
 5 cytosine deaminase-Cas9 nickase fusion protein, a deaminase-dCas9-UGI fusion protein, an UGI-deaminase-dCas9 fusion protein, an UGI-deaminase-Cas9 nickase fusion protein, an APOBEC1-dCas9-UGI fusion protein, an APOBEC1-Cas9 nickase-UGI fusion protein.

In certain embodiments, the inhibitor may comprise Cas12a/Cpf1, CasX, C2c1/2/3, mutants thereof, or variants thereof.

10 Various derivations of the base editor (BE) system, such as BE1, BE2 and BE3, may be used.

The methods and compositions of the present disclosure may be used with the single- or double-strand-inducing version of Cas9, as well as with other RNA-guided DNA nucleases, such as other bacterial Cas9-like systems. The sequence-specific nuclease of the present methods and
 15 compositions can be engineered, chimeric, or isolated from an organism. The nuclease can be introduced into the cell in form of a DNA, mRNA and protein.

Standard procedures may be used to express wildtype Cas9, nickase Cas9, or the base editor Cas9 which include transfection of plasmids, transduction of lentiviral particles, or nucleofection of protein.

20 In some embodiments, the Cas endonuclease is a Cas9 enzyme or variant thereof. In some embodiments, the Cas9 endonuclease is derived from *Streptococcus pyogenes*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Streptococcus thermophilus*, or *Treponema denticola*. In some embodiments, the nucleotide sequence encoding the Cas endonuclease may be codon optimized for expression in a host cell. In some embodiments, the endonuclease is a
 25 Cas9 homolog or ortholog.

In some embodiments, the nucleotide sequence encoding the Cas9 endonuclease is further modified to alter the activity of the protein. In some embodiments, the Cas9 endonuclease is a catalytically inactive Cas9. For example, dCas9 contains mutations of catalytically active residues (D10 and H840) and does not have nuclease activity. Alternatively
 30 or in addition, the Cas9 endonuclease may be fused to another protein or portion thereof. In some embodiments, dCas9 is fused to a repressor domain, such as a KRAB domain. In some

embodiments, such dCas9 fusion proteins are used with the constructs described herein for multiplexed gene repression (e.g. CRISPR interference (CRISPRi)). In some embodiments, dCas9 is fused to an activator domain, such as VP64 or VPR. In some embodiments, dCas9 is fused to an epigenetic modulating domain, such as a histone demethylase domain or a histone acetyltransferase domain. In some embodiments, dCas9 is fused to a LSD1 or p300, or a portion thereof. In some embodiments, the dCas9 fusion is used for CRISPR-based epigenetic modulation. In some embodiments, dCas9 or Cas9 is fused to a Fok1 nuclease domain. In some embodiments, Cas9 or dCas9 fused to a Fok1 nuclease domain is used for genome editing. In some embodiments, Cas9 or dCas9 is fused to a fluorescent protein (e.g., GFP, RFP, mCherry, etc.). In some embodiments, Cas9/dCas9 proteins fused to fluorescent proteins are used for labeling and/or visualization of genomic loci or identifying cells expressing the Cas endonuclease.

Alternatively or in addition, the Cas endonuclease is a Cpf1 nuclease. In some embodiments, the host cell expresses a Cpf1 nuclease derived from *Providentia spp.* or *Francisella spp.* In some embodiments, the nucleotide sequence encoding the Cpf1 nuclease may be codon optimized for expression in a host cell.

In addition to the CRISPR-Cas system, a new CRISPR enzyme, called Cpf1 (Cas protein 1 of PreFran subtype) has recently been described (Zetsche et al. Cell. pii: S0092-8674(15)01200-3. doi: 10.1016/j.cell.2015.09.038 (2015)). Cpf1 is a single RNA-guided endonuclease that lacks tracrRNA, and utilizes a T-rich protospacer-adjacent motif. The authors demonstrated that Cpf1 mediates strong DNA interference with characteristics distinct from those of Cas9. Thus, in one embodiment of the present invention, CRISPR-Cpf1 system can be used to cleave a desired region within the targeted gene.

Single guide RNA(s) used in the methods of the present disclosure can be designed so that they direct binding of the Cas-sgRNA complexes to pre-determined cleavage sites in a genome.

For Cas family enzyme (such as Cas9) to successfully bind to DNA, the target sequence in the genomic DNA should be substantially complementary to the sgRNA sequence and may be immediately followed by the correct protospacer adjacent motif or PAM sequence.

“Complementarity” refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. A

percent complementarity indicates the percentage of residues in a nucleic acid molecule, which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex. A target sequence may

5 comprise any polynucleotide, such as DNA or RNA polynucleotides. The Cas9 protein can tolerate mismatches distal from the PAM, however, mismatches within the 12 base pairs (bps) of sequence next to the PAM sequence can dramatically decrease the targeting efficiency. The PAM sequence is present in the DNA target sequence but not in the sgRNA sequence. Any DNA sequence with the correct target sequence followed by the PAM sequence will be bound by Cas9.

10 The PAM sequence varies by the species of the bacteria from which Cas9 was derived. The most widely used CRISPR system is derived from *S. pyogenes* and the PAM sequence is NGG located on the immediate 3' end of the sgRNA recognition sequence. The PAM sequences of CRISPR systems from exemplary bacterial species include: *Streptococcus pyogenes* (NGG), *Neisseria meningitidis* (NNNNGATT), *Streptococcus thermophilus* (NNAGAA) and *Treponema denticola*

15 (NAAAAC).

sgRNA(s) used in the present disclosure can be between about 5 and 100 nucleotides long, or longer (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100

20 nucleotides in length, or longer). In one embodiment, sgRNA(s) can be between about 15 and about 30 nucleotides in length (e.g., about 15-29, 15-26, 15-25; 16-30, 16-29, 16-26, 16-25; or about 18-30, 18-29, 18-26, or 18-25 nucleotides in length).

To facilitate sgRNA design, many computational tools have been developed (See

25 Prykhodzhiy et al. (PLOS ONE, 10(3): (2015)); Zhu et al. (PLOS ONE, 9(9) (2014)); Xiao et al. (Bioinformatics. Jan 21 (2014)); Heigwer et al. (Nat Methods, 11(2): 122–123 (2014)). Methods and tools for guide RNA design are discussed by Zhu (Frontiers in Biology, 10 (4) pp 289-296 (2015)), which is incorporated by reference herein. Additionally, there is a publically available software tool that can be used to facilitate the design of sgRNA(s)

30 (<http://www.genscript.com/gRNA-design-tool.html>).

In further embodiment, the DNA digesting agent is a transcription activator-like effector nuclease (TALEN). TALENs are composed of a TAL effector domain that binds to a specific nucleotide sequence and an endonuclease domain that catalyzes a double strand break at the target site (PCT Patent Publication No. WO2011072246; Miller et al., Nat. Biotechnol. 29, 143-148 (2011); Cermak et al., Nucleic Acid Res. 39, e82 (2011)). Sequence-specific endonucleases may be modular in nature, and DNA binding specificity is obtained by arranging one or more modules. Bibikova et al., Mol. Cell. Biol. 21, 289-297 (2001). Boch et al., Science 326, 1509-1512 (2009).

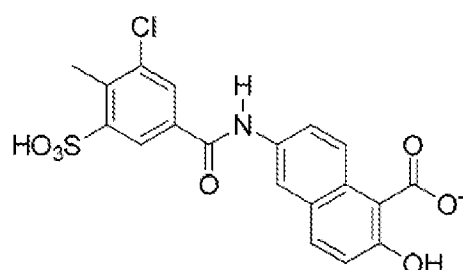
ZFNs can be composed of two or more (e.g., 2 – 8, 3 – 6, 6 – 8, or more) sequence-specific DNA binding domains (e.g., zinc finger domains) fused to an effector endonuclease domain (e.g., the FokI endonuclease). Porteus et al., Nat. Biotechnol. 23, 967-973 (2005). Kim et al. (2007) Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain, Proceedings of the National Academy of Sciences of USA, 93: 1156–1160.

The sequence-specific nuclease can be introduced into the cell in the form of a protein or in the form of a nucleic acid encoding the sequence-specific nuclease, such as an mRNA or a cDNA. Nucleic acids can be delivered as part of a larger construct, such as a plasmid or viral vector, or directly, e.g., by electroporation, lipid vesicles, viral transporters, microinjection, and biolistics. Similarly, the construct containing the one or more transgenes can be delivered by any method appropriate for introducing nucleic acids into a cell.

Small Molecule Inhibitors

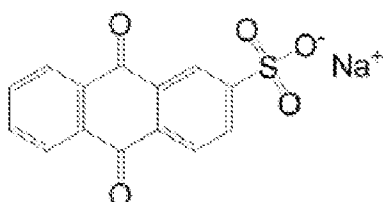
As used herein, the term "small molecules" encompasses molecules other than proteins or nucleic acids without strict regard to size. Non-limiting examples of small molecules that may be used according to the methods and compositions of the present invention include, small organic molecules, peptide-like molecules, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules.

In one embodiment, the inhibitor of a FOXO3A antagonist has the below structure. See, He et al., Characterization of Small Molecules Inhibiting the Pro-Angiogenic Activity of the Zinc Finger Transcription Factor Vezf1, Molecules 2018, 23, 1615, the content of which is incorporated herein by reference in its entirety.

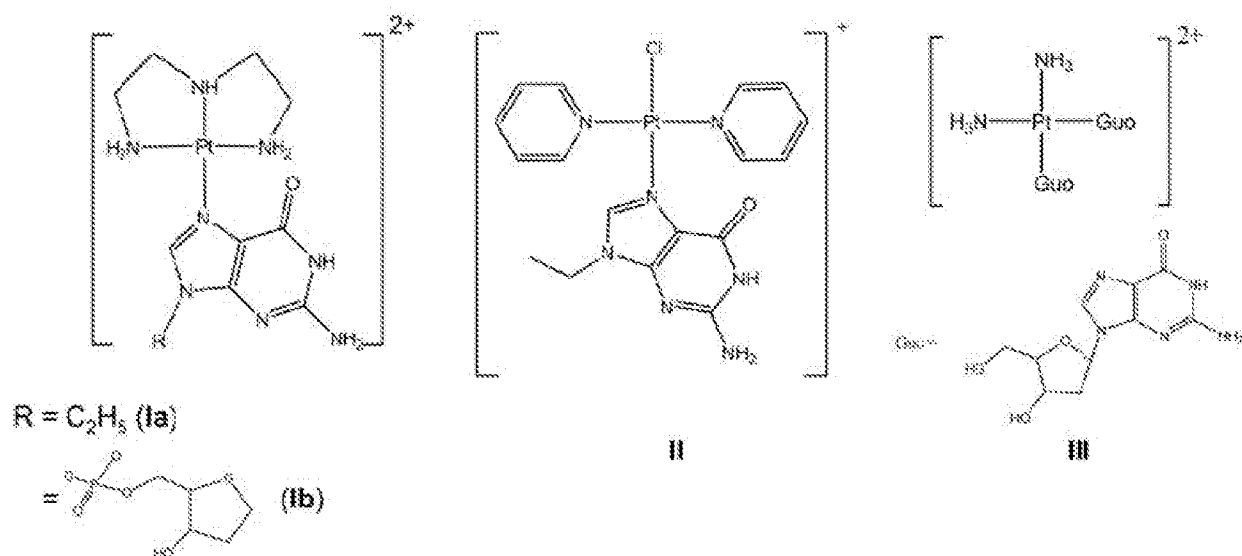


In another embodiment, the inhibitor of a FOXO3A antagonist has the below structure.

See, Farina et al., Targeting zinc finger domains with small molecules: solution structure and
 5 binding studies of the RanBP2-type zinc finger of RBM5, *Chembiochem.*, 2011 December 16;
 12(18): 2837–2845, the content of which is incorporated herein by reference in its entirety.



In yet another embodiment, the inhibitor of a FOXO3A antagonist is a small molecule
 containing platinated purine nucleobases, such as compounds Ia, Ib, II and III shown below. See,
 10 Anzellotti et al., Targeting Retroviral Zn Finger-DNA Interactions: A Small-Molecule Approach
 Using the Electrophilic Nature of trans-Platinum-Nucleobase Compounds, *Chemistry & Biology*
 13, 539–548, May 2006, the content of which is incorporated herein by reference in its entirety.



See also, Khedkar et al., Discovery of Small Molecule Inhibitors to Krüppel-like Factor 10 (KLF10): Implications for Modulation of T Regulatory Cell Differentiation, J. Med. Chem., 2015, 58 (3), pp 1466–1478, the content of which is incorporated herein by reference in its entirety.

5

Polypeptides

The present inhibitors can also be a polypeptide exhibiting inhibitory activity toward a FOXO3A antagonist (e.g., ZNF274).

Various means for delivering polypeptides to a cell can be utilized to carry out the methods of the subject invention. For example, protein transduction domains (PTDs) can be fused to the polypeptide, producing a fusion polypeptide, in which the PTDs are capable of transducing the polypeptide cargo across the plasma membrane (Wadia, J. S. and Dowdy, S. F., Curr. Opin. Biotechnol., 2002, 13(1)52-56).

Antibodies

The present inhibitors can be an antibody or antigen-binding portion thereof that is specific to a FOXO3A antagonist (e.g., ZNF274).

The antibody or antigen-binding portion thereof may be the following: (a) a whole immunoglobulin molecule; (b) an scFv; (c) a Fab fragment; (d) an F(ab')₂; and (e) a disulfide linked Fv. The antibody or antigen-binding portion thereof may be monoclonal, polyclonal, chimeric and humanized. The antibodies may be murine, rabbit or human/humanized antibodies.

In one embodiment, the inhibitor of NR3C1 is the GRp20 antibody.

Combination Therapy

The invention also provides for methods of using an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) to treat a disease, such as cancer. An inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) may be administered alone, or in combination with radiation, surgery or a chemotherapeutic agent (or a cytotoxic agent). An inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) may also be co-administered with antiviral agents, anti-inflammatory agents or antibiotics. The agents may be administered concurrently or sequentially. An inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) can be

administered before, during or after radiation, surgery, or the administration of the other active agent(s) described herein.

The inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) may be used in combination with radiation therapy. In one embodiment, the present disclosure provides for a method of treating tumor cells with radiation, where the cells are treated with an effective amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274), and then exposed to radiation. The inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) treatment may be before, during and/or after radiation.

The present disclosure provides for a method of treating tumor cells with chemotherapy or cytotoxic agent treatment, where the cells are treated with an effective amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274), and then exposed to chemotherapy or a cytotoxic agent. The inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) treatment may be before, during and/or after chemotherapy or cytotoxic agent treatment.

The present method for treating cancer may comprise the step of administering to a subject an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274).

The present method for treating cancer may comprise the step of administering to a subject an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a cytotoxic agent (e.g., a chemotherapeutic agent).

This may be achieved by administering a pharmaceutical composition that includes both agents (an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a cytotoxic agent (e.g., a chemotherapeutic agent)), or by administering two pharmaceutical compositions, at the same time or within a short time period, wherein one composition comprises an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274), and the other composition includes a cytotoxic agent (e.g., a chemotherapeutic agent).

The combination of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and a cytotoxic agent (e.g., a chemotherapeutic agent) may produce an additive or synergistic effect (i.e., greater than additive effect) in treating the cancer compared to the effect of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone or the cytotoxic agent (e.g., a chemotherapeutic agent) alone. For example, the combination may result in a synergistic increase in apoptosis of cancer cells, and/or a synergistic reduction in tumor volume. In different embodiments, depending on the combination and the effective amounts used, the combination of

compounds can inhibit tumor growth, achieve tumor stasis, or achieve substantial or complete tumor regression. In some embodiments, the combination therapy results in a synergistic effect, for example, the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and a cytotoxic agent (e.g., a chemotherapeutic agent) act synergistically, for example, in the apoptosis of cancer cells, inhibition of proliferation/survival of cancer cells, in the production of tumor stasis.

In various embodiments, the present invention provides methods to reduce cancer cell growth, proliferation, and/or metastasis, as measured according to routine techniques in the diagnostic art. Specific examples of relevant responses include reduced size, mass, or volume of a tumor, or reduction in cancer cell number.

The present compositions and methods can have one or more of the following effects on cancer cells or the subject: cell death; decreased cell proliferation; decreased numbers of cells; inhibition of cell growth; apoptosis; necrosis; mitotic catastrophe; cell cycle arrest; decreased cell size; decreased cell division; decreased cell survival; decreased cell metabolism; markers of cell damage or cytotoxicity; indirect indicators of cell damage or cytotoxicity such as tumor shrinkage; improved survival of a subject; preventing, inhibiting or ameliorating the cancer in the subject, such as slowing progression of the cancer, reducing or ameliorating a sign or symptom of the cancer; reducing the rate of tumor growth in a patient; preventing the continued growth of a tumor, reducing the size of a tumor; and/or disappearance of markers associated with undesirable, unwanted, or aberrant cell proliferation.

Methods and compositions of the present invention can be used for prophylaxis as well as amelioration of signs and/or symptoms of cancer.

As used herein, the term "synergy" (or "synergistic") means that the effect achieved with the methods and combinations of this disclosure is greater than the sum of the effects that result from using the individual agents alone, e.g., using the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone and the cytotoxic agent (e.g., a chemotherapeutic agent) alone. For example, the effect (e.g., apoptosis of cells, a decrease in cell viability, cytotoxicity, a decrease in cell proliferation, a decrease in cell survival, inhibition of tumor growth, a reduction in tumor volume, and/or tumor stasis, etc. as described herein) achieved with the combination of an inhibitor of ZNF274 and a cytotoxic agent (e.g., a chemotherapeutic agent) is about 1.1 fold, about 1.2 fold, about 1.3 fold, about 1.4 fold, about 1.5 fold, about 1.6 fold, about 1.7 fold, about

1.8 fold, about 1.9 fold, about 2 fold, about 2.5 fold, about 3 fold, about 3.5 fold, about 4 fold, about 4.5 fold, about 5 fold, about 5.5 fold, about 6 fold, about 6.5 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 12 fold, about 15 fold, about 20 fold, about 25 fold, about 30 fold, about 50 fold, about 100 fold, at least about 1.2 fold, at least about 1.5 fold, at least about 2 fold, at least about 2.5 fold, at least about 3 fold, at least about 3.5 fold, at least about 4 fold, at least about 4.5 fold, at least about 5 fold, at least about 5.5 fold, at least about 6 fold, at least about 6.5 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, of the sum of the effects that result from using the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone and the inhibitor of the cytotoxic agent (e.g., a chemotherapeutic agent) alone.

Synergistic effects of the combination may also be evidenced by additional, novel effects that do not occur when either agent is administered alone, or by reduction of adverse side effects when either agent is administered alone.

In vitro efficacy of the present composition/agent can be determined using methods well known in the art. For example, the cytotoxicity of the present composition/agent and/or the therapeutic agents may be studied by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytotoxicity assay. MTT assay is based on the principle of uptake of MTT, a tetrazolium salt, by metabolically active cells where it is metabolized into a blue colored formazon product, which can be read spectrometrically. J. of Immunological Methods 65: 55 63, 1983. The cytotoxicity of the present composition/agent and/or the therapeutic agents may be studied by colony formation assay. Functional assays for inhibition of VEGF secretion and IL-8 secretion may be performed via ELISA. Cell cycle block by the present composition/agent and/or the therapeutic agents may be studied by standard propidium iodide (PI) staining and flow cytometry. Invasion inhibition may be studied by Boyden chambers. In this assay a layer of reconstituted basement membrane, Matrigel, is coated onto chemotaxis filters and acts as a barrier to the migration of cells in the Boyden chambers. Only cells with invasive capacity can cross the Matrigel barrier. Other assays include, but are not limited to cell viability assays, apoptosis assays, and morphological assays.

Cytotoxicity effects can be determined by any suitable assay *in vitro*, including, but not limited to, assessing cell membrane integrity (using, e.g., dyes such as trypan blue or propidium

iodide, or using lactate dehydrogenase (LDH) assay), measuring enzyme activity, measuring cell adherence, measuring ATP production, measuring co-enzyme production, measuring nucleotide uptake activity, crystal violet method, Tritium-labeled Thymidine uptake method, measuring lactate dehydrogenase (LDH) activity, 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or MTS assay, sulforhodamine B (SRB) assay, WST assay, clonogenic assay, cell number count, monitoring cell growth, etc.

Apoptosis of cells may be assayed by any suitable method, including, but not limited to, TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay, assaying levels of cytochrome C release, assaying levels of cleaved/activated caspases, assaying 5-bromo-2'-deoxyuridine labeled fragmented DNA, assaying levels of survivin etc.

Other methods that can be used to show the synergistic effects of the present methods, pharmaceutical compositions and combinations include, but are not limited to, clonogenic assay (colony formation assay) to show decrease in cell survival and/or proliferation, studying tumor volume reduction in animal models (such as in mice, etc.)

In one embodiment, advantageously, such synergy provides greater efficacy at the same doses, lower side effects, and/or prevents or delays the build-up of multi-drug resistance.

The cytotoxic agent (e.g., a chemotherapeutic agent) and the inhibitor of ZNF274 may be administered simultaneously, separately or sequentially. They may exert an advantageously combined effect (e.g., additive or synergistic effects).

For sequential administration, either an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) is administered first and then a cytotoxic agent (e.g., a chemotherapeutic agent), or a cytotoxic agent (e.g., a chemotherapeutic agent) is administered first and then an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274). In embodiments where an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a cytotoxic agent (e.g., a chemotherapeutic agent) are administered separately, administration of a first agent can precede administration of a second agent by seconds, minutes, hours, days, or weeks. The time difference in non-simultaneous administrations may be greater than 1 minute, and can be, for example, precisely, at least, up to, or less than 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 36 hours, or 48 hours, or more than 48 hours. The two or more agents can be administered within minutes of each other or within about 0.5, about 1, about 2, about 3, about 4, about 6, about 9, about 12, about 15, about

18, about 24, or about 36 hours of each other or within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 days of each other or within about 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks of each other. In some cases, longer intervals are possible.

The present invention also provides for a pharmaceutical composition comprising (i) the
5 inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274); (ii) a cytotoxic agent (e.g., a chemotherapeutic agent); and (iii) at least one pharmaceutically acceptable excipient.

Combinations may be administered either concomitantly, e.g., as an admixture,
separately but simultaneously or concurrently; or sequentially. This includes presentations in
which the combined agents are administered together as a therapeutic mixture, and also
10 procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration in combination further includes the separate administration of one of the compounds or agents given first, followed by the second.

This may be achieved by administering a pharmaceutical composition that includes both
15 agents, or by administering two pharmaceutical compositions, at the same time or within a short time period.

In certain embodiments, the combination of the present inhibitor and the second
treatment produces an additive or synergistic effect (i.e., greater than additive effect) in treating a
disorder as discussed herein, compared to the effect of the inhibitor alone or the second treatment
20 alone.

As used herein, the term “synergy” (or “synergistic”) means that the effect achieved with
the methods and combinations of the combination therapy is greater than the sum of the effects
that result from using the individual agents alone, e.g., using the inhibitor of a FOXO3A
antagonist (e.g., the ZNF274 inhibitor) alone and the second treatment alone. For example, the
25 effect achieved with the combination of the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) and the second treatment is about 1.1 fold, about 1.2 fold, about 1.3 fold, about 1.4 fold, about 1.5 fold, about 1.6 fold, about 1.7 fold, about 1.8 fold, about 1.9 fold, about 2 fold, about 2.5 fold, about 3 fold, about 3.5 fold, about 4 fold, about 4.5 fold, about 5 fold, about 5.5 fold, about 6 fold, about 6.5 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 12
30 fold, about 15 fold, about 20 fold, about 25 fold, about 30 fold, about 50 fold, about 100 fold, at least about 1.2 fold, at least about 1.5 fold, at least about 2 fold, at least about 2.5 fold, at least

about 3 fold, at least about 3.5 fold, at least about 4 fold, at least about 4.5 fold, at least about 5 fold, at least about 5.5 fold, at least about 6 fold, at least about 6.5 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, of the sum of the effects that result from using the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) alone or the
5 second treatment alone.

In one embodiment, advantageously, such synergy provides greater efficacy at the same doses, and/or lower side effects.

For sequential administration, either an inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) is administered first and then a second treatment, or the second treatment is
10 administered first and then an inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor). In embodiments where the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) and the second treatment are administered separately, administration of a first agent can precede administration of a second agent by seconds, minutes, hours, days, or weeks. The time difference in non-simultaneous administrations may be greater than 1 minute, and can be, for example,
15 precisely, at least, up to, or less than 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, 2 hours, 3 hours, 6 hours, 9 hours, 12 hours, 24 hours, 36 hours, or 48 hours, or more than 48 hours. The two or more agents can be administered within minutes of each other or within about 0.5, about 1, about 2, about 3, about 4, about 6, about 9, about 12, about 15, about 18, about 24, or about 36 hours of each other or within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14
20 days of each other or within about 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks of each other. In some cases, longer intervals are possible.

The present disclosure may provide for a pharmaceutical composition comprising a first amount of an inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) and a second amount of a second agent. The combination of the first amount of an inhibitor of a FOXO3A antagonist
25 (e.g., a ZNF274 inhibitor) and the second amount of the second agent may produce a synergistic effect on a cancer cell compared to the effect of the first amount of the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) alone or the effect of the second amount of the second agent alone.

The amount of the inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) or the
30 amount of the second agent that may be used in the combination therapy may be a

therapeutically effective amount, a sub-therapeutically effective amount or a synergistically effective amount.

The inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor), and/or the second agent may be present in the pharmaceutical composition in an amount ranging from about 0.005% (w/w) to about 100% (w/w), from about 0.01% (w/w) to about 90% (w/w), from about 0.1% (w/w) to about 80% (w/w), from about 1% (w/w) to about 70% (w/w), from about 10% (w/w) to about 60% (w/w), from about 0.01% (w/w) to about 15% (w/w), or from about 0.1% (w/w) to about 20% (w/w).

The inhibitor of a FOXO3A antagonist (e.g., a ZNF274 inhibitor) and the second agent may be present in two separate pharmaceutical compositions to be used in a combination therapy.

The effective amount of the inhibitor of a FOXO3A antagonist (e.g., the ZNF274 inhibitor) or the second agent for the combination therapy may be less than, equal to, or greater than when the agent is used alone.

Cytotoxic Agents

The present method for treating cancer may comprise the step of administering to a subject having cancer an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a cytotoxic agent. The combination of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and the cytotoxic agent may produce a synergistic effect or additive effect on the cancer compared to the effect of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone or the effect of the cytotoxic agent alone.

The cytotoxic agent may be any chemotherapeutic agents including, but not limited to, alkylating agents, anti-metabolites, anti-microtubule agents, topoisomerase inhibitors, cytotoxic antibiotics, endoplasmic reticulum stress inducing agents, platinum compounds, vincalkaloids, taxanes, epothilones, enzyme inhibitors, receptor antagonists, tyrosine kinase inhibitors, boron radiosensitizers (i.e. velcade), and chemotherapeutic combination therapies.

Non-limiting examples of DNA alkylating agents are nitrogen mustards, such as Cyclophosphamide (Ifosfamide, Trofosfamide), Chlorambucil (Melphalan, Prednimustine), Bendamustine, Uramustine and Estramustine; nitrosoureas, such as Carmustine (BCNU), Lomustine (Semustine), Fotemustine, Nimustine, Ranimustine and Streptozocin; alkyl

sulfonates, such as Busulfan (Mannosulfan, Treosulfan); Aziridines, such as Carboquone, Triaziquone, Triethylenemelamine; Hydrazines (Procarbazine); Triazines such as Dacarbazine and Temozolomide (TMZ); Altretamine and Mitobronitol.

Non-limiting examples of Topoisomerase I inhibitors include Campothecin derivatives
 5 including SN-38, APC, NPC, camptothecin, topotecan, exatecan mesylate, 9-nitrocamptothecin, 9-aminocamptothecin, lurtotecan, rubitecan, silatecan, gimatecan, diflomotecan, extatecan, BN-80927, DX-8951f, and MAG-CPT as described in Pommier Y. (2006) *Nat. Rev. Cancer* 6(10):789-802 and U.S. Patent Publication No. 200510250854; Protoberberine alkaloids and derivatives thereof including berberrubine and coralyne as described in Li et al. (2000)
 10 *Biochemistry* 39(24):7107-7116 and Gatto et al. (1996) *Cancer Res.* 15(12):2795-2800; Phenanthroline derivatives including Benzo[i]phenanthridine, Nitidine, and fagaronine as described in Makhey et al. (2003) *Bioorg. Med. Chem.* 11 (8): 1809-1820; Terbenzimidazole and derivatives thereof as described in Xu (1998) *Biochemistry* 37(10):3558-3566; and Anthracycline derivatives including Doxorubicin, Daunorubicin, and Mitoxantrone as described
 15 in Foglesong et al. (1992) *Cancer Chemother. Pharmacol.* 30(2):123-125, Crow et al. (1994) *J. Med. Chem.* 37(19):3191-3194, and Crespi et al. (1986) *Biochem. Biophys. Res. Commun.* 136(2):521-8. Topoisomerase II inhibitors include, but are not limited to Etoposide and Teniposide. Dual topoisomerase I and II inhibitors include, but are not limited to, Saintopin and other Naphthecenediones, DACA and other Acridine-4-Carboxamides, Intoplicine and other
 20 Benzopyridoindoles, TAS-I03 and other 7H-indeno[2,1-c]Quinoline-7-ones, Pyrazoloacridine, XR 11576 and other Benzophenazines, XR 5944 and other Dimeric compounds, 7-oxo-7H-dibenz[f,ij]Isoquinolines and 7-oxo-7H-benzo[e]pyrimidines, and Anthracenyl-amino Acid Conjugates as described in Denny and Baguley (2003) *Curr. Top. Med. Chem.* 3(3):339-353. Some agents inhibit Topoisomerase II and have DNA intercalation activity such as, but not
 25 limited to, Anthracyclines (Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, Zorubicin) and Anthracenediones (Mitoxantrone and Pixantrone).

Examples of endoplasmic reticulum stress inducing agents include, but are not limited to, dimethyl-celecoxib (DMC), nelfinavir, celecoxib, and boron radiosensitizers (i.e. velcade
 30 (Bortezomib)).

Platinum based compounds are a subclass of DNA alkylating agents. Non-limiting examples of such agents include Cisplatin, Nedaplatin, Oxaliplatin, Triplatin tetranitrate, Satraplatin, Aroplatin, Lobaplatin, and JM-216. (See McKeage et al. (1997) J. Clin. Oncol. 201 :1232-1237 and in general, CHEMOTHERAPY FOR GYNECOLOGICAL NEOPLASM, CURRENT THERAPY AND NOVEL APPROACHES, in the Series Basic and Clinical Oncology, Angioli et al. Eds., 2004).

"FOLFOX" is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. It includes 5-FU, oxaliplatin and leucovorin.

"FOLFOX/BV" is an abbreviation for a type of combination therapy that is used to treat colorectal cancer. This therapy includes 5-FU, oxaliplatin, leucovorin and Bevacizumab. Furthermore, "XELOX/BV" is another combination therapy used to treat colorectal cancer, which includes the prodrug to 5-FU, known as Capecitabine (Xeloda) in combination with oxaliplatin and bevacizumab.

Non-limiting examples of antimetabolite agents include folic acid based, i.e. dihydrofolate reductase inhibitors, such as Aminopterin, Methotrexate and Pemetrexed; thymidylate synthase inhibitors, such as Raltitrexed, Pemetrexed; Purine based, i.e. an adenosine deaminase inhibitor, such as Pentostatin, a thiopurine, such as Thioguanine and Mercaptopurine, a halogenated/ribonucleotide reductase inhibitor, such as Cladribine, Clofarabine, Fludarabine, or a guanine/guanosine: thiopurine, such as Thioguanine; or Pyrimidine based, i.e. cytosine/cytidine: hypomethylating agent, such as Azacitidine and Decitabine, a DNA polymerase inhibitor, such as Cytarabine, a ribonucleotide reductase inhibitor, such as Gemcitabine, or a thymine/thymidine: thymidylate synthase inhibitor, such as a Fluorouracil (5-FU). Equivalents to 5-FU include prodrugs, analogs and derivative thereof such as 5'-deoxy-5-fluorouridine (doxifluoridine), 1-tetrahydrofuran-5-yl-5-fluorouracil (ftorafur), Capecitabine (Xeloda), S-I (MBMS-247616, consisting of tegafur and two modulators, a 5-chloro-2,4-dihydroxypyridine and potassium oxonate), raltitrexed (tomudex), nolatrexed (Thymitaq, AG337), LY231514 and ZD9331, as described for example in Papamichael (1999) The Oncologist 4:478-487.

Examples of vincalkaloids, include, but are not limited to Vinblastine, Vincristine, Vinflunine, Vindesine and Vinorelbine.

Examples of taxanes include, but are not limited to docetaxel, Larotaxel, Ortataxel, Paclitaxel and Tesetaxel. An example of an epothilone is iabepilone.

Examples of enzyme inhibitors include, but are not limited to farnesyltransferase inhibitors (e.g., Tipifarnib); CDK inhibitors (e.g., Alvocidib, Seliciclib); proteasome inhibitors (e.g., Bortezomib); phosphodiesterase inhibitors (e.g., Anagrelide; rolipram); IMP dehydrogenase inhibitors (e.g., Tiazofurine); and lipoxigenase inhibitors (e.g., Masoprocil).

Chemotherapeutic agents may also include amsacrine, Trabectedin, retinoids (Alitretinoin, Tretinoin), Arsenic trioxide, asparagine depleter Asparaginase/ Pegaspargase), Celecoxib, Demecolcine, Elesclomol, Elsamitrucin, Etoglucid, Lonidamine, Lucanthone, Mitoguazone, Mitotane, Oblimersen, Temsirolimus, and Vorinostat.

Conditions to be treated

The present disclosure provides for methods for inhibiting the growth, or increasing cell death, of a tumor cell. The method may comprise contacting the tumor cell with an effective amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274). The contacting may be *in vitro* or *in vivo*.

The tumor cell may be a cell of any cancer described herein.

Cancers treated using methods and compositions described herein are characterized by abnormal cell proliferation including, but not limited to, pre-neoplastic hyperproliferation, cancer in-situ, neoplasms and metastasis.

Cancers that can be treated by the present compositions and methods include, but are not limited to, melanoma, breast cancer, colorectal cancer, pancreatic cancer, cervical cancer, thyroid cancer, bladder cancer, non-small cell lung cancer, liver cancer, prostate cancer, muscle cancer, hematological malignancies, endometrial cancer, lymphomas, sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, synovioma, mesothelioma, lymphangioendotheliosarcoma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, ovarian cancer, gastric cancer, esophageal cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma,

embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, non-small cell lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, ear, nose and throat cancer, hematopoietic cancer, biliary tract cancer; bladder cancer; bone cancer; choriocarcinoma; connective tissue cancer; cancer of the digestive system; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; intra-epithelial neoplasm; kidney cancer; larynx cancer; leukemia including acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia; lymphoma including Hodgkin's and Non-Hodgkin's lymphoma; myeloma; fibroma, oral cavity cancer (e.g., lip, tongue, mouth, and pharynx); prostate cancer; retinoblastoma; rhabdomyosarcoma; rectal cancer; renal cancer; cancer of the respiratory system; skin cancer; stomach cancer; testicular cancer; uterine cancer; cancer of the urinary system, as well as other carcinomas and sarcomas.

The present compositions may be administered alone, or in combination with radiation, surgery or chemotherapeutic agents. The present compositions may be administered before, during or after the administration of radiation, surgery or chemotherapeutic agents.

Pharmaceutical Compositions

The present disclosure provides for a pharmaceutical composition comprising (consisting essentially of, or consisting of) the inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274).

The present disclosure provides for a pharmaceutical composition comprising a first amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and a second amount of a cytotoxic agent. The combination of the first amount of an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and the second amount of a cytotoxic agent produces a synergistic effect on cancer (or in treating other disorders) compared to the effect of the first

amount of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) alone or the effect of the second amount of the cytotoxic agent alone.

The amount of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) or the amount of the cytotoxic agent that may be used in the pharmaceutical composition may be a therapeutically effective amount, a sub-therapeutically effective amount or a synergistically effective amount.

An inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) and/or a cytotoxic agent may be present in the pharmaceutical composition in an amount ranging from about 0.005% (w/w) to about 100% (w/w), from about 0.01% (w/w) to about 90% (w/w), from about 0.1% (w/w) to about 80% (w/w), from about 1% (w/w) to about 70% (w/w), from about 10% (w/w) to about 60% (w/w), from about 0.01% (w/w) to about 15% (w/w), or from about 0.1% (w/w) to about 20% (w/w).

The present agents or pharmaceutical compositions may be administered by any route, including, without limitation, oral, transdermal, ocular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous, implant, sublingual, subcutaneous, intramuscular, intravenous, rectal, mucosal, ophthalmic, intrathecal, intra-articular, intra-arterial, sub-arachinoid, bronchial and lymphatic administration. The present composition may be administered parenterally or systemically.

The pharmaceutical compositions of the present invention can be, e.g., in a solid, semi-solid, or liquid formulation. Intranasal formulation can be delivered as a spray or in a drop; inhalation formulation can be delivered using a nebulizer or similar device; topical formulation may be in the form of gel, ointment, paste, lotion, cream, poultice, cataplasm, plaster, dermal patch aerosol, etc.; transdermal formulation may be administered via a transdermal patch or iontophoresis. Compositions can also take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, emulsions, suspensions, elixirs, aerosols, chewing bars or any other appropriate compositions.

The composition may be administered locally via implantation of a membrane, sponge, or another appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed release bolus, or continuous administration.

To prepare such pharmaceutical compositions, one or more of compound of the present invention may be mixed with a pharmaceutical acceptable excipient, e.g., a carrier, adjuvant and/or diluent, according to conventional pharmaceutical compounding techniques.

Pharmaceutically acceptable carriers that can be used in the present compositions
5 encompass any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions can additionally contain solid pharmaceutical excipients such as starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk
10 and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols. For examples of carriers, stabilizers, preservatives and adjuvants, see Remington's Pharmaceutical Sciences,
15 edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990). Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutically acceptable excipient may be selected from the group consisting of fillers, e.g. sugars and/or sugar alcohols, e.g. lactose, sorbitol, mannitol, maltodextrin, etc.; surfactants, e.g. sodium lauryl sulfate, Brij 96 or Tween 80; disintegrants, e.g. sodium starch
20 glycolate, maize starch or derivatives thereof; binder, e.g. povidone, crosspovidone, polyvinylalcohols, hydroxypropylmethylcellulose; lubricants, e.g. stearic acid or its salts; flowability enhancers, e.g. silicon dioxide; sweeteners, e.g. aspartame; and/or colorants. Pharmaceutically acceptable carriers include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and
25 the like.

The pharmaceutical composition may contain excipients for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable excipients include, but are not limited to, amino acids (such as glycine, glutamine, asparagine,
30 arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen sulfite); buffers (such as borate, bicarbonate, Tris HCl, citrates, phosphates, other

organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta cyclodextrin or hydroxypropyl beta cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (in one aspect, sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company, 1990).

Oral dosage forms may be tablets, capsules, bars, sachets, granules, syrups and aqueous or oily suspensions. Tablets may be formed from a mixture of the active compounds with fillers, for example calcium phosphate; disintegrating agents, for example maize starch, lubricating agents, for example magnesium stearate; binders, for example microcrystalline cellulose or polyvinylpyrrolidone and other optional ingredients known in the art to permit tableting the mixture by known methods. Similarly, capsules, for example hard or soft gelatin capsules, containing the active compound, may be prepared by known methods. The contents of the capsule may be formulated using known methods so as to give sustained release of the active compounds. Other dosage forms for oral administration include, for example, aqueous suspensions containing the active compounds in an aqueous medium in the presence of a non-toxic suspending agent such as sodium carboxymethylcellulose, and oily suspensions containing the active compounds in a suitable vegetable oil, for example arachis oil. The active compounds may be formulated into granules with or without additional excipients. The granules may be ingested directly by the patient or they may be added to a suitable liquid carrier (e.g. water)

before ingestion. The granules may contain disintegrants, e.g. an effervescent pair formed from an acid and a carbonate or bicarbonate salt to facilitate dispersion in the liquid medium. U.S. Patent No. 8,263,662.

Intravenous forms include, but are not limited to, bolus and drip injections. Examples of
5 intravenous dosage forms include, but are not limited to, Water for Injection USP; aqueous
vehicles including, but not limited to, Sodium Chloride Injection, Ringer's Injection, Dextrose
Injection, Dextrose and Sodium Chloride Injection, and Lactated Ringer's Injection; water-
miscible vehicles including, but not limited to, ethyl alcohol, polyethylene glycol and
polypropylene glycol; and non-aqueous vehicles including, but not limited to, corn oil,
10 cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate and benzyl benzoate.

Additional compositions include formulations in sustained or controlled delivery, such as
using liposome or micelle carriers, bioerodible microparticles or porous beads and depot
injections.

The present compound(s) or composition may be administered as a single dose, or as two
15 or more doses (which may or may not contain the same amount of the desired molecule) over
time, or as a continuous infusion via implantation device or catheter. The pharmaceutical
composition can be prepared in single unit dosage forms.

Appropriate frequency of administration can be determined by one of skill in the art and
can be administered once or several times per day (e.g., twice, three, four or five times daily).
20 The compositions of the invention may also be administered once each day or once every other
day. The compositions may also be given twice weekly, weekly, monthly, or semi-annually. In
the case of acute administration, treatment is typically carried out for periods of hours or days,
while chronic treatment can be carried out for weeks, months, or even years. U.S. Patent No.
8,501,686.

25 Administration of the compositions of the invention can be carried out using any of
several standard methods including, but not limited to, continuous infusion, bolus injection,
intermittent infusion, inhalation, or combinations of these methods. For example, one mode of
administration that can be used involves continuous intravenous infusion. The infusion of the
compositions of the invention can, if desired, be preceded by a bolus injection.

As used herein, the term "therapeutically effective amount" is an amount sufficient to treat a specified disorder or disease or alternatively to obtain a pharmacological response treating a disorder or disease.

5 Methods of determining the most effective means and dosage of administration can vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. The specific dose level for any particular subject depends upon a variety of factors including the activity of the specific peptide, the age, body weight, general health, sex, diet, time of administration, route of administration, 10 and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

For example, the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) or the cytotoxic agent may be administered at about 0.0001 mg/kg to about 500 mg/kg, about 0.01 mg/kg to about 200 mg/kg, about 0.01 mg/kg to about 0.1 mg/kg, about 0.1 mg/kg to about 100 mg/kg, about 10 mg/kg to about 200 mg/kg, about 10 mg/kg to about 20 mg/kg, about 5 mg/kg to about 15 mg/kg, about 0.0001 mg/kg to about 0.001 mg/kg, about 0.001 mg/kg to about 0.01 mg/kg, about 0.01 mg/kg to about 0.1 mg/kg, about 0.1 mg/kg to about 0.5 mg/kg, about 0.5 mg/kg to about 1 mg/kg, about 1 mg/kg to about 2.5 mg/kg, about 2.5 mg/kg to about 10 mg/kg, about 10 mg/kg to about 50 mg/kg, about 50 mg/kg to about 100 mg/kg, about 100 mg/kg to about 250 mg/kg, about 0.1 µg/kg to about 800 µg/kg, about 0.5 µg/kg to about 500 µg/kg, about 1 µg/kg to about 20 µg/kg, about 1 µg/kg to about 10 µg/kg, about 10 µg/kg to about 20 µg/kg, about 20 µg/kg to about 40 µg/kg, about 40 µg/kg to about 60 µg/kg, about 60 µg/kg to about 100 µg/kg, about 100 µg/kg to about 200 µg/kg, about 200 µg/kg to about 300 µg/kg, or about 400 µg/kg to about 600 µg/kg. In some embodiments, the dose is within the range of about 250 mg/kg to about 500 mg/kg, about 0.5 mg/kg to about 50 mg/kg, or any other suitable amounts. 25

The effective amount of the inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) or the cytotoxic agent for the combination therapy may be less than, equal to, or greater than when the agent is used alone.

The amount or dose of the inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274) or a cytotoxic agent may range from about 0.01 mg to about 10 g, from about 0.1 mg to about 9 g, from about 1 mg to about 8 g, from about 1 mg to about 7 g, from about 5 mg to about 30

6 g, from about 10 mg to about 5 g, from about 20 mg to about 1 g, from about 50 mg to about 800 mg, from about 100 mg to about 500 mg, from about 600 mg to about 800 mg, from about 800 mg to about 1 g, from about 0.01mg to about 10 g, from about 0.05 μ g to about 1.5 mg, from about 10 μ g to about 1 mg protein, from about 0.1mg to about 10 mg, from about 2 mg to about 5 mg, from about 1 mg to about 20 mg, from about 30 μ g to about 500 μ g, from about 40 pg to about 300 pg, from about 0.1 μ g to about 200 mg, from about 0.1 μ g to about 5 μ g, from about 5 μ g to about 10 μ g, from about 10 μ g to about 25 μ g, from about 25 μ g to about 50 μ g, from about 50 μ g to about 100 μ g, from about 100 μ g to about 500 μ g, from about 500 μ g to about 1 mg, from about 1 mg to about 2 mg.

Different dosage regimens may be used. In some embodiments, a daily dosage, such as any of the exemplary dosages described above, is administered once, twice, three times, or four times a day for at least three, four, five, six, seven, eight, nine, or ten days. Depending on the stage and severity of the cancer, a shorter treatment time (e.g., up to five days) may be employed along with a high dosage, or a longer treatment time (e.g., ten or more days, or weeks, or a month, or longer) may be employed along with a low dosage. In some embodiments, a once- or twice-daily dosage is administered every other day.

The composition may be in the form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a solid form (e.g., a dry powder) to be reconstituted with water or another suitable vehicle before use. The compositions may be in the form of an oil emulsion, water-in-oil emulsion, water-in-oil-in-water emulsion, site-specific emulsion, long-residence emulsion, sticky emulsion, microemulsion, nanoemulsion, liposome, microparticle, microsphere, nanosphere, nanoparticle and various natural or synthetic polymers, such as nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and Hytrel® copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures, that allow for sustained release of the vaccine.

Methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in detail. Bai, J. Neuroimmunol. 80: 65-75, 1997. Warren, J. Neurol. Sci. 152: 31-38, 1997. Tonegawa, J. Exp. Med. 186: 507-515, 1997.

Formulations for parenteral administration may, for example, contain excipients, sterile water, saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or

hydrogenated naphthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the present agent. Nanoparticulate formulations (e.g., biodegradable nanoparticles, solid lipid nanoparticles, liposomes) may be used to control the biodistribution of the present agent. Other potentially useful delivery systems include ethylene-vinyl acetate copolymer particles, osmotic pumps, intrathecal pumps, implantable infusion systems, and liposomes. The concentration of the agent in the formulation varies depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

10 **Kits**

The present disclosure also provides for a kit for use in the treatment or prevention of cancer or other conditions. Kits according to the invention include package(s) (e.g., vessels) comprising agents or compositions of the invention. The kit may contain an inhibitor of a FOXO3A antagonist (e.g., an inhibitor of ZNF274). The kit may further contain a cytotoxic agent. The inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and/or the cytotoxic agent may be present in the pharmaceutical compositions as described herein. The inhibitor of a FOXO3A antagonist (e.g., the inhibitor of ZNF274) and/or the cytotoxic agent may be present in unit dosage forms.

Examples of pharmaceutical packaging materials include, but are not limited to, bottles, tubes, inhalers, pumps, bags, vials, containers, syringes, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

Kits can contain instructions for administering agents or compositions of the invention to a patient. Kits also can comprise instructions for uses of the present agents or compositions. Kits also can contain labeling or product inserts for the present agent/composition. The kits also can include buffers for preparing solutions for conducting the methods.

Subjects, which may be treated according to the present disclosure include all animals which may benefit from administration of the agents of the present invention. Such subjects include mammals, preferably humans, but can also be an animal such as dogs and cats, farm animals such as cows, pigs, sheep, horses, goats and the like, and laboratory animals (e.g., rats, mice, guinea pigs, and the like).

The term "pharmaceutically acceptable carrier", as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent. The diluent or carrier ingredients should not be such as to diminish the therapeutic effects of the active compound(s).

The term "composition" as used herein means a product which results from the mixing or combining of more than one element or ingredient.

"Treating" or "treatment" of a state, disorder or condition includes:

(1) preventing or delaying the appearance of clinical symptoms of the state, disorder, or condition developing in a person who may be afflicted with or predisposed to the state, disorder or condition but does not yet experience or display clinical symptoms of the state, disorder or condition; or

(2) inhibiting the state, disorder or condition, i.e., arresting, reducing or delaying the development of the disease or a relapse thereof (in case of maintenance treatment) or at least one clinical symptom, sign, or test, thereof; or

(3) relieving the disease, i.e., causing regression of the state, disorder or condition or at least one of its clinical or sub-clinical symptoms or signs.

The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician.

"Treat" or "treating" may refer to administering a therapeutic agent, such as a composition containing any of the tissue-specific, e.g., neuronal or ocular targeted viral vectors, RNAi, shRNA or other ZNF274 inhibitors, combinations thereof, or similar compositions described herein, internally or externally to a subject or patient having one or more disease symptoms, or being suspected of having a disease or being at elevated at risk of acquiring a disease, for which the agent has therapeutic activity. Gene editing technology such as CRISPR/Cas9 methods may also be utilized to carry out tissue-specific reduction of ZNF274 or a combination thereof. Typically, the agent is administered in an amount effective to alleviate one or more disease symptoms in the treated subject or population, whether by inducing the regression of or inhibiting the progression of such symptom(s) by any clinically measurable degree. The amount of a therapeutic agent that is effective to alleviate any particular disease symptom (also referred to as the "therapeutically effective amount") may vary according to factors such as the disease state, age, and weight of the

patient, and the ability of the drug to elicit a desired response in the subject. Whether a disease symptom has been alleviated can be assessed by any clinical measurement typically used by physicians or other skilled healthcare providers to assess the severity or progression status of that symptom. While an embodiment of the present invention (*e.g.*, a treatment method or article of manufacture) may not be effective in alleviating the target disease symptom(s) in every subject, it should alleviate the target disease symptom(s) in a statistically significant number of subjects as determined by any statistical test known in the art such as the Student's t-test, the χ^2 -test, the U-test according to Mann and Whitney, the Kruskal-Wallis test (H-test), Jonckheere-Terpstra-test and the Wilcoxon-test.

"Treatment," as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment" as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, encompasses transfection of any of the tissue-targeted viral vectors, delivery of RNAi, shRNA or other ZNF274 inhibitors, combinations thereof, or similar compositions, including gene editing technology such as CRISPR/cas9 methods, which may be utilized to carry out tissue specific reduction of ZNF274, combinations thereof or related methods described herein as applied to a human or animal subject, a cell, tissue, physiological compartment, or physiological fluid.

A "therapeutically effective amount" means the amount of a compound that, when administered to an animal for treating a state, disorder or condition, is sufficient to effect such treatment. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the age, weight, physical condition and responsiveness of the animal to be treated.

"Patient" or "subject" refers to mammals and includes human and veterinary subjects.

Acceptable excipients, diluents, and carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington: The Science and Practice of Pharmacy. Lippincott Williams & Wilkins (A. R. Gennaro edit. 2005). The choice of pharmaceutical excipient, diluent, and carrier can be selected with regard to the intended route of administration and standard pharmaceutical practice.

As used herein, the phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are "generally regarded as safe", *e.g.*, that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and

the like, when administered to a human. Preferably, as used herein, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopeias for use in animals, and more particularly in humans.

5 The dosage of the therapeutic formulation will vary widely, depending upon the nature of the disease, the patient's medical history, the frequency of administration, the manner of administration, the clearance of the agent from the host, and the like. The initial dose may be larger, followed by smaller maintenance doses. The dose may be administered as infrequently as weekly or biweekly, or fractionated into smaller doses and administered daily, semi-weekly, etc., to
10 maintain an effective dosage level. In some cases, oral administration will require a higher dose than if administered intravenously. In some cases, topical administration will include application several times a day, as needed, for a number of days or weeks in order to provide an effective topical dose.

 The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the
15 compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, olive oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Alternatively, the carrier can be a solid dosage form carrier,
20 including but not limited to one or more of a binder (for compressed pills), a glidant, an encapsulating agent, a flavorant, and a colorant. Suitable pharmaceutical carriers are described in “Remington's Pharmaceutical Sciences” by E. W. Martin.

 As used herein, the term “adjuvant” refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the
25 antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., Immunology, Second Ed., 1984, Benjamin/Cummings: Menlo Park, Calif., p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum
30 hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, and potentially useful human

adjuvants such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine, and BCG (bacille Calmette-Guerin). Preferably, the adjuvant is pharmaceutically acceptable.

5

The following are examples of the present invention and are not to be construed as limiting.

Example 1

10 We identified transcription factor ZNF274 that plays a key role in activating the latter processes, and showed that reducing the activity of ZNF274 leads to activation of FoxO3A. More specifically, we have shown that targeting the gene that encodes ZNF274 using RNA interference in cell culture leads to an increase in the nuclear concentration of FoxO3A as indicated by microscopy, and to activation of the known target genes of FoxO3A, as indicated by
15 RNA-seq assays. Since we find that ZNF274 is an activator of the growth processes that spin out of control in cancer, targeting ZNF274 can be a natural strategy for getting cancer under control, or pushing the cell towards a regulatory state associated with extended life span.

Our integrative analysis of functional genomics data (mRNA expression and ChIP-seq) identified the human zinc finger protein ZNF274, as well as several other human transcription
20 factors, as a FOXO3A antagonist. Our data in human cells show that ZNF274 competes with FOXO3A for nuclear localization.

Summary

In *C. elegans*, the nuclear localization of DAF-16/FOXO is specifically affected by loss
25 of *pqm-1*, which promotes transcription of DAF-16's transcriptional targets (Tepper et al., Cell, 2013; PMID 23911329). In human cells, we observed that knockdown of ZNF274 induces a subset of FOXO3a transcriptional targets, similar to PQM-1 in *C. elegans*. To determine if ZNF274 knockdown leads to nuclear translocation of FOXO3a, siRNA-treated HepG2 cells were examined for FOXO3a localization. To reduce the level of ZNF274, a pool of three stealth
30 siRNAs targeting ZNF274, or a negative control containing a pool of three non-targeting siRNAs, were reverse transfected into HepG2 cells. As a positive control for nuclear

translocation, a PI3K inhibitor (LY2940020) was separately added to drive FOXO3a into the nucleus, and the ratios of nuclear/cytoplasmic fluorescence intensity were determined and compared for each sample. As expected, the PI3K inhibitor-treated cells exhibited a higher ratio of nuclear : cytoplasmic FOXO3a compared to DMSO-treated cells ($p=0.0002$, t-test; see Figure 1A). Similarly, cells treated with ZNF274 siRNAs had a higher nuclear : cytoplasmic FOXO3a ratio than the controls ($p<0.0001$, t-test; see Figure 1B). These data suggest that the relationship between ZNF274 and FOXO3a in humans is similar to that of PQM-1 and DAF-16 in *C. elegans*.

10 SiRNA treatment

To knock down ZNF274, HepG2 cells were reverse transfected at 3×10^5 cells/mL with 40 nM of a pool of three stealth ZNF274 siRNAs (Life Technologies Stealth RNAi ZNF274 siRNA: #HSS116638, #HSS116639, #HSS116640), or a negative control containing a pool of three non-targeting siRNAs (Life Technologies Stealth RNAi™ Negative Control Kit siRNA: #12935100) using Lipofectamine RNAiMAX Transfection Reagent per manufacturer instructions. Cells were plated into a 6-well dish containing Corning BioCoat Poly-D-Lysine 12mm #1 glass round coverslips (#354086) with antibiotic-free serum. On the following day, the media was replaced. 48 hrs later, the PI3k inhibitor (LY294002) was added at a final concentration of 50 uM. DMSO was added as a negative control. 24 hrs after drug treatment, cover slips were washed in ice-cold PBS and cells fixed using 4% paraformaldehyde for 10 minutes at room temperature followed by permeabilization with 1X PBS/0.3% Triton X-100. The cells were then incubated with Anti-FKHRL1 (FOXO3a) rabbit anti-human primary antibody, Sigma #F2178-200µL (1:200), overnight at 4°C. On the following day, they were washed with PBS and incubated with Alexa Fluor 488 goat anti-rabbit secondary antibody, Abcam #ab150077 (1:1000) for 2 hours at room temperature. The coverslips were mounted using ProLong Diamond Antifade with DAPI (#P36966). The cells were imaged using fluorescence microscopy on a Nikon Eclipse Ti inverted microscope. Threshold analysis and intensity quantification were done using NIS-Elements software. Thresholds for each channel, FITC or DAPI, were determined to calculate fluorescence intensities by dividing the sum intensity by area. The DAPI channel threshold was used to represent the nuclear area. The FITC channel (whole cell) minus the nuclear region was used as representative for the cytoplasmic area. The

ratio of nuclear FITC intensity over cytoplasm FITC intensity was then determined for each image.

5 The scope of the present invention is not limited by what has been specifically shown and described hereinabove. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions and dimensions. Numerous references, including patents and various publications, are cited and discussed in the description of this invention. The citation and discussion of such references is provided merely to clarify the
10 description of the present invention and is not an admission that any reference is prior art to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entirety. Variations, modifications and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention. While certain embodiments of the
15 present invention have been shown and described, it will be obvious to those skilled in the art that changes and modifications may be made without departing from the spirit and scope of the invention. The matter set forth in the foregoing description and accompanying drawings is offered by way of illustration only and not as a limitation.

What is claimed is:

1. A method for inhibiting growth, or increasing cell death, of a tumor cell, the method comprising contacting the tumor cell with an effective amount of an inhibitor of ZNF274.
2. The method of claim 1, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
3. The method of claim 2, wherein the polynucleotide is a small interfering RNA (siRNA).
4. The method of claim 1, wherein the inhibitor comprises a CRISPR/Cas9 system.
5. The method of claim 1, wherein the tumor cell is a breast cancer cell, a liver cancer cell, a colon cancer cell, a prostate cancer cell, a bladder cancer cell, or a nasopharyngeal cancer cell.
6. The method of claim 1, wherein the contacting is *in vitro* or *in vivo*.
7. The method of claim 1, further comprising contacting the tumor cell with a cytotoxic agent.
8. The method of claim 7, wherein the cytotoxic agent is an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, or an endoplasmic reticulum stress inducing agent.
9. A method of modulating subcellular localization of, and/or regulating activity/expression of, FOXO3A in a cell, the method comprising contacting the cell with an inhibitor of ZNF274.
10. The method of claim 9, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
11. The method of claim 10, wherein the polynucleotide is a small interfering RNA (siRNA).
12. The method of claim 9, wherein the inhibitor comprises a CRISPR/Cas9 system.

13. The method of claim 9, wherein the cell is a breast cancer cell, a liver cancer cell, a colon cancer cell, a prostate cancer cell, a bladder cancer cell, or a nasopharyngeal cancer cell.
14. The method of claim 9, wherein the contacting is *in vitro* or *in vivo*.
15. A method of treating cancer in a subject, comprising administering to the subject an effective amount of an inhibitor of ZNF274.
16. The method of claim 15, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
17. The method of claim 16, wherein the polynucleotide is a small interfering RNA (siRNA).
18. The method of claim 15, wherein the inhibitor comprises a CRISPR/Cas9 system.
19. The method of claim 15, wherein the cancer is breast cancer, liver cancer, colon cancer, prostate cancer, bladder cancer, or nasopharyngeal cancer.
20. The method of claim 15, further comprising administering to the subject a cytotoxic agent.
21. The method of claim 20, wherein the cytotoxic agent is an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, or an endoplasmic reticulum stress inducing agent.
22. The method of claim 20, wherein the administration of the inhibitor of ZNF274 and the cytotoxic agent results in a synergistic increase in apoptosis of cancer cells.
23. The method of claim 20, wherein the administration of the inhibitor of ZNF274 and the cytotoxic agent results in a synergistic reduction in tumor volume.
24. The method of claim 20, wherein the inhibitor of ZNF274 and the cytotoxic agent are administered simultaneously, sequentially or separately.

25. The method of claim 15, further comprising treating the subject with radiation.
26. The method of claim 15, further comprising administering to the subject a chemotherapeutic agent.
27. The method of claim 26, wherein the chemotherapeutic agent is selected from the group consisting of a DNA alkylating agent, a topoisomerase inhibitor, an endoplasmic reticulum stress inducing agent, a platinum compound, an antimetabolite, an enzyme inhibitor, a receptor antagonist, and a therapeutic antibody.
28. The method of any of claims 4, 12 and 18, wherein the inhibitor comprises Cas9, dCas9, and/or a dCas9 fusion protein.
29. A method for inhibiting growth, or increasing cell death, of a tumor cell, the method comprising contacting the tumor cell with an effective amount of an inhibitor of a FOXO3A antagonist.
30. The method of claim 29, wherein the FOXO3A antagonist is ZNF274, $ERR\alpha$, NR3C1, GATAD1, ZKSCAN1, PGC-1 α , HDAC8, or combinations thereof.
31. The method of claim 29, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
32. The method of claim 31, wherein the polynucleotide is a small interfering RNA (siRNA).
33. The method of claim 29, wherein the inhibitor comprises a CRISPR/Cas9 system.
34. The method of claim 33, wherein the inhibitor comprises Cas9, dCas9, and/or a dCas9 fusion protein.

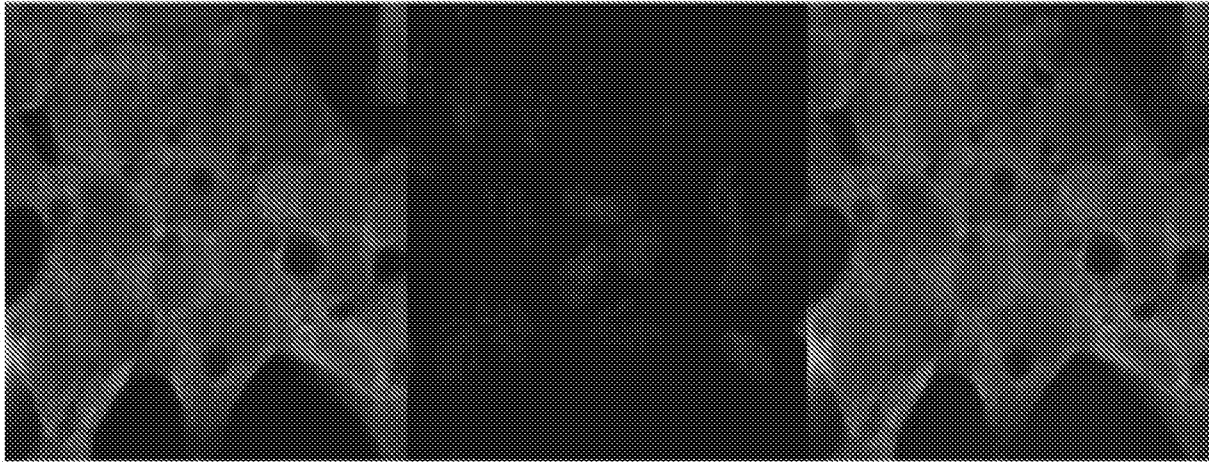
35. The method of claim 29, wherein the tumor cell is a breast cancer cell, a liver cancer cell, a colon cancer cell, a prostate cancer cell, a bladder cancer cell, or a nasopharyngeal cancer cell.
36. The method of claim 29, wherein the contacting is *in vitro* or *in vivo*.
37. The method of claim 29, further comprising contacting the tumor cell with a cytotoxic agent.
38. The method of claim 37, wherein the cytotoxic agent is an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, or an endoplasmic reticulum stress inducing agent.
39. A method of modulating subcellular localization of, and/or regulating activity/expression of, FOXO3A in a cell, the method comprising contacting the cell with an inhibitor of a FOXO3A antagonist.
40. The method of claim 39, wherein the FOXO3A antagonist is ZNF274, $ERR\alpha$, NR3C1, GATAD1, ZKSCAN1, PGC-1 α , HDAC8, or combinations thereof.
41. The method of claim 39, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
42. The method of claim 41, wherein the polynucleotide is a small interfering RNA (siRNA).
43. The method of claim 39, wherein the inhibitor comprises a CRISPR/Cas9 system.
44. The method of claim 43, wherein the inhibitor comprises Cas9, dCas9, and/or a dCas9 fusion protein.
45. The method of claim 39, wherein the cell is a breast cancer cell, a liver cancer cell, a colon cancer cell, a prostate cancer cell, a bladder cancer cell, or a nasopharyngeal cancer cell.
46. The method of claim 39, wherein the contacting is *in vitro* or *in vivo*.

47. A method of treating cancer in a subject, comprising administering to the subject an effective amount of an inhibitor of a FOXO3A antagonist.
48. The method of claim 47, wherein the FOXO3A antagonist is ZNF274, ERR α , NR3C1, GATAD1, ZKSCAN1, PGC-1 α , HDAC8, or combinations thereof.
49. The method of claim 47, wherein the inhibitor is a small molecule, a polynucleotide, or an antibody or antigen-binding portion thereof.
50. The method of claim 49, wherein the polynucleotide is a small interfering RNA (siRNA).
51. The method of claim 47, wherein the inhibitor comprises a CRISPR/Cas9 system.
52. The method of claim 51, wherein the inhibitor comprises Cas9, dCas9, and/or a dCas9 fusion protein.
53. The method of claim 47, wherein the cancer is breast cancer, liver cancer, colon cancer, prostate cancer, bladder cancer, or nasopharyngeal cancer.
54. The method of claim 47, further comprising administering to the subject a cytotoxic agent.
55. The method of claim 54, wherein the cytotoxic agent is an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, or an endoplasmic reticulum stress inducing agent.
56. The method of claim 54, wherein the administration of the inhibitor of a FOXO3A antagonist and the cytotoxic agent results in a synergistic increase in apoptosis of cancer cells.
57. The method of claim 54, wherein the administration of the inhibitor of a FOXO3A antagonist and the cytotoxic agent results in a synergistic reduction in tumor volume.
58. The method of claim 54, wherein the inhibitor of a FOXO3A antagonist and the cytotoxic agent are administered simultaneously, sequentially or separately.

59. The method of claim 47, further comprising treating the subject with radiation.
60. The method of claim 47, further comprising administering to the subject a chemotherapeutic agent.
61. The method of claim 60, wherein the chemotherapeutic agent is selected from the group consisting of a DNA alkylating agent, a topoisomerase inhibitor, an endoplasmic reticulum stress inducing agent, a platinum compound, an antimetabolite, an enzyme inhibitor, a receptor antagonist, and a therapeutic antibody.

Figure 1A

Negative control siRNA treated with DMSO



Negative control siRNA treated with PI3k inhibitor

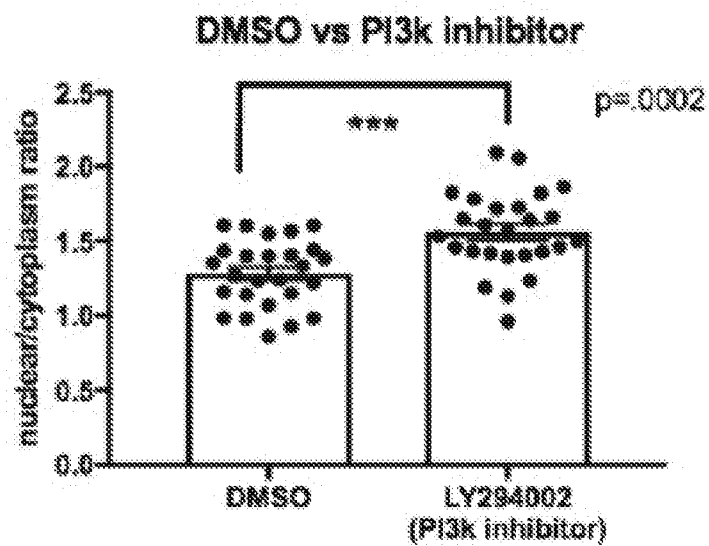
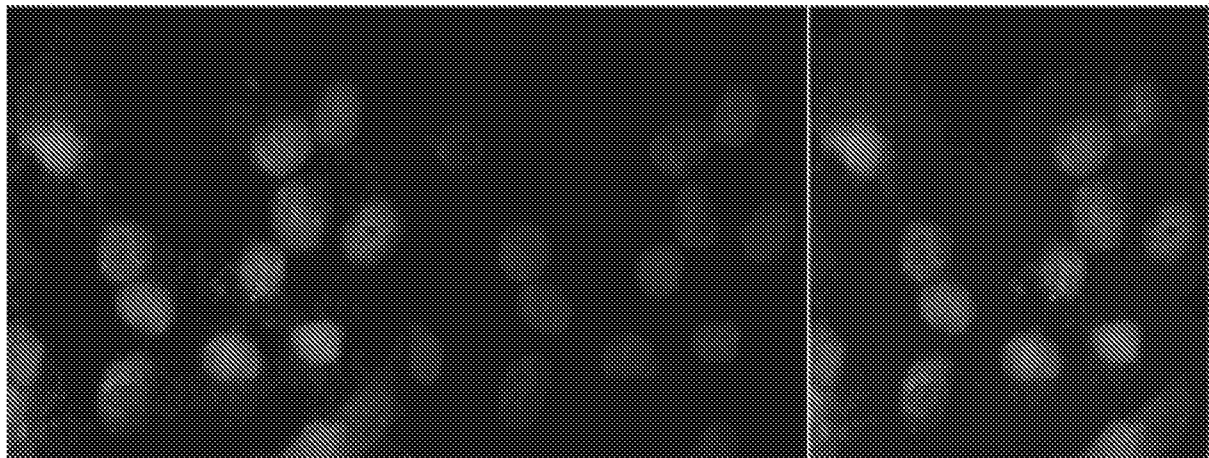
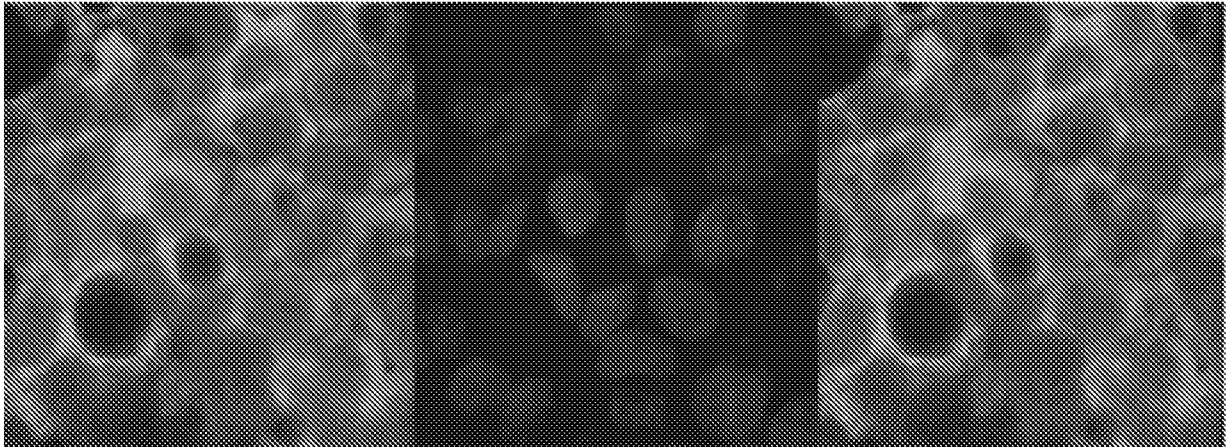
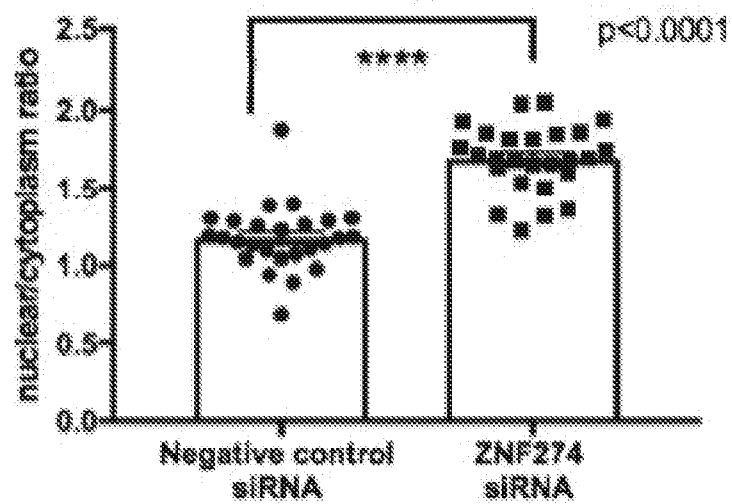


Figure 1B

Negative control siRNA



ZNF274 siRNA

**ZNF274 siRNA vs Negative control siRNA**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/52046

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/00, A61K 48/00, C12Q 1/68 (2019.01)

CPC - C07K 14/4705, C12N 15/8509, A61K 48/00, C12Q 2600/158

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)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	SUNTERS et al. Paclitaxel-Induced Nuclear Translocation of FOXO3a in Breast Cancer Cells Is Mediated by c-Jun NH2-Terminal Kinase and Akt. Cancer Research, 1 January 2006, Vol 66, No 1, pp 212-20 Especially Abstract; pg 218, col 1, para 2	29, 31, 35, 36, 39, 41, 45-47, 49, 53 ----- 9-14, 28/12, 30, 32, 33, 34, 37, 38, 40, 42-44, 48, 50-52, 54-61
Y	US 2010/0215588 A1 (SKALITER) 26 August 2010 (26.08.2010) Especially Abstract, para [0017], [0061], [0065]	1-8, 15-27, 28/(4,18)
Y	Li et al. Investigating the specific core genetic-and-epigenetic networks of cellular mechanisms involved in human aging in peripheral blood mononuclear cells. Oncotarget, 23 February 2016, Vol 7, No 8, pp 8556-79 Especially Abstract; pg 8571, col 2, para 1; pg 8574, col 1, para 1; Figure 10	1-28, 30, 40, 48
Y	US 2008/0125447 A1 (WEDGE) 29 May 2008 (29.05.2008) Especially Abstract, para [0100], [0190]-[0191]	7, 8, 20-27, 37, 38, 54-61
Y	US 2014/0170753 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.) 19 June 2014 (19.06.2014) Especially Abstract, para [0009], [0111], TABLE A	33, 34, 43, 44, 51, 52
Y	US 2011/0038792 A1 (XING) 17 February 2011 (17.02.2011) Especially Abstract, para [0027], [0052]	32, 42, 50

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

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

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"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

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Date of the actual completion of the international search

7 November 2019

Date of mailing of the international search report

06 DEC 2019

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/52046

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LANGOUET et al. Zinc finger protein 274 regulates imprinted expression of transcripts in Prader-Willi syndrome neurons. 1 February 2018, Vol 27, No 3, pp 505-515 Especially Abstract; pg 505, col 1, para 2	4, 12, 18, 28
Y	CRUVINEL et al. Reactivation of maternal SNORD116 cluster via SETDB1 knockdown in Prader-Willi syndrome iPSCs. Human Molecular Genetics, 1 September 2014, Vol 23, No 17, pp 4674-85 Especially Abstract; pg 4675, col 2, para 3-pg 4676, col 2, para 1	10, 11