

ANTIMITOSCINS: TARGETED INHIBITORS OF MITOCHONDRIAL BIOGENESIS FOR ERADICATING CANCER STEM CELLS

FIELD

[0001] The present disclosure relates to “antimitoscins,” antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria, and includes methods for synthesizing antimitoscins, methods of using antimitoscins to target cancer stem cells, and pharmaceutical compositions for both treating cancer and reducing drug resistance in cancer cells, the pharmaceutical compositions containing one or more antimitoscins as the active ingredient.

BACKGROUND

[0002] Researchers have struggled to develop new anti-cancer treatments. Conventional cancer therapies (e.g. irradiation, alkylating agents such as cyclophosphamide, and anti-metabolites such as 5-Fluorouracil) have attempted to selectively detect and eradicate fast-growing cancer cells by interfering with cellular mechanisms involved in cell growth and DNA replication. Other cancer therapies have used immunotherapies that selectively bind mutant tumor antigens on fast-growing cancer cells (e.g., monoclonal antibodies). Unfortunately, tumors often recur following these therapies at the same or different site(s), indicating that not all cancer cells have been eradicated. Relapse may be due to insufficient chemotherapeutic dosage and/or emergence of cancer clones resistant to therapy. Hence, novel cancer treatment strategies are needed.

[0003] Advances in mutational analysis have allowed in-depth study of the genetic mutations that occur during cancer development. Despite having knowledge of the genomic landscape, modern oncology has had difficulty with identifying primary driver mutations across

cancer subtypes. The harsh reality appears to be that each patient's tumor is unique, and a single tumor may contain multiple divergent clone cells. What is needed, then, is a new approach that emphasizes commonalities between different cancer types. Targeting the metabolic differences between tumor and normal cells holds promise as a novel cancer treatment strategy. An analysis of transcriptional profiling data from human breast cancer samples revealed more than 95 elevated mRNA transcripts associated with mitochondrial biogenesis and/or mitochondrial translation. Sotgia et al., *Cell Cycle*, 11(23):4390-4401 (2012). Additionally, more than 35 of the 95 upregulated mRNAs encode mitochondrial ribosomal proteins (MRPs). Proteomic analysis of human breast cancer stem cells likewise revealed the significant overexpression of several mitoribosomal proteins as well as other proteins associated with mitochondrial biogenesis. Lamb et al., *Oncotarget*, 5(22):11029-11037 (2014).

[0004] Functional inhibition of mitochondrial biogenesis using the off-target effects of certain bacteriostatic antibiotics or OXPHOS inhibitors provides additional evidence that functional mitochondria are required for the propagation of cancer stem cells. The inventors recently showed that a mitochondrial fluorescent dye (MitoTracker) could be effectively used for the enrichment and purification of cancer stem-like cells (CSCs) from a heterogeneous population of living cells. Farnie et al., *Oncotarget*, 6:30272-30486 (2015). Cancer cells with the highest mitochondrial mass had the strongest functional ability to undergo anchorage-independent growth, a characteristic normally associated with metastatic potential. The 'Mito-high' cell sub-population also had the highest tumor-initiating activity in vivo, as shown using pre-clinical models. The inventors also demonstrated that several classes of non-toxic antibiotics could be used to halt CSC propagation. Lamb et al., *Oncotarget*, 6:4569-4584 (2015). Because of the conserved evolutionary

similarities between aerobic bacteria and mitochondria, certain classes of antibiotics or compounds having antibiotic activity can inhibit mitochondrial protein translation as an off-target side-effect.

SUMMARY

[0005] In view of the foregoing background, it is an object of this disclosure to demonstrate that existing antibiotics having intrinsic anti-mitochondrial properties can be chemically modified to target the mitochondria and thus can be used to eradicate CSCs. In one aspect, there is provided an antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline. In another aspect, there is provided use of a pharmaceutically effective amount of an antimitoscin and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating cancer, the antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline. In another aspect, there is provided use of a pharmaceutical composition in the manufacture of a medicament for treating cancer, said pharmaceutical composition containing, as the active ingredient, at least one antimitoscin, the antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline. In a further aspect, there is provided an antimitoscin synthesis method comprising: chemically modifying an antibiotic having anti-cancer properties with a membrane targeting signal comprising a fatty acid, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline. In a further aspect, there is provided a method for improving the anti-cancer properties of an antibiotic, the method comprising: chemically modifying an antibiotic having anti-cancer properties with a membrane targeting signal comprising a fatty acid, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline. In a further aspect, there is provided a method for monitoring the effectiveness of an antimitoscin therapy, the method comprising: determining the level of at least one CSC marker in a tissue sample obtained from a patient; and classifying the antimitoscin therapy as effective if the sample is determined to have a decreased level of at least one CSC marker relative to a threshold level, wherein the antimitoscin is as disclosed herein.

Described herein are examples of existing antibiotics having intrinsic anti-mitochondrial properties that have been chemically modified with one or more mitochondria-targeting signals that, as a result, have enhanced anti-cancer properties. The term “antimitoscin” used herein broadly refers to an antibiotic having intrinsic anti-mitochondrial properties that is chemically modified to target the antibiotic to mitochondria. The contemporary art considers intrinsic anti-mitochondrial activity in antibiotics to be an unwanted side-effect. Indeed, some potential antibiotics have been excluded from trials due to excessive anti-mitochondrial properties, and researchers have viewed anti-mitochondrial activity as a potential drawback. However, under the present approach, an antibiotic’s intrinsic anti-mitochondrial activity can become the basis for an entirely new therapeutic. The inventors have determined that these anti-mitochondrial properties may be harnessed and enhanced through chemical modification. As a result, antibiotics with intrinsic anti-mitochondrial activity may be re-purposed as novel therapeutics for, among other potential therapies, anti-cancer treatments. These compounds may bind to either the large sub-unit or the small sub-unit of the mitochondrial ribosome (or in some instances, both) and inhibit mitochondrial biogenesis. Alternatively, these compounds may bind to the inner mitochondrial membrane to block the OXPHOS pathway and thus inhibit mitochondrial metabolism. The present disclosure further describes methods of

synthesizing antimitoscins, methods of using antimitoscins to target cancer stem cells, and pharmaceutical compositions for both treating cancer and for reducing drug resistance, the pharmaceutical compositions containing one or more antimitoscins as the active ingredient. The present disclosure further describes methods for monitoring the effectiveness of an antimitoscin therapy. The methods may include obtaining a tissue sample from a patient, determining the level of at least one CSC marker in the sample, and classifying the antimitoscin therapy as effective if the sample is determined to have a decreased level of at least one CSC marker relative to a threshold level. The CSC marker may be at least one of CD44, Sox2, Nanog, Oct 4, MYC, and ALDH.

[0006] The present disclosure may, in some examples, take the form of an antimitoscin. Exemplar antimitoscins are disclosed herein. In some examples, the antimitoscin comprises an antibiotic having intrinsic anti-mitochondrial properties and a mitochondria-targeting compound. In some examples, the antibiotic is a member of the tetracycline family, the erythromycin family, chloramphenicol, pyriminidyl pamoate, atovaquone, and bedaquiline. The mitochondria-targeting compound may be a chemical modification to the antibiotic. In some examples, the mitochondria-targeting compound is at least one compound selected from the group comprising a membrane targeting signal and a mitochondrial ribosome-targeting signal. In some examples, the membrane targeting signal is a compound selected from the group comprising palmitic acid, stearic acid, myristic acid, and oleic acid. In some examples, the mitochondrial targeting signal is selected from the group comprising tri-phenyl-phosphonium and guanidinium. In some examples, the antimitoscin possesses anti-cancer activity. In some examples, the antimitoscin binds to the large sub-unit or the small sub-unit of the mitochondrial ribosome. In some examples, the antimitoscin binds to at least one of the large

sub-units of the mitochondrial ribosome and the small sub-unit of the mitochondrial ribosome. In some examples, the antimitoscin binds to the inner mitochondrial membrane. In some examples, an antimitoscin possesses radiosensitizing activity, photosensitizing activity, sensitizes cancer cells to chemotherapeutic agents, sensitizes cancer cells to natural substances, and/or sensitizes cancer cells to caloric restriction. In some examples, the present disclosure relates to methods of treating cancer comprising administering to a patient in need thereof of a pharmaceutically effective amount of an antimitoscin and a pharmaceutically acceptable carrier. In some examples, the present disclosure relates to a pharmaceutical composition for treating cancer containing, as the active ingredient, at least one antimitoscin. In some examples, the pharmaceutical composition comprises a plurality of antimitoscins. Examples of the present approach may take the form of methods of synthesizing antimitoscins. Examples of the present approach may also take the form of improving the anti-cancer properties of an antibiotic.

[0007] The inventors analyzed phenotypic properties of CSCs that could be targeted across a wide range of cancer types, and identified a strict dependence of CSCs on mitochondrial biogenesis for the clonal expansion and survival of a CSC. Previous work by the inventors demonstrated that different classes of FDA-approved antibiotics, and in particular tetracyclines such as doxycycline and erythromycin, have an off-target effect of inhibiting mitochondrial biogenesis. As a result, such compounds have efficacy for eradicating CSCs. However, these common antibiotics were not designed to target the mitochondria, and therefore their anti-cancer efficacy is limited. Under the present approach, existing antibiotics having intrinsic anti-mitochondrial properties may be chemically modified to form antimitoscins, to target the mitochondria, and inhibit mitochondrial biogenesis and metabolism. Antimitoscins selectively inhibit CSCs because mitochondrial biogenesis is upregulated in CSCs and is required for

propagation and survival. As a result of their ability to inhibit mitochondrial biogenesis, antimitoscins have enhanced anti-cancer properties.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1 illustrates members of the tetracycline family, including (A) tetracycline, (B) doxycycline, (C) tigecycline, and (D) minocycline.

[0009] FIG. 2 illustrates members of the erythromycin family, including (A) erythromycin, (B) clarithromycin, and (C) azithromycin.

[0010] FIG. 3 illustrates other antibiotics known to inhibit the mitochondrial ribosome or mitochondrial protein translation via off-target side-effects, including (A) chloramphenicol, (B) actinonin, and (C) levofloxacin.

[0011] FIG. 4 illustrates other antibiotics known to inhibit the mitochondrial ribosome or mitochondrial protein translation via direct effects on mitochondrial oxygen consumption, including (A) pyrvinium pamoate, (B) atovaquone, and (C) bedaquiline.

[0012] FIG. 5 shows the structures of membrane-targeting signals including the fatty acids (A) stearic acid, (B) myristic acid, (C) palmitic acid, and (D) oleic acid.

[0013] FIG. 6 shows the structures of mitochondria-targeting signals including (A) tri-phenyl-phosphonium (TPP) and (B) guanidinium.

[0014] FIG. 7 shows a diagram of methods of converting antibiotics to antimitoscins by means of attachment (covalent or non-covalent) of a membrane or mitochondrial targeting signal to an antibiotic.

[0015] FIG. 8 shows the structures of two antimitoscins.

DESCRIPTION

[0016] The following description illustrates embodiments of the present approach in sufficient detail to enable practice of the present approach. Although the present approach is described with reference to these specific embodiments, it should be appreciated that the present approach can be embodied in different forms, and this description should not be construed as limiting any appended claims to the specific embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the present approach to those skilled in the art.

[0017] The mitochondria is an untapped gateway for treating a number of afflictions, ranging from cancer to bacterial and fungal infections to aging. Functional mitochondria are required for the propagation of cancer stem cells. Inhibiting mitochondrial biogenesis and metabolism in cancer cells impedes the propagation of those cells. Mitochondrial inhibitors therefore represent a new class of anti-cancer therapeutics.

[0018] As disclosed herein, existing antibiotics having intrinsic anti-mitochondrial properties may be chemically-modified with at least one mitochondria-targeting compound. The mitochondria-targeting compound may be a chemical modification to the antibiotic, and the chemical modification may be made according to chemical synthesis methods as are known in the art. The mitochondria-targeting compound may be one of a membrane-targeting signal and a mitochondrial-ribosome targeting signal. In some embodiments, the antibiotic having intrinsic anti-mitochondrial properties may be chemically-modified with at least one membrane-targeting signal and at least one mitochondrial-targeting signal. The resulting antimitoscin may be used as an anti-cancer therapeutic, as well as to target bacteria and pathogenic yeast, provide anti-aging benefits, function as radiosensitizers and/or photo-sensitizers, and/or sensitize bulk cancer cells

and cancer stem cells to chemotherapeutic agents, pharmaceuticals, and/or other natural substances.

[0019] Novel antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria, referred to herein as “antimitoscins,” may be formed by the addition of at least one membrane-targeting signal and/or at least one mitochondrial-targeting signal to an antibiotic having intrinsic anti-mitochondrial properties. Such chemical modifications increase the efficiency of the specific targeting of these compounds to the mitochondria and in particular the mitochondrial ribosome. The resulting compound, an antimitoscin, has dramatically enhanced therapeutic properties, including anti-cancer properties.

[0020] FIGS. 1-4 provide examples of known antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria to form an antimitoscin under the present approach. Antibiotics in the tetracycline family are examples of antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria to form antimitoscins having efficacy as anti-cancer therapeutics. FIG. 1 illustrates members of the tetracycline family, including (A) tetracycline, (B) doxycycline, (C) tigecycline, and (D) minocycline. Each of these broad-spectrum antibiotics may be chemically modified with at least one mitochondrial ribosome-targeting compound to form an antimitoscin. It should be appreciated that the specific antibiotics shown are demonstrative, and that the scope of the present approach is not limited to only those structures shown. For example, other members of the tetracycline family not specifically identified herein may be used as an initial compound for forming an antimitoscin. This may include, as a non-exhaustive list of examples only, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, chlortetracycline, omadacycline, and sarecycline.

[0021] Antibiotics in the erythromycin family are additional examples of antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria to form antimitoscins having efficacy as anti-cancer therapeutics. FIG. 2 shows the chemical structures for sample erythromycin family members, including (A) erythromycin, (B) azithromycin, and (C) clarithromycin. Each of these antibiotics may be chemically modified with at least one mitochondria-targeting compound to form an antimitoscin. It should be appreciated that the specific antibiotics shown are demonstrative, and that the scope of the present approach is not limited to only those structures shown. For example, other members of the tetracycline family not specifically identified herein may be used as an initial compound for forming an antimitoscin. This may include, for example, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, minocycline, rolitetracycline, tigecycline, omadacycline, and sarecycline, to name a few further examples.

[0022] Other known antibiotics having intrinsic anti-mitochondrial properties that are chemically modified to target the antibiotics to mitochondria may be antimitoscins. FIG. 3 shows other antibiotics known to inhibit the mitochondrial ribosome or mitochondrial protein translation as an off-target side-effect. These examples include chloramphenicol, actinonin, and levofloxacin. Each of these compounds may be chemically-modified with at least one mitochondria-targeting compound to form an antimitoscin. FIG. 4 shows other antibiotics known to impact mitochondrial oxygen consumption by interfering with mitochondrial complexes I, II, III, IV, and/or V. These examples include pyrvinium pamoate, atovaquone, and bedaquiline. Each of these compounds may be chemically-modified with at least one mitochondria-targeting compound to form an antimitoscin.

[0023] Unlike antibiotics, antimitoscins are specifically designed to target mitochondria by attachment of at least one membrane-targeting signal and/or at least one mitochondrial-targeting signal. FIG. 5 provides examples of membrane-targeting signals, including fatty acids such as palmitate, stearate, myristate, and oleate. It should be appreciated that this is not a comprehensive list of membrane-targeting signals, and that an unlisted membrane-targeting signal may be used without departing from the present approach. FIG. 6 provides examples of mitochondria-targeting signals, including tri-phenyl-phosphonium (TPP) and guanidinium-based moieties. It should be appreciated that this is not a comprehensive list of mitochondria-targeting signals, and that an unlisted mitochondria-targeting signal may be used without departing from the present approach.

[0024] FIG. 7 shows a diagram of methods of converting antibiotics to antimitoscins by means of attachment (covalent or non-covalent) of a membrane or mitochondrial targeting signal 701 to one or more of antibiotics 703, nutraceuticals 705, conventional chemotherapies 707 as are known in the art, and other compounds or therapies 709.

[0025] As described herein, an antimitoscin may be formed by chemically-modifying an antibiotic having intrinsic anti-mitochondrial properties with at least one membrane-targeting signal and/or at least one mitochondria-targeting signal. FIG. 8 shows two examples of antimitoscins. In these examples, the side chain of a tetracycline family member has been replaced with (A) palmitic acid and (B) a carbon-spacer-arm and TPP. It should be appreciated that the mitochondria-targeting compound(s) may be linked to the antibiotic in other locations without departing from the present approach.

[0026] The specific antimitoscin formulas shown in FIG. 8 are examples of antimitoscins formed from the exemplar antibiotics identified in FIGS. 1-4. It should be appreciated that an

antimitoscin may be selected for therapeutic use individually, or in combination with one or more antimitoscin, and/or with other substances to enhance the efficacy of other therapeutics. For example, antimitoscins formed from different antibiotics may be used together in a therapeutic formulation. Further, antimitoscins formed from the antibiotic but having different mitochondria-targeting compounds (such as the structures shown in FIG. 8) may be used together in a therapeutic formulation. The therapeutics may be used in the form of usual pharmaceutical compositions which may be prepared using one or more known methods. For example, a pharmaceutical composition may be prepared by using diluents or excipients such as, for example, one or more fillers, bulking agents, binders, wetting agents, disintegrating agents, surface active agents, lubricants, and the like as are known in the art. Various types of administration unit forms can be selected depending on the therapeutic purpose(s). Examples of forms for pharmaceutical compositions include, but are not limited to, tablets, pills, powders, liquids, suspensions, emulsions, granules, capsules, suppositories, injection preparations (solutions and suspensions), topical creams, and other forms as may be known in the art. For the purpose of shaping a pharmaceutical composition in the form of tablets, any excipients which are known may be used, for example carriers such as lactose, white sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, cyclodextrins, crystalline cellulose, silicic acid and the like; binders such as water, ethanol, propanol, simple syrup, glucose solutions, starch solutions, gelatin solutions, carboxymethyl cellulose, shelac, methyl cellulose, potassium phosphate, polyvinylpyrrolidone, etc. Additionally, disintegrating agents such as dried starch, sodium alginate, agar powder, laminaria powder, sodium hydrogen carbonate, calcium carbonate, fatty acid esters of polyoxyethylene sorbitan, sodium laurylsulfate, monoglyceride of stearic acid, starch, lactose, etc., may be used. Disintegration inhibitors such as white sugar, stearin, coconut butter, hydrogenated

oils; absorption accelerators such as quaternary ammonium base, sodium laurylsulfate, etc., may be used. Wetting agents such as glycerin, starch, and others known in the art may be used. Adsorbing agents such as, for example, starch, lactose, kaolin, bentonite, colloidal silicic acid, etc., may be used. Lubricants such as purified talc, stearates, boric acid powder, polyethylene glycol, etc., may be used. If tablets are desired, they can be further coated with the usual coating materials to make the tablets as sugar coated tablets, gelatin film coated tablets, tablets coated with enteric coatings, tablets coated with films, double layered tablets, and multi-layered tablets. Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, foams, sprays, aerosols, or oils. Such pharmaceutical compositions may include conventional additives which include, but are not limited to, preservatives, solvents to assist drug penetration, co-solvents, emollients, propellants, viscosity modifying agents (gelling agents), surfactants, and carriers.

[0027] Antimitoscins may also be used to reverse drug resistance in cancer cells. Drug resistance is thought to be based, at least in part, on increased mitochondrial function in cancer cells. In particular, cancer cells demonstrating resistance to endocrine therapies, such as tamoxifen, are expected to have increased mitochondrial function. Antimitoscins inhibit mitochondrial function, and therefore are useful in reducing and, in some cases reversing, drug resistance in cancer cells. Additionally, previously generated data suggests that inhibitors of mitochondrial function that target the mitochondrial ribosome, referred to as “mitoriboscins,” may be used to target bacteria and pathogenic yeast, provide anti-aging benefits, function as radiosensitizers and/or photo-sensitizers, sensitize bulk cancer cells and cancer stem cells to chemotherapeutic agents, pharmaceuticals, and/or other natural substances, such as dietary supplements and caloric restriction. Given their mitochondrial-inhibition properties, antimitoscins may similarly be used to

target bacteria and pathogenic yeast, provide anti-aging benefits, function as radiosensitizers and/or photo-sensitizers, sensitize bulk cancer cells and cancer stem cells to chemotherapeutic agents, pharmaceuticals, and/or other natural substances.

[0028] In addition to antibiotics, other compounds having antibiotic activity may be modified with a membrane or mitochondria-targeting signal to have enhanced anti-cancer activity. For example, nutraceuticals and conventional chemotherapies may be modified with at least one mitochondria-targeting compound(s) to specifically target the mitochondria. The efficacy of such compounds may be increased when specifically targeting the mitochondria. Examples of nutraceuticals having antibiotic activity that may be modified to target the mitochondria include caffeic acid phenethyl ester (found in bee propolis), ascorbic acid (vitamin C) and other vitamins and trace minerals, polyphenols, epigallocatechin-3-gallate, resveratrol, and quercetin. It should be appreciated that this is not a comprehensive list of nutraceuticals having antibiotic activity, and that an unlisted nutraceutical may be used without departing from the present approach.

[0029] The table below summarizes demonstrative antibiotics and chemical targeting signals that may be linked to create an antimitoscin.

FDA-Approved Antibiotics Tetracycline Family <ul style="list-style-type: none"> • tetracycline • minocycline • doxycycline • tigecycline • among others Erythromycin Family <ul style="list-style-type: none"> • erythromycin • clarithromycin • azithromycin • among others Other(s) & OXPHOS <ul style="list-style-type: none"> • chloramphenicol • pyrvinium pamoate • atovaquone • Bedaquiline • among others 	Chemical Modifications Membrane-Targeting-Signals <ul style="list-style-type: none"> • palmitic acid • stearic acid • myristic acid • oleic acid • among others Mitochondrial-Targeting-Signals <ul style="list-style-type: none"> • tri-phenyl-phosphonium (TPP) • guanidinium-related groups • choline esters • among others Other Applications Nutraceuticals Conventional chemotherapies Newly Discovered Compounds	Specific Formulations Inert ingredients Cyclodextrins <ul style="list-style-type: none"> • alpha • beta • Gamma Combination Therapies
--	---	--

Table 1. Demonstrative constituents for creating an antimitoscin.

[0030] The present disclosure also relates to methods of monitoring the effectiveness of antimitoscin therapies. In some embodiments, one or more CSC markers may be monitored to determine the effectiveness of treatment with one or more antimitoscins. Relative levels of CSC markers may decrease in response to antimitoscin treatment, as compared to an untreated control. In some embodiments, the CSC marker is at least one of CD44, Sox2, Nanog, Oct4, MYC, and ALDH. The relative levels of one or more CSC markers may be measured in a tumor tissue sample. The relative levels of one or more CSC markers may be measured by any number of ways known in the art for measuring RNA, DNA, and/or protein levels of a marker including, without limitation, quantitative PCR and/or RT-PCR kits, microarrays, Northern blots, and Western blots.

[0031] The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. The invention includes numerous alternatives, modifications, and equivalents as will become apparent from consideration of the following detailed description.

[0032] It will be understood that although the terms “first,” “second,” “third,” “a),” “b),” and “c),” etc. may be used herein to describe various elements of the invention should not be limited by these terms. These terms are only used to distinguish one element of the invention from another. Thus, a first element discussed below could be termed an element aspect, and similarly, a third without departing from the teachings of the present invention. Thus, the terms “first,” “second,” “third,” “a),” “b),” and “c),” etc. are not intended to necessarily convey a sequence or other hierarchy to the associated elements but are used for identification purposes only. The sequence of operations (or steps) is not limited to the order presented in the claims.

[0033] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents

and other references mentioned herein are incorporated by reference in their entirety. In case of a conflict in terminology, the present specification is controlling.

[0034] Also, as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0035] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed.

[0036] As used herein, the transitional phrase “consisting essentially of” (and grammatical variants) is to be interpreted as encompassing the recited materials or steps “and those that do not materially affect the basic and novel characteristic(s)” of the claimed invention. Thus, the term “consisting essentially of” as used herein should not be interpreted as equivalent to “comprising.”

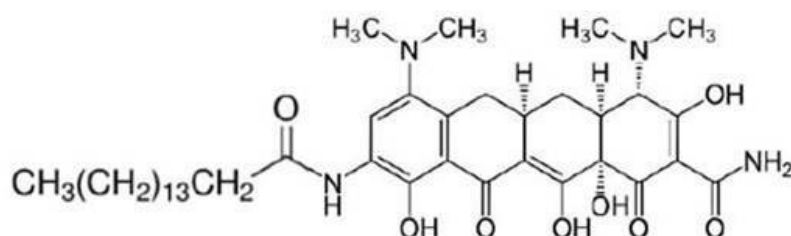
[0037] The term “about,” as used herein when referring to a measurable value, such as, for example, an amount or concentration and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount. A range provided herein for a measurable value may include any other range and/or individual value therein.

[0038] Having thus described certain embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited by particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope thereof as hereinafter claimed.

CLAIMS

What is claimed is:

1. An antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline.
2. The antimitoscin of claim 1, wherein the antimitoscin comprises the formula:



, or pharmaceutically

acceptable salts thereof.

3. The antimitoscin of claim 1, wherein the antibiotic is doxycycline or tigecycline.
4. The antimitoscin of claim 1, wherein the membrane targeting signal is one of palmitic acid, stearic acid, myristic acid, and oleic acid.
5. The antimitoscin of claim 3, wherein the membrane targeting signal is one of palmitic acid, stearic acid, myristic acid, and oleic acid.
6. The antimitoscin of claim 1, wherein the antimitoscin possesses anti-cancer activity.
7. The antimitoscin of claim 1, wherein the antimitoscin binds to the large sub-unit of the mitochondrial ribosome.
8. The antimitoscin of claim 1, wherein the antimitoscin binds to the small sub-unit of the mitochondrial ribosome.

9. The antimitoscin of claim 1, wherein the antimitoscin binds to at least one of the large sub-unit of the mitochondrial ribosome or the small sub-unit of the mitochondrial ribosome.
10. The antimitoscin of claim 1, wherein the antimitoscin binds to a mitochondrial membrane.
11. The antimitoscin of claim 1, wherein the antimitoscin possesses radiosensitizing activity.
12. The antimitoscin of claim 1, wherein the antimitoscin possesses photosensitizing activity.
13. The antimitoscin of claim 1, wherein the antimitoscin sensitizes cancer cells to chemotherapeutic agents.
14. The antimitoscin of claim 1, wherein the antimitoscin sensitizes cancer cells to natural substances.
15. The antimitoscin of claim 1, wherein the antimitoscin sensitizes cancer cells to caloric restriction.
16. Use of a pharmaceutically effective amount of an antimitoscin and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating cancer, the antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline.
17. Use of a pharmaceutical composition in the manufacture of a medicament for treating cancer, said pharmaceutical composition containing, as the active ingredient, at least one antimitoscin, the antimitoscin comprising an antibiotic having intrinsic anti-mitochondrial properties chemically modified with a membrane targeting signal comprising a fatty acid moiety, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline,

oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline.

18. The use of claim 17, wherein the pharmaceutical composition comprises a plurality of said antimitoscins.

19. An antimitoscin synthesis method comprising:

chemically modifying an antibiotic having anti-cancer properties with a membrane targeting signal comprising a fatty acid, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline.

20. The method of claim 19, wherein the antibiotic is one of doxycycline, tigecycline and minocycline.

21. The method of claim 19, wherein the fatty acid is selected from palmitic acid, stearic acid, myristic acid, and oleic acid.

22. The method of claim 20, wherein the antibiotic is selected from doxycycline, tigecycline and minocycline, and the fatty acid is selected from palmitic acid, stearic acid, myristic acid, and oleic acid.

23. A method for improving the anti-cancer properties of an antibiotic, the method comprising:

chemically modifying an antibiotic having anti-cancer properties with a membrane targeting signal comprising a fatty acid, wherein the antibiotic is selected from doxycycline, tigecycline, minocycline, chlortetracycline, oxytetracycline, demeclocycline, lymecycline, meclocycline, methacycline, rolitetracycline, omadacycline, and sarecycline.

24. The method of claim 23, wherein the antibiotic is selected from doxycycline, tigecycline and minocycline.
25. The method of claim 23, wherein the fatty acid is selected from palmitic acid, stearic acid, myristic acid, and oleic acid.
26. The method of claim 24, wherein the antibiotic is selected from doxycycline, tigecycline and minocycline, and the fatty acid is selected from palmitic acid, stearic acid, myristic acid, and oleic acid.
27. A method for monitoring the effectiveness of an antimitoscin therapy, the method comprising:
determining the level of at least one CSC (cancer stem cell) marker in a tissue sample obtained from a patient;
and classifying the antimitoscin therapy as effective if the sample is determined to have a decreased level of at least one CSC marker relative to a threshold level,
wherein the antimitoscin is as set forth in any one of claims 1 to 15.
28. The method of claim 27, wherein the CSC marker is at least one of CD44, Sox2, Nanog, Oct 4, MYC, and ALDH.