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(54) Title: COMPOSITIONS OF TIGECYCLINE AND USES THEREOF

(57) Abstract: Compositions for stabilizing tigecycline are provided, the compositions including one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers. Compositions of stabilized tigecycline are also provided comprising tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers. The compositions can also include a cyclodextrin. Methods of preparing, uses and kits are also provided.



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Title: Compositions of Tigecycline and Uses Thereof**Related Applications**

[0001] This is a Patent Cooperation Treaty Application which claims the benefit of 35 U.S.C. § 119 based on the priority of U.S. Provisional Patent Application No. 61/566,377, filed December 2, 2011 which is incorporated herein by reference in its entirety.

Field

[0002] The present application relates to tigecycline compositions with improved stability, the compositions comprising tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof, a cyclodextrin and ascorbic acid and methods for their preparation and their use, for example, as medicaments.

15 Introduction

[0003] Tigecycline is a glycylicycline antibiotic, i.e., a t-butylglycyl substituted naphthacenecarboxamide free base, and an analog of the semisynthetic tetracycline, minocycline. Tetracyclines such as chlortetracycline hydrochloride (Aureomycin) and oxytetracycline (Terramycin) are safe and have been used therapeutically as broad-spectrum antibiotics since 1948 (1). However, the emergence of resistance to these antibiotics has limited their continued widespread usage. Tigecycline was thus developed as an agent to potentially restore therapeutic utility to tetracyclines by overcoming tetracycline resistance mechanisms. Tigecycline may also provide activity against emerging multi-drug resistant pathogens.

25 [0004] Currently, for its use as an anti-bacterial, tigecycline can be formulated as a powder with no excipients. The drug is reconstituted in a vial at a concentration of 10 mg/mL in saline, 5% dextrose, or Ringer's lactate. In this form, the drug is stable for only 6 hours at room temperature. Tigecycline can be further diluted to a concentration of 1 mg/mL in the above solutions. When reconstituted in
30 the above conditions, the drug is oxidized over time (2) At the above concentration, the drug is stable for 24 hours at room temperature. (3). Given the poor stability of the drug, patients must be hospitalized to receive this therapy or return daily to the hospital. Moreover, continuous infusion pumps with this formulation are not practical.

- [0005] US Patent Serial No. 7,879,828 issued February 1, 2011 to Wyeth relates to compositions of tigecycline with improved stability comprising a suitable carbohydrate, such as lactose, an acid and a buffer. Currently, tigecycline is supplied as a vial containing 50 mg of lyophilized powder with 100 mg lactose monohydrate,
5 hydrochloric acid and sodium hydroxide (4).
- [0006] US Patent Application Publication No. 2008/0014256 describes pharmaceutical compositions comprising tigecycline for oral administration. The composition can comprise tigecycline having at least one enteric coating.
- [0007] US Patent Application Publication No. 2005/0130252 describes
10 compositions and methods for determining susceptibility of aerobic pathogens to antibiotics. The compositions can comprise tigecycline, media and an adjuvant.
- [0008] US Patent Application Publication No. 2009/0275660 describes stable parenteral formulations comprising tigecycline, an edetate, a pH-modifying agent or an antioxidant and optionally, other pharmaceutically acceptable excipients.
- 15 [0009] US Patent Application Publication No. 2010/0035845 is directed to a frozen pharmaceutical formulation suitable for administration to a subject parenterally, comprising a therapeutically effective amount of tigecycline and an agent selected from the group consisting of lactose, dextrose, glucose, mannose, sucrose, ribose, xylose and a combination thereof, wherein the formulation in a pre-
20 frozen state at about 22°C or in an unfrozen state at about 22 °C has a pH in the range of from 4.0 to 5.5.
- [0010] US Patent Application Publication No. 2011/0118216 relates to tigecycline compositions with improved stability in both solid and solution states and processes for making these compositions. These compositions comprise tigecycline,
25 a suitable carbohydrate, and an acid or buffer.

Summary

- [0011] The present application relates to compositions for increasing tigecycline stability as well as compositions of tigecycline comprising one or more
30 additives that increase tigecycline stability and methods of preparing and using said compositions for example to treat bacterial infections and/or cancer.

[0012] An aspect of the application includes a composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. In an embodiment, the composition further comprises a chelator.

[0013] Another aspect of the application includes a composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid, and optionally one or more excipients, diluents or buffers.

10 [0014] In another embodiment, the composition is substantially free of EDTA. In an embodiment, the composition is comprised in a light blocking vessel.

[0015] In an embodiment, the composition comprises a pH modifying agent, that for example adjusts the pH of a liquid formulation and/or a reconstituted formulation to about pH 4 to about pH 8, for example, about pH 7. In an embodiment, the composition further comprises a cyclodextrin.

[0016] Another aspect of the application includes a composition comprising, consisting of, or consisting essentially of pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. Such a composition can be used for example, for reconstituting and/or diluting a tigecycline formulation. Accordingly, it is an embodiment that the composition is a liquid formulation for formulating, reconstituting and/or diluting a tigecycline formulation.

[0017] A further aspect of the application includes a composition comprising, consisting of, or consisting essentially of two or more of a chelator, pyruvic acid, or a salt or ester thereof, ascorbic acid and a cyclodextrin. Such a composition can be used, for example, for formulating, reconstituting and/or diluting a tigecycline formulation.

[0018] In an embodiment, the chelator is selected from EGTA, citrates, penicillamine, dimercapto-propane sulfonate and tartrates. In another embodiment, the chelator is EDTA or derivatives thereof, for example, disodium edetate, trisodium edetate, tetrasodium edetate, disodium calcium edetate, and the like. In a further embodiment, the pyruvic acid, or salt or ester thereof is a pyruvate, such as sodium pyruvate, calcium pyruvate, potassium pyruvate, magnesium pyruvate, or

dihydroxyacetone pyruvate. In yet a further embodiment, the cyclodextrin is selected from 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD), alpha-cyclodextrin, beta-cyclodextrin and gamma-cyclodextrin.

[0019] The tigecycline compositions of the application can comprise, for
5 example a composition containing a therapeutically effective amount of tigecycline.

[0020] In an embodiment, the composition is a liquid formulation. In another embodiment, the formulation is a lyophilized formulation for reconstitution. Where the formulation is a lyophilized formulation for example, the ranges and concentrations provided describe the ranges and concentrations of the reconstituted formulation.

10 [0021] In an embodiment, the composition comprises when in a liquid such as reconstituted formulation about 0.05 mg/mL to about 100 mg/mL tigecycline. In another embodiment, the composition comprises about 0.1 mg/mL to about 50 mg/mL tigecycline. In a further embodiment, the composition comprises about 0.5 mg/mL to about 10 mg/mL tigecycline. In another embodiment, the composition
15 comprises about 1 mg/mL to about 5 mg/mL tigecycline.

[0022] In an embodiment, the tigecycline in the composition is in a concentration of about 0.5 mg/mL to about 100 mg/mL; about 1 mg/mL to about 50 mg/mL or about 1 mg/mL to about 10 mg/mL.

[0023] In an embodiment, the composition is a lyophilized composition
20 and/or in powder form, and the composition components are for reconstitution in a suitable diluent, to give a reconstituted formulation. In an embodiment, the reconstituted formulation comprises tigecycline and is substantially free of EDTA. In an embodiment, the reconstituted formulation is comprised in a light blocking vessel.

[0024] In an embodiment, the composition comprises about 0.6 mg/mL to
25 about 300 mg/mL pyruvate; about 0.06 mg/mL to about 30 mg/mL EDTA, about 0.5 mg/mL to about 250 mg/mL of 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) and/or about 0.3 mg/mL to about 150 mg/mL ascorbic acid.

[0025] In an embodiment, the composition comprises about 0.6 mg/mL to
30 about 300 mg/mL pyruvate, about 0.5 mg/mL to about 250 mg/mL of 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) and/or about 0.3 mg/mL to about 150 mg/mL ascorbic acid.

[0026] In another embodiment, the composition comprises about 0.6 mg/mL to about 300 mg/mL pyruvate and/or about 0.3 mg/mL to about 150 mg/mL ascorbic acid.

5 [0027] In an embodiment, the formulating agent(s) are effective to limit degradation of the tigecycline in the liquid composition to not more than about 20% over a period of 7 days at 25°C

[0028] In an embodiment, the composition is comprised in a unit dosage form and the amount of tigecycline in the unit dosage form is selected from about 5 mg to about 2000 mg, from about 10 mg to about 1500 mg, from about 25 mg to about 10
10 1500 mg, from about 25 mg to about 1000 mg, from about 25 mg to about 700 mg, from about 25 mg to about 500 mg, from about 25 mg to about 350 mg, from about 25 mg to about 300 mg or from about 25 mg to about 250 mg of tigecycline.

[0029] In another embodiment, the composition is a liquid formulation and the volume of the formulation is selected from about 0.5 mL to 5mL, from about 5 mLs to about 10 mLs, from about 10mL to about 10000 mL, from about 100 mL to about 200
15 mL, from about 200 to about 300mL, about 300 mL to about 400 ml, about to 400 to about 500 mL and from about 500 mL to about 2000 mL.

[0030] In an embodiment, wherein the composition components are for example lyophilized and/or in powder form, the composition components can be reconstituted, for example in a suitable diluent to give a reconstituted formulation. Non-limiting examples of suitable diluents include saline, water, 5% dextrose, Hartmann's solution, Ringer's solution and Ringer's lactate solution. Other diluents suitable for intravenous administration known in the art can also be used. The one or more formulating agents selected from pyruvic acid, or a salt or ester thereof, ascorbic acid, a chelator and a cyclodextran; as well as optionally one or more
20 25 excipients, diluents or buffers can for example be added to a diluent such as the above diluents (e.g. Ringer's solution) and for reconstituting tigecycline formulations such as lyophilized formulations.

[0031] Where such a reconstituted formulation comprises tigecycline and is
30 substantially free of EDTA, it is an embodiment that the reconstituted formulation is comprised in a light blocking vessel.

[0032] In another embodiment, the composition comprises a pH modifying agent such as a buffering agent such as 0.1 N NaOH or an acidifying agent such as 0.1 N HCL.

[0033] In a further embodiment, the pH of a liquid formulation of the composition is about 7. In an embodiment, the solid formulation comprises a pH
5 modifying agent that produces a reconstituted formulation with a pH of about 7.

[0034] The compositions of the application can include additional excipients including for example, lactose such as lactose monohydrate.

[0035] It is an embodiment that the compositions of the application are
10 comprised in a light blocking vessel.

[0036] Another aspect includes a storage-stable, liquid, pharmaceutical composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid or a salt or ester thereof, and ascorbic acid; and optionally one or more pharmaceutically
15 excipients, diluents, or buffers, wherein the formulating agent(s) are effective to limit degradation of the tigecycline in the liquid composition to not more than about 20% over a period of 7 days at 25°C.

[0037] The tigecycline compositions of the application are suitably formulated into pharmaceutical compositions for administration to subjects in a biologically
20 compatible form suitable for administration *in vivo*. Accordingly, another aspect includes a pharmaceutical composition comprising a tigecycline composition of the application and a pharmaceutically acceptable carrier. It is an embodiment that the pharmaceutical composition is comprised in a light blocking vessel.

[0038] The tigecycline compositions of the application are suitably formulated
25 into dermatological or cosmetic compositions suitable for topical administration. Accordingly, another aspect includes a dermatological or cosmetic composition comprising a tigecycline composition of the application and a dermatologically or cosmetically acceptable excipient. It is an embodiment that the dermatological or cosmetic composition is comprised in a light blocking vessel.

[0039] The tigecycline compositions of the application are suitably formulated
30 into dermatological or cosmetic compositions suitable for topical administration. Accordingly, another aspect includes a dermatological or cosmetic composition

comprising a tigecycline composition of the application and a dermatologically or cosmetically acceptable excipient. It is an embodiment that the dermatological or cosmetic composition is comprised in a light blocking vessel.

5 [0040] Also provided in another aspect is a method for preparing said compositions.

[0041] An embodiment includes a method for preparing a composition described herein, comprising resuspending and/or diluting each component of the composition in a suitable diluent and combining the resuspended and/or diluted components to provide the desired concentrations or blending the components of the
10 composition together to provide a solid formulation comprising each of the components of the composition and optionally subsequently diluting the solid formulation with a suitable diluent such as water or saline.

[0042] In an embodiment, the method comprises:

i) resuspending and/or diluting tigecycline and one or more
15 formulating agent(s) selected from pyruvic acid, or a salt or ester thereof, a chelator, a cyclodextrin and ascorbic acid; and optionally one or more excipients or buffers in a suitable solvent;

ii) filtering the solution of step i) under aseptic conditions;

iii) filling a light blocking vessel with the filtered solution of ii)

20 iv) optionally lyophilizing the solution in iii) and

v) stoppering and sealing the solution in step iii) or the lyophilizate in step iv).

[0043] In another embodiment, the composition is a liquid formulation comprising tigecycline and is substantially free of EDTA.

25 [0044] Another aspect of the disclosure includes a method of treating a condition or disease comprising administering a tigecycline composition of the application to a subject in need thereof. In an embodiment, the condition or disease is a bacterial infection. In another embodiment, the condition or disease is cancer. In yet a further embodiment, the condition is a dermatological condition such as acne,
30 scarring and/or skin aging.

[0045] A further aspect includes a method of treating a bacterial infection or a cancer comprising administering to a subject in need thereof an effective amount of a tigecycline composition of the application.

5 [0046] Another aspect includes a use of a tigecycline composition of the application for treating a bacterial infection, cancer or a dermatological condition.

[0047] In an embodiment, the composition is for use in treating a cosmetic condition and/or the pharmaceutical composition is for use in treating a bacterial infection and/or cancer.

10 [0048] In an embodiment, the cancer is a hematological cancer or a solid cancer.

[0049] In an embodiment, the hematological cancer is leukemia. In a further embodiment, the leukemia is acute myeloid leukemia (AML).

[0050] In another embodiment, the dermatological condition is acne, scarring and/or skin aging.

15 [0051] Another aspect includes a kit comprising tigecycline or a first composition comprising tigecycline and a second composition comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers.

20 [0052] In an embodiment, the second composition further comprises a chelator. In another embodiment, the second composition is substantially free of EDTA, and if the second composition is a liquid formulation, the second composition is comprised optionally in a light blocking vessel. In a further embodiment, the tigecycline or the first composition comprising tigecycline and/or the second composition are comprised in a light blocking vessel, for example a light blocking
25 vial.

[0053] Another aspect includes a kit comprising the a composition a tigecycline stabilized composition or a pharmaceutical composition comprising a tigecycline composition, and a vessel such as a vial, for example a light blocking vessel such as a light blocking vial, and optionally instructions for use.

[0054] In an embodiment, the composition, the pharmaceutical composition or the kit described herein, is formulated into a unit or multi-dosage form. In an embodiment, the composition is a formulated into liquid unit or multi dosage forms.

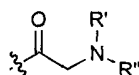
- 5 [0055] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become
10 apparent to those skilled in the art from this detailed description.

Detailed description

I. Definitions

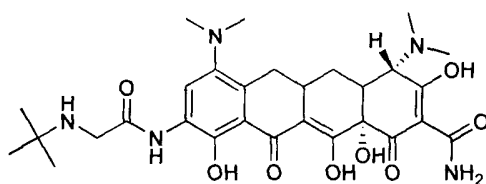
- [0056] The term "glycylcycline" as used herein means any glycyl derivative of
15 any tetracycline, for example a tert-butyl-glycylamido derivative, and includes any salt forms, such as any pharmaceutically acceptable salt, enantiomer, stereoisomer, solvate, prodrug or mixtures thereof. For example, see^{5,6}. In an embodiment, the glycyl derivative is a tetracycline wherein a glycyl group is attached at the 9 position of the tetracyclic structure.

- 20 [0057] The term "glycyl" as used herein means a group of the formula:



- wherein R' and R'' are independently selected from the group consisting of H, C₁₋₂₀alkyl, C₆₋₁₀aryl and C₃₋₁₀cycloalkyl, or R' and R'' are joined to form, together with the nitrogen to which they are attached, a 3 to 10 membered ring. In an embodiment,
25 one of R' and R'' is H and the other of R' and R'' is C₁₋₆alkyl (branched or unbranched).

[0058] The term "tigecycline" as used herein means a compound having the structure:

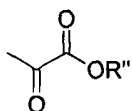


or pharmaceutically acceptable salts, solvates or prodrugs thereof as well as mixtures thereof. Tigecycline can be produced according to methods known in the art for example as described in U.S. Patent Application Publication Nos.: 2006-0247181, 5 titled "Tigecycline compositions and methods of preparation"; and 2007-0026080, titled "Manufacturing process for tigecycline".

[0059] The term "mixture" as used herein, means a composition comprising two or more compounds. In an embodiment a mixture is a mixture of two or more distinct compounds. In a further embodiment, when a compound is referred to as a 10 "mixture", this means that it can comprise two or more "forms" of the compounds, such as, salts, solvates, prodrugs or, where applicable, stereoisomers of the compound in any ratio. A person of skill in the art would understand that a compound in a mixture can also exist as a mixture of forms. For example, a compound may exist as a hydrate of a salt or as a hydrate of a salt of a prodrug of the compound. All 15 forms of the compounds disclosed herein are within the scope of the present application.

[0060] The term "chelator" as used herein is a compound that binds for example cations, for example, mono- and/or divalent cations, removing them from solution. Suitable chelators for use in the compositions of the application include 20 without limitation EGTA, penicillamine, dimercapto-propane sulfonate citrates, and tartrates. In another embodiment, the chelator is EDTA and derivatives thereof, for example, disodium edetate, trisodium edetate, tetrasodium edetate, disodium calcium edetate, and the like.

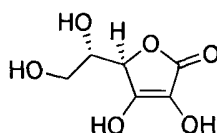
[0061] The term "pyruvic acid, or an ester or salt thereof" as used herein 25 means a compound having the structure



wherein R''' is selected from H, a cation, C₁₋₂₄alkyl, C₆₋₁₀aryl and C₃₋₁₀cycloalkyl, or pharmaceutically acceptable solvates or prodrugs thereof, as well as mixtures

thereof. The carboxylate (COOH) anion of pyruvic acid, $\text{CH}_3\text{COCOO}^-$, is pyruvate, which can be for example sodium pyruvate, calcium pyruvate, potassium pyruvate, magnesium pyruvate, or dihydroxyacetone pyruvate, or any other suitable salt.

[0062] The term "ascorbic acid" as used herein means a compound having
5 the structure



or pharmaceutically acceptable solvates or prodrugs thereof as well as mixtures thereof.

[0063] The term "cyclodextrin" as used herein refers to a family of
10 compounds that are made up of sugar molecules bound together in a ring. Non-limiting examples include 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD), alpha-cyclodextrin, beta-cyclodextrin and gamma-cyclodextrin.

[0064] The term "compositions of the application" as used herein includes a composition comprising one or more formulating agent(s) selected from pyruvic acid,
15 or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, wherein the composition is for use in formulating tigecycline. In an embodiment, the composition further comprises a chelator. In another embodiment, the composition further comprises a cyclodextrin. In yet a further embodiment, the composition further comprises tigecycline. A "tigecycline composition of the
20 application" is a composition comprising tigecycline and one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, optionally further comprising a chelator, optionally further comprising a cyclodextrin. Wherein a tigecycline composition is substantially free of EDTA and is a liquid formulation, it is an
25 embodiment that the composition is comprised in a light blocking vessel. In an embodiment, compositions of the application not comprising tigecycline, comprise pyruvic acid, or a salt or ester thereof, and ascorbic acid, and optionally one or more excipients, diluents or buffers, optionally further comprising a chelator, optionally further comprising a cyclodextrin.

[0065] The term "cancer" as used herein means a metastatic and/or a non-metastatic cancer, and includes primary and secondary cancers. Reference to cancer includes reference to cancer cells.

5 [0066] The term "hematological cancer" as used herein refers to cancers of blood and bone marrow, such as leukemia, multiple myeloma and lymphoma and includes primary and secondary cancers. Reference to hematological cancer includes reference to hematological cancer cells.

10 [0067] The term "leukemia" as used herein means any disease involving the progressive proliferation of abnormal leukocytes found in hematopoietic tissues, other organs and usually in the blood in increased numbers. Leukemia includes, but is not limited to, acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML).

15 [0068] The term "myeloma" and/or "multiple myeloma" as used herein means any tumor or cancer composed of cells derived from the hematopoietic tissues of the bone marrow. Multiple myeloma is also known as MM and/or plasma cell myeloma.

[0069] The term "lymphoma" as used herein means any disease involving the progressive proliferation of abnormal lymphoid cells. For example, lymphoma includes mantle cell lymphoma, Non-Hodgkin's lymphoma, and Hodgkin's lymphoma. Non-Hodgkin's lymphoma includes indolent and aggressive Non-Hodgkin's lymphoma. Aggressive Non-Hodgkin's lymphoma includes intermediate and high grade lymphoma. Indolent Non-Hodgkin's lymphoma includes low grade lymphomas.

25 [0070] The term "solid tumour cancer" as used herein refers to a cancer resulting in one or more solid tumours composed of cancer cells and includes, for example, a cancer of the lung, brain (glioblastomas, medulloblastoma, astrocytoma, oligodendroglioma, ependymomas), liver, thyroid, bone, adrenal, spleen, kidney, lymph node, small intestine, pancreas, colon, stomach, breast, endometrium, prostate, testicle, ovary, skin, head and neck, and esophagus.

[0071] The term "pharmaceutically acceptable" means compatible with the treatment of subjects, in particular humans.

30 [0072] The term "pharmaceutically acceptable salt" means an acid addition salt or a base addition salt which is suitable for, or compatible with, the treatment of subjects.

[0073] An acid addition salt which is suitable for, or compatible with, the treatment of subjects as used herein means any non-toxic organic or inorganic salt of any basic compound. Basic compounds that form an acid addition salt include, for example, compounds comprising an amine group. Illustrative inorganic acids which
5 form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and
10 salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, acid addition salts are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free
15 base forms. The selection of the appropriate salt will be known to one skilled in the art.

[0074] A base addition salt which is suitable for, or compatible with, the treatment of subjects as used herein means any non-toxic organic or inorganic base addition salt of any acidic compound. Acidic compounds that form a basic addition
20 salt include, for example, compounds comprising a carboxylic acid group. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic or aromatic organic amines such as methylamine, trimethylamine and picoline, alkylammonias or ammonia. The selection
25 of the appropriate salt will be known to a person skilled in the art.

[0075] The formation of a desired compound salt is achieved using standard techniques. For example, the neutral compound is treated with an acid or base in a suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

30 [0076] The term "prodrug" as used herein refers to a derivative of an active form of a known compound or composition which derivative, when administered to a subject, is gradually converted to the active form to produce a better therapeutic response and/or a reduced toxicity level. In general, prodrugs will be functional derivatives of the compounds disclosed herein which are readily convertible *in vivo*

into the compound from which it is notionally derived. Prodrugs include, without limitation, acyl esters, carbonates, phosphates, and urethanes. These groups are exemplary and not exhaustive, and one skilled in the art could prepare other known varieties of prodrugs. Prodrugs may be, for example, formed with available hydroxy, 5 thiol, amino or carboxyl groups. For example, the available OH and/or NH₂ in the compounds of the disclosure may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g. an acid chloride in pyridine). Some common esters which have been utilized as prodrugs are phenyl esters, aliphatic (C₁-C₂₄) esters, acyloxymethyl esters, carbamates and amino acid esters. In 10 certain instances, the prodrugs of the compounds of the disclosure are those in which the hydroxy and/or amino groups in the compounds is masked as groups which can be converted to hydroxy and/or amino groups *in vivo*. Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in "Design of Prodrugs" ed. H. Bundgaard, Elsevier, 1985.

15 [0077] Where the compounds according to the disclosure possess one or more than one asymmetric centre, they may exist as "stereoisomers", such as enantiomers and diastereomers. It is to be understood that all such stereoisomers and mixtures thereof in any proportion are encompassed within the scope of the present disclosure. It is to be understood that, while the stereochemistry of the 20 compounds of the disclosure may be as provided for in any given compound shown herein, such compounds may also contain certain amounts (e.g. less than 20%, less than 10%, less than 5%) of compounds having alternate stereochemistry.

[0078] The term "solvate" as used herein means a compound or its pharmaceutically acceptable salt, wherein molecules of a suitable solvent are 25 incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate". The formation of solvates will vary depending on the compound and the solvate. In general, solvates are formed by dissolving the compound in the appropriate solvent and 30 isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

[0079] The term "subject" as used herein includes all members of the animal kingdom including mammals, and suitably refers to humans.

[0080] The term "treating" or "treatment" as used herein and as is well understood in the art, means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, 5 diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. "Treating" and "treatment" can also mean prolonging survival as compared to expected survival 10 if not receiving treatment. "Treating" and "treatment" as used herein also include prophylactic treatment. For example, a subject with early stage leukemia can be treated to prevent progression or metastases, or alternatively a subject in remission can be treated with a composition described herein to prevent recurrence. Treatment methods comprise administering to a subject a therapeutically effective amount of a 15 composition described herein and optionally consists of a single administration, or alternatively comprises a series of applications. For example, the compositions described herein may be administered at least once a week. However, in another embodiment, the compositions of the application may be administered to the subject from about one time per three weeks, or about one time per week to about once daily 20 for a given treatment. In another embodiment, the composition of the application is administered twice daily. The length of the treatment period depends on a variety of factors, such as the severity of the disease, the age of the subject, the concentration, the activity of the compositions described herein, and/or a combination thereof. It will also be appreciated that the effective dosage of the composition used for the 25 treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the subject.

30 [0081] As used herein, the term "dosage form" refers to the physical form of a dose for example comprising a composition of the application, and includes without limitation liquid and solid dosage forms including, for example tablets, including enteric coated tablets, caplets, gelcaps, capsules, ingestible tablets, buccal tablets, troches, elixirs, suspensions, syrups, wafers, resuspendable powders, liquids,

solutions as well as injectable dosage forms, including, for example, sterile solutions and sterile powders for reconstitution, and the like, that are suitably formulated for injection.

[0082] As used herein, the term "effective amount" in terms of stabilizing
5 tigecycline, means an amount effective to inhibit degradation (e.g. compared to an initial amount or concentration) of tigecycline in a liquid formation for example by at least 10%, 20%, 30%, 40%, 50% or more for at least 2, 3, 4, 5, 6 or 7 days at 25°C.

[0083] As used herein, the term "therapeutically effective amount" means an
10 amount effective, at dosages and for periods of time necessary to achieve the desired result. For example in the context of treating a hematological malignancy, an effective amount is an amount that, for example, induces remission, reduces tumor burden, and/or prevents tumor spread or growth compared to the response obtained without administration of the composition. In the context of treating a bacterial
15 infection, an effective amount is an amount that for example, reduces and/or limits bacterial growth and/or spread, compared to the response obtained without administration of the composition. Effective amounts may vary according to factors such as the disease state, age, sex, and/or weight of the subject. The amount of a given composition that will correspond to such an amount will vary depending upon
20 various factors, such as the pharmaceutical formulation, the route of administration, the type of disease or condition, the identity of the subject or host being treated, and the like, but can nevertheless be routinely determined by one skilled in the art.

[0084] The term "administered" as used herein means administration of a therapeutically effective dose of a composition of the application to a cell either in cell culture or in a subject.

25 [0085] In understanding the scope of the present application, the term "comprising" and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also
30 applies to words having similar meanings such as the terms, "including" and "having", and their derivatives. The term "consisting" and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the

presence of other unstated features, elements, components, groups, integers and/or steps. The term "consisting essentially of", as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel
5 characteristic(s) of features, elements, components, groups, integers, and/or steps.

[0086] Further, terms of degree such as "substantially", "about" and "approximately" as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation of at least $\pm 5\%$ or at least $\pm 3\%$
10 of the modified term if this deviation would not negate the meaning of the word it modifies.

[0087] More specifically, the term "about" means plus or minus 0.1 to 50%, 5-50%, or 10-40%, 10-20%, 10%-15%, preferably 5-10%, most preferably about 5% or about 3% of the number to which reference is being made.

15 [0088] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural references unless the content clearly dictates otherwise. Thus for example, a composition containing "a compound" includes a mixture of two or more compounds. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates
20 otherwise.

[0089] In compositions comprising an "additional" or "second" component, the second component as used herein is chemically different from the other components or first component. A "third" component is different from the other, first, and second components, and further enumerated or "additional" components are
25 similarly different.

[0090] The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about."

30 The term "light blocking vessel" as used herein refers, for example to a vessel that at least substantially reduces or eliminates the penetration of light to the contents comprised therein. The expression "at least substantially reduces or eliminates the penetration of light to the contents comprised therein" as used herein refers, for

example to reducing or eliminating at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% of the light such as ambient light, UV light and/or specific wavelengths thereof from penetrating to the contents comprised in the vessel. The term "light blocking vessel" includes, for example, a vessel that is itself
5 comprised at least partially of a light blocking material, or a vessel which is not comprised of a light blocking material which has been adapted to be light blocking through, for example the addition of a light blocking material such as a light blocking paint, coating or chemical treatment, a light blocking sleeve, or a light blocking foil, for example an aluminum foil. The light blocking vessel can for example be a
10 coloured vessel such as a brown (or other coloured) glass vial that filters out a range of wavelengths. A vessel can for example be made light blocking after reconstitution into a liquid formulation. Light blocking vessels may be obtained through a commercial source, for example from Schott which produces vials and bottles that block light up to for example 500 nm or they may be prepared by methods known in
15 the art, for example, covering a vessel with an aluminum foil. The selection of a suitable light blocking vessel is within the ability of a person skilled in the art. In an embodiment, the light blocking vessel is a light blocking vial.

[0091] Further, the definitions and embodiments described in particular sections are intended to be applicable to other embodiments herein described for
20 which they are suitable as would be understood by a person skilled in the art. For example, in the above passages, different aspects of the invention are defined in more detail. Each aspect so defined can be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous can be combined with any other feature or features
25 indicated as being preferred or advantageous.

II. Methods and Compositions

[0092] Tigecycline, which is for example sold under the brand name Tygacil®, is presently used for the treatment of certain infections. Recently, the
30 inventors demonstrated that tigecycline displays preclinical activity against acute myeloid leukemia (AML) *in vitro* and *in vivo*. Mechanistically, the drug's anti-leukemia efficacy was related, for example, to its ability to inhibit mitochondrial protein synthesis.

[0093] Disclosed herein are compositions comprising tigecycline with improved stability and which retain, for example, anti-leukemic activity and anti-bacterial activity. For example, a formulation of tigecycline in the presence of EDTA, sodium pyruvate, and 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) remained
5 stable and active when stored at a concentration of 1 mg/mL for up to 7 days for example at 25°C. It was also demonstrated that the addition of ascorbic acid permitted increased concentrations of tigecycline in the solution. For example, compositions comprising tigecycline at drug concentrations of up to 5 mg/mL were stable and retained activity when stored at room temperature for at least 7 days. It
10 was further found that by keeping compositions comprising tigecycline at a pH of about 7 in the dark, EDTA could be eliminated from the composition, while maintaining the stability of the tigecycline.

[0094] Accordingly, an aspect of the application includes a composition comprising, consisting of, or consisting essentially of, tigecycline and one or more
15 formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. In an embodiment, the composition further comprises a chelator.

[0095] Another aspect of the present application is a composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating
20 agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers.

[0096] In another embodiment, the composition is substantially free of EDTA. In embodiments the composition is comprised in a light blocking vessel.

[0097] In an embodiment, the composition further comprises a cyclodextrin.

25 [0098] The components can be combined in various combinations.

[0099] In an embodiment, the composition comprises, consists of, or consists essentially of:

- (1) tigecycline and a chelator;
 - (2) tigecycline and pyruvic acid, or a salt or ester thereof; or
 - 30 (3) tigecycline and ascorbic acid;
- and optionally one or more excipients, diluents or buffers.

[00100] In another embodiment, the composition comprises, consists of, or consists essentially of:

(1) tigecycline, a chelator and pyruvic acid, or a salt or ester thereof;

(2) tigecycline, a chelator and a cyclodextrin; or

5 (3) tigecycline, a chelator and ascorbic acid;

and optionally one or more excipients, diluents or buffers.

[00101] In another embodiment, the composition comprises, consists of, or consists essentially of:

10 (1) tigecycline, pyruvic acid, or a salt or ester thereof and ascorbic acid; or

(2) tigecycline, pyruvic acid, or a salt or ester thereof and a cyclodextrin;

and optionally one or more excipients, diluents or buffers.

15 [00102] In yet another embodiment, the composition comprises, consists of, or consists essentially of tigecycline, pyruvic acid, or a salt or ester thereof, ascorbic acid and a cyclodextrin; and optionally one or more excipients, diluents or buffers.

[00103] In embodiments comprising, consisting of or consisting essentially of tigecycline and a chelator, the chelator is not EDTA or a derivative thereof.

20 [00104] Compositions combining tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally a cyclodextrin and/or one or more excipients, diluents or buffers result in stabilized tigecycline compositions. For example, the compositions in liquid form have increased stability when compared to liquid compositions comprising tigecycline alone. As demonstrated, compositions comprising the formulating agents
25 described herein and comprising tigecycline are stable for at least 7 days at 25 °C as demonstrated in Table 1 and 2. The increased stability at ambient temperature permits, for example, tigecycline processing and formulation without expensive low temperature manufacturing processes. Further, it has been found that reconstituted compositions are stable at 4 °C for at least 7 days (Example 2; 100 microliter
30 volumes tested in vitro and 5 mL volumes tested in vivo). Accordingly, in an embodiment, the disclosure includes a stabilized composition comprising tigecycline

and one or more formulating agents selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally cyclodextrin. In an embodiment, one or more formulating agent(s) is/are in an amount or concentration effective to limit degradation of the tigecycline in the liquid composition to for example less than 50%, 40%, 30%, 20%, or 10% (e.g. compared to an initial amount or concentration) after storage for example for at least 2, 3, 4, 5, 6 or 7 days at 25°C. In another embodiment, the composition comprising one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally cyclodextrin, in an amount or concentration effective to limit degradation of the tigecycline in the liquid composition to for example less than 50%, 40%, 30%, 20%, or 10% (e.g. compared to an initial amount or concentration) after storage for example for at least 2, 3, 4, 5, 6 or 7 days at 4 °C. As another example, the composition exhibits less than 50%, 40%, 30%, 20%, or 10% degradation over a minimum of 2, 3, 4, 5, 6, or 7 days at 25 °C, for example compared to a fresh tigecycline comprising preparation as determined by HPLC. Stability is also maintained at 4°C. For example, the composition exhibits less than 50%, 40%, 30%, 20%, or 10% degradation over a minimum of 2, 3, 4, 5, 6, or 7 days at 4 °C. In an embodiment the reconstituted solution can be stored at a temperature from about 2 °C to about 35 °C or any 0.1 °C in between or from about 4 °C to about 25 °C or any 0.1 °C in between.

[00105] In another embodiment the composition is a storage-stable, liquid, pharmaceutical composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agents selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents, or buffers, wherein the one or more formulating agents are effective to limit degradation of the tigecycline in the liquid composition to not more than about 20% over a period of 7 days at 25 °C. In another embodiment, the one or more formulating agents are effective to limit degradation of the tigecycline in the liquid composition to not more than about 10% over a period of 7 days at 25 °C.

[00106] It has further been found that by keeping compositions comprising tigecycline at a pH of about 7 in the dark at room temperature or 4 °C, compositions having increased stability of tigecycline for up to 7 days can be prepared that do not comprise EDTA (Example 2).

[00107] Accordingly, in an embodiment, the composition comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally cyclodextrin, increases tigecycline stability for at least 2, 3, 4, 5, 6 or 7 days at 25 °C. In another embodiment, the composition comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally cyclodextrin, increases tigecycline stability for at least 2, 3, 4, 5, 6 or 7 days at 4 °C. In another embodiment, the composition exhibits less than 50%, 40%, 30%, 20%, or 10% degradation over a minimum of 2, 3, 4, 5, 6, or 7 days at 25 °C. In a further embodiment, the composition exhibits less than 50%, 40%, 30%, 20%, or 10% degradation over a minimum of 2, 3, 4, 5, 6, or 7 days at 4 °C. It is an embodiment that the temperature is from about 2 °C to about 35 °C or any 0.1 °C in between or from about 4 °C to about 25 °C or any 0.1 °C in between.

[00108] The combination of, for example, one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid, optionally a chelator, and optionally cyclodextrin, inhibits the oxidative degradation of tigecycline. Glycylcyclines are a structurally related class of compounds and other glycylcyclines are sensitive to oxidation. Accordingly, the compositions can comprise another glycylcycline instead of tigecycline.

[00109] The components can also be combined without tigecycline, and subsequently combined with tigecycline, for example to reconstitute a solid and/or powder formulation (e.g. lyophilized) of tigecycline.

[00110] Accordingly, another aspect of the application includes a composition comprising, consisting of, or consisting essentially of pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. Such a composition can be used for example, for reconstituting and/or diluting a tigecycline formulation. It is a further embodiment that the composition is a liquid formulation for reconstituting a tigecycline formulation.

[00111] A further aspect of the present application is a composition for reconstituting a solid or powder formulation of tigecycline. In an embodiment, the composition comprises, consists of, or consists essentially of two or more of a chelator, pyruvic acid, or a salt or ester thereof, ascorbic acid and a cyclodextrin; and optionally one or more excipients, diluents or buffers.

[00112] In another embodiment, the composition comprises, consists of, or consists essentially of:

(1) a chelator and pyruvic acid, or a salt or ester thereof;

(2) a chelator and a cyclodextrin; or

5 (3) a chelator and ascorbic acid;

and optionally one or more excipients, diluents or buffers.

[00113] In another embodiment, the composition comprises consists of, or consists essentially of:

(1) pyruvic acid, or a salt or ester thereof and ascorbic acid; or

10 (2) pyruvic acid, or a salt or ester thereof and a cyclodextrin;

and optionally one or more excipients, diluents or buffers.

[00114] In an embodiment, the diluent is water, saline or other suitable diluents for intravenous administration.

[00115] In an embodiment, the composition is for reconstituting a tigecycline
15 composition for example, a powder (e.g. lyophilized) tigecycline formulation available from Pfizer (previously Wyeth Pharmaceuticals), for example sold under the brand name Tygacil™ to give a reconstituted formulation. It is an embodiment where the reconstituted formulation comprises tigecycline and is substantially free of EDTA, the reconstituted formulation is optionally comprised in a light blocking vessel.

20 [00116] The compositions, for example, inhibit the oxidative degradation of tigecycline. As mentioned, glycylicyclines are structurally related and other glycylicyclines are sensitive to oxidation. Accordingly, such compositions can also be used for reconstituting and/or diluting other glycylicycline formulations. Similarly the methods of making and using embodiments described for tigecyclines are applicable
25 to other oxidation sensitive glycylicyclines. An oxidation sensitive glycylicycline is for example a glycylicycline that exhibits oxidation induced degradation in a saline formulation of at least, 30%, 40%, 50%, 60%, 70% or more after 7 days at 25°C.

[00117] A number of compositions comprising different combinations and concentrations of components disclosed herein were tested, (see Examples 1-3).

[00118] For example, the following concentrations of additives were tested: 0.6 to 300 mg/mL (sodium pyruvate), 0.06 to 30 mg/mL (EDTA), 0.5 to 250 mg/mL (2-hydroxypropyl-beta-cyclodextrin) and 0.3 to 150 mg/mL (L-ascorbic acid). After 7 day-incubation the intact tigecycline was measured by HPLC with an established method. TEX leukemia cells were treated with increasing concentration of tigecycline in the different formulations at 37 °C in a CO₂ incubator and after seventy two hours of incubation, cell viability was determined. Tigecycline concentration varied from 0.5 to 50 microM (e.g. 1 – 5 mg/mL)

[00119] As described for example in Example 1, compositions comprising tigecycline and one or more formulating agent(s) selected from pyruvate, EDTA, and ascorbic acid optionally in combination with 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) showed increased tigecycline stability. For example, tigecycline dissolved in saline and incubated at room temperature for 7 days, results in total degradation of the drug as measured by HPLC and loss of activity as an antimicrobial and an anti-leukemic agent. When reconstituted for example with EDTA, pyruvate, and 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD), the drug retained stability, anti-leukemic activity, and anti-bacterial activity after incubation at room temperature for 7 days when dissolved at a concentration of 1 mg/mL. The addition of ascorbic acid up to 3 mg/L further improved the stability and anti-leukemic activity and anti-bacterial activity when dissolved at drug concentrations up to 5 mg/mL and stored at room temperature for 7 days. Formulations that retained stability and anti-leukemic activity also continued to inhibit the enzymatic activity of the Complex IV respiratory chain enzyme in the mitochondria.

[00120] Compositions are described in Table 2 that also increased tigecycline stability. In an embodiment, the composition is a composition described in Table 1 or 2.

[00121] In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid, is present in the composition in a concentration of about 0.06% to about 30% (w/v), or any 0.01% increment in between. In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid, is present in the composition in a concentration of about 1% to about 25% (w/v). In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid is present in the composition in a concentration of at least about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10% (w/v). In another embodiment, the pyruvic acid, or salt or ester of pyruvic acid, is

present in the composition in a concentration of at most about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29% or
5 about 30% (w/v).

[00122] In an embodiment, the chelator is present in the composition in a concentration of about 0.006% to about 3% (w/v), or any 0.001% increment in between. In an embodiment, the chelator is present in the composition in a concentration of about 0.1% to about 2.5% (w/v). In an embodiment, the chelator is
10 present in the composition in a concentration of at least about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9% or about 1% (w/v). In another embodiment, the chelator is present in the composition in a concentration of at most about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about
15 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9% or about 3.0% (w/v).

[00123] In an embodiment, the cyclodextrin is present in the composition in a concentration of about 0.05% to about 25% (w/v), or any 0.01% increment in between. In an embodiment, the cyclodextrin is present in the composition in a concentration of about 1% to about 25% (w/v). In an embodiment, the cyclodextrin is
20 present in the composition in a concentration of at least about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10% (w/v). In another embodiment, the cyclodextrin is present in the composition in a concentration of at most about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, about 22%, about 23%, about 24% or about 25%
25 (w/v).

[00124] In an embodiment, ascorbic acid is present in the composition in a concentration of about 0.003% to about 15% (w/v), or any 0.001% increment in between. In an embodiment, the ascorbic acid is present in the composition in a concentration of about 0.006% to about 15% (w/v). In an embodiment, ascorbic acid
30 is present in the composition in a concentration of at least about 0.01%, about 0.025%, about 0.05%, about 0.075%, about 0.1%, about 0.3%, about 0.6%, about

0.9%, about 1.2%, about 1.5%, about 1.75%, about 2%, about 2.25%, about 2.5%, about 2.75% or about 3 (w/v). In another embodiment, the ascorbic acid is present in the composition in a concentration of at most about 4%, about 5%, about 6%, about 7%, about 8%, about 9% or about 10% (w/v).

5 [00125] In an embodiment, for every about 1 to about 5 g/L of tigecycline, the composition comprises about 6 to about 600 g/L of pyruvic acid, or salt or ester of pyruvic acid, about 5 to about 500 g/L of a cyclodextran, and/or about 0.3 to about 300 g/L of ascorbic acid. A person skilled in the art would be familiar with calculating quantities for a desired quantity of tigecycline for example based on these ratios.

10 [00126] Tigecycline can be administered in formulations described herein as an intravenous infusion. A concentrated tigecycline solution can be prepared comprising for example about 1 mg/ml to about 100 mg/ml, optionally about 5 mg/ml to about 25 mg/ml or about 10 mg/ml tigecycline.

[00127] The concentrated solution can be diluted prior to administration for
15 example to about 200 mL, 500 mL, 1000mL or 2000 mL and comprise for example about 50 mg to about 2000 mg, or for example about 50 mg to about 500 mg of tigecycline.

[00128] The amount of formulating agent depends for example on the final
20 volume of reconstitution. As the volume is increased, additional formulating agent is need to maintain the concentration of formulating agent.

[00129] The weight per volume ranges for each of the components provided
can pertain for example to a reconstituted liquid formulation of tigecycline wherein the concentration of tigecycline is from about 0.025 mg/ml to about 25 mg/ml, optionally from about 0.1 mg/ml to about 20 mg/mL, from about 0.5 mg/mL to about
25 10 mg/mL or from about 1 mg/mL to 10 mg/mL in the reconstituted formulation. The weight per volume ranges can also for example pertain to compositions without tigecycline which are used for formulating tigecycline. A person skilled in the art would through routine calculations and/or testing be able to determine scaled quantities.

30 [00130] In an embodiment, tigecycline compositions of the application are liquid formulations and the volume of the formulation is selected from about 0.5 mL to 5mL, from about 5 mLs to about 10 mLs, from about 10mL to about 100 mL, from about 100 mL to about 200 mL, from about 200 to about 300mL, about 300 mL to

about 400 ml, about 400 to about 500 mL and from about 500 mL to about 2000 mL.

[00131] The volume of compositions for reconstituting tigecycline can be any convenient volume, for example comprised in a 5 mLs vessel for reconstituting
5 tigecycline solid formulations of for example 100 mg, or larger volumes for diluting tigecycline for patient administration, in an embodiment, about 100 mLs, about 200 mLs, about 300 mLs, about 400 mLs, about 500 mLs, about 1000mLs or about 2000 mLs.

[00132] In an embodiment, the weight per volume concentrations of the one or
10 more formulating agents is the concentration in a solution for administration. In another embodiment, the weight per volume concentrations of the one or more formulating agents is the concentration in a concentrated solution which is to be diluted prior to administration.

[00133] In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid, in
15 solid formulation is present in the composition in a concentration of about 20% to about 99% (w/w), or any 1% increment in between. In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid, is present in the composition in a concentration of about 30% to about 95% (w/w). In an embodiment, the pyruvic acid, or salt or ester of pyruvic acid is present in the composition in a concentration of at least about
20 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90% or about 98% (w/w).

[00134] In an embodiment, the chelator in solid formulation is present in the composition in a concentration of about 0.04% to about 86% (w/w), or any 0.1% increment in between. In an embodiment, the chelator is present in the composition
25 in a concentration of about 0.1% to about 56% (w/w). In an embodiment, the chelator is present in the composition in a concentration of at least about 0.4%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20% 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75% or about 80% (w/w).

30 [00135] In an embodiment, the cyclodextrin, in solid formulation is present in the composition in a concentration of about 20% to about 99% (w/w), or any 1% increment in between. In an embodiment, the cyclodextrin, is present in the composition in a concentration of about 30% to about 95% (w/w). In an embodiment,

the cyclodextrin is present in the composition in a concentration of at least about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90% or about 98% (w/w).

[00136] In an embodiment, ascorbic acid in solid formulation is present in the composition in a concentration of about 0.02% to about 98% (w/v), or any 0.01% increment in between. In an embodiment, the ascorbic acid is present in the composition in a concentration of about 0.06% to about 75% (w/v). In an embodiment, ascorbic acid is present in the composition in a concentration of at least about 1%, about 2.5%, about 5%, about 7.5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 60%, about 70% or about 75 (w/w).

[00137] In an embodiment, the formulation is a solid formulation such as a lyophilized powder. In an embodiment, for every about 1 to about 5 grams of tigecycline, the composition comprises about 6 to about 600 grams of pyruvic acid and/or a salt thereof, about 5 to about 500 grams of a cyclodextran, and/or about 0.3 to about 300 grams of ascorbic acid. A person skilled in the art would be familiar with calculating quantities for a desired quantity of tigecycline for example based on these ratios.

[00138] In an embodiment, the weights are based on the total weight of a tigecycline composition) of the application (e.g. a composition of tigecycline and one or more formulating agents or a composition for formulating tigecycline disclosed herein.

[00139] As mentioned above, the solid formulations can be reconstituted to provide liquid formulations.

[00140]

[00141] The molecular weight of tigecycline is about 585.65 g/mol. The molecular weight of pyruvic acid is about 88.06 g/mol. The molecular weight of sodium pyruvate is about 110.04 g/mol. The molecular weight of ascorbic acid is about 176.12 g/mol. Commercially available 2-hydroxypropyl-beta-cyclodextrins are available having varied molecular weight depending on the degree of substitution of 2-hydroxypropyl (C_3H_7O) per glucose unit. For example, a 2-hydroxypropyl-beta-cyclodextrin having 0.6 molar substitution is reported to have an average molecular weight of about 1,380 g/mol; a 2-hydroxypropyl-beta-cyclodextrin having 0.6-0.8

molar substitution is reported to have an average molecular weight of about 1,396 g/mol, a 2-hydroxypropyl-beta-cyclodextrin having 0.8 molar substitution is reported to have an average molecular weight of about 1,460 g/mol; and a 2-hydroxypropyl-beta-cyclodextrin having 1.0 molar substitution is reported to have an average
5 molecular weight of about 1,540 g/mol. A person skilled in the art could determine the molar ratios of tigecycline to each of these components to for example identify the molar ratios of the ranges provided and to determine the amount of a related compound that could be used. For example, the molecular weight ratio of sodium pyruvate to tigecycline for the ranges provided could be computed and used to
10 determine the amount of a different pyruvate, such as a pyruvate ester that should be included in a composition.

[00142] The molecular weight of tigecycline is about 585.65 g/mol and a concentration of 1 mg/mL is about 0.0017 mol/L, 2.5 mg/mL is about 0.004 mol/L and 5 mg/mL is about 0.008 mol/L. The molecular weight of sodium pyruvate is about
15 110.04 g/mol and a concentration of about 60 mg/mL is about 0.544 mol/L. The molar ratio of the components is readily calculated and a person skilled in the art would readily be able to calculate the weight of for example pyruvic acid to give a similar molar ratio.

[00143] The molar concentrations of the components tested in Example 1 are
20 provided therein.

[00144] In an embodiment, the chelator is selected from EDTA, EGTA, citrates, and tartrates. In another embodiment, the chelator is selected from EGTA, citrates, and tartrates. In a further embodiment, the chelator is penicillamine or dimercapto-propane sulfonate. In another embodiment, the salt or ester of pyruvic
25 acid is a pyruvate, such as sodium pyruvate, calcium pyruvate, potassium pyruvate, magnesium pyruvate, or dihydroxyacetone pyruvate. In a further embodiment, the cyclodextrin is selected from 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD), alpha-cyclodextrin, beta-cyclodextrin and gamma-cyclodextrin.

[00145] In an embodiment, the composition is a liquid formulation, including a
30 liquid formulation prior to lyophilizing comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. In another embodiment, the composition is a liquid formulation, including a liquid formulation prior to lyophilizing comprising

one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers. In an embodiment, the liquid formulation further comprises a cyclodextrin, and/or optionally tigecycline. For example, the composition comprising one or more
5 formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers and/or cyclodextrin or the composition comprising one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers and/or cyclodextrin, can be
10 provided as a liquid which is used as is to reconstitute tigecycline.

[00146] Alternatively, the composition can further comprise tigecycline and be provided as a liquid formulation, for example for administration.

[00147] In an embodiment, the composition is a powder comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and
15 ascorbic acid; and optionally one or more excipients, diluents or buffers (in dry form), a cyclodextrin and/or tigecycline. In another embodiment, the composition is a powder comprising one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers, a cyclodextrin and/or tigecycline.

20 [00148] In yet another embodiment, the powder is a lyophilized formulation for reconstitution. For example, the composition can comprise tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers and/or cyclodextrin, wherein the composition is a powder, for example to be
25 reconstituted with water and/or saline, or other suitable diluent. In another embodiment the composition can comprise tigecycline and one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers and/or cyclodextrin, wherein the composition is a powder, for example to be reconstituted with water and/or saline, or
30 other suitable diluent.

[00149] Alternatively, the composition can comprise one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, wherein the composition is a

powder, for example to be reconstituted with water or saline or other suitable diluent. In another embodiment the composition can comprise one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, wherein the composition is a powder, for example to be reconstituted with water or saline or other suitable diluent. Once reconstituted, the composition comprising the one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, and/or cyclodextrin or the composition comprising the one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally one or more excipients, diluents or buffers, and/or cyclodextrin can be used to reconstitute a powder formulation of tigecycline to give a reconstituted formulation. Where such a reconstituted formulation comprises tigecycline and is substantially free of EDTA, it is an embodiment that the reconstituted formulation is comprised in a light blocking vessel.

[00150] As disclosed elsewhere, the composition can be reconstituted with saline, water or other suitable diluents for parenteral administration. Where the composition of the application is a lyophilized formulation for example, the ranges and concentrations provided can describe the ranges and concentrations corresponding to the reconstituted or liquid formulation.

[00151] In an embodiment, the composition comprises the components described in Table 1 or Table 2, with or without tigecycline.

[00152] Accordingly, in another embodiment, tigecycline is present in the composition in a concentration of about 0.05 mg/mL to about 100 mg/mL or any 0.01 mg/mL increment in between. In another embodiment, tigecycline is present in the composition in a concentration of about 0.1 mg/mL to about 50 mg/mL or any 0.1 mg/mL increment in between. In a further embodiment, tigecycline is present in the composition in a concentration of about 0.5 mg/mL to about 10 mg/mL or any 0.1 mg/mL increment in between. In another embodiment, tigecycline is present in the composition in a concentration of about 1 mg/mL to about 5 mg/mL or any 0.1 mg/mL increment in between.

[00153] In an embodiment, pyruvate is present in the composition in a concentration of about 0.6 mg/mL to about 300 mg/mL, or any 0.1 mg/mL increment

in between. In another embodiment, EDTA is present in the composition in a concentration of about 0.06 mg/mL to about 30 mg/mL or any 0.01 mg/mL increment in between. In yet another embodiment, 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) is present in the composition in a concentration of about 0.5 mg/mL to
5 about 250 mg/mL or any 0.1 mg/mL increment in between. In another embodiment, ascorbic acid is present in the composition in a concentration of about 0.03 mg/mL to about 150 mg/mL or any 0.01 mg/mL increment in between.

[00154] It was found that by keeping tigecycline compositions of the application at a pH of about 7 in the dark, EDTA can be eliminated from the
10 composition, while maintaining the stability of the tigecycline. Accordingly, in another embodiment, the composition is substantially free of EDTA.

[00155] In yet another embodiment, the composition comprises from about 0.6 mg/mL to about 300 mg/mL pyruvate or any 0.1 mg/mL increment in between, from about 0.06 mg/mL to about 30 mg/mL EDTA or any 0.01 mg/mL increment in
15 between, from about 0.5 mg/mL to about 250 mg/mL of 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) or any 0.1 mg/mL increment in between; and/or from about 0.03 mg/mL to about 150 mg/mL ascorbic acid or any 0.01 mg/mL increment in between.

[00156] In an embodiment, the composition comprises from about 0.6 mg/mL
20 to about 300 mg/mL pyruvate or any 0.1 mg/mL increment in between, from about 0.5 mg/mL to about 250 mg/mL of 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD) or any 0.1 mg/mL increment in between and/or from about 0.03 mg/mL to about 150 mg/mL ascorbic acid or any 0.01 mg/mL increment in between.

[00157] The tigecycline compositions of the application can comprise, for
25 example a composition containing a therapeutically effective amount of tigecycline.

[00158] Tigecycline can be administered in formulations described herein as an intravenous infusion. A concentrated solution can be prepared comprising for example about 1 mg/ml to about 100 mg/ml, optionally about 5 mg/ml to about 25 mg/ml or about 10 mg/ml.

30 [00159] In an embodiment, the composition of the application is formulated into a unit dosage form, such as dosage form reconstitutable for IV infusion.

[00160] For example an intravenous unit dosage form comprises an amount of the composition for example in a vial that can be reconstituted and administered by infusion, for example over a period of time such as 30 – 60 minutes one or twice daily.

5 [00161] For example, compositions comprising for example 100 mg or 50 mg, useful for example for treating bacterial infections, tigecycline can be administered during for example an about 30 minute to about one-hour infusion. Twice daily administration of 25-50 mg in 100-200mL infusions over 30 minutes to one hour can also be administered, for example every 12 hours. A single infusion of 100 mg for
10 example can result in peak serum concentrations of 0.9 to 1.1 micrograms/mL. (see for example WO2005/023263).

[00162] For example, the recommended dosage regimen of Tygacil® is an initial dose of 100 mg (typically diluted in 100 mLs), followed by 50 mg (typically diluted in 100 mLs) every 12 hours. Intravenous infusion should be administered over
15 approximately 30 to 60 minutes every 12 hours. The recommended duration of treatment for complicated skin infections is 5 -14 days and for community acquired infections is 7 -14 days.

[00163] In mammals, for example methicillin-resistant *S. aureus* may be treated with tigecycline in the range of 5 mg/kg to 60 mg/kg twice daily, more
20 preferably 10 mg/kg to 40 mg/kg, more preferably 12 mg/kg to 20 mg/kg. Appropriate dosages for treatment of other pathogens will be apparent to those skilled in the art. In its approved form (as Tygacil®), and in a specific embodiment, tigecycline is present in the composition at a unit dose of about 100 mg or about 50 mg.

[00164] As well, tigecycline doses that are subantibacterial can be useful to
25 improve the appearance of skin aging and scarring when applied topically to the affected area (see WO 2012/052563).

[00165] .According to the product monograph, diluted Tygacil can be store at room temperature for up to 24 hours, or up to 48 hours at 2-8C. Increased stabilization of tigecycline as demonstrated herein can allow for other unit dosage
30 forms administered over an increased number of days.

[00166] In an embodiment, the application provides dosage forms for treating cancer, wherein the dose is expected to be greater than the dose required for bacterial infection treatment.

[00167] In an embodiment, each unit dosage form of tigecycline present in a
5 tigecycline composition comprises from about 5 mg to about 2000 mg, from about 25 mg to about 1500 mg, from about 25 mg to about 1500 mg, from about 25 mg to about 1000 mg, from about 25 mg to about 700 mg, from about 25 mg to about 500 mg, from about 25 mg to about 350 mg, from about 25 mg to about 300 mg or from about 25 mg to about 250 mg of tigecycline.

10 [00168] In another embodiment, each unit dosage form of tigecycline present in a tigecycline composition comprises from about 100 mg to about 2000 mg, from about 100 mg to about 1500 mg, from about 100 mg to about 1000 mg, from about 100 mg to about 700 mg, from about 100 mg to about 500 mg, from about 100 mg to about 400 mg, from about 100 mg to about 350 mg, from about 100 mg to about 250
15 mg or from about 100 mg to about 200 mg of tigecycline.

[00169] In an embodiment, the tigecycline composition of the application is provided in a unit dosage form sufficient to produce a peak serum concentration (i.e. C_{max}) of tigecycline from about 0.5 micrograms/mL to about 100 micrograms/mL, from about 0.5 micrograms/mL to about 80 micrograms/mL, from about 0.5
20 micrograms/mL to about 60 micrograms/mL, from about 0.5 micrograms/mL to about 40 micrograms/mL, from about 0.5 micrograms/mL to about 20 micrograms/mL, or from about 0.5 micrograms/mL to about 10 micrograms/mL.

[00170] In another embodiment, the unit dosage form comprises sufficient tigecycline to produce a peak serum concentration (i.e. C_{max}) from about 1
25 micrograms/mL to about 100 micrograms/mL, from about 10 micrograms/mL to about 100 micrograms/mL, from about 25 micrograms/mL to about 100 micrograms/mL, from about 40 micrograms/mL to about 100 micrograms/mL, from about 60 micrograms/mL to about 100 micrograms/mL, or from about 80 micrograms/mL to about 100 micrograms/mL.

30 [00171] It should be understood, that all of these dosages are exemplary, and any dosage in-between these points is also expected to be of use in the methods described herein.

[00172] The tigecycline compositions of the application are suitably formulated into dermatological or cosmetic compositions suitable for topical administration. Accordingly, another aspect includes a dermatological or cosmetic composition comprising a tigecycline composition of the application and a dermatologically or
5 cosmetically acceptable excipient. It is an embodiment that the dermatological or cosmetic composition is comprised in a light blocking vessel.

[00173] In an embodiment, the composition is a pharmaceutical composition.

[00174] The compositions are suitably formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form
10 suitable for administration *in vivo*.

[00175] Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more than one pharmaceutically acceptable vehicles or diluents, and contained in
15 buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

[00176] Pharmaceutical compositions include, without limitation, lyophilized powders or aqueous or non-aqueous sterile injectable solutions or suspensions, which optionally further contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially compatible with the tissues or the blood of an
20 intended recipient. Other components that are optionally present in such compositions include, for example, water, surfactants (such as TweenTM), alcohols, polyols, glycerin and vegetable oils. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, tablets, or concentrated solutions or suspensions. The composition can be supplied, for
25 example, but not by way of limitation, as a lyophilized powder which is reconstituted with sterile water or saline prior to administration to the subject.

[00177] Suitable pharmaceutically acceptable carriers include essentially chemically inert and nontoxic compositions that do not interfere with the effectiveness of the biological activity of the pharmaceutical composition. Examples
30 of suitable pharmaceutical carriers include, but are not limited to, water, saline solutions, glycerol solutions, ethanol, N-(1(2,3-dioleoyloxy)propyl)N,N,N-trimethylammonium chloride (DOTMA), diolesyl-phosphotidyl-ethanolamine (DOPE), and liposomes. Such compositions should contain a therapeutically effective amount

of the compound(s), together with a suitable amount of carrier so as to provide the form for direct administration to the subject.

[00178] In an embodiment, the composition is a sterile parenteral formulation.

[00179] In an embodiment, the compositions described herein are
5 administered parenterally, for example, by infusion, inhalation or injection by a route such as intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, or intranasal. The composition can also be administered as an, aerosol. In the alternative, the tigecycline composition can be administered enterally such as byoral
10 administration.

[00180] In an embodiment, the composition is administered by intravenous infusion. In an embodiment, for example where the cancer is a solid tumour, the composition is administered by direct intratumoral injection. In an embodiment, the composition is administered by injection into tumour vasculature.

15 [00181] In an embodiment, where the composition components are for example lyophilized and/or in solid or powder form, the composition components are reconstituted in a suitable diluent. Non-limiting examples of suitable diluents include saline, such as 0.9% saline, water, 5% dextrose, Hartmann's solution, Ringer's solution and Ringer's lactate solution. Other diluents suitable for intravenous
20 administration known in the art can also be used including combinations of the foregoing.

[00182] To maintain its pH, the composition may include a pH modifying agent such as buffering agent, for example to maintain the pH of the composition in a solution between about 3 to about 8, about 5 to about 8, about 6 to about 8 or about
25 7. In another embodiment, the pH of the composition is about 4, in yet another embodiment the pH is about 5. The pH modifying agent can include any pharmaceutically acceptable acid or base capable of adjusting the pH of a composition of the application to between about 3 to about 8. Examples of pH modifying agents include but are not limited to sodium hydroxide, including 0.1 N
30 NaOH and 1N NaOH as well as other suitable bases, citric acid, acetic acid, lactic acid, hydrogenophosphoric acid, diethylamine, hydrochloric acid, including 1.0 N HCl, gentisic acid, lactic acid, and phosphoric acid.

[00183] The compositions of the application can include additional excipients including for example, a carbohydrate such as lactose for example lactose monohydrate. Suitable carbohydrates are disclosed in US Patent No. 7,879,828, herein incorporated by reference.

5 [00184] Also provided in another aspect is a method for preparing said compositions.

[00185] The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions that can be administered to subjects, such that an effective quantity of the active substance is
10 combined in a mixture with a pharmaceutically acceptable vehicle.

[00186] The compositions can be prepared using a number of acceptable methods. The methods described below are exemplary and non-limiting.

[00187] In an embodiment, the method is for preparing a liquid formulation. Liquid formulations can be prepared using methods known in the art. For example,
15 liquid formulations can be prepared by resuspending and/or diluting each component of the composition in a suitable diluent and combining the resuspended and/or diluted components to provide the desired concentrations. Alternatively, the components can be blended together to provide a solid formulation comprising each of the desired components of the composition of the application and subsequently
20 diluted with a suitable diluent such as water or saline.

[00188] For example, each of the one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers and/or cyclodextrin, can be resuspended and/or diluted with water or saline, such as sterile saline or intravenous
25 grade saline and subsequently combined to provide the desired concentrations. Alternatively, the one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers and/or cyclodextrin, can be combined and subsequently diluted with saline, such as sterile saline or intravenous grade saline to
30 provide the desired concentrations.

[00189] In an embodiment, stock solutions of tigecycline (Sequoia), sodium pyruvate (Bioshop), ethylenediaminetetraacetic acid, disodium salt (FisherBioTech),

2-hydroxypropyl-beta-cyclodextrin (Sigma) and L-ascorbic acid (Sigma) are prepared by dissolving in saline for example, at the following concentrations of 25 mg/mL, 300 mg/mL, 30 mg/mL, 250 mg/mL and 150 mg/mL, respectively. Formulations are prepared to obtain desired concentrations, for example

5 concentrations shown in Table 1 2 or 3.

[00190] An embodiment includes a method for preparing a composition described herein, comprising resuspending and/or diluting each component of the composition in a suitable diluent and combining the resuspended and/or diluted components to provide the desired concentrations or blending the components of the

10 composition together to provide a solid formulation comprising each of the components of the composition and optionally subsequently diluting the solid formulation with a suitable diluent such as water or saline.

[00191] In an embodiment, the method comprises:

- i) resuspending and/or diluting tigecycline and one or more
- 15 formulating agent(s) selected from one or more formulating agent(s) selected from a pyruvic acid, or a salt or ester thereof, a chelator, a cyclodextrin and ascorbic acid; and optionally one or more excipients or buffers in a suitable solvent;
- ii) filtering the solution of step i) under aseptic conditions;
- iii) filling a light blocking vessel with the filtered solution of ii)
- 20 iv) optionally lyophilizing the solution in iii) and
- v) stoppering and sealing the solution in step iii) or the lyophilizate in step iv).

[00192] In another embodiment, the composition is a liquid formulation comprising tigecycline and is substantially free of EDTA.

25 In an embodiment the method comprise adjusting the pH of the solution by adding a pH modulating agent, for example in an amount sufficient to render the pH of the solution in step i) or a reconstituted solution between 5 and 8, for example about 7.

[00193] In an embodiment, the method is for preparing a powder formulation. In an embodiment, the powder formulation comprises tigecycline and one or more

30 formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers and/or

cyclodextrin. A typical process for preparing a powder composition comprising tigecycline can involve dissolving the composition of the application comprising tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more
5 excipients, diluents or buffers and/or cyclodextrin in water and lyophilizing (freeze-drying) the solution to dryness to form solid cakes of amorphous tigecycline containing compositions. Prior to being administered to subjects, the cakes are reconstituted, often in saline, for example, 0.9% saline, to provide a tigecycline concentration of, for example, about 10 mg/mL, optionally 5 mg/mL, optionally 1
10 mg/mL. Lyophilizing may be accomplished by any pharmaceutically acceptable means.

[00194] The pH of tigecycline liquid formulations is adjusted by adding sufficient acid or buffering agent as described supra to the aqueous solution containing tigecycline to obtain a pH from about 3.0 to about 8.0, or any 0.1
15 increment in between. In an embodiment, the pH is about 7. In another embodiment, the pH of the tigecycline composition is at least mildly acidic having a pH in the range from 4 to 7.

[00195] The compositions of the application may be prepared for single-dosage use, wherein solutions of the application are lyophilized in individual vials, for
20 example light blocking vials. The vials can be reconstituted by adding sufficient diluent to achieve the desired concentration of tigecycline. Any pharmaceutically acceptable diluent may be used, for example diluents acceptable for intravenous administration.

[00196] Reconstituted solutions can be further diluted for example by mixing in
25 an intravenous bag containing a pharmaceutically acceptable diluent such as saline solution or 5% dextrose solution. The admixture may be administered to a subject alone or together with another pharmaceutical agent or composition.

[00197] Timed-release compositions can be formulated, e.g. liposomes or those wherein the active compound is protected with differentially degradable
30 coatings, such as by microencapsulation, multiple coatings, etc. As mentioned, it is also possible to freeze-dry the compounds described herein and use the lyophilizates obtained, for example, for the preparation of products for injection.

[00198] In another embodiment, the application describes a pharmaceutical composition wherein the dosage form is a liquid dosage form. A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[00199] In another embodiment, the disclosure describes a pharmaceutical composition wherein the dosage form is an injectable dosage form. An injectable dosage form is to be understood to refer to liquid dosage forms suitable for, but not limited to, intravenous, subcutaneous, intramuscular, or intraperitoneal administration. Solutions of compounds described herein can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Or for example, can be prepared in a sodium chloride solution, for example a 0.9% sodium chloride solution or a dextrose solution for example a 5% dextrose solution.

[00200] Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (2003 - 20th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

[00201] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that syringability exists.

[00202] Another aspect of the disclosure includes a method of treating a condition or disease comprising administering a tigecycline composition of the application to a subject in need thereof. In an embodiment, the condition or disease is a bacterial infection. In another embodiment, the condition or disease is cancer. In yet a further embodiment, the condition is a dermatological condition such as acne, scarring and/or skin aging.

[00203] Accordingly, a further aspect includes a method of treating a bacterial infection or a cancer comprising administering to a subject in need thereof an effective amount of a tigecycline composition of the application.

5 [00204] Another aspect includes a use of a tigecycline composition of the application for treating a bacterial infection or cancer.

[00205] A further aspect includes a tigecycline composition of the application for use in treating a bacterial infection or cancer.

10 [00206] A still further aspect includes a use of a tigecycline composition of the application for the manufacture of a medicament for the treatment of a bacterial infection or cancer.

[00207] Accordingly, an aspect of the present application includes a method of treating a bacterial infection comprising administering to a subject in need thereof an effective amount of a composition comprising tigecycline and one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; tigecycline and a chelator; tigecycline and pyruvic acid, or a salt or ester thereof; or tigecycline and ascorbic acid; and optionally one or more excipients, diluents or buffers. In an embodiment, the composition administered further comprises cyclodextrin and/or a chelator. Any of the compositions described herein comprising tigecycline can be administered.

20 [00208] In another aspect, the disclosure includes use of a tigecycline comprising composition of the application for treating a bacterial infection. Another aspect includes use of a tigecycline comprising composition of the application for the manufacture of a medicament for the treatment of a bacterial infection. In yet a further aspect, the disclosure includes a tigecycline comprising composition of the application for use in the treatment of a bacterial infection.

[00209] In an embodiment, the bacterial infection is a skin and/or skin structure infection or an intra-abdominal infection, a bacterial pneumonia, febrile neutropenia or sepsis.

30 [00210] In an embodiment, the composition is used to treat a skin and/or skin structure infection or an intra-abdominal infection. In yet another embodiment, the composition is used to treat a bacterial pneumonia. In an embodiment, the composition is used to treat febrile neutropenia or sepsis.

[00211] In an embodiment, the bacterial infection comprises a drug-resistant bacteria, for example a methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci or a tetracycline resistant organism.

5 [00212] In another embodiment, the bacterial infection is selected from an *Escherichia coli*, *Enterococcus faecalis* (e.g. vancomycin-susceptible strains), *Staphylococcus aureus* (e.g. methicillin-susceptible and -resistant strains), *Streptococcus agalactiae*, *Streptococcus anginosus*, *Streptococcus pyogenes* and *Bacteroides fragilis*. For example, the foregoing can cause complicated skin and skin
10 structure infections.

[00213] In another embodiment, the bacterial infection is selected from *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Enterococcus faecalis* (e.g. vancomycin-susceptible strains), *Staphylococcus aureus* (e.g. methicillin-susceptible strains), *Streptococcus*
15 *anginosus* grp. (includes, for example *S. anginosus*, *S. intermedius*, and *S. constellatus*), *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides vulgatus*, *Clostridium perfringens*, and *Peptostreptococcus micros*. For example, the foregoing can cause complicated intra-abdominal infections.

20 [00214] Accordingly, another aspect of the present application includes a method of treating a cancer comprising administering to a subject in need thereof an effective amount of a composition comprising tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid, or a salt or ester thereof and ascorbic acid; tigecycline and a chelator; tigecycline and pyruvic acid, or a salt or
25 ester thereof; or tigecycline and ascorbic acid; and optionally one or more excipients, diluents or buffers. In an embodiment, the composition administered further comprises cyclodextrin.

[00215] In another aspect, the disclosure includes use of a tigecycline comprising composition of the application for treating a cancer. Another aspect
30 includes use of a tigecycline comprising composition of the application for the manufacture of a medicament for the treatment of a cancer.

[00216] In yet a further aspect, the disclosure includes a tigecycline comprising composition of the application for use in the treatment of a cancer.

- [00217] In an embodiment, the cancer is a hematological cancer or a solid cancer.
- [00218] Cancers and cancer cells that can be treated include, but are not limited to, hematological cancers, including leukemia, lymphoma and myeloma, and
5 solid cancers, including for example tumors of the brain (glioblastomas, medulloblastoma, astrocytoma, oligodendroglioma, ependymomas), lung, liver, thyroid, bone, adrenal, spleen, kidney, lymph node, small intestine, pancreas, colon, stomach, breast, endometrium, prostate, testicle, ovary, skin, head and neck, and esophagus.
- 10 [00219] In an embodiment, the cancer is a hematological cancer. In an embodiment, the hematological cancer is a leukemia. In another embodiment, the hematological cancer is a myeloma. In an embodiment, the hematological cancer is a lymphoma.
- [00220] In an embodiment, the leukemia is selected from acute myeloid
15 leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML). In an embodiment, the leukemia is AML. In an embodiment, the leukemia is ALL. In an embodiment, the leukemia is CLL. In a further embodiment, the leukemia is CML. In an embodiment, the cancer cell is a leukemic cell, for example, but not limited to, an AML cell, an ALL cell, a CLL
20 cell or a CML cell.
- [00221] In a further embodiment, the hematological cancer is a myeloma. In another embodiment, the hematological cancer cell is a myeloma cell.
- [00222] In yet a further embodiment, the hematological cancer is a lymphoma. In an embodiment, the hematological cancer cell is a lymphoma cell.
- 25 [00223] In an embodiment, the cancer is a solid tumour cancer. In an embodiment, the solid tumour cancer is selected from ovarian cancer, prostate cancer, pancreas cancer and lung cancer. In an embodiment, the cancer cell is an ovarian cancer cell, a prostate cancer cell or a lung cancer cell.
- [00224] For example, pancreatic cancer cell lines and a lung cancer cell line
30 have been found to be responsive to tigecycline (IC 50 of about 5-10 μ M).
- [00225] It is demonstrated in PCT/CA2011/00258, Use of Tigecycline for Treatment of Cancer, herein incorporated by reference, that AML samples have

increased mitochondrial mass and/or DNA copy number of ND1 relative to human globin DNA. For example, ND1/human globulin (HGB) ratio is significantly increased in AML samples.

[00226] Cancer cells such as leukemia cells for example, with a mitochondrial
5 DNA copy number and/or mitochondrial mass that is at least 2-fold increased compared to the mitochondrial DNA copy number and/or mitochondrial mass of the control may be sensitive to tigecycline treatment.

[00227] A further aspect includes a method of treating a cancer such as a leukemia with an at least 2-fold increased mitochondrial DNA copy number and/or
10 mitochondrial mass compared to a control comprising administering to the subject in need thereof, an effective amount of a glycylicycline such as tigecycline.

[00228] A further aspect includes use of tigecycline for treating a cancer such as a leukemia with an at least 2-fold increased mitochondrial DNA copy number and/or mitochondrial mass compared to a control. Another aspect includes use of
15 tigecycline for the manufacture of a medicament for treating a cancer with an at least 2-fold increased mitochondrial DNA copy number and/or mitochondrial mass compared to a control. Yet another aspect includes tigecycline for treating a cancer with an at least 2-fold increased mitochondrial DNA copy number and/or mitochondrial mass compared to a control. For example, a cancer or cell
20 mitochondrial mass is assessed by taking a biopsy sample e.g. a test sample from a subject and determining the mitochondrial mass of the test sample cancer cells using for example a method known in the art and/or described in PCT/CA2011/00258, Use of Tigecycline for Treatment of Cancer, herein incorporated by reference.

[00229] Another aspect includes a method of treating a cancer such as a leukemia with an at least 2-fold increased mitochondrial DNA copy number and/or
25 mitochondrial mass compared to a control comprising administering to the subject in need thereof, an effective amount of a glycylicycline composition such as a tigecycline composition of the application.

[00230] A further aspect includes use of a tigecycline composition of the application for treating a cancer such as a leukemia with an at least 2-fold increased
30 mitochondrial DNA copy number and/or mitochondrial mass compared to a control. Another aspect includes use of a tigecycline composition of the application for the manufacture of a medicament for treating a cancer with an at least 2-fold increased

mitochondrial DNA copy number and/or mitochondrial mass compared to a control. Yet another aspect includes a tigecycline composition of the application for treating a cancer with an at least 2-fold increased mitochondrial DNA copy number and/or mitochondrial mass compared to a control. For example, a cancer or cell
5 mitochondrial mass is assessed by taking a biopsy sample e.g. a test sample from a subject and determining the mitochondrial mass of the test sample cancer cells using for example a method known in the art and/or described in PCT/CA2011/00258, Use of Tigecycline for Treatment of Cancer, herein incorporated by reference.

[00231] In an embodiment, the cancer e.g. leukemia and/or cancer cells e.g.
10 leukemia cells have at least a 3-fold increase, at least a 4-fold increase and/or at least a 5-fold increase in mitochondrial DNA copy number and/or mitochondrial mass compared to a control.

III. Kits

[00232] Another aspect of the disclosure is a kit comprising tigecycline and/or
15 a composition comprising tigecycline, optionally in a vessel such as a vial, for example a light blocking vessel such as a light blocking vial, described herein, optionally lyophilized, and a composition for reconstituting the tigecycline and/or the composition comprising tigecycline comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid and optionally
20 one or more excipients, diluents or buffers, also optionally lyophilized. In an embodiment, the composition further comprises a cyclodextrin and/or a chelator.

[00233] Each component of the reconstitution composition can be provided separately, for example to be combined at time of tigecycline and/or composition comprising tigecycline reconstitution. Alternatively, the components can be blended
25 together in a single formulation. The reconstitution composition can be a solid, for example a powder to be dissolved, with for example water, saline or other suitable diluent, and used to reconstitute the tigecycline and/or composition comprising tigecycline. The reconstitution composition can also be provided as a liquid, each component separately and/or combined in a solution.

30 [00234] The kit can comprise for example any of the compositions described herein where the components are comprised in a single vessel and/or in two or more vessels comprising one or more of the stabilizing components described herein.

[00235] The kit can further comprise instructions for use, for example indicating how the compositions are to be reconstituted and/or administered, including for example instructions for further diluting, such as mixing in an IV bag. In an embodiment, the instructions for reconstitution include instruction to store the
5 solution in the dark for example by adding a sleeve or foil to the reconstituted vial or vessel.

[00236] In another embodiment, the kit comprises a composition of the application in a vessel such as a vial, such as a sterile vial and/or a light blocking vessel, and optionally instructions for use.

10 [00237] In an embodiment, the vessel at least substantially reduces or eliminates at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.9% of the light from penetrating to the contents comprised in the vessel. In an embodiment, the vessel itself is comprised at least partially of a light blocking material, or a vessel which is not comprised of a light blocking material which has
15 been adapted to be light blocking through, for example the addition of a light blocking material such as a light blocking paint, coating or chemical treatment, a light blocking sleeve, or a light blocking foil, for example an aluminum foil. A vessel can for example be made light blocking after reconstitution into a liquid formulation. Light blocking vessels may be obtained through a commercial source or they may be
20 prepared by methods known in the art, for example, covering a vessel with an aluminum foil. The selection of a suitable light blocking vessel is within the ability of a person skilled in the art. In an embodiment, the light blocking vessel is a light blocking vial.

[00238] In an embodiment, the kit comprises multiple unit dosage forms for
25 example 2, 3, 4, 5, 6, 7, 8, 9 or 10 unit dosage forms, each comprising the same or different compositions described herein, for example tigecycline containing compositions of the application and/or formulations for reconstituting tigecycline.

[00239] The above disclosure generally describes the present application. A more complete understanding can be obtained by reference to the following specific
30 examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the application. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

[00240] The following non-limiting examples are illustrative of the present disclosure:

Examples

Example 1

5 Reagents

[00241] Stock solutions of Tigecycline (Sequoia), Sodium Pyruvate (Bioshop), Ethylenediaminetetraacetic acid, Disodium Salt (FisherBioTech), 2-hydroxypropyl-beta-cyclodextrin (Sigma) and L-Ascorbic Acid (Sigma) were dissolved in saline at the following concentrations: 25 mg/mL (0.043 mol/L), 300 mg/mL (2.7 mol/L), 30
10 mg/mL, 250 mg/mL (0.16 mol/L) and 150 mg/mL (0.85 mol/L), respectively. Formulations were prepared in Eppendorf tubes to obtain corresponding concentrations shown in Table 1. Formulations were stored at room temperature (25 °C) for 7 days. A fresh solution of tigecycline in saline was prepared just before analysis as a control.

15 [00242] The following concentrations of additives were tested: 0.6 to 300 mg/mL (0.0055 to 2.7 mol/L) of Sodium Pyruvate; 0.06 to 30 mg/mL (EDTA), 0.5 to 250 mg/mL (0.00032 to 0.16 mol/L) of 2-hydroxypropyl-beta-cyclodextrin; and 0.3 to 150 mg/mL (0.0017 to 0.85 mol/L) of L-Ascorbic Acid.

[00243] All in vitro experiments were conducted with 100 uL.

20

Drug stability

[00244] After 7-day-incubation the intact tigecycline was measured by HPLC with an established method. Samples were diluted 140-fold with mobile phase and subjected to HPLC-UV. Details are the following: Symmetry C18 Column (3.9*150
25 mm, 5 µm), mobile phase of 4 mM 1-Octanesulfonic Acid in Acetonitrile:Monosodium Phosphate, pH 3 (25/75), UV detection at 244 nm, flow 1 ml/min, injection volume 5 µL.

Cell viability

30 [00245] TEX leukemia cells were treated with increasing concentration of tigecycline in the different formulations at 37 °C in a CO₂ incubator.

[00246] Seventy-two hours after incubation, cell viability was determined by Alamar Blue and Sulforhodamine B assay methods. Tigecycline concentration varied from 0.5 to 50 μ M. TEX cells were treated for 72 hours

5 Bacterial growth

[00247] *E. coli* bacteria were treated with increasing concentrations of tigecycline in the different formulations for 18 hours. The minimum inhibitory concentration of tigecycline was determined by visual inspection and comparison to the untreated control.

10

Complex IV activity

[00248] TEX leukemia cells were treated with formulations having a 5 μ M concentration of tigecycline which were prepared from the different stock formulations for 72 hours. After treatment, mitochondria were isolated from the TEX
15 cells and the enzymatic activity of Complex IV (cytochrome C oxidase) and Citrate Synthase were determined by spectrophotometric methods as previously described (Skrtec et al., Cancer Cell 2011).

Results

20 [00249] After dissolving tigecycline in saline and incubation at room temperature for 7 days, all of the drug had degraded as measured by HPLC and the drug lost activity as an antimicrobial and an anti-leukemic agent. When reconstituted with EDTA, pyruvate, and 2-hydroxypropyl-beta-cyclodextrin (2-HP-beta-CD), the drug retained stability, anti-leukemic activity, and anti-bacterial activity after
25 incubation at room temperature for 7 days when dissolved at a concentration of 1 mg/mL. However, upon increasing the concentration of the drug in solution, stability and activity was lost. Therefore, the formulation was supplemented with ascorbic acid. The addition of ascorbic acid up to 3 mg/L improved the stability and anti-leukemic activity and anti-bacterial activity when dissolved at drug concentrations up
30 to 5 mg/mL and stored at room temperature for 7 days. Formulations that retained stability and anti-leukemic activity also continued to inhibit the enzymatic activity of the Complex IV respiratory chain enzyme in the mitochondria.

[00250] It was demonstrated that a formulation of tigecycline in the presence of EDTA (6 g/L), sodium pyruvate (60 g/L), and 2-hydroxylpropyl-beta-cyclodextrin (2-HP-beta-CD) (50 g/L) remained stable and active when stored at 1 mg/mL for up to 7 days. The addition of Ascorbic acid (3 mg/mL) permitted increased concentrations of tigecycline in the solution.

Example 2

[00251] It has been shown that as the concentration of tigecycline in solution increases, its stability generally decreases (see Table 1, also as indicated above 10 mg/mL tigecycline reconstituted in for example saline, 5% dextrose, or Ringer's lactate is stable for about 6 hours at room temperature whereas 1 mg/mL tigecycline so formulated is stable for 24 hours at room temperature). Due to stability issues noted at higher concentrations a typical concentration of tigecycline in i.v. infusions administered to humans is about 1 mg/mL. Increased concentration may be desirable. Also when tigecycline is administered to mice, the concentration of tigecycline is generally increased because only small volumes of fluid can be administered to mice (e.g. a higher concentration is needed to provide the desired dose). Accordingly, compositions comprising increased tigecycline concentrations were tested

[00252] All mice experiments were done with 5 mL aliquots.

[00253] The toxicity of each of the components in the compositions was tested for toxicity as well as all of the components together in mice without adjusting for pH. The mice used in the toxicity study received either a single or a repeat dose of the composition being tested in saline that was not adjusted for pH. Toxicity was observed in the mice for each of the compositions not adjusted for pH that were tested. The compositions were found to be acidic.

[00254] More specifically, toxicity experiments were performed with each component of the compositions of Table 2 and with all composition components together. SCID mice received either single doses daily- doses x 4- for each component alone or a mixture of the components dissolved in saline and then adjusted to a pH of 7. Mice were observed for signs of distress by cage-side exams. It was found that with dosing daily for a few days, neither the components nor the composition of the components was toxic.

[00255] The pH of the compositions was adjusted to 7 and toxicity was retested using mice. Compositions comprising EDTA (either alone or in combination with the other components) were observed to be toxic. However, compositions comprising the other components either alone, or in combination with each other, 5 including in combination with tigecycline were no longer observed to be toxic in mice under the conditions used.

[00256] Accordingly, compositions not comprising EDTA were prepared and studied. Table 2 shows the results of the stability of tigecycline kept at ambient temperature (25°C) in the dark monitored by HPLC for the various compositions 10 listed therein. Table 3 shows the results of the stability of tigecycline kept at 4 °C in the dark monitored by HPLC for the various compositions listed therein.

[00257] It was found that by keeping liquid formulations of the compositions not comprising EDTA at a pH of about 7 in the dark at either ambient temperature or at 4 °C, EDTA can be eliminated from the composition, while maintaining the stability 15 of the tigecycline. It is expected that brief exposure of such liquid formulations to light would not have a substantial effect on the maintenance of the stability of the tigecycline.

Table 1

Tigecycline mg/mL	Composition				Conditions		Intact Tigecycline after incubation by HPLC, %	IC ₅₀ of Tigecycline for TEX cell line, μ M		MIC, μ M	Complex IV in TEX cells, %
	Sodium Pyruvate 50g/L	EDTA [*] , 6g/L	HP- β -CD ^{**} , 50 g/L	L-Ascorbic Acid	Incubation Temperature, $^{\circ}$ C	Incubation period, days		Alamar Blue	Sulfohodamit, g B		
1	-	-	-	-	Fresh, no incubation	-	100	3.48 \pm 0.20	3.10 \pm 0.60	0.104 \pm 0.05	37 \pm 10
1	-	-	-	-	25	7	0.4	>20		>3.4	
1	+	-	-	-	25	7	40.1	6.74 \pm 0.32			
1	-	+	-	-	25	7	53.2	6.09 \pm 0.38			
1	-	-	+	-	25	7	1.7	>20			
1	-	-	-	+	25	7	87.9	4.93 \pm 0.30	4.19 \pm 0.52		
1	-	-	-	+(0.3g/L)	25	7	61.9	6.52 \pm 0.53	5.86 \pm 0.82		
1	-	-	-	+(3 g/L)	25	7	35.7	4.22 \pm 0.40	4.14 \pm 1.33		85 \pm 3
1	-	-	-	+(30 g/L)	25	7	94.6	3.73 \pm 0.18	3.03 \pm 0.29		
1	+	+	+	-	25	7	66.1	4.79 \pm 0.32			
1	+	+	+	-	25	7	72.8	5.58 \pm 0.31			
1	+	+	+	-	25	7	91.8 \pm 0.05	3.59 \pm 0.14	2.17 \pm 0.25		51 \pm 3
1	+	+	+	+(0.3g/L)	25	7	88.9	3.56 \pm 0.22	2.99 \pm 0.38		
1	+	+	+	+(3g/L)	25	7	65.3	2.81 \pm 0.20	3.46 \pm 0.52		42 \pm 2
1	+	+	+	+(3g/L)	25	7	72.4				
2.5	+	+	+	-	25	7	92.0 \pm 3.0	4.43 \pm 0.64	3.94 \pm 0.70	0.104 \pm 0.05	
2.5	+	+	+	+(3g/L)	25	7	67.3	4.52 \pm 0.13	2.81 \pm 0.35	0.104 \pm 0.05	
3.5	+	+	+	-	25	7	73.3	6.74 \pm 0.50			
5	+	+	+	-	25	7	44.7	9.14 \pm 0.40	7.61 \pm 1.47		39
5	+	+	+	-	25	7	62.5 \pm 5.5	6.60 \pm 0.12	3.67 \pm 0.15		45 \pm 12
5	-	-	-	+(0.3g/L)	25	7	0.8	>20			
5	-	-	-	+(3g/L)	25	7	56.2	6.07 \pm 0.25	5.76 \pm 0.54		
5	+	+	+	+(0.3g/L)	25	7	53.4	ND	ND		
5	+	+	+	+(3g/L)	25	7	63.4	6.07 \pm 0.34	5.23 \pm 0.61		45 \pm 5
5	+	+	+	+(0.3g/L)	25	7	60.0	6.24 \pm 0.31	3.99 \pm 0.63		
5	+	+	+	+(3 g/L)	25	7	67.4 \pm 1.3	5.35 \pm 0.36	3.58 \pm 0.29		32 \pm 5

* Ethylenediaminetetraacetic acid, Disodium Salt

** 2-Hydroxypropyl-beta-cyclodextrin

*** Minimum inhibitory concentration

Table 2

[illegible]

1	60	—	2	100 ± 8.7	101.7 ± 10.8	97.0 ± 7.5	96.0 ± 2.8			89.7 ± 8.7	4.76 ± 0.28	4.74 ± 0.68	125	42.2 ± 8.3
1	60	—	3	100 ± 7.1	101.2 ± 4.1	105.0 ± 7.8	99.7 ± 2.3			92.5 ± 6.1	4.25 ± 0.26		125	
1	—	50	0.3	100 ± 7.5	51.1 ± 7.3			35.7 ± 5.1						
1	—	50	0.6	100 ± 11.4	65.4	56.7	48.2			48.1				
1	—	50	1	100 ± 4.4	84.3 ± 6.3	71.4 ± 2.9	65.2 ± 1.8			55.2 ± 5.9				
1	—	50	2	100 ± 0.9	69.9 ± 4.1	58.1 ± 3.8	56.2 ± 5.2			48 ± 22				
1	—	50	3	100 ± 3.3	53.0 ± 8.8	66.5 ± 10.7	44.7 ± 8.8			44 ± 29				
1	60	50	—	100 ± 7.1	82.9 ± 9.3			67.5 ± 8.1		58.6 ± 2.2				
1	60	50	0.3	100 ± 10.2	72.2 ± 8.6			67.4 ± 5						
1	60	50	0.6	100 ± 13.2	90.9 ± 4.0	74.8 ± 6.8	89 ± 0.3			76.5 ± 0.2				
1	60	50	1	100 ± 1.12	94.9 ± 9.0	98.5 ± 7.5	94.0 ± 8.1			80.5 ± 7.8		4.50 ± 0.60	125	
1	60	50	2	100 ± 1.13	97.5 ± 7.1	99.6 ± 3.5	93.6 ± 3.1			83.0 ± 8.2	4.68 ± 0.28	4.16 ± 0.51	125	39.7 ± 5.7
1	60	50	3	100 ± 4.04	97.4 ± 2.6	99.9 ± 3.1	104.5 ± 0.6			88.4 ± 8.1	4.32 ± 0.28		125	
2.5	60	50	0.3	100 ± 15.2	74.1 ± 1.1			51.8 ± 5.8						
2.5	60	50	0.6	100 ± 1.4	78 ± 0.1			56 ± 13.1						
2.5	60	50	2	100 ± 15.2	90.8 ± 13.1	93.7 ± 6.1	87.1 ± 11.1			68				
2.5	60	50	3	100 ± 1.4	79.2 ± 0.2	84.0 ± 2.4	73.3 ± 8.0			78				

Samples were incubated in the dark at ambient temp (25°C) and the pH of the final solution was adjusted to 7

*ComplexIV was measured after 48-hr treatment TEX with 5 µM TIG that has been incubated for 5 days at ambient temperature, data are normalized to

5 Citrate Synthase content.

Tig: tigecycline; Pyr: sodium pyruvate; CD: 2-hydroxypropyl-beta-cyclodextrin; AA: ascorbic acid; MIC: minimum inhibitory concentration

Table 3

Composition					Conditions		Intact Tigecycline after incubation by HPLC, %
Tigecycline, mg/mL	Sodium Pyruvate, 60g/L	EDTA, 6g/L	HP-β-CD, 50 g/L	L-Ascorbic Acid	Incubation Temperature, °C	Incubation period, days	
1	-	-	-	-	Fresh, no incubation		100
1	-	-	-	-	4	7	3.7
1	+	+	+	-	4	7	95.6
1	+	+	+	+(3g/L)	4	7	96.2
2.5	+	+	+	-	4	7	89.1
2.5	+	+	+	+(3g/L)	4	7	92.7
5	+	+	+	-	4	7	77.2
5	+	+	+	+(3 g/L)	4	7	95.4

10 [00258] While the present disclosure has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the disclosure is not limited to the disclosed examples. To the contrary, the disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

15 [00259] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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Claims:

1. A composition comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers.
- 5 2. The composition of claim 1, the composition further comprising a chelator.
3. The composition of claim 1, wherein the composition is substantially free of EDTA.
4. The composition of any one of claims 1 to 3, the composition further comprising a cyclodextrin.
- 10 5. A composition comprising, consisting of, or consisting essentially of pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers, optionally wherein the composition is for use in formulating tigecycline.
6. The composition of claim 5, the composition further comprising a chelator.
7. The composition of claim 5, wherein the composition is substantially free of EDTA.
- 15 8. The composition of any one of claims 5 to 7, the composition further comprising a cyclodextrin.
9. The composition of any one of claims 5 to 8, wherein the composition is a liquid formulation for reconstituting a tigecycline formulation.
10. The composition of any one of claims 1 to 4, containing a therapeutically effective
20 amount of tigecycline.
11. The composition of any one of claims 1-8 and 10, wherein the composition is a liquid formulation or a lyophilized formulation for reconstitution.
12. The composition of any one of claims 1-4, 10 and 11, wherein the tigecycline in the composition is in a concentration of about 0.5 mg/mL to about 100 mg/mL; about
25 1 mg/mL to about 50 mg/mL or about 1 mg/mL to about 10 mg/mL.

13. The composition of any one of claims 1 to 12, wherein the composition comprises about 0.6 mg/mL to about 300 mg/mL pyruvate and/or about 0.003 mg/mL to about 150 mg/mL ascorbic acid.
14. The composition of any one of claims 1-8 and 10-13, wherein the composition
5 components are lyophilized and/or in powder form, and the composition components are reconstituted in a suitable diluent, to give a reconstituted formulation, wherein if the reconstituted formulation comprises tigecycline and is substantially free of EDTA, the reconstituted formulation is comprised in a light blocking vessel.
15. The composition of any one of claims 1 to 14, the composition further comprising
10 a pH modifying agent.
16. The composition of any one of claims 2, 4, 6 and 8-14, wherein the chelator is selected from EGTA, citrates, and tartrates.
17. The composition of any one of claims 1 to 16, wherein the salt or ester of pyruvic acid is a pyruvate, selected from sodium pyruvate, calcium pyruvate, potassium
15 pyruvate, magnesium pyruvate, or dihydroxyacetone pyruvate.
18. The composition of any one of claims 4 and 8-17, wherein the cyclodextrin is selected from 2-hydroxylpropyl-beta-cyclodextrin (2-HP-beta-CD), alpha-cyclodextrin, beta-cyclodextrin and gamma-cyclodextrin.
19. The composition of any one of claims 1 to 18, wherein the pH of a liquid
20 formulation of the composition is about 7.
20. The composition of any one of claims 1 to 19, wherein the composition is comprised in a light blocking vessel.
21. The composition of any one of claims 1 to 20, wherein the formulating agent(s)
25 are effective to limit degradation of the tigecycline in the liquid composition to not more than about 20% over a period of 7 days at 25°C.
22. The composition of any one of claims 1 to 21, wherein the composition is comprised in a unit dosage form and the amount of tigecycline in the unit dosage form is selected from about 5 mg to about 2000 mg, from about 10 mg to about 1500 mg, from about 25 mg to about 1500 mg, from about 25 mg to about 1000 mg, from

about 25 mg to about 700 mg, from about 25 mg to about 500 mg, from about 25 mg to about 350 mg, from about 25 mg to about 300 mg or from about 25 mg to about 250 mg of tigecycline.

23. The composition of any one of claims 1 to 22, wherein the composition is a liquid
5 formulation and the volume of the formulation is selected from about 0.5 mL to 5mL, from about 5 mLs to about 10 mLs, from about 10mL to about 10000 mL, from about 100 mL to about 200 mL, from about 200 to about 300mL, about 300 mL to about 400 ml, about 400 to about 500 mL and from about 500 mL to about 2000 mL.

24. A pharmaceutical composition comprising the composition of any one of claims
10 1-4 and 10-21 and a pharmaceutically acceptable carrier.

25. The pharmaceutical composition of claim 24, wherein the pharmaceutical composition is comprised in a light blocking vessel.

26. A storage-stable, liquid, pharmaceutical composition of any one of claims 24 to
15 26 comprising, consisting of, or consisting essentially of, tigecycline and one or more formulating agent(s) selected from a chelator, pyruvic acid or a salt or ester thereof, and ascorbic acid; and optionally one or more pharmaceutically excipients, diluents, or buffers, wherein the formulating agent(s) are effective to limit degradation of the tigecycline in the liquid composition to not more than about 20% over a period of 7 days at 25°C.

20 27. The composition of any one of claims 1-4 and 10-23 for use in treating a cosmetic condition or the pharmaceutical composition of any one of claims 24 to 26 for use in treating a bacterial infection and/or cancer.

28. A method for preparing a composition of any one of claims 1 to 27, comprising
25 resuspending and/or diluting each component of the composition in a suitable diluent and combining the resuspended and/or diluted components to provide the desired concentrations or blending the components of the composition together to provide a solid formulation comprising each of the components of the composition and optionally subsequently diluting the solid formulation with a suitable diluent such as water or saline.

29. The method of claim 28, wherein the composition is comprised in a light blocking vessel.

30 The method of claim 28 or 29, wherein the method comprises

5 i) resuspending and/or diluting tigecycline and one or more
formulating agent(s) selected from a pyruvic acid, or a salt or ester thereof, a
chelator, a cyclodextrin and ascorbic acid; and optionally one or more excipients or
buffers in a suitable solvent;

ii) filtering the solution of step i) under aseptic conditions;

iii) filling a light blocking vessel with the filtered solution of ii);

10 iv) optionally lyophilizing the solution in iii); and

v) stoppering and sealing the solution in step iii or the lyophilizate in
step iv).

31. The method of any one of claims 28 to 30, wherein the composition is a liquid
formulation comprising tigecycline and is substantially free of EDTA.

15 32. The method of any one of claims 28 to 31, further comprising adjusting the pH of
the solution using a pH modifying agent.

33. A method of treating a bacterial infection and/or a cancer comprising
administering a composition of any one of claims 1-4, 10-23 or a pharmaceutical
composition of any one of claims 24 to 27 comprising an effective amount of
20 tigecycline, to a subject in need thereof.

34. The method of claim 33, wherein the composition or pharmaceutical composition
is for treating a skin and/or skin structure infection, an intra-abdominal infection, a
bacterial pneumonia, febrile neutropenia or sepsis.

25 35. The method of claim 34, wherein the cancer is a hematological cancer or a solid
cancer.

36. The method of claim 35, wherein the hematological cancer is a leukemia.

37. Use of the composition of any one of claims 1-4 and 10-23 for treating a cosmetic condition or the pharmaceutical composition of any one of claims 24 to 26 for use in treating a bacterial infection and/or cancer.
38. A kit comprising tigecycline or a first composition comprising tigecycline and a
5 second composition comprising one or more formulating agent(s) selected from pyruvic acid, or a salt or ester thereof and ascorbic acid; and optionally one or more excipients, diluents or buffers.
39. The kit of claim 38, wherein the second composition further comprises a chelator and/or cyclodextran.
- 10 40. The kit of claim 39, wherein the second composition is substantially free of EDTA, and wherein if the second composition is a liquid formulation, the second composition is comprised in a light blocking vessel.
41. The kit of any one of claims 38 to 40, wherein the tigecycline or the first composition comprising tigecycline and/or the second composition are comprised in a
15 light blocking vessel, for example a light blocking vial.
42. A kit comprising the composition of any one of claims 1 to 23 or a pharmaceutical composition of any one of claims 24 to 27, and a vessel such as a vial, for example a light blocking vessel such as a light blocking vial, and optionally instructions for use.
- 20 43. The composition of any of claims 1 to 23, the pharmaceutical composition of any one of claims 24 to 27, or the kit claims of any one of claims 38 to 42, wherein the composition is formulated into a unit dosage form or multi-dosage form.
44. The composition of any one of claims 1 to 23, or the pharmaceutical composition of any one of claims 24 to 27, wherein the composition or pharmaceutical
25 composition is stored in a light blocking vessel.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/001114

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 47/22** (2006.01) , **A61K 31/65** (2006.01) , **A61K 47/12** (2006.01) , **A61K 47/14** (2006.01) ,
A61P 31/04 (2006.01) , **A61P 35/00** (2006.01) , **A61P 35/02** (2006.01) , **C07C 237/26** (2006.01)

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)

IPC: **A61K 47/22** (2006.01) , **A61K 31/65** (2006.01) , **A61K 47/12** (2006.01) , **A61K 47/14** (2006.01) ,
A61P 31/04 (2006.01) , **A61P 35/00** (2006.01) , **A61P 35/02** (2006.01) , **C07C 237/26** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
STN CAPlus (keywords = tetracycline, tigecycline, pyruvic/pyruvate, ascorbic, stablization, and cyclodextrin), Canadian Patent Database (IPC and keywords)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/0130252 A1 (Bradford et al.) 16 June 2005 (16-06-2005) *Claims 1-7 & paragraph [0092]*	1, 3, 5, 10, 12
X	WO 2011/141708 A2 (Turner et al.) 17 November 2011 (17-11-2011) *Claims 1-22*	1, 3, 10, 33-38
A	CA 2,722,602 A1 (Kipp et al.) 19 November 2009 (19-11-2009) *entire document*	1-44
A	US 2010/0278894 A1 (Burgmeier) 4 November 2010 (04-11-2010) *entire document*	1-44

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 February 2013 (19-02-2013)

Date of mailing of the international search report

07 March 2013 (07-03-2013)

Name and mailing address of the ISA/CA
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Facsimile No.: 001-819-953-2476

Authorized officer

Tung Siu (819) 934-6735

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2012/001114**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 33-36

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 33-36 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 33-36.

2. ☐ Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. ☐ Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2012/001114

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