

Anticancer combinations of artemisinin-based drugs and other chemotherapeutic agents

FIELD OF THE INVENTION

5 The present invention relates to new combinations of artemisinin-based drugs selected from the group consisting of artemisinin, dihydroartemisinin and artemether, together with chemotherapeutic agents, pharmaceutical compositions containing them and their use as cytotoxic compositions.

BACKGROUND OF THE INVENTION

10 Besides their well known antimalarial activities, artemisinin-based drugs such as artemisinin (ART), dihydroartemisinin (DHA), artemether (ARM), and artesunate (ARS) have been reported recently to be also endowed of cytotoxic properties through induction of apoptosis (Singh N.P., *et al.*, *Anticancer Res.*, **2004**, *24*, 2277; Nam W., *et al.*, *Head Neck*, **2007**, *29*, 335).

15 Even if DHA possesses some cytotoxic properties, the latter are usually observable at relatively high drug concentration. Over the past few years, ART derivatives have emerged because endowed of more pronounced cytotoxic properties than DHA itself, against a wide range of cancer cells suggesting some potential for the treatment of various cancers such as human hepatocellular carcinoma (Hou J., *et al.*, *Clin. Cancer Res.*, **2008**, *14*, 17, 5519), leukemia (Lu J.J., *et al.*, *Canc. Biol. Ther.*, **2008**, *7*, 7, 1017), prostate cancer, non-small cell lung cancer (Lu Y.Y., *et al.*, *J. Biomed. Sci.*, **2009**, *Feb 2*, 16:16), pancreas (Chen H., *et al.*, *Anti-Cancer Drugs*, **2009**, *20*, 2, 131) and cervical cancer (Disbrow G.L., *et al.*, *Cancer Res.*, **2005**, *65*, 23, 10854).

25 It has been shown that compositions of some known antimalarial and various chemotherapeutics can lead to improved cytotoxic combinations. For example, ARS and tyrosine kinase inhibitor OSI-774 produce mainly additive effect on glioblastoma multiforme cell lines (Efferth T., *et al.*, *Biochem. Pharmacol.*, **2004**, *67*, 9, 1689); ARS and the anthracycline intercalating agent doxorubicin showed
30 synergistic activity on leukemic T-cells (Efferth T., *et al.*, *PLoS One*, **2007**, *2*, 1, e693), the anti-CD20 antibody rituximab potentiates the cytotoxic effect of ARS at high concentrations (Sieber S., *et al.*, *Int. J. Oncol.*, **2009**, *35*, 1, 149). Lately, a clinical trial aimed at comparing the efficacy of a cocktail containing vinorelbine and cisplatin with that of said cocktail in combination with ARS was reported. The

combination treatment did not provide any improvement in the short term survival rate, nor in the mean survival time or in the one year survival rate, thus failing to demonstrate any benefit over the single cocktail regimen on these important end point (Zhang Z.Y., *et al.*, *J. Integrative Med.*, **2008**, *6*, 2, 134).

5 WO2004/071506 reported that ART could be used for treating tumours induced by oncogenic viruses and for treating viral infections. The inventors also reported that combinations of ART with further anti-cancer drugs could be used favourably. However, no biological data regarding such combination treatment were reported to support the alleged synergistic activity.

10 On another side, DHA and gemcitabine showed a modest 1.2 fold increase in inhibition of proliferation of HepG2 and Hep3B hepatoma cells (Hou J., *et al.*, *Clin. Cancer Res.*, **2008**, *14*, 17, 5519).

Besides those disclosures, some contradictory data also appeared in the literature. Synergistic effects between DHA and the well known HDAC inhibitor sodium
15 butyrate have been reported (Singh N.P., *et al.*, *Anticancer Res.*, **2005**, *25*, 6B, 4325). It is noteworthy that these experiments were all conducted in the presence of 12 μ M of holotransferrin, which, as an iron-carrying protein, acted to enhance iron penetration into the cells. This phenomenon is known to improve DHA reactivity by enhancing radical generation from the peroxy moiety (Disbrow G.L., *et al.*, *Cancer*
20 *Res.*, **2005**, *65*, 23, 10854). However, the same authors have also disclosed previously in a patent application (WO199634602) that DHA and holotransferrin alone proved to lead to enhanced cytotoxic activity on MOLT-4 lymphoblastoid cells as well as in a canine mast cells carcinoma. Strangely, if the paper from 2005 highlights the fact that 10 μ M DHA in the presence of 12 μ M of holotransferrin had
25 no effect on Molt-4 cells (Figure 1C, page 4327), the same experimental conditions used in the patent application were reported to lead to 75% reduction in cell count at 8 hours. US5578637 also reported combinations involving endoperoxide-containing compounds wherein the presence of an iron enhancing agent was mandatory.

30 EP1658844 reports the outcome of a treatment therapy of two patients affected by uveal melanoma involving the use of ARS in combination with dacarbazin in the presence or absence of a concomitant iron therapy, the latter was aimed at increasing the efficacy of ARS. Meanwhile the first patient, who did not receive the

supplementary iron medication, died 23 months after entry into stage IV according to AJCC (Balch C.M., *et al.*, *J. Clin. Oncol.*, **2001**, *19*, 16, 3635), the second patient, who received the supplementary iron medication, was still alive at the time this patent application was filed.

- 5 WO200213826 disclosed combinations of the anti-malarial agents chloroquine, hydroxychloroquine and primaquine with the anti-cancer agents cisplatin and doxorubicin. Such combinations resulted in a better anti-cancer efficacy on few cell-lines, above all when the anti-malarial drug was used in high concentration. Quinine derivatives, and in particular hydroxychloroquine have recently been
- 10 reported to block autophagy, the latter being recognized to be a tumour resistance mechanism allowing cancer cells to survive in stress conditions (Rubinsztein D.C., *et al.*, *Nature Rev. Drug Disc.*, **2007**, *6*, 304). Furthermore, the difference in the mechanism of action of quinine-like drugs and artemisinin-like drugs is reported (Jung M., *et al.*, *Curr. Med. Chem.*, **2004**, *11*, 10, 1265; Meshinick S.R., *et al.*,
- 15 *Microbiol. Rev.*, **1996**, *60*, 301; Wu W.M., *et al.*, *Chem. Soc. Chem. Commun.*, **1996**, 2213; Wu W.M., *et al.*, *J. Am. Chem. Soc.*, **1998**, *120*, 3316). Jung M., *et al.* cited herein above also reported that novel C-12 non-acetal type deoxyartemisinin were found to possess exceptionally high *in vitro* antitumour activity. Such derivatives are structurally unrelated to the compounds of the present invention.
- 20 Nevertheless, other pharmaceutical combinations involving either ART, DHA or ARM and a second chemotherapeutic agent chosen from the group consisting of a camptothecin derivative, or a PARP-1 inhibitor, or an intercalating DNA agent, or an alkylating agent have never been reported to produce synergistic effects toward the treatment of cancer diseases.
- 25 Camptothecin derivatives originated from the discovery more than forty years ago of the alkaloid camptothecin, further referred to as CPT. The latter is reputed to be endowed of potent and wide spectrum anti-cancer activity. A lot of efforts, from numerous medicinal chemistry groups, have been devoted at improving the physico-chemical properties of CPT itself. Potent CPT derivatives can be found for example
- 30 in EP1044977 (filed in the name of the Applicant). Zunino F., *et al.* also nicely reviewed the latest advances in the field of CPT derivatives (Zunino F., *et al.*, *Curr. Pharm. Des.*, **2002**, *8*, 2505).

A promising role of PARP-1 inhibition in oncology was established on the basis that BRCA1 and BCRA2 knock-out cell lines were found to be highly sensitive to PARP-1 inhibitors, the latter provoking cell death. BRCA1 and BCRA2 protein mutations can lead to a major risk of breast, ovary prostate and pancreas cancers (e.g., MK-4827, Jones P., *et al.*, *J. Med. Chem.*, **2009**, 52, 22, 7170). WO2006110816 reported PARP inhibitors, among which ABT-888, endowed with anti-inflammatory properties besides possessing anti-proliferative properties.

PARP-1 inhibitors have also been reported to enhance the cytotoxic activities of anti-cancer drugs such as topoisomerase-I inhibitors (Delaney C.A., *et al.*, *Clin. Cancer Res.*, **2000**, 6, 2860–2867) and cisplatin (Miknyoczki S.J., *et al.*, *Mol. Cancer Ther.*, **2003**, 2, 371). Combination therapy on BRCA2/p53 deficient mice developing mammary tumours involving the use of PARP-1 inhibitor AZD2281 (Menear K.A., *et al.*, *J. Med. Chem.*, **2008**, 51, 6581) together with carboplatin was reported lately. Such a study showed no advantage over carboplatin monotherapy. Only the time to tumour relapse was increased when the PARP-1 inhibition was prolonged (Hay T., *et al.*, *Cancer Res.*, **2009**, 69, 9, 3850). The patent application WO2008063644 (Cephalon) reports that some carbazole derivatives as PARP-1 inhibitors cause radio-sensitization in human glioblastoma related xenograf model. A clinical trial (NCT00920595) is currently recruiting patient for a study regarding a combination therapy against solid tumours involving a PARP inhibitor and the methylating agent temozolomide.

Intercalating DNA agents such as anthracycline or acridine derivatives have been known and used for years in the treatment of various form of cancers; the best known derivatives being doxorubicin, daunorubicin, and dactinomycin.

Treatment of cancer remains largely unsatisfactory because of drug resistance phenomenon or dose-limiting cytotoxicity. Therefore, new treatments involving potent and safer drugs are highly desired to further increase the chance of finding an adequate therapies against cancer diseases.

DESCRIPTION OF THE INVENTION

Notwithstanding uncertainty about alleged activity of DHA-containing compositions coupled to an unknown mechanism of action, rendering the usefulness of DHA and/or its analogues as cytotoxic agents rather hypothetical, we surprisingly found that artemisinin-based potent anti-malarial agents, selected from the group

consisting of ART, DHA and ARM, when combined with a camptothecin derivative, or a PARP-1 inhibitor, or an intercalating DNA agent, or an alkylating agent, provoked medium to strong synergism in various models of cancer, in particular in NSCL.

- 5 One embodiment of the present invention relates to a chemotherapeutic combination consisting of: a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents; wherein within said combination, each
10 ingredient (a) and (b) are formulated separately from each other or are formulated in a single dosage form.

A further embodiment of the present invention relates to a chemotherapeutic combination consisting of: a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from
15 the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents; wherein within said combination, pharmaceutical agent (a) and chemotherapeutic agent (b) are formulated in a single dosage form suitable for concomitant use of both agents.

A still further embodiment of the present invention relates to a chemotherapeutic
20 combination consisting of: a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents; wherein within said combination, pharmaceutical agent (a) and chemotherapeutic agent (b) are formulated
25 separately, which is suitable for separate, concomitant or sequential use.

In a preferred embodiment, within said combination the pharmaceutical agent (a) is DHA.

In a further preferred embodiment, within said combination the chemotherapeutic agent (b) is a camptothecin derivative.

- 30 In a more preferred embodiment, the camptothecin derivative is one of those disclosed in US patent N° 6242457 or is irinotecan or its active metabolite SN-38. Preferred camptothecin derivatives are selected from the group consisting of 7-methoxyiminomethylcamptothecin, 7-methoxyiminomethyl-10-

hydroxycamptothecin, 7-(ter-butoxycarbonyl-2-propoxy)-iminomethylcamptothecin, 7-ethoxyiminomethylcamptothecin, 7-isopropoxy-iminomethylcamptothecin, 7-(2-methylbutoxy)-iminomethylcamptothecin, 7-t-butoxyiminomethylcamptothecin, 7-t-butoxyiminomethyl-10-hydroxycamptothecin, 7-t-butoxyiminomethyl-10-methoxycamptothecin, 7-(4-hydroxybutoxy)-iminomethylcamptothecin, 7-triphenylmethoxyiminomethylcamptothecin, 7-carboxymethoxyiminomethylcamptothecin, 7-(2-amino)-ethoxyiminomethylcamptothecin, 7-(2-N,N-dimethylamino)-ethoxyiminomethylcamptothecin, 7-allyloxyiminomethylcamptothecin, 7-cyclohexyloxyiminomethylcamptothecin, 7-cyclohexylmethoxyiminomethylcamptothecin, 7-cyclooctyloxyiminomethylcamptothecin, 7-cyclooctylmethoxyiminomethylcamptothecin, 7-benzyloxy-iminomethylcamptothecin, 7-[(1-benzyloxyimino)-2-phenylethyl]camptothecin, 7-(1-benzyloxyimino)-ethylcamptothecin, 7-phenoxyiminomethylcamptothecin, 7-(1-t-butoxyimino)-ethylcamptothecin, 7-p-nitrobenzyloxyiminomethylcamptothecin, 7-p-methylbenzyloxyiminomethylcamptothecin, 7-pentafluorobenzyloxyiminomethylcamptothecin, 7-p-phenylbenzyloxyiminomethylcamptothecin, 7-[2-(2,4-difluorophenyl)-ethoxy]iminomethylcamptothecin, 7-(4-t-butylbenzyloxy)-iminomethylcamptothecin, 7-(1-adamantyloxy)-iminomethylcamptothecin, 7-(1-adamantylmethoxy)-iminomethylcamptothecin, 7-(2-naphthyloxy)-iminomethylcamptothecin, 7-(9-anthrylmethoxy)-iminomethylcamptothecin, 7-oxiranylmethoxyiminomethylcamptothecin, 7-(6-uracyl)-methoxyiminomethylcamptothecin, 7-[2-(1-uracyl)-ethoxy]-iminomethylcamptothecin, 7-(4-pyridyl)-methoxyiminomethylcamptothecin, 7-(2-thienyl)-methoxyiminomethylcamptothecin, 7-[(N-methyl)-4-piperidiny]-methoxyiminomethylcamptothecin, 7-[2-(4-morpholinyl)-ethoxy]-iminomethylcamptothecin, 7-(benzoyloxyiminomethyl)camptothecin, 7-[(1-hydroxyimino)-2-phenylethyl]-camptothecin, 7-ter-butyloxyiminomethylcamptothecin N-oxide, 7-methoxyiminomethylcamptothecin N-oxide, irinotecan and its active metabolite SN-38.

In an even more preferred embodiment, the camptothecin derivative is selected from the group consisting of 7-t-butoxyiminomethylcamptothecin, 7-benzyloxyiminomethylcamptothecin, 7-(2-amino)-ethoxyiminomethylcamptothecin, irinotecan and its active metabolite SN-38.

In another preferred embodiment, within said combination the chemotherapeutic agent (b) is a PARP-1 inhibitor.

In a more preferred embodiment, the PARP-1 inhibitor is chosen from the group consisting of AZD2281, ABT 888 and MK-4827.

In another further preferred embodiment, within said combination the chemotherapeutic agent (b) is an intercalating DNA agent chosen between
5 doxorubicin, dactinomycin and daunorubicin.

In another further preferred embodiment, within said combination the chemotherapeutic agent (b) is an alkylating agent chosen from the group consisting of cisplatin, carboplatin, nedaplatin, oxaliplatin and satraplatin.

A more preferred embodiment consists of the use of a pharmaceutical agent (a)
10 comprising at least one artemisinin-based compound selected from the group consisting of ART, DHA and ARM in combination with a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents, wherein such use is separate, concomitant or sequential, as a medicament.

15 A further preferred embodiment consists of the use of a pharmaceutical agent (a) comprising at least one artemisinin-based compound selected from the group consisting of ART, DHA and ARM in combination with a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents; wherein such use is
20 separate, concomitant or sequential, for the prevention and/or treatment of neoplasms.

Another more preferred embodiment consists of the use of a pharmaceutical agent (a) which is DHA in combination with a pharmaceutical agent (b) which is 7-(2-amino)-ethoxyiminomethylcamptothecin, 7- t-butoxyiminomethylcamptothecin,
25 irinotecan or its active metabolite SN-38, wherein such use is separate, concomitant or sequential, for the prevention and/or treatment of neoplasms.

A further more preferred embodiment consists of the use of a pharmaceutical agent (a) which is DHA in combination with a pharmaceutical agent (b) which is doxorubicin, wherein such use is separate, concomitant or sequential, for the
30 prevention and/or treatment of neoplasms.

An even more preferred embodiment consists of the use of a pharmaceutical agent (a) comprising at least one artemisinin-based compound selected from the group consisting of ART, DHA and ARM in combination with a chemotherapeutic agent

(b) selected from the group consisting of PARP-1 inhibitors, wherein such use is separate, concomitant or sequential, for the prevention and/or treatment of neoplasms.

A still further preferred embodiment consists of the use of a pharmaceutical agent

5 (a) comprising at least one artemisinin-based compound selected from the group consisting of ART, DHA and ARM in combination with a pharmaceutical agent (b) which is an alkylating agent, wherein such use is separate, concomitant or sequential, for the prevention and/or treatment of neoplasms.

A more preferred embodiment consists of the use of a pharmaceutical agent (a)
10 which is DHA in combination with a pharmaceutical agent (b) which is cisplatin or carboplatin, wherein such use is separate, concomitant or sequential, for the prevention and/or treatment of neoplasms.

The expressions "separate use" or "use separately" indicate that each agent (a) and (b) can follow a proper administration schedule which can be different from the one
15 of the other agent.

The expressions "concomitant use" or "use concomitantly" indicate that each agent are administered at the same time, said agents being formulated in one single dosage from or separately, whatever their specific dosages are.

The expressions "sequential use" or "use sequentially" indicate that each agent
20 dosage schedule is administered on a periodic basis, involving only one agent, (a) or (b) at any one time.

The term "neoplasm" indicates an abnormal mass of tissue as a result of neoplasia. Neoplasia consists of the abnormal proliferation of cells. The growth of this clone of cells exceeds, and is uncoordinated with that of the normal tissues around it. It
25 usually causes a tumour. Neoplasms may be benign, pre-malignant or malignant. Benign neoplasms include for example uterine fibroids and melanocytic nevi and do not transform into cancer. Potentially malignant neoplasms include carcinoma in situ. They do not invade and destroy the surrounding tissue but, given enough time, will transform into a cancer. Malignant neoplasms are commonly called cancer.
30 They invade and destroy the surrounding tissue, may form metastases and eventually kill the host. Metastasis consists of the spread of a disease from one organ or part to another non-adjacent organ or part. Only malignant tumour cells and infections have the established capacity to metastasize.

Cancer cells can break away, leak, or spill from a primary tumour, enter lymphatic and blood vessels, circulate through the bloodstream, and be deposited within normal tissues elsewhere in the body. Metastasis is one of three hallmarks of malignancy. Most tumours can metastasize, although in varying degrees (e.g., glioma and basal cell carcinoma rarely metastasize). When tumour cells metastasize, the new tumour is called a secondary or metastatic tumour, and its cells are like those in the original tumour.

According to an embodiment of the present invention the neoplasm to be treated is a primary tumour, selected from the group comprising sarcoma, carcinoma, melanoma, bone tumour, neuroendocrine tumour, lymphoid leukaemia, myeloid leukaemia, monocytic leukaemia, megakaryocytic leukaemia, acute promyelocytic leukaemia or Hodgkin's disease.

The above mentioned sarcoma and carcinoma consist of the group comprising: breast cancer; lung cancer, including non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC); gastrointestinal cancer, including esophageal, gastric, small bowel, large bowel, rectal and colon cancer; glioma, including glioblastoma; ovarian cancer, cervical cancer, endometrial cancer, mesothelioma; renal cancer; prostate cancer and skin cancers.

The neoplasm can also refer to a paediatric cancer. For example paediatric cancers that can be treated or where the progression of the condition can be delayed according to the present invention are selected from the group consisting of: acute lymphoblastic leukaemia, acute myeloid leukaemia, adrenocortical carcinoma, astrocytomas, bladder cancer, brain stem glioma, central nervous system atypical teratoid/rhabdoid cancer, brain cancer, central nervous system embryonal cancers, brain cancer, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, childhood medulloblastoma, medulloepithelioma, pineal parenchymal cancers of intermediate differentiation, supratentorial primitive neuroectodermal cancers and pineoblastoma, breast cancer, bronchial cancers, carcinoid cancer, cervical cancer, chordoma, colorectal cancer, oesophageal cancer, extra cranial germ cell cancer, gastric cancer, glioma, hepatocellular (liver) cancer, Hodgkin lymphoma, kidney cancer, laryngeal cancer, leukaemia, acute lymphoblastic/myeloid leukaemia, liver cancer, non-Hodgkin lymphoma, medulloblastoma, mesothelioma, multiple endocrine neoplasia syndrome, nasopharyngeal cancer, oral cancer, ovarian cancer,

pancreatic cancer, papillomatosis, renal cell cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma, skin cancer, thymoma and thymic carcinoma, thyroid cancer and vaginal cancer.

The expression "prevention of neoplasms" relates to the use of the combination of the present invention to prevent the development of malignant tumours whenever pre-malignant (also called potentially malignant) neoplasm are detected.

According to a further embodiment of the present invention the neoplasm to be treated is a malignant neoplasm, also called cancer, or a potentially malignant neoplasm.

A further embodiment of the present invention is related to the use of a chemotherapeutic combination consisting of a pharmaceutical agent (a) comprising at least one artemisinin-based compound selected from the group consisting of ART, DHA and ARM in combination with a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents, for the preparation of a medicament useful for the prevention and/or treatment of neoplasms wherein the antitumoural activity is derived from the cytotoxic, and/or apoptotic properties of the chemotherapeutic composition.

A still further embodiment of the present invention is related to the use of said chemotherapeutic composition wherein the tumour is selected from the group comprising sarcoma, carcinoma, melanoma, bone tumour, neuroendocrine tumour, lymphoid leukaemia, myeloid leukaemia, monocytic leukaemia, megakaryocytic leukaemia, acute promyelocytic leukaemia or Hodgkin's disease.

A still further embodiment consists of a pharmaceutical composition comprising a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents; which further comprises a pharmaceutically acceptable carrier and/or excipient and/or diluent.

A further object of the invention is a process for the preparation of a pharmaceutical composition as above defined characterised by mixing a pharmaceutical agent (a) as above defined and at least one chemotherapeutic agent (b) as above defined with at

least one suitable pharmaceutically acceptable carrier and/or excipient and/or diluent.

In a further preferred embodiment, the agents (a) and (b) can be administered together or one after the other in one combined unit dosage form or in two separate unit dosage forms respectively.

In an even further preferred embodiment, chemotherapeutic agent (b) is administered straight after administration of pharmaceutical agent (a).

Another even further preferred embodiment of the present invention relates to a commercial package or product comprising a pharmaceutical formulation of a pharmaceutical agent (a); and a pharmaceutical formulation of a chemotherapeutic agent (b) for separate, concomitant or sequential use.

A still further preferred embodiment of the present invention relates to a commercial package or product wherein pharmaceutical agent (a) is formulated together with chemotherapeutic agent (b).

In an even most preferred embodiment of the present invention, the agents (a) and (b) demonstrate additive or even preferably synergistic effects.

Another embodiment of the present invention relates to a method for the prevention and/or treatment of neoplasms which comprises treating the patient concurrently or sequentially with a combination of at least one artemisinin-based compound (a) selected from the group consisting of ART, DHA and ARM and at least one chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives, PARP-1 inhibitors, intercalating DNA agents and alkylating agents.

The term “pharmaceutical agent(s)” or “compound”, as used herein, is meant to include the single agents (a) and (b), their pharmaceutically acceptable salts and, according to the context, can also be referred to the combination of the two.

The term “unit dosage form” refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active ingredient (a) or (b) calculated to produce the desired therapeutic effect when administered concomitantly or sequentially, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include refilled, pre-measured ampoules or syringes of the liquid compositions; or pills, tablets, capsules or the like in the case of solid compositions. In such compositions, the compound (a) and compound (b) of the invention are usually a

minor component (from about 0.1 to about 50% by weight or preferably from about 1 to about 40% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form. Dosage treatment may be a single dose schedule or a multiple dose schedule.

5 The term “combined unit dosage form” refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active ingredient (a) and (b) calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipients. As above disclosed, the combination of active ingredients (a) and (b) of the present
10 invention is useful as a medicament for the treatment of cancer diseases where the cancer disease is a cancer of the breasts, pancreas, lung, pleura, peritoneum, face and neck, bladder, brain, prostate, ovaries, eyes or a metastatic cancer.

The term “commercial package” or “product”, as used herein, defines a kit of parts allowing separate appropriate dosages of pharmaceutical agents (a) and (b) for
15 concurrent or subsequent use.

The compositions covered by the present invention are entirely conventional and are obtained with methods which are common practice in the pharmaceutical industry, such as, for example, those illustrated in Remington's Pharmaceutical Science Handbook, Mack Pub. N.Y. – last edition. According to the administration
20 route chosen, the compositions will be in solid or liquid form, suitable for oral, parenteral or topical administration. The compositions according to the present invention contain, along with the active ingredients, at least one pharmaceutically acceptable vehicle or excipient. These may be particularly useful formulation coadjuvants, e.g. solubilising agents, dispersing agents, suspension agents, and
25 emulsifying agents.

The amount of the pharmaceutical agents actually administered will typically be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compounds administered, drug combination, the age, body weight, and response of the
30 individual patient, the severity of the patient's symptoms, and the like. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rats, guinea pigs, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate

concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. In calculating the Human Equivalent Dose (HED) it is recommended to use the conversion table provided in Guidance for Industry and Reviewers document (2002, U.S. Food and Drug Administration, Rockville, Maryland, USA).

Generally, an effective dose will be from 0.01 mg/kg to 2000 mg/kg of pharmaceutical agents, preferably from 0.05 mg/kg to 500 mg/kg of pharmaceutical agent. The precise effective dose for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician.

The medicament of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal or transcutaneous applications, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal means.

The compositions for oral administration may take the form of bulk liquid solutions or suspensions, or bulk powders. More commonly, however, the compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The medicament may also contain a pharmaceutically acceptable carrier, for administration of a therapeutic agent. Such carriers include antibodies and other polypeptides, genes and other therapeutic agents such as liposomes, provided that the carrier does not induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity.

Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles. A thorough discussion of

pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol.

5 Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

10 The term "therapeutically effective dose" refers specifically to the cumulative dose of the combination involving pharmaceutical agent (a) and (b).

A further object of the invention is a process for the preparation of pharmaceutical compositions characterised by mixing at least one pharmaceutical agent (a) and a pharmaceutical agent (b) with suitable excipients, stabilizers and/or
15 pharmaceutically acceptable diluents.

BIOLOGICAL EXPERIMENTS

Example 1

Anti-proliferative activity of DHA and ART on different tumour cells

The biological activity of DHA and ART on tumour cells survival was evaluated.
20 The latter were seeded in 96-well tissue culture plates at approximately 10% confluence and were allowed to attach and recover for at least 24 h before being exposed to DHA or ART for 24 h at 37°C. They were then washed to remove DHA and ART and left to recover for 48 hours in medium culture. The medium culture was then removed and cells were washed three times with PBS. The plates were
25 then incubated for on ice for 1 hour with 200 µl of PBS and 50 µl of cold 80% TCA before being washed with distilled water, dried on paper and finally at 40°C for 5 minutes. 200 µl of 0.4% sulphorodamine B in 1% acetic acid were added and the incubation was prolonged for 30 minutes. Sulphorodamine B was removed, and the plates were washed 3 times with 1%acetic acid. They were finally dried on paper
30 followed by standing at 40°C for 5 minutes. Then 200 µl Tris 10 mM were added, the plates were kept under stirring for 20 minutes. Cell survival was determined as optical density at 540 nm by means of a Multiskan spectrofluorimeter. The amount of cells killed was expressed as the percentage decrease in sulphorodamine B

binding compared to control cultures. IC₅₀ were generated with their standard deviations by the ALLFIT program.

Results

ART showed a very weak antiproliferative effect on tumour cells. With the exception of the NSCLC line (i.e., NCI-H460), DHA showed to be able to inhibit non-resistant tumour cell lines in sub-micromolar range or even at lower concentrations. DHA was indeed, unexpectedly equally effective on the resistant breast tumour cell line MCF-7/Dx MDR and on the non-resistant one (MCF-7). The same analysis could be elaborated with regard to the colon carcinoma cell lines (i.e., LoVo). The resistant LoVo/Dx MDR cell line proved to be 63 times less sensitive to doxorubicin than its non resistant cell line counterpart. Interestingly, the resistant cell lines showed to be not resistant to DHA (resistant index values inferior to 1). Detailed results are shown in table 1 underneath.

Table 1

Tumour cell lines	IC ₅₀ ± SD (μM)	
	ART	DHA
HCT116	257 ± 28.8	0.37 ± 0.08
SW620	ND	1.8 ± 0.1
HT29	ND	5.8 ± 0.5
LoVo	> 200	2.57 ± 0.8
LoVo/Dx MDR	> 200	1.18 ± 0.3 (0.46)
MCF-7	45.8 ± 11.5	1.05 ± 0.08
MCF-7/Dx MDR	187 ± 70 (4)	0.97 ± 0.3 (0.92)
A2780	ND	0.23 ± 0.02
NCI-H460	ND	8.16 ± 0.5

ND: Not determined, in brackets, are reported the resistance index values calculated.

Example 2

Synergistic interaction between DHA and various chemotherapeutic drugs on tumour cells

Tumour cells were grown in RPMI 1640 containing 10% foetal bovine serum and treated as described in example 1 with the exception that instead of being exposed only to ART or DHA, the cell lines were treated with the drugs reported in table 2 in

combination with DHA (from 1.0 μ M to 20 μ M, table 2). Tumour cells were treated for 2 h with DHA prior to exposure to further chemotherapeutic agents for 72h. Cell survival was then evaluated through sulphorodamine B test. Data gathered from the various combination experiments were analyzed using CalcuSyn software (Biosoft, Ferguson, MO) through the elaboration of isobolograms to determine if the combinations of DHA and the various chemotherapeutic agents were additive, synergistic or antagonistic. Viability assay results (SRB) were expressed as the fraction of cells killed when exposed to a single drug or to a drug combination with respect to the untreated cells. A combination index value (CI) was determined by means of the Chou-Talalay median effect method (T-C Chou, *et al.*, *Trends Pharmacol. Sci.*, **1983**, 4, 450), wherein synergism can be defined when $CI < 1$ and the lower the CI parameter value, the stronger the synergism (as defined by CalcuSyn 2.0 analyzer).

Results

As shown in the table 2, good to very good synergistic interactions were found between DHA and different chemotherapeutic agents (PARP-1 inhibitors, camptothecin derivatives, anthracyclines and platinum compounds) on several tumour cell lines of various hystotypes (i.e., non-small cell lung carcinoma, ovarian, colon, breast, epidermoid skin, pancreas carcinoma and glioblastoma).

Depending on the cell type, particularly interesting synergistic combinations were found.

Table 2

Entry	Drugs	Cell lines	Combination index values		
			ED ₅₀	ED ₇₅	ED ₉₀
1	Cisplatin	NCI-H460	0.79	0.88	0.98
2	Phenanthridinone	NCI-H460	0.19	0.20	0.22
3		A2780	0.003	0.05	0.67
4	AZD2281	NCI-H460	0.62	0.48	0.42
5		HCT116	0.36	0.34	0.31
6		SW620	0.60	0.55	0.66
7		A431	0.37	0.02	0.02
8		A2780/DDP	0.63	0.68	0.63
9		CAPAN-1	0.35	0.49	0.73
10		MDA-MB436	0.28	0.30	0.68
11	ABT-888	NCI-H460	0.78	0.61	0.49
12		HCT116	0.66	0.66	0.66
13		A431	0.45	0.49	0.37
14		MDA-MB436	0.42	0.43	0.47
15		CAPAN-1	0.59	0.32	0.22
16	MK-4827	NCI-H460	0.83	0.83	0.84
17	SN-38	NCI-H460	0.81	0.68	0.59
18	Doxorubicin	NCI-H460	0.76	0.53	0.37
19	Carboplatin	NCI-H460	0.53	0.71	0.95
20	Cisplatin	NCI-H460	0.79	0.88	0.98
21	7-(2-amino)-ethoxyiminomethyl-camptothecin	NCI-H460	0.39	0.24	0.16
22	Temozolomide	U87MG	0.24	0.35	0.72
23		Capan-1	0.52	0.58	0.66
24		MDA-MB436	0.75	0.79	0.89

Example 3In vivo antitumor activity of the combination involving DHA and the PARP-1 inhibitor (AZD2281)

NCI-H460 non-small cell lung carcinoma cells were inoculated subcutaneously (s.c.) in the right flank of CD1 nude mice (3x10⁶/0.1 ml of Medium 199). Treatments started three days after tumour injection. Mice were subdivided (10 mice/group) in the following experimental groups:

a) Vehicle (DMSO 10%) 10 ml/kg, i.p.;

b) DHA 200 mg/kg, p.o. qdx5/wx3w;

10 c) AZD2281 100 mg/kg, i.p. qdx5/wx3w;

d) AZD2281 + DHA (doses and schedules as above).

For the latter experiment DHA was administered immediately before AZD2281.

To evaluate the antitumour activity, tumour diameters were measured with a Vernier calliper according to the formula

15
$$TV = d^2 \times D/2$$

where d and D are the shortest and longest diameters, respectively.

When tumours reached a volume of about 1500 mm³, mice were sacrificed by cervical dislocation. The efficacy of the drug was assessed as the tumour volume inhibition according to the formula reported underneath:

20
$$TVI\% = 100 - \left[\left(\frac{(\text{mean TV of treated group})}{(\text{mean TV of control group})} \right) \times 100 \right]$$

Body weight recording was carried out to evaluate body weight loss as calculated in the equation underneath:

$$\%BWL = 100 - \left[\frac{BW_{day_x}}{BW_{day1}} \right] \times 100$$

Wherein BW day x corresponds to the mean weight at day x of the experiment meanwhile BW day 1 corresponds to the mean weight at the first day of the experiment.

Results

Both compounds (i.e., DHA and AZD2281) were well-tolerated since mice did not show any body weight loss during the treatment. When DHA was given orally at 200 mg/kg according to the schedule qdx5/wx3w prior to administration of AZD2281 (i.p. at 100 mg/kg) according to the same schedule, the combination produced a

substantial tumour growth inhibition of 31% against NCI-H460 non-small cell lung carcinoma xenografted, meanwhile no antitumor effect was seen when the molecules were given singularly. (Table 3).

Table 3

Drugs	Dose (mg/10 ml/kg)	BWL %	TV±SE	TVI %
Vehicle	-	0	1615±266	-
DHA	200 p.o.	0	1575±191	3
AZD2281	100 i.p.	1	1603±171	1
DHA+ AZD2281	200 p.o. + 100 i.p.	0	*1120±135	31

5 *P<0.05 *versus* vehicle treated group (Mann-Whitney).

Example 4

In vivo antitumour activity of the combination involving DHA and the PARP-1 inhibitors AZD2281 and ABT-888

10 HCT116 colon cancer cells were inoculated subcutaneously (s.c.) in the right flank of CD1 nude mice (5x10⁶/0.1 ml of Medium 199). Treatments started three days after tumour injection. Mice were subdivided (8 mice/group) in the following experimental groups:

- a) Vehicle (DMSO 10%) 10 ml/kg, i.p.;
- b) DHA 200 mg/kg, p.o. qdx5/wx4w;
- 15 c) AZD2281 100 mg/kg, i.p. qdx5/wx4w;
- d) AZD2281 + DHA (doses and schedules as above);
- e) ABT-888 50 mg/kg, i.p. qdx5/wx4w;
- f) ABT-888 +DHA (doses and schedules as above).

DHA was administered immediately before AZD2281 or ABT-888.

20 The antitumour activity was evaluated according to the protocol reported hereinabove in example 3 when tumours reached a volume of about 500 mm³.

Body weight recording was carried out to evaluate body weight loss according to the protocol reported hereinabove in example 3.

Results

25 All compounds (i.e., DHA, AZD2281 and ABT-888) were well-tolerated since mice did not show any body weight loss during the treatment. None of them when given singularly did show any antitumor effect on this tumour hystotype. When DHA was

given orally at 200 mg/kg according to the schedule qdx5/wx4w prior to administration of AZD2281 (i.p. at 100 mg/kg) according to the same schedule, the combination gave rise to a substantial tumour growth inhibition of 37%. Such a synergistic effect was found to be even more pronounced when DHA was combined with another PARP-1 inhibitor, ABT-888 (p.o. at 50 mg/kg) showing a tumour volume inhibition of 49%. (Table 4).

Table 4

Drugs	Dose (mg/10 ml/kg)	BWL %	TV±SE	TVI %
Vehicle	-	0	401±22	-
DHA	200 p.o.	1	447±39	0
AZD2281	100 i.p.	1	328±52	18
DHA+ AZD2281	200 p.o. + 100 i.p.	3	*252±46	37
ABT-888	50 i.p.	0	395±56	2
DHA+ABT-888	200 p.o. + 50 i.p.	0	**205±45	49

*P<0.05 and **P<0.01 *versus* vehicle treated group (Mann-Whitney).

CLAIMS

1. A chemotherapeutic combination consisting of: a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives which are themselves selected from the group consisting of 7-methoxyiminomethylcamptothecin, 7-methoxyiminomethyl-10-hydroxycamptothecin, 7-(ter-butoxycarbonyl-2-propoxy)-iminomethylcamptothecin, 7-ethoxyiminomethylcamptothecin, 7-isopropoxy-iminomethylcamptothecin, 7-(2-methylbutoxy)-iminomethylcamptothecin, 7-t-butoxyiminomethylcamptothecin, 7-t-butoxyiminomethyl-10-hydroxycamptothecin, 7-t-butoxyiminomethyl-10-methoxycamptothecin, 7-(4-hydroxybutoxy)-iminomethylcamptothecin, 7-triphenyl-methoxyiminomethylcamptothecin, 7-carboxymethoxyiminomethylcamptothecin, 7-(2-amino)-ethoxyiminomethylcamptothecin, 7-(2-N,N-dimethylamino)-ethoxyiminomethylcamptothecin, 7-allyloxyiminomethylcamptothecin, 7-cyclohexyl-oxyiminomethylcamptothecin, 7-cyclohexylmethoxyiminomethylcamptothecin, 7-cyclooctyloxyiminomethylcamptothecin, 7-cyclooctylmethoxyiminomethylcamptothecin, 7-benzyloxy-iminomethylcamptothecin, 7-[(1-benzyloxyimino)-2-phenylethyl]camptothecin, 7-(1-benzyloxyimino)-ethylcamptothecin, 7-phenoxy-iminomethylcamptothecin, 7-(1-t-butoxyimino)-ethylcamptothecin, 7-p-nitrobenzyl-oxyiminomethylcamptothecin, 7-p-methylbenzyloxyiminomethylcamptothecin, 7-pentafluorobenzyloxyiminomethylcamptothecin, 7-p-phenylbenzyloxyiminomethylcamptothecin, 7-[2-(2,4-difluorophenyl)-ethoxy]iminomethylcamptothecin, 7-(4-t-butylbenzyloxy)-iminomethylcamptothecin, 7-(1-adamantyloxy)-iminomethylcamptothecin, 7-(1-adamantylmethoxy)-iminomethylcamptothecin, 7-(2-naphthyloxy)-iminomethylcamptothecin, 7-(9-anthrylmethoxy)-iminomethylcamptothecin, 7-oxiranylmethoxyiminomethylcamptothecin, 7-(6-uracyl)-methoxy-iminomethylcamptothecin, 7-[2-(1-uracyl)-ethoxy]-iminomethylcamptothecin, 7-(4-pyridyl)-methoxyiminomethylcamptothecin, 7-(2-thienyl)-methoxyiminomethylcamptothecin, 7-[(N-methyl)-4-piperidinyll]-methoxyiminomethylcamptothecin, 7-[2-(4-morpholininyl)-ethoxy]-iminomethylcamptothecin, 7-(benzoyloxyiminomethyl)-camptothecin, 7-[(1-hydroxyimino)-2-phenylethyl]-camptothecin, 7-ter-butyloxy-iminomethylcamptothecin N-oxide, 7-methoxyiminomethylcamptothecin N-oxide, irinotecan and its active metabolite

SN-38; PARP-1 inhibitors, intercalating DNA agents and alkylating agents; wherein within said combination, each ingredient (a) and (b) are formulated separately from each other or are formulated in a single dosage form.

2. The chemotherapeutic combination as defined in claim 1 wherein the
5 pharmaceutical agent (a) and chemotherapeutic agent (b) are formulated in a single dosage form.
3. The chemotherapeutic combination as defined in claim 1 wherein the pharmaceutical agent (a) and chemotherapeutic agent (b) are formulated separately.
- 10 4. The chemotherapeutic combination according to any of claims 1 to 3 wherein the pharmaceutical agent (a) is DHA.
5. The chemotherapeutic combination according to any of claims 1 to 4, wherein the chemotherapeutic agent (b) is a camptothecin derivative as claimed in claim 1.
6. The chemotherapeutic combination according to claim 5, wherein the
15 camptothecin derivative is chosen from the group consisting of 7-t-butoxyiminomethylcamptothecin, 7-benzyloxyiminomethylcamptothecin, 7-(2-amino)-ethoxyiminomethylcamptothecin, irinotecan and its active metabolite SN-38.
7. The chemotherapeutic combination according to any of claims 1 to 4, wherein the
20 chemotherapeutic agent (b) is a PARP-1 inhibitor.
8. The chemotherapeutic combination according to claim 7, wherein the PARP-1 inhibitor is phenanthridinone, AZD2281 or ABT-888.
9. The chemotherapeutic combination according to any of claims 1 to 4, wherein the chemotherapeutic agent (b) is an intercalating DNA agent or an alkylating
25 agent.
10. Use of a chemotherapeutic combination consisting of: a pharmaceutical agent (a) selected from the group consisting of ART, DHA and ARM; and a chemotherapeutic agent (b) selected from the group consisting of camptothecin derivatives as claimed in claim 1, PARP-1 inhibitors, intercalating DNA agents
30 and alkylating agents, as medicament.
11. The use according to claim 10 for the preparation of a medicament for the prevention and/or treatment of a neoplasm.

12. The use according to claim 11, wherein agents (a) and (b) are administered separately, concomitantly or sequentially.

13. The use according to claim 12 wherein the neoplasm is selected from the group consisting of: sarcoma, carcinoma, bone tumour, neuroendocrine tumour, lymphoid leukaemia, myeloid leukaemia, monocytic leukaemia, megakaryocytic leukaemia or Hodgkin's disease; wherein sarcoma and carcinoma are chosen from the group consisting of: breast cancer; lung cancer, including non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC); gastrointestinal cancer, including esophageal, gastric, small bowel, large bowel, rectal and colon cancer; glioma, including glioblastoma; ovarian cancer, cervical cancer, endometrial cancer, mesothelioma; renal cancer; prostate cancer and skin cancers; or the tumour relates to a paediatric cancer selected from the group consisting of: acute lymphoblastic leukaemia, acute myeloid leukaemia, adrenocortical carcinoma, astrocytomas, bladder cancer, brain stem glioma, central nervous system atypical teratoid/rhabdoid cancer, brain cancer, central nervous system embryonal cancers, brain cancer, astrocytomas, craniopharyngioma, ependymoblastoma, ependymoma, childhood medulloblastoma, medulloepithelioma, pineal parenchymal cancers of intermediate differentiation, supratentorial primitive neuroectodermal cancers and pineoblastoma, breast cancer, bronchial cancers, carcinoid cancer, cervical cancer, chordoma, colorectal cancer, oesophageal cancer, extra cranial germ cell cancer, gastric cancer, glioma, hepatocellular (liver) cancer, Hodgkin lymphoma, kidney cancer, laryngeal cancer, leukaemia, acute lymphoblastic/myeloid leukaemia, liver cancer, non-Hodgkin lymphoma, medulloblastoma, mesothelioma, multiple endocrine neoplasia syndrome, nasopharyngeal cancer, oral cancer, ovarian cancer, pancreatic cancer, papillomatosis, renal cell cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma, skin cancer, thymoma and thymic carcinoma, thyroid cancer and vaginal cancer.

14. A pharmaceutical composition comprising a chemotherapeutic combination as defined in any of claims 1 to 9 and at least one pharmaceutically acceptable carriers and/or excipient and/or diluent; wherein pharmaceutical agent (a) and chemotherapeutic agent (b) are present in a single dosage form or in two separate dosage forms.

15. A process for preparing a pharmaceutical composition according to claim 14 comprising mixing at least a pharmaceutical agent (a) as defined in claim 1 with pharmaceutically acceptable carriers and/or excipients and/or diluent; and a chemotherapeutic agent (b) as defined in claim 1 with pharmaceutically acceptable carriers and/or excipients and/or diluent; wherein pharmaceutical agent (a) and chemotherapeutic agent (b) are present in a single dosage form or in two separate dosage forms.
16. Use of a pharmaceutical composition as defined in claim 14, wherein agents(a) and (b) are administered separately, concomitantly or sequentially.
- 10 17. A method for the prevention and/or treatment of neoplasms consisting of administering a pharmaceutical composition as defined in claim 16.