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(54) **Title:** TREATMENT OF CARDIAC ARRHYTHMIAS

(57) **Abstract:** The present invention is directed to a pharmaceutical combination comprising amiodarone and afamelanotide, to methods for symptomatic or prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in a subject suffering from cardiac arrhythmia, to methods for the prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity and/or cytotoxicity, and to further medical uses of afamelanotide in the treatment of cardiac arrhythmia and predisposed to amiodarone-induced photo-sensitivity and/or cytotoxicity.



Title: Treatment of cardiac arrhythmias

FIELD OF THE INVENTION

5 The invention is in the field of medicine. In particular, the invention is in the field of therapeutic treatment of cardiac arrhythmias using amiodarone. The present invention pertains to pharmaceutical combinations for prophylactic or symptomatic treatment of amiodarone-induced photosensitivity, and to methods of preventive and symptomatic
10 treatment of amiodarone-induced photosensitivity and other toxic effects of amiodarone in amiodarone recipients.

BACKGROUND OF THE INVENTION

 The treatment of cardiac arrhythmias can be a clinical challenge ,
15 because of a limited efficacy of and unwanted effects of the currently available pharmacological and non-pharmacological treatment modalities. Amiodarone (2-4-((2-butyl-1-benzofuran-3-yl)carbonyl]-2,6-diiodophenoxy ethyl)diethylamine; C₂₈H₂₉I₂N₃O₃) is one of the most frequently prescribed antiarrhythmic medications and is considered the most effective
20 antiarrhythmic drug. It is currently used most frequently but not exclusively in patients with supraventricular arrhythmias (including atrial fibrillation) and left ventricular dysfunction, in patients with acute sustained ventricular arrhythmias, patients who undergo cardiac surgery and patients with an internal cardioverter defibrillator and appropriate or
25 inappropriate shocks. Amiodarone was discovered in 1961 and currently generic products of multiple companies are available. It is a very effective antiarrhythmic drug, but has a number of serious adverse effects including photosensitivity. Photosensitivity caused by amiodarone occurs in 25% to 75% of the patients using this medication, and has a severe impact on the
30 quality of life. The photosensitivity in patients using amiodarone is a frequent complaint and may range from an increased propensity to tan to

intense erythema and swelling of sun-exposed areas. Generally the photosensitivity is not dose-related, and patients using amiodarone are advised to avoid direct sunlight and use a sunblocker and an umbrella. This photosensitivity clearly reduces the quality of life and a substantial proportion of the patients need to stop the amiodarone treatment. When patients need to stop treatment with amiodarone because of skin problems, there is a high likelihood of recurrence of cardiac arrhythmias. The subsequent treatment of these patients is difficult. The recurrence of supraventricular cardiac arrhythmias including atrial fibrillation may lead to heart failure and thrombo-embolic events like stroke. The recurrence of ventricular arrhythmias may lead to hemodynamic instability and cardiac death. After the discontinuation of amiodarone other antiarrhythmic drugs are hardly effective. Patients with symptomatic recurrence of atrial fibrillation may need invasive treatment.

One invasive treatment option is pulmonary vein ablation or surgical ablation. The second invasive treatment option is Hisbundle ablation with the implantation of a permanent pacemaker. These invasive treatment options are associated with a increased morbidity and substantial costs. Patients with recurrent ventricular arrhythmias may need invasive treatment with transcutaneous or surgical ablation. A substantial proportion of patients with recurrent ventricular arrhythmias have an indication for an internal cardioverter defibrillator, which is also associated with substantial morbidity (including inappropriate shocks) and is an expensive treatment option. There is thus an urgent need to provide for an effective treatment of cardiac arrhythmias wherein the side effect of photosensitivity is decreased.

It is an aim of the present invention to provide an effective treatment for cardiac arrhythmias with a decreased number of unwanted side-effects. It is an aim of the present invention to prevent or decrease the photosensitivity induced by amiodarone, to thereby improving the tolerance

of sun light exposure so as to contribute to an improvement in the quality of life of patients treated with amiodarone.

SUMMARY OF THE INVENTION

5 The present invention now provides, in a first aspect, a pharmaceutical combination comprising amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, and optionally one or more pharmaceutically acceptable excipients, carriers or diluents. In preferred embodiments of this aspect of the invention the
10 amiodarone and/or afamelanotide, or their pharmaceutically acceptable derivatives or solvates, are formulated for oral or parenteral administration.

 It is also an aspect of the present invention to provide a method for symptomatic or prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in a subject suffering from cardiac
15 arrhythmia, comprising the simultaneous, separate or sequential co-administration of a therapeutically effective amount of amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof.

 It is furthermore an aspect of the present invention to provide a
20 method for the prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity and/or cytotoxicity, comprising the administration to said subject of a therapeutically effective amount of afamelanotide, or a
25 pharmaceutically acceptable derivative or solvate of either thereof prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity and/or cytotoxicity.

 Also an aspect of this invention is a pharmaceutical combination as described above for use in the symptomatic or prophylactic treatment of
30 amiodarone-induced photosensitivity and/or cytotoxicity in a subject

suffering from cardiac arrhythmia, comprising the simultaneous, separate or sequential co-administration of a therapeutically effective amount of amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof.

5 A further aspect of this invention is afamelanotide, or a pharmaceutically acceptable derivative or solvate of thereof, for use in the prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity and/or cytotoxicity.

10 In a preferred embodiment of this aspect, said prophylactic treatment is in accordance with a treatment regimen involving the administration to said recipient of a therapeutically effective amount of afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof prior to the clinical manifestation of a symptom associated with said amiodarone-

15 induced photosensitivity and/or cytotoxicity.

 In another aspect, the present invention provides the use of a pharmaceutical combination as described above in the preparation of a medicament for the symptomatic or prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in a subject suffering from

20 cardiac arrhythmia.

 In yet another aspect, the present invention provides the use of afamelanotide, or a pharmaceutically acceptable derivative or solvate of thereof, in the preparation of a medicament for the prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in an amiodarone

25 recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity and/or cytotoxicity, preferably wherein the preparation is for administration to said recipient of a therapeutically effective amount of afamelanotide, prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity and/or

30 cytotoxicity.

It is an advantage of the above indicated (i) pharmaceutical combinations, (ii) methods, (iii) afamelanotide for use and (iv) uses that more patients can continue optimal and cheap antiarrhythmic treatment with amiodarone. They will further reduce the number of patients that are forced to discontinue amiodarone treatment due to intolerance to this drug, resulting in the development of adverse skin reactions. Another advantage is that they substantially improve the cost-effectiveness of the treatment of patients with arrhythmias. It was further unexpectedly found that afamelanotide has a protective effect on amiodarone-induced cell toxicity.

The present invention, in another aspect, further provides an anticytotoxic agent comprising afamelanotide.

Also provides, is a pharmaceutical combination of a toxic pharmaceutically active agent the toxicity of which is due to lowering of the mitochondrial membrane potential, preferably selected from phenytoin, lidocaine, quinidine, isoproterenol, clopidogrel, acetyl-salicylic acid and molsidomine, said combination further comprising afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, and optionally one or more pharmaceutically acceptable excipients, carriers or diluents.

DESCRIPTION OF THE DRAWINGS

Figure 1 frames A and B show the in vitro cytotoxicity of increasing concentrations of amiodarone, with (square; upper line) or without (dot; lower line) 1.0 microgram/ml afamelanotide, on human C5RO fibroblast cells (A) and human HepG2 hepatoma cells (B). The cytotoxicity tests show that there is no toxic interaction between amiodarone and afamelanotide on the human cells tested, and that the combination of these drugs is expected to be safe for humans. Panels A and B show the unexpected protective effect of afamelanotide on amiodarone-induced toxicity in C5RO and HepG2 cells. Figure 1 frames C and D show the in

vitro cytotoxicity of increasing concentrations of afamelanotide, with (square) or without (dot) 250 microgram/ml amiodarone, on C5RO cells (C) and HepG2 cells (D). Both panels show that cytotoxicity is induced by afamelanotide only at high concentrations of afamelanotide.

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DETAILED DESCRIPTION OF THE INVENTION

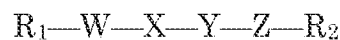
Definitions

The term “amiodarone” as used herein refers to the Class III antiarrhythmic agent (2-{4-[(2-butyl-1-benzofuran-3-yl)carbonyl]-2,6-diiodophenoxy}ethyl)diethylamine. Amiodarone is approved for the treatment of life-threatening ventricular tachyarrhythmias, and is used for various types of cardiac dysrhythmias, both ventricular and atrial. Despite relatively common side-effects, it is used in arrhythmias that are otherwise difficult to treat with medication. Administration of amiodarone is associated with photosensitivity resulting in erythema and pruritus, and patients are advised to avoid exposure to sunlight and use sunscreens comprising UVA and UVB blockers. Amiodarone induces photosensitivity in 25% to 75% of the patients treated with this drug. Another adverse skin reaction associated with long-term administration of amiodarone (usually more than 18 months) is a blue-grey discoloration of the skin. Although skin discoloration generally improves upon cessation of the drug, skin color may not return completely to normal. Amiodarone is commercially available in oral and intravenous formulations. As used herein, the term amiodarone includes all neutral and salt forms of the same. N-desethylamiodarone (DEA) is the major active metabolite of amiodarone in humans. The term amiodarone also includes DEA and all of its neutral and salt forms.

The term “afamelanotide” as used herein refers to any alpha-MSH analogue. The term “alpha-MSH analogue” as used herein is defined as a derivative of alpha-MSH which exhibits agonist activity for the melanocortin-1 receptor (MC1R), the receptor to which alpha-MSH binds to

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initiate the production of melanin within a melanocyte. Such derivatives include derivatives in which (i) one or more amino acid residues are deleted from the native alpha-MSH molecule at the N-terminal end, the C-terminal end, or both; and/or (ii) one or more amino acid residues of the native alpha-MSH molecule are replaced by another natural, non-natural or synthetic amino acid residue; and/or (iii) an intramolecular interaction forms as a cyclic derivative. The use of any alpha-MSH analogue is contemplated in the methods described herein. Several derivatives of alpha-MSH have been synthesized. In one aspect of the present invention, the alpha-MSH analogues described in U.S. Pat. Nos. 4,457,864, 4,485,039, 4,866,038, 4,918,055, 5,049,547, 5,674,839 and 5,714,576 and U.S. Pat. Nos. 4,918,055 and 5,683,981, which are herein incorporated by reference for their teachings with respect to alpha-MSH analogues and their synthesis thereof, can be used herein. In one aspect of the present invention, the alpha-MSH analogue may be a compound as disclosed in Australian Patent No. 597630, selected from compounds of the formula:



wherein

R_1 is absent, n-Pentadecanoyl, Ac, 4-phenylbutyryl, Ac-Gly-, Ac-Met-Glu, Ac-Nle-Glu-, or Ac-Tyr-Glu-;

W is -His- or -D-His-;

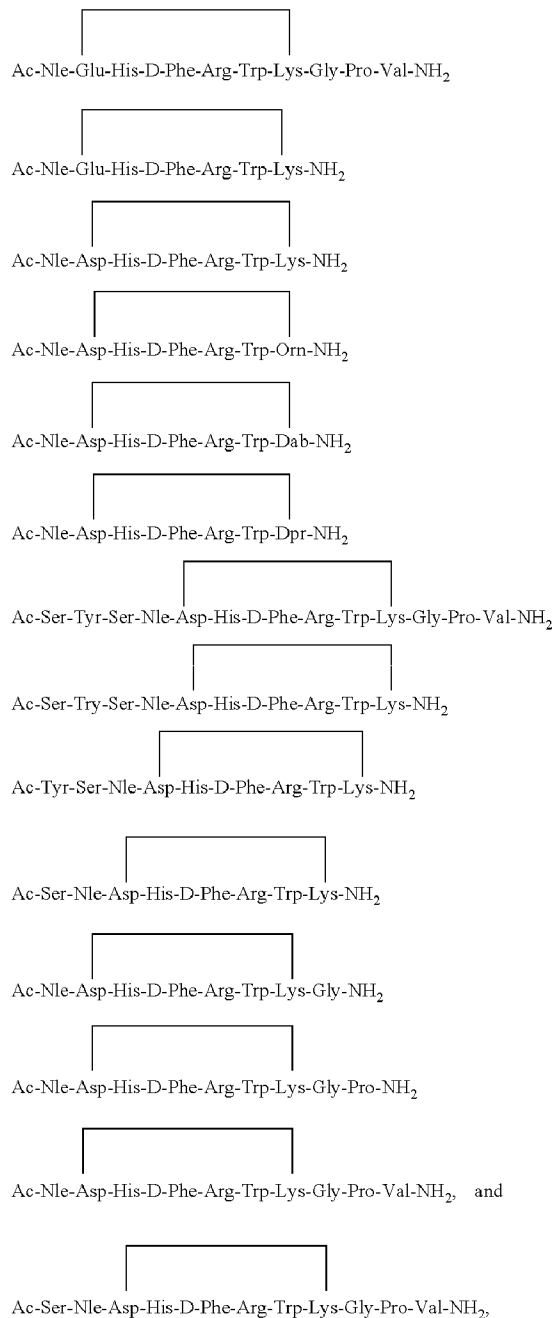
X is -Phe-, -D-Phe-, -Tyr-, -D-Tyr-, or -(pNO₂)D-Phe⁷-;

Y is -Arg- or -D-Arg-; Z is -Trp- or -D-Trp-; and R_2 is —NH₂; -Gly-NH₂; or -Gly-Lys-NH₂.

In another aspect, the alpha-MSH analogue maybe selected from cyclic analogues which are disclosed in Australian Patent No. 618733 where an intramolecular interaction (such as a disulfide or other covalent bond) exists (1) between the amino acid residue at position 4 and an amino acid residue at position 10 or 11, and/or (2) between the amino acid residue at position 5 and the amino acid residue at position 10 or 11.

The alpha-MSH analogue may be a linear analogue as disclosed in U.S. Pat. No. 5,674,839, selected from the group consisting of:

- Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Lys-Gly-Pro-Val-NH₂, Ac-Ser-Tyr-Ser-Nle-Asp-His-D-Phe-Arg-Trp-Lys-Gly-Pro-Val-NH₂, Ac-Nle-Glu-His-
5 D-Phe-Arg-Trp-Lys-Gly-Pro-Val-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-Gly-Pro-Val-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-Gly-NH₂, Ac-Nle-Glu-His-D-Phe-Arg-Trp-Lys-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH₂, Ac-Nle-Glu-His-D-Phe-Arg-Trp-Orn-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-Orn-NH₂,
Ac-Nle-Glu-His-D-Phe-Arg-Trp-Dab-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-
10 Dab-NH₂, Ac-Nle-Asp-His-D-Phe-Arg-Trp-Dpr-NH₂, Ac-Nle-Glu-His-Phe-Arg-Trp-Lys-NH₂, and Ac-Nle-Asp-His-Phe-Arg-Trp-Lys-NH₂. The alpha-MSH analogue may also be a cyclic analogue as disclosed in U.S. Pat. No. 5,674,839, selected from the group consisting of:



wherein Ala=alanine, Arg=arginine, Dab=2,4-diaminobutyric acid, Dpr=2,3-diaminopropionic acid, Glu=glutamic acid, Gly=glycine, His=histidine, Lys=lysine, Met=methionine, Nle=norleucine, Orn=ornithine,

- 5 Phe=phenylalanine, (pNO₂)Phe=paranitrophenylalanine, Plg=phenylglycine, Pro=proline, Ser=serine, Trp=tryptophan, TrpFor=N¹-formyl-tryptophan, Tyr=tyrosine, Val=valine.

All peptides are written with the acyl-terminal end at the left and the amino terminal end to the right; the prefix “D” before an amino acid designates the D-isomer configuration, and unless specifically designated otherwise, all amino acids are in the L-isomer configuration.

5 In one aspect of the present invention, the alpha-MSH analogue can be

[D-Phe⁷]-alpha-MSH, [Nle⁴, D-Phe⁷]-alpha-MSH,
 [D-Ser¹, D-Phe⁷]-alpha-MSH, [D-Tyr², D-Phe⁷]-alpha-MSH,
 [D-Ser³, D-Phe⁷]-alpha-MSH, [D-Met⁴, D-Phe⁷]-alpha-MSH,
 [D-Glu⁵, D-Phe⁷]-alpha-MSH, [D-His⁶, D-Phe⁷]-alpha-MSH,
 [D-Phe⁷, D-Arg⁸]-alpha-MSH, [D-Phe⁷, D-Trp⁹]-alpha-MSH,
 [D-Phe⁷, D-Lys¹¹]-alpha-MSH, [D-Phe⁷, D-Pro¹²]-alpha-MSH,
 [D-Phe⁷, D-Val¹³]-alpha-MSH, [D-Ser¹, Nle⁴, D-Phe⁷]-alpha-MSH,
 [D-Tyr², Nle⁴, D-Phe⁷]-alpha-MSH,
 [D-Ser³, Nle⁴, D-Phe⁷]-alpha-MSH,
 [Nle⁴, D-Glu⁵, D-Phe⁷]-alpha-MSH,
 [Nle⁴, D-His⁶, D-Phe⁷]-alpha-MSH,
 [Nle⁴, D-Phe⁷, D-Arg⁸]-alpha-MSH,
 [Nle⁴, D-Phe⁷, D-Trp⁹]-alpha-MSH,
 [Nle⁴, D-Phe⁷, D-Lys¹¹]-alpha-MSH,
 [Nle⁴, D-Phe⁷, D-Pro¹²]-alpha-MSH,
 [Nle⁴, D-Phe⁷, D-Val¹³]-alpha-MSH,


 [Cys⁴, Cys¹⁰]-alpha-MSH [Cys⁴, D-Phe⁷, Cys¹⁰]-alpha-MSH


 [Cys⁴, Cys¹¹]-alpha-MSH [Cys⁵, Cys¹⁰]-alpha-MSH


 [Cys⁵, Cys¹¹]-alpha-MSH [Cys⁴, Cys¹⁰]-alpha-MSH₄₋₁₃


 [Cys⁴, Cys¹⁰]-alpha-MSH₄₋₁₂ [Nle⁴, D-Phe⁷]-alpha-MSH₄₋₁₀

[Nle⁴, D-Phe⁷]-alpha-MSH₄₋₁₁, [D-Phe⁷]-alpha-MSH₅₋₁₁,
 [Nle⁴, D-Tyr⁷]-alpha-MSH₄₋₁₁, [(pNO₂)D-Phe⁷]-alpha-MSH₄₋₁₁,
 [Tyr⁴, D-Phe⁷]-alpha-MSH₄₋₁₀, [Tyr⁴, D-Phe⁷]-alpha-MSH₄₋₁₁,
 [Nle⁴]-alpha-MSH₄₋₁₁, [Nle⁴, (pNO₂)D-Phe⁷]-alpha-MSH₄₋₁₁,
 [Nle⁴, D-His⁶]-alpha-MSH₄₋₁₁,
 [Nle⁴, D-His⁶, D-Phe⁷]-alpha-MSH₄₋₁₁,
 [Nle⁴, D-Arg⁸]-alpha-MSH₄₋₁₁, [Nle⁴, D-Trp⁹]-alpha-MSH₄₋₁₁,
 [Nle⁴, D-Phe⁷, D-Trp⁹]-alpha-MSH₄₋₁₁,
 [Nle⁴, D-Phe⁷]-alpha-MSH₄₋₉, and
 [Nle⁴, D-Phe⁷, D-Trp⁹]-alpha-MSH₄₋₉

In a further aspect, the alpha-MSH analogue is:

[Nle⁴, D-Phe⁷]-alpha-MSH₄₋₁₀,
[Nle⁴, D-Phe⁷]-alpha-MSH₄₋₁₁,
[Nle⁴, D-Phe⁷, D-Trp⁹]-alpha-MSH₄₋₁₁, or
[Nle⁴, D-Phe⁷]-alpha-MSH₄₋₉.

5 In most preferred embodiments, the term “afamelanotide” refers to [Nle⁴, D-Phe⁷]-α-MSH, the synthetic analog of the naturally occurring melanocortin peptide hormone alpha-melanocyte stimulating hormone (α-MSH). The chemical name is also indicated as melanotan-1, or N-acetyl-L-seryl-L-tyrosyl-L-seryl-L-norleucyl-L-α-glutamyl-L-histidyl-D-phenylalanyl-
10 L-arginyl-L-tryptophylglycyl-L-lysyl-L-prolyl-L-valinamide. Its amino acid sequence is Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂. Nle⁴-D-Phe⁷-alpha-MSH, can induce melanin synthesis in human volunteers. Nle⁴-D-Phe⁷-alpha-MSH contains two amino acid substitutions and is approximately 10 to 1,000-fold more potent than the native hormone
15 at inducing pigmentation in experimental systems such as the frog skin bioassay or in cultured human keratinocytes. Afamelanotide received Investigational New Drug (IND) status from the US Food and Drug Administration allowing clinical trials for its effectiveness as photoprotective drug in patients diagnosed with erythropoietic
20 protoporphyria (EPP). EPP is a metabolic disorder characterised by absolute intolerance to light (blue spectrum), caused by the accumulation of protoporphyrin IX. Its use in metabolic cardiovascular diseases has not been investigated. The use of afamelanotide is presently restricted to symptomatic patients, i.e., patients who present with skin disorders, and
25 has until now only been used for the treatment of very rare skin diseases including erythropoietic protoporphyria (severe photodermatosis), vitiligo (depigmentation of skin), solar urticarial (skin rash induced by light), actinic keratosis, squamous cell carcinoma in organ transplant patients, Hailey-Hailey disease and PLE (skin irritation induced by sunlight) and associated
30 photosensitivity. Afamelanotide has not been used or studied for the

treatment of photosensitivity that is not associated with these skin diseases. In particular, afamelanotide has not been used before to treat patients with amiodarone-induced photosensitivity. Moreover, afamelanotide has not been considered in preventive strategies. The present invention aims to prevent
5 or at least reduce subsequent photosensitive reactions during amiodarone use. It is an aspect of the present invention that afamelanotide is *inter alia* used as part of a prophylactic, i.e. preventive, treatment regimen of patients receiving amiodarone for the treatment of cardiac dysrhythmias.

In aspects of this invention, all administration forms of both
10 amiodarone and afamelanotide are foreseen as embodiments. Amiodarone and/or afamelanotide may be administered in free form or in pharmaceutically acceptable salt form.

As used herein, the phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions,
15 and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. As used herein, a "pharmaceutically acceptable liquid carrier" is any aqueous
20 medium used in the pharmaceutical sciences for dilution or dissolution of parenteral formulations. As used herein, a pharmaceutically acceptable carrier may be a liquid carrier, which may be any aqueous medium used in the pharmaceutical sciences for dilution or dissolution of pharmaceutical formulations, specifically for parenteral use. In a specific embodiment, the
25 term "pharmaceutically acceptable" means generally accepted by or approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a formulation
30 of the invention is administered. Such pharmaceutical carriers can be

liquids, such as water, saline, aqueous solutions and the like. When administered to a patient, the formulations of the invention and pharmaceutically acceptable vehicles are preferably sterile. Water is a preferred vehicle when the compound of the invention is administered intravenously. Saline solutions and aqueous dextrose solutions can also be employed as liquid vehicles, particularly for injectable solutions. The present compositions, if desired, can also contain minor amounts of wetting agents, or pH buffering agents.

The term "combination", "therapeutic combination" or "pharmaceutical combination", as used herein, defines either a fixed combination in one dosage unit form or a kit of parts for the combined administration where Compound A and Compound B may be administered independently at the same time or separately within time intervals that allow that the combination partners show a cooperative, e.g., synergistic, effect.

The term "a combined preparation" is defined herein to refer to especially a "kit of parts" in the sense that the combination partners (a) and (b) as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners (a) and (b), i.e., simultaneously or at different time points. The parts of the kit of parts can then e.g., be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the combination partner (a) to the combination partner (b) to be administered in the combined preparation can be varied, e.g., in order to cope with the needs of a patient sub-population to be treated or the needs of the single patient.

The term "co-administration" or "combined administration" as used herein is defined to encompass the administration of the selected therapeutic agents to a single patient, and are intended to include

treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

As used herein, the terms "patient", "subject" or "recipient" refers to a warm blooded animal such as a mammal, for example, a cat, a dog, a mouse, a guinea pig, a horse, a bovine cow, a sheep, and, preferably, a human.

The term "jointly therapeutically active" or "joint therapeutic effect" means that the therapeutic agents may be given separately (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that they prefer, in the warm-blooded animal, especially human, to be treated, still show a (preferably synergistic) interaction (joint therapeutic effect). Whether this is the case can, inter alia, be determined by following the blood levels, showing that both compounds are present in the blood of the human to be treated at least during certain time intervals.

The term "pharmaceutically effective amount" or "clinically effective amount" or "therapeutically effective amount" of a combination of therapeutic agents is an amount sufficient to provide an observable improvement over the baseline clinically observable signs and symptoms of the disorder treated with the combination. In the context of this specification, the term "therapeutically effective amount" includes within its meaning a non-toxic amount of the active compound sufficient to provide the desired therapeutic effect. The exact amount will vary from subject to subject depending on the age of the subject, their general health, the severity of the disorder being treated and the mode of administration. It is therefore not possible to specify an exact "therapeutically effective amount", however one skilled in the art would be capable of determining a "therapeutically effective amount" by routine trial and experimentation.

Pharmaceutically acceptable salts include, where applicable, acid addition salts derived from pharmaceutically acceptable inorganic and organic acids such as a chloride, bromide, sulphate, phosphate, maleate,

fumarate, tartrate, citrate, benzoate, 4-methoxybenzoate, 2- or 4-hydroxybenzoate, 4-chlorobenzoate, p-toluenesulphonate, methanesulphonate, ascorbate, acetate, succinate, lactate, glutarate, gluconate, tricarballylate, hydroxynaphthalene-carboxylate or oleate salt; and salts prepared from pharmaceutically acceptable inorganic and organic bases. Salts derived from inorganic bases include aluminium, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium, zinc and bismuth salts. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic bases include salts of primary, secondary and tertiary amines, cyclic amines like arginine, betaine, choline and the like. Examples of pharmaceutically acceptable solvates include hydrates.

In the context of the present specification, unless otherwise stated, a pharmaceutically acceptable derivative of amiodarone and/or afamelanotide means a pharmaceutically acceptable ester, salt or solvate of amiodarone and/or afamelanotide or a pharmaceutically acceptable solvate of such an ester or salt.

It is one objective of this invention to provide a pharmaceutical composition, comprising the combination of the invention which is jointly therapeutically effective.

The present invention relates to a method of treating a subject having a proliferative disease comprising administering to said subject a combination of the invention in a quantity, which is jointly therapeutically effective.

The amiodarone recipient suffering from cardiac arrhythmia may be treated with an effective amount of afamelanotide prior to receiving the amiodarone. This embodiment is specifically foreseen in aspects relating to a method for the prophylactic treatment of amiodarone-induced photosensitivity and/or cytotoxicity in an amiodarone recipient suffering

from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity and/or cytotoxicity. It is envisioned that the administration of a therapeutically effective amount of afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof to said
5 amiodarone recipient suffering from cardiac arrhythmia occurs prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity and/or cytotoxicity.

The pharmaceutical combination of the present invention may comprise amiodarone and afamelanotide in an amount of 10-1000 mg of
10 amiodarone per unit dosage and 0.01 to 10 mg of afamelanotide per unit dosage in a combine preparation according to the invention. Preferred unit dosage forms comprise about 50-500 mg of amiodarone, preferably 100-300 mg of amiodarone, more preferably 150-250 mg of amiodarone, such as about 200 mg of amiodarone in a unit dosage, which unit dosage is for daily
15 administration or delivery. Preferred unit dosage forms comprise about 0.05 to 1 mg of afamelanotide, preferably 0.1-0.5 mg, more preferably 0.2-0.4 mg of afamelanotide, such as about 0.25 mg of afamelanotide in a unit dosage, which unit dosage is for daily administration or delivery. Preferred dosages for administration include an amount of amiodarone of 200 mg/day, and
20 afamelanotide of 0.25 mg/day.

The toxic effects of amiodarone and its toxic metabolite desethylamiodarone on different off-target organs (cornea, liver, skin, thyroid, and lung) may share a common mechanism: reducing mitochondrial membrane potential, stimulating the reactive oxygen species production and
25 stimulating apoptosis. In our *in vitro* experiment, we confirm that amiodarone induces apoptotic cell death of human cells. Moreover, we found an improved survival of cells after adding afamelanotide, which may be the result of suppression of the apoptotic pathway. *In vivo*, this may contribute to immunosuppression. Hence, such protective effects against amiodarone-
30 induced toxicity of afamelanotide will be larger in the context of the immune

system of the patient. Afamelanotide has a protective effect on amiodarone-induced cell toxicity, in line with the findings of our experiment in which specifically hepatotoxicity or fibroblast toxicity were tested without the presence of the immune system. This protective effect was not previously
5 known. Hence, it is an aspect of this invention to provide protective combinations of amiodarone and afamelanotide as described herein. Preferably, the protective combination in aspects of this invention provides blood plasma levels of amiodarone below 250 microgram/ml, more preferably below 150 microgram/ml, most preferably below, 125, 100, 75, or 50
10 microgram/ml. The blood plasma level of amiodarone is preferably at least 1 microgram/ml, more preferably at least 5, 10 or 15 microgram/ml. The present invention thus provides for pharmaceutical combinations comprising amiodarone for use in the treatment of arrhythmia, wherein the negative side effects of amiodarone, such as amiodarone-induced
15 photosensitivity and/or amiodarone-induced cytotoxicity, are prevented or at least diminished due to the presence in said pharmaceutical combination of a therapeutically effective amount of afamelanotide.

The pharmaceutically acceptable derivative or solvate of either amiodarone and afamelanotide can for instance be a pharmaceutically
20 acceptable salt. Amiodarone hydrochloride is a preferred embodiment.

The pharmaceutical combination of the present invention may further comprise pharmaceutically acceptable excipients, carriers or diluents. In preferred embodiments, said carrier is for instance selected from the group consisting of aqueous, non-aqueous and nanoparticulate
25 suspensions, solutions, creams, ointments, gels, syrups, suppositories and micro-droplet sprays. In yet other preferred embodiments, said carrier may include an adjuvant selected from the group consisting of builders, stabilizers, emulsifiers, dispersants, preservatives, buffers, electrolytes, tissue penetrating agents and tissue softening agents.

In aspects of this invention, amiodarone and/or afamelanotide, or their pharmaceutically acceptable derivatives or solvates, may be formulated for administration in any suitable form. Pharmaceutical compositions include those suitable for oral, parenteral (including
5 subcutaneous, intradermal, intramuscular, intravenous and intra-articular), inhalation (including use of metered dose pressurised aerosols, nebulisers or insufflators), rectal and topical (including dermal, buccal, sublingual and intraocular) administration. Hence, the pharmaceutical compositions and pharmaceutical combinations of this invention may be in liquid, semi solid,
10 or solid form.

Very suitable administration forms for afamelanotide include providing the afamelanotide in the form of a subcutaneous controlled release formulation administering a fixed, subcutaneous daily dose for between 1 to 30 days, for instance using a monthly implant comprising from
15 10 to 25 mg of afamelanotide (preferably 16 mg) that is released over a period of between 7 and 10 days after implantation providing blood plasma levels between 0.001 and 10 ng/ml during this period.

The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of
20 pharmacy. All methods include the step of bringing afamelanotide or the combination of amiodarone and afamelanotide as defined herein into association with a carrier, which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the afamelanotide or the combination of
25 amiodarone and afamelanotide with a liquid carrier or finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired composition.

Generally, an effective dosage of afamelanotide or the combination of amiodarone and afamelanotide present in pharmaceutical
30 compositions of the present invention is expected to be in the range of about

0.0001mg to about 1000mg per kg body weight per 24 hours; about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours, or about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range of about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours, or about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage may be up to about 500mg/m². Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m², about 25 to about 350mg/m², about 25 to about 300mg/m², about 25 to about 250mg/m², about 50 to about 250mg/m², or about 75 to about 150mg/m².

Compositions suitable for buccal (sublingual) administration include lozenges comprising afamelanotide or the combination of amiodarone and afamelanotide in a flavoured base, usually sucrose and acacia or tragacanth; and pastilles comprising afamelanotide or the combination of amiodarone and afamelanotide in an inert base such as gelatine and glycerin or sucrose and acacia.

Compositions comprising afamelanotide or the combination of amiodarone and afamelanotide suitable for oral administration may be presented as discrete units such as gelatine or HPMC capsules, cachets or tablets, each containing a predetermined amount of afamelanotide or the combination of amiodarone and afamelanotide, as a powder, granules, as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion.

Afamelanotide or the combination of amiodarone and afamelanotide may also be present in a paste.

When the compositions comprising afamelanotide or the combination of amiodarone and afamelanotide are formulated for oral or other forms of administration, the afamelanotide or the combination of amiodarone and afamelanotide may be formulated with one or more pharmaceutically acceptable carriers such as starch, lactose, microcrystalline cellulose, silicon dioxide and/or a cyclic oligosaccharide such as cyclodextrin. Additional ingredients, in this or other embodiments of this invention may include lubricants such as magnesium stearate and/or calcium stearate. Suitable cyclodextrins include α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, 2-hydroxyethyl- β -cyclodextrin, 2-hydroxypropyl-cyclodextrin, 3-hydroxypropyl- β -cyclodextrin and tri-methyl- β -cyclodextrin. The cyclodextrin may be hydroxypropyl- β -cyclodextrin. Suitable derivatives of cyclodextrins include Captisol® a sulfobutyl ether derivative of cyclodextrin and analogues thereof as described in US patent No. 5,134,127. Suitable cyclodextrin derivatives of amiodarone having improved solubilization characteristics and suitable for oral, intranasal, or parenteral administration are indicated in US patent No. 5,134,127. Very suitably, increase the solubility of amiodarone may be accomplished as described in US patent No. 7,635,773 which discloses sulfoalkyl ether cyclodextrin ("SAE-CD") derivatives of amiodarone.

Tablets may be prepared by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine afamelanotide or the combination of amiodarone and afamelanotide in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant (for example magnesium stearate or calcium stearate), inert diluent or a surface active/dispersing agent. Moulded tablets may be made by moulding a mixture of the powdered afamelanotide or the combination of amiodarone

and afamelanotide moistened with an inert liquid diluent, in a suitable machine. The tablets may optionally be coated, for example, with an enteric coating and may be formulated so as to provide slow or controlled release of afamelanotide or the combination of amiodarone and afamelanotide therein.

5 Very suitable administration forms include subcutaneous controlled release formulations administering a fixed, subcutaneous daily dose of afamelanotide or the combination of amiodarone and afamelanotide for between 1 to 30 days, for instance using monthly implants comprising multiple daily dosages (e.g. 1-60) that are controllably released over a period
10 of, e.g., 1-60 days.

 Compositions for parenteral administration include aqueous and non-aqueous sterile injectable solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient, and which may include suspending
15 agents and thickening agents. A parenteral composition may comprise a cyclic oligosaccharide such as hydroxypropyl- β -cyclodextrin. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for
20 example saline or water-for-injection, immediately prior to use.

 Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably comprise afamelanotide or the combination of amiodarone and
25 afamelanotide as an optionally buffered aqueous solution of, for example, 0.1 M to 0.2 M concentration with respect to the compound.

 Compositions suitable for transdermal administration may also be delivered by iontophoresis, and typically take the form of an optionally buffered aqueous solution of the active compound. Suitable compositions
30 may comprise citrate or Bis/Tris buffer (pH 6) or ethanol/water and contain

from 0.1 M to 0.2 M of afamelanotide or the combination of amiodarone and afamelanotide.

Spray compositions for topical delivery to the lung by inhalation may, for example be formulated as aqueous solutions or suspensions or as
5 aerosols, suspensions or solutions delivered from pressurized packs, such as a metered dose inhaler, with the use of a suitable liquefied propellant. Suitable propellants include a fluorocarbon or a hydrogen-containing chlorofluorocarbon or mixtures thereof, particularly hydrofluoroalkanes, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane,
10 especially 1,1,1,2-tetrafluoroethane, 1,1,2,2,3,3,3-heptafluoro-n-propane or a mixture thereof. Carbon dioxide or other suitable gas may also be used as propellant. The aerosol composition may be excipient free or may optionally contain additional composition excipients well known in the art, such as surfactants e.g. oleic acid or lecithin and cosolvents e.g. ethanol. Pressurised
15 compositions will generally be retained in a canister (e.g. an aluminium canister) closed with a valve (e.g. a metering valve) and fitted into an actuator provided with a mouthpiece.

Medicaments for administration by inhalation desirably have a controlled particle size. The optimum particle size for inhalation into the
20 bronchial system is usually 1-10 μm , preferably 2-5 μm . Particles having a size above 20 μm are generally too large when inhaled to reach the small airways. When the excipient is lactose it will typically be present as milled lactose, wherein not more than 85% of lactose particles will have a MMD of 60-90 μm and not less than 15% will have a MMD of less than 15 μm .

25 Compositions for rectal administration may be presented as a suppository with carriers such as cocoa butter or polyethylene glycol, or as an enema wherein the carrier is an isotonic liquid such as saline. Additional components of the compositions may include a cyclic oligosaccharide, for example, a cyclodextrin, as described above, such as hydroxypropyl- β -

cyclodextrin, one or more surfactants, buffer salts or acid or alkali to adjust the pH, isotonicity adjusting agents and/or anti-oxidants.

Compositions suitable for topical administration to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include Vaseline, lanoline, polyethylene glycols, alcohols, and combination of two or more thereof. Afamelanotide or the combination of amiodarone and afamelanotide is generally present at a concentration of from 0.1% to 20% w/w, or from 0.5% to 5% w/w. Examples of such compositions include cosmetic skin creams.

The composition may also be administered or delivered to target cells in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Specific examples of liposomes that may be used to administer or deliver a compound formula (I) include synthetic cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine, 3-N-[(methoxy poly(ethylene glycol)2000)carbamoyl]-1,2-dimyrestyloxy-propylamine (PEG-cDMA) and 1,2-di-o-octadecenyl-3-(N,N-dimethyl)aminopropane (DODMA).

The compositions may also be administered in the form of microparticles. Biodegradable microparticles formed from polylactide (PLA), polylactide-co-glycolide (PLGA), and ϵ -caprolactone have been extensively used as drug carriers to increase plasma half life and thereby prolong efficacy (R. Kumar, M., 2000, J Pharm Pharmaceut Sci. 3(2) 234-258).

The compositions may incorporate a controlled release matrix that is composed of sucrose acetate isobutyrate (SAIB) and organic solvent or organic solvent mixtures. Polymer additives may be added to the vehicle as a release modifier to further increase the viscosity and slow down the release rate. Afamelanotide or the combination of amiodarone and afamelanotide may be added to the SAIB delivery vehicle to form SAIB solution or suspension compositions. When the formulation is injected

subcutaneously, the solvent diffuses from the matrix allowing the SAIB-drug or SAIB-drug-polymer mixtures to set up as an in situ forming depot.

In the treatment of cardiac arrhythmias, therapeutic advantages may be obtained through combination treatment regimens as disclosed
5 herein. As such, methods of treatment according to the present invention also include co-administration regimens. The co-administration of amiodarone and afamelanotide may be simultaneous or sequential. Simultaneous administration may be effected by afamelanotide being in the same unit dose as amiodarone, or afamelanotide and amiodarone may be
10 present in individual and discrete unit doses administered at the same, or at a similar time. Sequential administration may be in any order as required. Preferably in preventive photosensitivity treatment regimens, afamelanotide (co-)administration starts prior to the onset of the amiodarone regime.

15 In aspects of the present invention, the invention provides improved methods for treating cardiac arrhythmia in a subject in need thereof. In particular, the present invention provides improved methods for treating cardiac arrhythmia using amiodarone in subjects suffering from amiodarone-induced photosensitivity and/or cytotoxicity or likely to suffer
20 from amiodarone-induced photosensitivity and/or other toxic effects of amiodarone. The methods of the present invention envisage the co-administration of a therapeutically effective amount of amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof. As indicated above, the co-administration may be
25 simultaneous, separate or sequential. In aspects of this invention, a subject suffering from amiodarone-induced photosensitivity may be symptomatic, in which case the subject shows signs of amiodarone-induced photosensitivity, such as erythema and pruritus. In aspects of this invention, a subject suffering from amiodarone-induced photosensitivity may be asymptomatic,
30 in which case no obvious symptoms of photosensitivity develop upon

amiodarone treatment. However, asymptomatic patients are included in aspects of this invention, as it is an aspect of this invention that negative side-effects of amiodarone may include symptoms not immediately diagnosed as photosensitivity, yet which can be attributed as being
5 amiodarone-induced, and which can be prevented or treated by the (co-) administration of afamelanotide.

Aspects of the present invention provide improved methods for treating cardiac arrhythmia in subjects in need thereof. In particular, the present invention provides improved methods for treating cardiac
10 arrhythmia using amiodarone in subjects likely to suffer from amiodarone-induced photosensitivity by co-administration of afamelanotide as indicated herein. The co-administration of afamelanotide in preventive treatment regimes involving treating a subject suffering from cardiac arrhythmias by administration of amiodarone, is suitably separated by sequential or
15 synchronous administration of amiodarone and afamelanotide in their own frequency of dosing. Our toxicity studies show that the dosages and the frequencies do not need adjustment when the two drugs are used in combination.

Afamelanotide has the advantage of reducing amiodarone-induced
20 cytotoxicity. This means that administration of the combination preparation is less toxic than administration of the amiodarone itself. Use of a particular dosage of amiodarone in the form of a combination preparation will be less toxic, and less inflammatory reactions coinciding with amiodarone use are to be expected. Pharmaceutical preparations comprising other active
25 compounds, such as phenytoin, lidocaine, quinidine, isoproterenol, clopidogrel, acetyl-salicylic acid and molsidomine, that also derive their toxicity from the same effect of lowering the mitochondrial membrane potential, may therefore also benefit from the anti-cytotoxic effect of afamelanotide. The present invention therefore also contemplates
30 afamelanotide as an anticytotoxic agent to the cytotoxicity of active agents

that reduce the membrane potential of mitochondria, including such agents as amiodarone and phenytoin (diphantoine). The present invention therefore also contemplates the provision of pharmaceutical combinations comprising afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, and an agent that is toxic due to lowering of the mitochondrial membrane potential, preferably selected from phenytoin, lidocaine, quinidine, isoproterenol, clopidogrel, acetyl-salicylic acid and molsidomine, and optionally one or more pharmaceutically acceptable excipients, carriers or diluents.

All publications mentioned in this specification are herein incorporated by reference. The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

For the purpose of clarity and a concise description, features are described herein as part of the same or separate aspects and preferred embodiments thereof, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

The invention will now be illustrated by the following example, which is provided by way of illustration and not of limitation and it will be understood that many variations in the methods described and the amounts indicated can be made without departing from the spirit of the invention and the scope of the appended claims.

EXAMPLES

Example 1: In vitro toxicity

General materials and methods

Cell culture

C5RO (primary human fibroblast) and HEPG2 (human liver hepatoma) cells were obtained in house (Erasmus MC, Rotterdam, The Netherlands). Frozen
5 cells were removed from the -140 °C storage freezer and thawed quickly in a water bath at 37 °C and subsequently seeded in a 75 cm² flask in culture medium (DMEM (GIBCO, Paisley, UK), supplemented with 10% (v/v) fetal bovine serum (FBS; GIBCO) and 1% (v/v) streptomycin/penicillin (LONZA, Basel, Switzerland). The flask was incubated for 24 h in a humidified
10 atmosphere at 37 °C at 5% CO₂ after which culture medium was aspirated and replaced with fresh medium. Cells were incubated until they were 80-90% confluent before they were passaged.

Cells were subcultured in a 1:3 ratio every 2-3 days. Cells were detached by incubation with a trypsin/EDTA solution (GIBCO) for 5
15 minutes. Cells were resuspended in culture medium, transferred to an appropriate container and mixed well. Before the cells had a chance to settle 10 µl of cell suspension was loaded on a haemocytometer chamber and cells were counted.

20 *In vitro toxicity*

For in vitro toxicity testing cells were seeded at 5.000 cells per well in 96-wells view plates (Greiner) in 100 µl culture medium and incubated for 24 h at 37 °C at 5% CO₂. Cells were incubated for 24 h with 15 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 250 µg/ml, 2.5 mg/ml, and 5 mg/ml of compound X
25 (amiodarone). Compound Y (afamelanotide; [Nle⁴, D-Phe⁷]-α-MSH) was added at 50 ng/ml, 100 ng/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml. For the interaction studies, amiodarone was cultured with the cells at 15 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 250 µg/ml, 2.5 mg/ml, and 5 mg/ml with 1 µg/ml afamelanotide. Afamelanotide was cultured with the cells at 50 ng/ml, 100
30 ng/ml, 1 µg/ml, 10 µg/ml, and 100 µg/ml with 250 µg/ml amiodarone. All

culture cells contained a blanco (100 µl medium with cells), positive control (5 M NaOH), and background read (100 µl medium). All experiments were performed in triplo and the laboratory performed the tests in a blinded way.

After 24 h in vitro toxicity was determined with the ATPlite
5 Luminescence ATP Detection Assay System (PerkinElmer) following the
manufacturers protocol. In short, 50 µl of mammalian cell lysis buffer
(ATPlite Luminescence ATP Detection Assay; Perkin Elmer) was added to
the cells and incubated for 24 h with the cytotoxic compounds. Following
this addition, the 96-wells plates were shaken at 400 rpm for 5 minutes
10 with an orbital shaker. Next 50 µl substrate solution was added to the wells
and the 96-wells plates were shaken at 700 rpm for 5 minutes with an
orbital shaker. The plates were dark adapted for at least 10 minutes and
luminescence was measured with a VICTOR3™ Multi Label Reader
(PerkinElmer).

15

Results

In Figure 1, the findings of the toxicity experiments are shown. No
toxic interaction between amiodarone and afamelanotide was shown on the
human cell lines tested, meaning that the combination of these drugs is safe
20 for humans. Afamelanotide had no apparent effect on cell death, but mild
effects were observed when applied in extreme high dosages. Amiodarone
showed its known severe toxicity inducing apoptosis/cell death already at low
concentrations. Unexpectedly, panels A and B of Figure 1 revealed a
protecting effect by afamelanotide on amiodarone-induced cell toxicity,
25 which was particularly visible at the lower amiodarone concentrations used.

Claims

1. A pharmaceutical combination comprising amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, and optionally one or more pharmaceutically acceptable excipients, carriers or diluents.

5

2. The pharmaceutical combination according to claim 1, wherein amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, are formulated for oral or parenteral administration.

10

3. A method for symptomatic or prophylactic treatment of amiodarone-induced photosensitivity in a subject suffering from cardiac arrhythmia, comprising the simultaneous, separate or sequential co-administration of a therapeutically effective amount of amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof.

15

4. A method for the prophylactic treatment of amiodarone-induced photosensitivity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity, comprising the administration to said recipient of a therapeutically effective amount of afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity.

20

25

5. A pharmaceutical combination according to claim 1 or 2 for use in the symptomatic or prophylactic treatment of amiodarone-induced

photosensitivity in a subject suffering from cardiac arrhythmia, comprising the simultaneous, separate or sequential co-administration of a therapeutically effective amount of amiodarone and afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof.

5

6. Afamelanotide, or a pharmaceutically acceptable derivative or solvate of thereof, for use in the prophylactic treatment of amiodarone-induced photosensitivity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity.

10

7. Afamelanotide, or a pharmaceutically acceptable derivative or solvate of thereof, for use according to claim 6, wherein said prophylactic treatment is in accordance with a treatment regimen involving the administration to said recipient of a therapeutically effective amount of afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity.

15

8. Use of a pharmaceutical combination according to claim 1 or 2 in the preparation of a medicament for the symptomatic or prophylactic treatment of amiodarone-induced photosensitivity in a subject suffering from cardiac arrhythmia.

20

9. Use of afamelanotide, or a pharmaceutically acceptable derivative or solvate of thereof, in the preparation of a medicament for the prophylactic treatment of amiodarone-induced photosensitivity in an amiodarone recipient suffering from cardiac arrhythmia and predisposed to amiodarone-induced photosensitivity, preferably wherein the preparation is for administration to said recipient of a therapeutically effective amount of

25

afamelanotide, prior to the clinical manifestation of a symptom associated with said amiodarone-induced photosensitivity.

10. Afamelanotide for use as an anticytotoxic agent

5

11. An anticytotoxic agent comprising afamelanotide.

12. A pharmaceutical combination of a toxic pharmaceutically active agent the toxicity of which is due to lowering of the mitochondrial

10 membrane potential, preferably selected from phenytoin, lidocaine, quinidine, isoproterenol, clopidogrel, acetyl-salicylic acid and molsidomine, said combination further comprising afamelanotide, or a pharmaceutically acceptable derivative or solvate of either thereof, and optionally one or more pharmaceutically acceptable excipients, carriers or diluents.

15

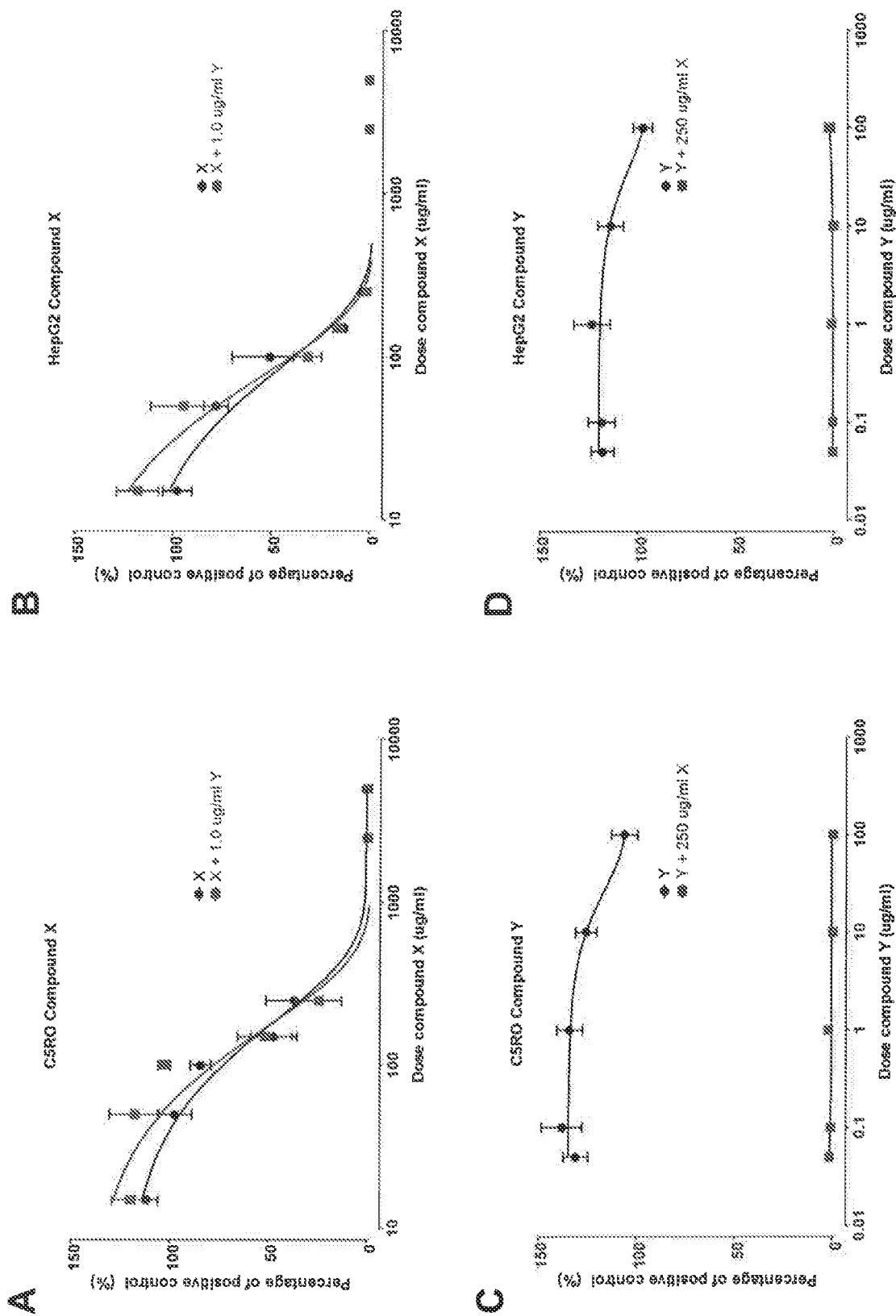


Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050389

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/34 A61P17/16
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, BIOSIS, EMBASE, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/103816 A1 (CLINUVEL PHARMACEUTICALS LTD [AU]; WOLGEN PHILIPPE [AU]) 27 August 2009 (2009-08-27) page 2, lines 8-13 - page 24, line 8 -----	1-9
A	WAITZER S ET AL: "Cutaneous ultrastructural changes and photosensitivity associated with amiodarone therapy", JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY, MOSBY, INC, US, vol. 16, no. 4, 1 April 1987 (1987-04-01), pages 779-787, XP022693595, ISSN: 0190-9622, DOI: 10.1016/S0190-9622(87)70101-7 [retrieved on 1987-04-01] abstract ----- -/--	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 July 2015

Date of mailing of the international search report

28/10/2015

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Cattell, James

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2015/050389

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANGAN ET AL: "Melanotropic peptides: more than just "Barbie drugs" and "sun-tan jabs"?", BRITISH J. OF DERMATOLOGY, vol. 163, 2010, - 2010, pages 451-455, XP002742578,	10,11
A	abstract -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2015/050389

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009103816 A1	27-08-2009	AU 2009216681 A1	27-08-2009
		CA 2716509 A1	27-08-2009
		EP 2259794 A1	15-12-2010
		WO 2009103816 A1	27-08-2009

INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims 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. ☐ Claims 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:

see additional sheet

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 an 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 claims 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 claims Nos.:

1-11

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11

The combination of Amiodarone and Afamelanotide.

2. claim: 12

Afamelanotide preferably combined with numerous other agents
