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(54) Title: USE OF ENDOTHELIN-1 ANTAGONISTS FOR IMPROVING CANCER THERAPY

(57) Abstract: The present invention relates to a method and composition for treating or for diagnosing cancer. In particular, the present invention relates to methods and compositions for improving the efficacy of conventional cancer therapy and diagnosis. The invention comprises the use of an endothelin antagonist, and in particular of a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist for treating or for diagnosing cancer or for improving the efficacy of conventional cancer therapy and diagnosis.

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Use of endothelin-1 antagonists for improving cancer therapy

Field of the invention

The present invention relates to the medical field. The present invention relates to a method and composition for treating cancer. The present invention also relates to a method and composition for diagnosing cancer. In particular, the present invention relates to methods and compositions for improving the efficacy of conventional cancer therapy and diagnosis.

Background

In mammals the endothelin (ET) family comprises three isoforms, ET-1, ET-2, and ET-3, mainly synthesized and released by endothelial cells. ET-1 is the principal isoform in the human cardiovascular system. Endothelins are produced by enzymatic cleavage of a precursor peptide by an endothelin converting enzyme (ECE). ET-1 is being released from a dual secretory pathway (Russel et al. 1998; Circ. Res 83:314-321). The peptide is continuously released from vascular endothelial cells by the constitutive pathway, contributing to vascular tone. The peptide is also released from endothelial cell specific storage granules (Weibel-Palade bodies) in response to external stimuli, to produce further vasoconstriction.

In humans, endothelin activity is mediated via binding with preferential affinities to two distinct G-coupled receptors, ET-A and ET-B, in an autocrine/paracrine manner (see, e. g., Hocher et al., 1997, Eur. J. Clin. Chem. Clin. Biochem. 35 (3): 175-189; Shichiri et al., 1991, J. Cardiovascular Pharmacol. 17: S76 S78). ET receptors are widely expressed in all tissues. In humans, ET-A receptors predominate on vascular smooth muscle cells, are linked to vasoconstriction and have been associated with cardiovascular, renal and central nervous system diseases; whereas ET-B receptors are located on the vascular endothelium, linked to vasodilation (Takayanagi et al. (1991) FEBS Lttrs. 282: 103-106) and have been associated with bronchoconstrictive disorders. In some vascular beds, ET-B receptors are also located on smooth muscle where they mediate vasoconstriction. Activation of endothelial ET-B receptors may lead to the release of endothelium-derived relaxing factors, e.g. nitric oxide and prostanoids. Non-vascular ET-B receptors in organs including kidney may beneficially clear ET-1 from the circulation.

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First discovered in vascular endothelial cells, ET and ET receptor binding are now known to modulate vasomotion, cell proliferation and differentiation, protein synthesis, and metabolic function in a variety of tissues and cell types such as ovary, prostate, skin, and brain. They are characterized by their potent vasoconstricting and angiogenic properties (see, e. g., Luscher et al., 1995, Agents Actions Suppl. (Switzerland) 45: 237-253; Yanagisawa et al., 1988, Nature 332: 411-415).

There are a variety of agonists and antagonists of endothelin receptors (Webb et al., 1997, Medicinal Research Reviews 17 (1): 17-67), which have been used to study the mechanism of action of the endothelins. Because endothelin is known to have powerful vasoconstrictive activity, endothelin antagonists in particular have been studied with regard to their possible role in treating human diseases, most notably, cardiovascular diseases such as hypertension, congestive heart failure, atherosclerosis, restenosis, and myocardial infarction (Mateo et al., 1997, Pharmacological Res. 36 (5): 339-351). In particular, the use of ET inhibitors in cardiovascular applications as anti-hypertension agents has been reported. However, different problems related to toxicity of the inhibitors have decreased the interest in this type of compound for cardiovascular applications.

More recently, endothelins and endothelin receptors have been implicated in a number of normal and pathological cell growth processes, e. g., cell cycle progression, cell growth, and cellular development (see, e. g., Parris et al., 1997, Vascular Medicine 2: 31-43; Markewitz et al., supra; Morbidelli et al., 1995, Am. J. Physiol. 269: H686-H695; and Battistini et al. 1993, Peptides 14: 385-399).

The use of ET inhibitors in cancer treatment as anti-proliferation agents has been reported. For example, EP 1,256,344 discloses a pharmaceutical composition, which comprises a therapeutic agent for suppressing the growth of the cancer cell of prostate cancer; and for suppressing the progress of prostate cancer. The composition comprises N-[6-Methoxy-5-(2-methoxyphenoxy)-2-(2-pyrimidinyl)-4-pyrimidinyl]-2-phenylethenesulfoneamide or a salt thereof. This compound is known to suppress ET-1 binding to endothelin ET-A receptor.

WO 02/11713 relates to methods for treating cancer and the pain related thereto using endothelin antagonists. In particular, the document relates to methods for the inhibition of

bone metastases, methods for the prevention of growth of new metastases, methods for the inhibition of bone turnover, and methods for the prevention of bone loss in patients, including cancer patients. The methods comprise administering a patient in need of such treatment a therapeutically effective composition comprising an endothelin ET-A receptor antagonist.

Also WO 01/00198 describes the use of endothelin receptor antagonists in the treatment of cancer. This document reports the use of endothelin receptor antagonists, in particular a selective inhibitor of an endothelin-B receptor activity, for reducing abnormal cell proliferation, for inhibiting cancerous growth, for inducing differentiation in a cell, for regressing cancer growth and for inducing apoptosis in a cancerous cell.

Cancer is now primarily treated with one or a combination of classical types of therapies, including surgery, radiation and chemotherapy. However, results of such therapies, while beneficial in some cancers, have had only marginal effects in many others. Furthermore, these therapies often are associated with unacceptable toxicity or poor efficacy. Radiation, as well as a great majority of the chemotherapeutic drugs used in the standard treatment of cancer have the drawback of being toxic or even highly toxic, i.e. involving many detrimental side-effects on healthy cells, tissues and organs, and this limits their clinical use to a relatively low number of administrations per patient.

Several new biological and gene-based therapies aim instead of killing cancer cells to enhance the body's natural defenses against invading cancers. One such cancer therapy includes immunotherapy. The human body has an extremely well developed immune system whose purpose is to repel/eliminate pathogens or foreign cells. Cancer cells are genetically different from the normal cells from which they derive. The immune system recognizes cancer cells as foreign because of the genetic changes that occur during malignant transformation. Cancer immunotherapy attempts to stimulate the reactivity of the individual's immune system towards eliminating the cancer cells. This can be done by the transfer of immunostimulants, i.e. non-specific agents that tune-up the body's immune defenses; antibodies or T-cells from an outside source to a patient to stimulate the patient's own immune defenses. Ideally, these agents would be targeted to molecules expressed on the cancer cells but not on healthy cells. However, such tumor-specific antigens have been hard to find, and so many of the immune agents now in use do target healthy cells as well.

Cancer gene therapy is another approach of cancer treatment. Gene therapy strategy must be based upon the identification or design of a gene that may aid in the management or correction of the cancer. To allow transfer of the therapeutic gene to the desired body cells and proper function in a patient the therapeutic gene must be built into a vector. Currentlmy often disabled viruses or liposomes (fat droplets) are used as vectors. However, a major problem associated with gene therapy is the effective delivery of the therapeutic gene with minimal side-effects, and proper control of expression of the inserted gene. Moreover, problems of this nature mostly need to be addressed specifically for each individual disease or target cell.

In addition, several methods exist for diagnosing cancer. Such methods may comprise, amongst other, the delivery of diagnostic compounds to tumors. However, a major challenge associated with this type of methods is the effective delivery of the diagnostic compounds with minimal side-effects to a targeted tumor.

It is clear that there remains a great need in the art for developing methods for improving conventional cancer therapies and for improving diagnosis methods. Therefore, it is an object of the present invention to provide methods and compositions for treating or diagnosing cancer. The present invention also aims to provide methods and compositions for improving conventional cancer therapies and for improving cancer diagnosis. In particular, it is an object of the present invention to provide methods and compositions for optimizing the supply of cancer therapies or diagnostic techniques.

Summary

In a first embodiment the present invention relates to a method for treating cancer comprising the steps of exposing an individual in need of said cancer treatment to a therapeutically effective amount of an endothelin antagonist, and exposing said individual to a cancer therapy.

The present invention also relates to a method for diagnosing cancer comprising the steps of exposing an individual in need of said cancer treatment to a suitable amount of an endothelin antagonist, and exposing said individual to a cancer diagnostic method.

The present invention is in part based on the Applicants' finding that endothelin-1 is a critical mediator of myogenic tone, i.e the ability of the blood vessels to contract when intraluminal pressure increases, in tumor arterioles. The Applicant has identified the mechanism whereby the myogenic tone of blood vessels, which irrigate tumors, is regulated through the up-regulation of the endothelin-1 (ET-1) signal pathway and of its receptor (ET-A).

Therefore, according to the present method, by administering an endothelin antagonist, i.e. a compound inhibiting endothelin activity, to a patient suffering from cancer, the myogenic tone of the tumor blood vessels can be blocked. Blocking of the myogenic tone enables to increase tumor perfusion and consecutively to increase the radiosensitivity of tumors and to more effectively deliver anti-cancer compounds, including chemotherapeutics, immunotherapeutics, vectors used in gene therapy as well as any other compounds needed to be addressed to the tumor, or diagnostic substances to tumors. In particular, increase in tumor blood flow leads to an increase in oxygenation of the tumor. As a result thereof, the tumor becomes more susceptible to ionizing radiation. Thus, improvement in the function of tumor blood vessels and tumor perfusion by administering an endothelin antagonist allows *potentiating the efficacy* of conventional cancer therapies including radio- and chemotherapy, immunotherapy or gene-based therapies as well as *the delivery* of any compounds needed to be addressed to a tumor.

The present method is in addition highly tumor-selective. This effect is mainly due to the specific phenotype of endothelial cells lining tumor blood vessels. Because of the very large increase in ET-1 abundance in tumors and the higher density of the ET-A receptors in tumor vessels, administration of ET antagonists will substantially increase tumor perfusion but will barely alter blood flow in the majority of healthy tissues. Also, the patient will only be exposed to the said ET antagonist at very specific times, thereby limiting the side effects of these drugs.

In another embodiment said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof. In a preferred embodiment, said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

In yet another embodiment said anti-cancer composition is selected from the group comprising chemotherapeutics, radiation, immunotherapeutics, vectors used in gene therapy or any combinations thereof.

In further embodiments, the present invention further relates to methods for optimizing the supply of an anti-cancer composition used in cancer therapy and methods for optimizing the supply of a diagnostic compound used in cancer diagnosis. The invention also relates to methods for reducing side-effects of an anti-cancer composition used in cancer therapy and to methods for reducing side-effects of a diagnostic compound used in cancer diagnosis. Also described in the present invention is a method for increasing the susceptibility of a tumor tissue to an anti-cancer composition or to a diagnostic compound, a method for preparing an anti-cancer composition or diagnostic composition having reduced toxicity, a method for preparing an anti-cancer composition or diagnostic composition having improved delivery to a tumor site, a method for adjusting the amount of an anti-cancer composition or a diagnostic compound supplied by a supplying device.

In another embodiment the invention relates to a composition containing an endothelin antagonist and an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy. The invention also relates to a composition containing an endothelin antagonist and a diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer diagnosis.

In yet another embodiment, the invention relates to the use of an endothelin antagonist for improving cancer therapy and to the use of an endothelin antagonist in one of the herein-described methods. Also, the invention provides for the use of an endothelin antagonist in the preparation of a medicament for improving cancer therapy and to the use of an endothelin antagonist in the preparation of a medicament for one of the herein-described treatment methods. The invention further relates to the use of an endothelin antagonist in cancer diagnosis and for improving cancer diagnosis.

The invention thus relates to methods and compositions for treating or diagnosis cancer and for improving cancer treatment or cancer diagnosis, which are effective, specific and which

have limited side-effects. Those skilled in the art will immediate recognize the many other effects and advantages of the present method and composition and the numerous possibilities for end uses of the present invention from the detailed description and examples provided below.

Detailed description of the figures

Figure 1 represents immunodetection of ET-1 in tumor and tumor vessels. ET-1 antibodies were used to immunostain cardiac tissues from healthy mice (cl indicates the lumen of a coronary artery) (Fig. 1a); TLT tumors (Fig. 1b); LLc tumors (Fig. 1c); and FSA-II tumors (Fig. 1d). Dashed lines delineate the healthy muscular tissue (m) surrounding tumors or the central necrotic core (nc). The bar representes 100 μm. Saphenous arterioles from healthy (Fig. 1e) or tumor-bearing (Fig. 1f) mice were also immunostained for ET-1 expression. FIG. 1g shows the anatomical location of the saphenous arteriole located between the saphenous vein (v) and nerve (n); the dashed line shows the limits of the tumor and the arrowhead indicates where the arteriole enters the tumor (e.g. where arterial segments were isolated for myograph studies).

Figure 2 illustrates that tumor arterioles are hyper-responsive to ET-1 and overexpress smooth muscle ET-A receptor. In Fig. 2a arterioles isolated from healthy (□) and from TLT tumor-bearing mice (■) were mounted on a pressure myograph and challenged with increasing doses of ET-1. Results are expressed as % of the maximal contraction obtained with 10^{-7} M ET-1. Sigmoidal fitting shows that the dose/effect curves for healthy and tumor vessels (R^2= 0.91 and 0.98, respectively) were significantly different ($P<0.01$; n = 5); note that some s.e.m. are smaller than symbols. In Fig. 2b pools of arterioles from healthy and TLT tumor-bearing mice were lysed (to obtain enough material) and were immunoblotted with ET_A and ET_B antibodies. Shown are a representative immunoblot and a bar graph depicting the densitometric analysis of two different immunoblots (2 pools of 10 arterioles per condition).

Figure 3 shows tumor arterioles exhibiting an ET-A-dependent myogenic tone. Isolated arterioles were mounted on a pressure myograph and allowed to respond to a step-by-step increase of the luminal pressure. Changes in vessel diameter were tracked in bathing media containing calcium (active diameter, ●, n=10) or not (passive diameter, ○, n=10). Saphenous arterioles were dissected from healthy mice (FIG. 3a) or were isolated from TLT tumor (after

cooption) (FIG. 3b). In some experiments, arterioles were pre-incubated with the ET_A antagonist BQ123 (1 μM) in the calcium-containing medium (active diameter, ▲, n=3)) (ns, not significantly different; **P<0.01 between the indicated dose/effect curves).

Figure 4 illustrates that ET-A inhibition increases tumor blood flow and oxygenation. Fig. 4a shows typical LDI pictures obtained in mice before (time 0) and after an IP injection of 1 mg/kg BQ123 (upper panels) or vehicle alone (lower panels). On each picture, the tumor-bearing leg is on the right (white circle) and the control leg on the left. Fig. 4b shows the time course of *global* changes in tumor perfusion (derived from LDI color pixel histograms) after BQ123 (●) (n=11) or saline (○) (n=10) injection; data are expressed as % of the basal perfusion. (**P<0.01 when comparing the two curves). Fig. 4c shows the time course of *local* changes in tumor perfusion determined with Laser Doppler needle microprobes (Oxyflo) after BQ123 (●) or saline (○) injection (n=12). Data are expressed as % of the basal perfusion at time 0. (**P<0.01 when comparing the two curves). Fig. 4d shows a bar graph representing the tumor pO_2 measured by EPR at baseline (open bars; n=14) and 45 min after saline (hatched; n=7) or BQ123 (black; n=7) administration (**P<0.01).

Figure 5 illustrates that ET-A inhibition radiosensitizes tumors. Growth of TLT tumors was determined by measuring tumor diameters in untreated mice (□), in mice treated with 1 mg/kg BQ123 alone (■), with irradiation and vehicle (○), and with the combination of BQ123 and irradiation (●).Irradiated mice received either (Fig. 5a) five fractions of 2 Gy (day 0 to day +4) or (Fig. 5b) two fractions of 6 Gy (day 0 to day +1); data are expressed as % of the initial tumor diameters (*n* = 8-11). (Fig. 5c) For the 2 x [6Gy+BQ123] condition, three doses of BQ123 were tested (0.5, 1 and 2 mg/kg) and mean tumor doubling times were determined (**P<0.01, n=6-8). Of note, the beneficial effect of the 2 mg/kg dose is underestimated since the doubling time was not determined for two mice (out of 6) which were in complete remission 5 weeks after the initiation of treatment. BQ123 alone (at each dose tested) failed to produce any significant delay in tumor growth. When co-administered with radiotherapy, BQ123 (or vehicle) was delivered i.p. 45-min before each irradiation.

Figure 6 illustrates that ET-A inhibition sensitizes tumors to chemotherapy. Growth of TLT tumors was determined by measuring tumor diameters in untreated mice (□), in mice treated with 1 mg/kg BQ123 alone (■), with 25 mg/kg cyclophosphamide and vehicle (○), and with

the combination of cyclophosphamide and BQ123 (●). Treated mice received two doses of cyclophosphamide at day 0 and 1; when co-treated with BQ123, the ETA antagonist was delivered i.p. 45-min before cyclophosphamide administration. Data are expressed as % of the initial tumor diameters (n = 6).

Detailed description of the invention

The term "endothelin" as used herein refers to a family of homologous 21-amino acid peptides found in 3 distinct isoforms: ET-1, ET2, and ET-3. Endothelin activity is mediated via binding with preferential affinities to two distinct G-coupled receptors, ET-A and ET-B.

The term "endothelin antagonist" as used herein refers to a compound inhibiting endothelin activity. In particular, the term "endothelin antagonist" encompasses "endothelin receptor antagonists", "endothelin converting enzyme inhibitors" or "endothelin neutralizing agents" or combinations thereof.

In the present invention, an "endothelin receptor antagonist" is defined as a substance that interferes with the action of endothelin peptides at an endothelin receptor. Such substances may act by (a) binding to the receptor, or (b) otherwise inhibiting it from binding or interacting with an endothelin peptide. This term includes peptide-based or non-peptide based compounds, which antagonize the ET-A receptor in a selective manner (these compounds are also referred to as specific ET-A receptors antagonists), peptide-based or non-peptide based compounds, which antagonize the ET-B receptor in a selective manner (these compounds are also referred to as specific ET-B receptors antagonists, as well as mixed antagonists that display affinities for ET-A and ET-B receptors (these compounds are also referred to as ET-A/ET-B receptors antagonists). An example of the latter type of compound is for instance a compound that antagonizes the ET-A receptor and also antagonizes the ET-B receptor.

Non-limiting examples of suitable endothelin receptor antagonists usable in the present invention are represented in Table 1, 2 and 3.

TABLE 1 Endothelin-A specific receptor antagonists

Company code	Company	Class name	CAS N°
A127722, ABT627, A216546, LU127043	Abbott (Knoll)		
A306552, ABT546	Abbott (Knoll)		
BSF208075	Abbott (Knoll)	ambrisentan	177036-94-1
ABT627	Abbott (Knoll)	atrasentan	173937-91-2
LU135252	Abbott (Knoll)	darusentan	171714-84-4
RPR118031	Aventis		
BMS182874, BMS184696	Bristol-Myers Squibb		
BMS184697, BMS193884	Bristol-Myers Squibb		
BMS207940	Bristol-Myers Squibb	endonentan	210891-04-6
IP1413	ImmunoPharmaceuticals Inc.		
PD155080, PD155719, PD180988	Pfizer (Parke-Davis)		
PD156707	Pfizer (Parke-Davis)		162412-70-6
PD163140	Pfizer (Parke-Davis)		187533-62-6
PD159433, PD164893	Pfizer (Parke-Davis)		
PD151242 PD147953	Pfizer (Parke-Davis)		
PD163070, PD163610	Pfizer (Parke-Davis)		
PED3512P1	Peptide International		
RO611790	Hoffman-LaRoche		180384-56-9
L744453, L754142,	Merck & Co		
L749329	Merck & Co		159590-37-1
BQ485, BQ153, BQ610	Merck & Co		
BQ123	Merck & Co		136553-81-6
EMD94246	Merck KGaA		
SB234551	GlaxoSmithKline		
34-sulfatobastadin	Novartis		
CGS27830	Novartis		155485-56-6
S-0139	Shionogi		193969-54-9
TTA101, TTA386	Takeda		
TBC11251	Texas Biotech Corp.	Sitaxsentan	184036-34-8
TBC3711	Texas Biotech Corp.		374680-51-0
WS7338A, WS009A, WS009B	Fujisawa		
FR139317	Fujisawa		142375-60-8
ZD1611, ZD2574, ZD4054	AstraZeneca		
YM-598	Yamanouchi		
TA0201	Taneba Seiyaku		188307-16-6

TABLE 2 Endothelin-B specific receptor antagonists

Company code	Company
BQ788	Merck & Co
IRL2500	
RES7011	
RO468443	Hoffman-LaRoche
A192621	Abbott (Knoll)

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TABLE 3 Endothelin-A/ endothelin-B non specific receptor antagonists

Company code	Company	Class name	CAS N°
Aselacins A, B and C; A182086	Abbott (Knoll)		
BSF420627	Abbott (Knoll)	feloprentan	204267-33-4
RO470203	Actelion		
RO610612	Actelion	tezosentan	180384-57-0
SB209670	GlaxoSmithKline		157659-79-5
SB217742	GlaxoSmithKline	enrasentan	
RO462005	Hoffman-LaRoche	bosentan	147536-97-8
Sulfamethoxazole	ImmunoPharmaceuticals Inc.		
Cochinmicin I, II and III	Merck & Co		
L749329, L751281, L754142	Merck & Co		
J104132	Merck & Co		198279-45-7
PD159020, PD160672 PD160874	Pfizer (Parke-Davis)		
PD156252	Pfizer (Parke-Davis)		175592-65-1
PD142893	Pfizer (Parke-Davis)		155893-16-6
PD145065	Pfizer (Parke-Davis)		153049-49-1
PD143296	Pfizer (Parke-Davis)		
TM-ET-1	Takeda		
TAK044	Takeda		157380-72-8
ATZ1993	Teikoku Hormone		219705-77-8

Other illustrative examples of non specific ET-A/ET-B receptors antagonists include the antagonists 6-(4-methoxymethoxytphenyl)-5-methylsulfonylaminomethyl-1-(2-methylthiobenzyl)-2,4-dioxo-1;2;3;4-tetrahydrothieno[2,3-d]-pyrimidine-3-acetic acid (Takeda) and 1,2-dihydro-1-(2-methyoxybenzyl)-4-(methoxyphenyl)-2-oxobenzofuro [3,2-b]carboxylic acid (Merck KgaA).

However, it is clear that also other examples of endothelin receptor antagonists, which are not provided in this table, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

In the present invention, an "endothelin converting enzyme inhibitor" is defined as a substance that inhibits the conversion of endothelin precursors to endothelin peptides. These substances include endothelin converting enzyme (ECE-1 & ECE-2) inhibitors such as but not limited to CGS26303 phosphoramidon, and Halistand Disulfate B.

In the present invention, an "endothelin neutralizing agent" is defined as a substance that binds to the endothelin peptides and effectively inactivates them, for instance a specific

binding partner such as an antibody, and more preferably a neutralizing antibody. Techniques for screening for endothelin peptide specific binding partners and producing antibodies capable of binding to and inactivating an endothelin peptide are well known in the art. Methods of producing antibodies include immunizing a mammal, e.g. mouse, rat, rabbit, horse, goat, sheep or monkey, with an endothelin peptide or a fragment thereof. Antibodies may be obtained from immunized animals using any of a variety of techniques known in the art, and screened, preferably using the binding of the antibody to an endothelin peptide of interest and/or to determine whether the antibody is a neutralizing antibody, that is it is capable of binding to and inactivating an endothelin peptide or inhibiting or preventing its interaction with a receptor. For instance, Western blotting techniques or immunoprecipitation may be used. Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

In a preferred embodiment, the endothelin antagonist is an endothelin receptor antagonist. In another preferred embodiment, the endothelin receptor antagonist is an ET-A specific endothelin antagonist and/or an ET-A/ET-B non-specific endothelin antagonist.

In a preferred embodiment, said endothelin receptor antagonist is a specific ET-A endothelin antagonist selected from the group comprising BQ123 (cyclo-(D-Alpha-aspartyl-L-Prolyl-D-Valyl-L-Leucyl-D-Tryptophyl), FR139317 (N-[N-[N-[(hexahydro-1H-azepin-1-yl)carbonyl]-L-Leucyl]-1-Methyl-D-Tryptophyl]-3-(2-pyridinyl)-D-alanine). In another preferred embodiment, said endothelin receptor antagonist is a specific ET-A endothelin antagonist selected form the group comprising BSF208075 ((+)-(2S)-2-[(4,6-dimethylpyirmidin-2-yl) oxy]-3-methoxy-3,3-diphenylpropanoic acid, also known in the art as ambrisentan), ABT627 (4-(1,3-benzodioxol-5-yl)-1-[2-(dibutylamino)-2-oxoethyl]-2-(4-methoxyphenyl)-(2R, 3R, 4S)-3-Pyrrolidinecarboxylic acid, also known in the art as atrasentan) and LU135252 (alpha-((4,6-dimethyoxy-2-puyrimidinyl-oxy)-beta-methoxy-beta-phenyl-benzenepropanoic acid, also known in the art as darusentan), BMS207940 (N-[[2'-[[4,5-dimethyl-3-isoxazoyl)amino]sulfonyl]-4-(2-oxazolyl)[1,1'-biphenyl]-2-yl]-methyl]-N,3,3-trimethyl-butanamide, also known in the art as endonentan), TBC11251 (N-(4chloro-3-methyl-5-isoxazolyl)-2-[[4,5-(methylenedioxy)-o-toly]-acetyl]-3-thiophenesulfonamide, also known in the art as sitaxsentan), PD156707 (alpha-[2-(4-methoxyphenyl)-2-oxo-1-[(3,4,5-rimethoxyphenyl)methyl]ethylidene]-1,3-benzodioxole-5-acetic acid), PD163140 (glycine, N-

[[[4-(1,3-benzodioxol-5-yl)-2,5-dihydro-2-(4-methoxyphenyl)-5-oxo-3-[(3,4,5-trimethoxyphenyl)methyl]-2-fuanyl]oxy]carbonyl]-, ethyl ester), RO611790 (N-[6-(2-hydroxyethoxy)-5(2-methoxyphenoxy)-2-[2-(1H-tetrazol-5-yl-4-pyridinyl]-4-pyrimidinyl]-5-methyl-2-pyridinesulfonamide), L749329 (4-[1-(1,3-benzodioxol-5-yl)-2-[[[4-(1-methylethyl)phenyl]sulfonyl]amino]-2-oxoethoxy]-3-propyl-benzoic acid), CGS27830 (1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-bimol.monoanhydride, dimethyl ester, (4R,4'S)-rel-3,5-pyridinedicarboxylic acid), S-0139 (olean-12-en-28-oic acid, 27-[[3-[5-hydroxy-2-[(4-methoxy-1,4-dioxo-2-butenyl)amino]phenyl]-1-oxo-2-propenyl]oxy]-3-oxo), TBC3711, or TA0201 (benzenesulfonamide, N-[6-[2-[(5-bromo-2-pyrimidinyl)oxy]ethoxy]-5-(4-methylphenyl)-4-pyrimidinyl]-4-(2-hydroxy-1,1-dimethylethyl)-).

In another preferred embodiment, said endothelin receptor antagonist is a non-specific ET-A/ET-B endothelin antagonist selected form the group comprising RO462005 (4-(1,1-dimethylethyl)-N-[6-(2-hydroxyethyoxy)-5-(2-methoxyphenoxy) [2,2'-bipyrimidin]-4-yl]-benzenesulfonamide, also known in the art as bosentan), SB2177242 (1S,2R,3S)-3-[2-(2-Hydroxy-ethoxy)-4-methoxyphenyl]-1-[3,4-(methylenedioxy)phenyl]-5-propoxy-2-indancarboxylic acid, also known in the art as enrasentan), RO610612 (N-(6(2-hydroxyethoxy)-5(2-methoxyphenoy)-2-(2-(1H-tetrazol-5-yl)-4-pyridinyl)-4-pyrimidinyl)-5-(1-methylethyl)-2-pyridinesulfonamide, also known in the art as tezosentan or BSF420627 ((2S)-3-[2(3,4-dimethyoxypheny)ethoxy]-2-[(4,6-dimethympyrimidin-2-yl)oxy]-3,3-diphenylpropanoic acid, also known in the art as feloprentan), J104132 (5H-cyclopenta(b)pyridine-6-carboxylic acid, 5-(1,3-benzodioxol-5-yl)-2-butyl-7-[2[(2S)-2-carboxypropyl]-4-methoxyphenyl]-6,7-dihydro-, (5S,6R,7R)-), PD156252 (L-tryptophan, (2R)-N-acetyl-2-(10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-yl)glycyl-L-leucyl-L-alpha-aspartyl-L-isoleycyl-N-methyl-L-isoleucyl), PD142893 (L-tryptophan, N-[N-[N-[N-[N-(N-acetyl-beta-phenyl-D-phenylalanyl)-L-leucyl]-L-alpha-aspartyl]-L-isoleucyl]-L-isoleucyl]-, mono(trifluoroacetate)-, PD145065 (L-tryptophan, N-[N-[N-[N-[N-acetyl-D-2-(10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5-yl)glycyl]-L-leucyl]-L-alpha-aspartyl]-L-isoleucyl]-L-isoleucyl]-), TAK044 (cyclo(4-oxo-4-(4-phenyl-1-piperazinyl)-L-2-aminobutanoyl-L-alpha-aspartyl-D-2-(2-thienyl)glycyl-L-leucyl-D-tryptophyl-D-alpha-aspartyl) disodium salt), or ATZ1993 (1H-benz(g)indazole-3-carboxylic acid, 1-(1-(3-ethoxyphenyl)propyl)-4,5-dihydro-7-(5-pyrimidinylmethoxy)-), or SB209670 (1H-indene-2-carboxylic acid, 1-(1,3-benzodioxol-5-yl)-3-[2-(carboxymethoxy)-4-methoxyphenyl]-2,3-dihydro-5-propoxy-, [1S-(1alpha, 2beta, 3alpha)].

Methods of treatment

In one aspect, the present invention relates to methods for treating cancer and to methods for improving cancer therapies.

In a first aspect, the present invention relates to a method for treating cancer. The method involves exposing an individual in need of said cancer treatment to a therapeutically effective amount of an endothelin antagonist, and exposing said individual to a cancer therapy.

The term "therapeutically effective amount" as used herein means that amount of active compound or component that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. In particular it refers to that amount of active compound or component that elicits an amelioration of blood flow and blood perfusion to a tumor as described herein.

As used herein the term "cancer therapy" is meant to encompass radiotherapy, chemotherapy, immunotherapy, gene-based therapy as well as combinations thereof. The term "radiotherapy" refers to the treatment of cancer using radiation. The term "chemotherapy" refers to the treatment of cancer with chemical substances, so-called chemotherapeutics. The term "immunotherapy" as used herein refers to the stimulation of the reactivity of the immune system towards eliminating the cancer cells by using immunotherapeutics. The term "gene-based therapy" refers to the treatment of cancer based upon the transfer of genetic material (DNA, or possibly RNA) into an individual.

The cancer types of which the treatment can be treated according to the present invention include, but are not limited to solid tumors or metastasis. Metastasis is the form of cancer wherein the transformed or malignant cells are traveling and spreading the cancer from one site to another. The cancer can be of the skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers may include, but are not limited to the following organs or systems: cardiac, lung, gastrointestinal, genitourinary tract, liver, bone, nervous system, gynecological, hematologic, skin, and adrenal glands. More particularly, the methods herein can be used for treating gliomas (Schwannoma, glioblastoma, astrocytoma), neuroblastoma, pheochromocytoma, paraganlioma, meningioma, adrenalcortical carcinoma,

kidney cancer, vascular cancer of various types, osteoblastic osteocarcinoma, prostate cancer, ovarian cancer, uterine leiomyomas, salivary gland cancer, choroid plexus carcinoma, mammary cancer, pancreatic cancer, colon cancer, and megakaryoblastic leukemia.

The "individual", or "patient", is generally a human subject, although as will be appreciated by those in the art, the patient may be animal as well. Thus other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of individual.

In a particularly preferred embodiment, said individual is exposed to said endothelin antagonist preferably prior to exposing said individual to a conventional cancer therapy. In a preferred embodiment, said individual is exposed to an endothelin antagonist between 10 minutes and 480 minutes, and more preferred between 15 minutes and 360 minutes, and even more preferred between 30 and 120 minutes prior to exposing said individual to a conventional cancer therapy.

"Tumor blood vessels" and "arterioles" are used herein as synonyms and both refer to blood vessels leading to tumor cells

The tumor vasculature has unique features. Although derived from the host tissue, the tumor vasculature is under the influence of the tumor microenvironment and needs to adapt to the resistance to blood flow inherent to the dynamics of tumor growth. The last fifteen years have witnessed the development of drugs targeting tumor vessels, namely the anti-angiogenic (Keshet & Sasson, J.Clin.Invest, 104: 1497-1501, 1999; Carmeliet, & Jain, Nature, 407: 249-257, 2000) and antivascular agents (Thorpe et al. Cancer Res., 63: 1144-1147, 2003). The goals of these approaches are different, e.g. to prevent new blood vessel formation and to occlude/destroy pre-existing vessels, respectively. The present invention relates to the opposite strategy of enhancing tumor perfusion in order to potentiate the efficacy of cancer therapy and diagnosis.

When using conventional vasomodulatory drugs in cancer treatments, their action can be controlled by adapting their protocol of administration, i.e. to the timing of exposure to conventional cancer therapies. However, the selectivity issue is limiting.

According to the present invention a qualitative improvement in the function of tumor blood vessels is obtained by administering an endothelin antagonist. Furthermore, the present method is highly tumor-selective. This effect is mainly due to the specific phenotype of endothelial cells lining tumor blood vessels. Because of the very large increase in ET-1 abundance in tumors and the higher density of the ET-A receptors in tumor vessels, administration of ET antagonists will substantially increase tumor perfusion but will barely alter blood flow in the majority of healthy tissues. Also, the patient will only be exposed to the said ET antagonist at very specific times, during the administration of chemotherapy, radiotherpy, immunotherapy or gene therapy, thereby limiting the potential side effects of ET antagonists as encountered when chronically delivered.

In various studies, endothelin-1 production was reported to be strongly raised in many tumor types where it plays crucial roles in proliferation and angiogenesis (Kurbel et al., Med.Hypotheses, 52: 329-333, 1999; Ashamet al., Br.J.Cancer, 85: 1759-1763, 2001; Bagnato & Spinella, Trends Endocrinol.Metab, 14: 44-50, 2002). However, in these studies, there is no mention of the powerful vasoconstrictive effects of endothelin-1 and its potential action on the contractile tone of tumor vessels. Tumor production of ET-1 has an impact on local vasomotion and blocking its effects has an impact on tumor blood flow. This effect can be exploited to promote conventional cancer therapies. Providing a endothelin antagonist to a patient in need of a conventional cancer treatment enables to improve the function of tumor blood vessels in this patient, which improves the efficacy of the conventional therapy. Therefore, in another aspect, the invention also relates to methods for improving cancer therapies.

The term "improving cancer therapy", as used herein, refers to an improvement of conventional cancer treatments and includes reduction of the amount of the anti-cancer composition which is applied during the conventional cancer treatment, e.g. amount of radiation in radiotherapy, of chemotherapeutics in chemotherapy, of immunotherapeutics in immunotherapy or of vectors in gene based therapies, and/or to an increase in efficacy of the

conventional therapy and the anti-cancer composition when applied at conventional doses or amounts during the conventional cancer therapy.

The reduction of the doses of radiations and chemotherapeutics applied during cancer therapy has several advantages. A reduction of the use of expensive anti-cancer compositions such as radiation or chemotherapeutics is not only cost-effective, but also allows reducing the toxicity of the applied compositions and the side-effects related thereto in the patient. Also, in general, an increase in the efficacy of the anti-cancer compositions applied at conventional doses enables to reduce the duration and the number of repetition of the conventional cancer therapy, which is also cost-effective and advantageous from a patient's point of view as to reduce toxicity and side-effect problems.

The term "anti-cancer composition", as used herein refers to composition used in conventional cancer therapy, and refers to chemotherapeutics, i.e. chemical agents applied during chemotherapy; radiation; immunotherapeutics, i.e. agents attacking cancer through the immune system such as immunostimulants, antibodies, lymphocytes, T-cells; vectors used in gene therapy as well as any other agent needed to be addressed to tumor during a cancer therapy, or any combinations thereof.

In another embodiment, the invention relates to a method for optimizing the supply of an anti-cancer used in cancer therapy for inhibiting the growth of a tumor cell, comprising the steps of

a) determining a suitable amount of an endothelin antagonist,
b) determining a suitable amount of an anti-cancer composition,
c) exposing said tumor to the amount of endothelin antagonist determined in step a),
d) supplying said exposed tumor with the amount of said anti-cancer composition, determined in step b) and
e) optionally reducing the amount of anti-cancer composition determined in step b) by amending the amount of said endothelin antagonist determined in step a).

In particular, the amount of the anti-cancer composition is determined in function of the amount of said endothelin antagonist. By adapting the amount of endothelin antagonist to a suitable value, the amount of an anti-cancer composition can be further reduced. In a

preferred embodiment, the amount of anti-cancer composition can be reduced with at least 10 %, and more preferred with at least 20% and even more preferred with at least 30 %.

As used herein the term "optimizing the supply of an anti-cancer composition" may refer to the optimization of the supply of radiotherapy, chemotherapy, immunotherapy, gene-based therapy or any combinations thereof. This term is meant to encompass a reduction of the amount of anti-cancer composition supplied during one or more of these therapies, and/or to an increase in efficacy of the anti-cancer composition when applied at conventional doses.

In a preferred embodiment, the method comprises optimising the supply of radiation. In a more preferred embodiment, administration of radiation comprises fractionated administration of radiation. Combined treatment of a patient with an endothelin antagonist and radiation significantly improves the efficacy of the radiation treatment. According to the invention, radiotherapy, combined with the pre-treatment with an endothelin antagonist, provides an important synergetic effect and induces an overall benefit on tumor growth retardation which is higher than the sum of the effects of the two treatments administered separately. The administration of the endothelin antagonist considerably improves tumor perfusion and tumor blood supply. Radiotherapy can take advantage of a better tumor oxygenation, which partly depends on the tumor blood supply. On one hand, administration of an endothelin antagonist to a patient in need of a cancer treatment enables to obtain more effective results of the radiotherapy at conventional radiation doses. On the other hand, the present method also allows reducing the amount of radiation, and thus also the toxic side effects thereof, without considerable loss of efficacy. In a preferred embodiment, the amount of radiation can be reduced with at least 10 %, and more preferred with at least 20% and even more preferred with at least 30 %.

In another preferred embodiment, the method comprises optimising the supply of chemotherapeutics. Combined treatment of a patient with an endothelin antagonist and chemotherapy significantly improves the efficacy of the chemotherapy and provides an important synergetic effect and induces an overall benefit on tumor growth retardation which is higher than the sum of the effects of the two treatments administered separately. Chemotherapy can, indeed, take advantage of an improved drug delivery, which is also partly dependent on the tumor blood supply. Chemotherapeutic drugs reach the tumor in a more

effective way, such that lower amount of chemotherapeutics need to be applied to obtain a same effect. In a preferred embodiment, the amount of chemotherapeutic can be reduced with at least 10 %, and more preferred with at least 20% and even more preferred with at least 30 %.

In yet another embodiment, the present invention provides a method for optimizing the supply of immunotherapeutics. A lack of efficacy in cancer immunotherapy can originate from defects in access of immunotherapeutics to the tumor. By providing a patient with an endothelin antagonist prior to or together with the immunotherapy, may significantly improves delivery of lymphocytes, T-cells, antibodies, immunostimulants or other immunotherapeutics to the tumor and may thus improve the efficacy of the therapy.

The invention also provides a method for optimising the supply of vectors used in gene-based therapies. A lack of efficacy in gene-based cancer therapies can originate from defects in access of a vector to the tumor. Gene-based cancer therapy can thus take advantage of a better delivery of the vectors, such as virusses or liposomes, to the tumor site.

In another preferred embodiment, the invention provides a method for delaying tumor re-growth after supply of a cancer therapy, including radiotherapy, chemotherapy, immunotherapy or gene-based therapy. In a preferred embodiment, tumor re-growth is delayed by more than 30 %, and preferably by more than 50% and even more preferred by more than 70 %.

Mature tumor vessels are often downplayed in the pathobiology of cancer although their existence probably represents a major drawback in the current clinical trial evaluating anti-angiogenic strategies. Indeed, although these treatments are particularly well suited for targeting rapidly proliferating, angiogenic (mouse) tumors, pre-existing (host) vessels and differentiated tumor vasculature, e.g. comprising functional smooth muscle layers, are poor responders to drugs targeting proliferating endothelial cells. Here, according to the present invention evidence is now provided that these mature tumor vessels, as observed in months/years old human tumors, are strongly influenced by the tumor environment and can constitute key targets for selective treatments. Accordingly, the tumor vessel selectivity of endothelin antagonist treatment offers strong bases for the establishment of an adjuvant

strategy aiming to increase the effectiveness of conventional anti-tumor treatments, by acting on tumor blood flow and oxygenation. Adequately scheduled combination of these latter approaches with anti-angiogenic drugs or anti-cancer compositions certainly represents a very attractive area of cancer therapy development.

In another preferred embodiment, the invention provides a method for reducing side-effects of an anti-cancer composition used in cancer therapy, comprising the steps of:

a) determining a suitable amount of an endothelin antagonist,
b) reducing the amount of the anti-cancer composition in function of the amount of endothelin antagonist determined in step a),
c) exposing an individual in need of said treatment to the amount of endothelin antagonist determined in step a), and
d) exposing said individual to the reduced amount of said anti-cancer composition determined in step b).

One of the major disadvantages of conventional anti-cancer compositions is their relatively high level of toxicity and their considerable lack of selectivity. Conventional anti-cancer compositions generally have detrimental effect(s) on healthy cells, tissues or organs. By combining conventional anti-cancer compositions with an endothelin antagonist, side effects induced by conventional cancer therapies can be considerably reduced according to the invention. This effect is mainly based on the fact that in combination with endothelin antagonists, lower doses of anti-cancer compositions than those conventionally applied, are sufficient for obtaining good tumor growth inhibition.

In another further embodiment, the invention relates to a method for increasing the susceptibility of a tumor tissue to an anti-cancer composition. In such method the tumor tissue is exposed to a suitable amount of an endothelin antagonist. Simultaneously or subsequently, the exposed tumor tissue is provided with a suitable amount of an anti-cancer composition, such as radiation, a chemotherapeutic, an immunotherapeutics, a vector using in gene therapy, or combinations thereof. The increase in susceptibility of the tumor tissue depends in particular on the amount of endothelin antagonist exposed to the tissue. Treatment with an endothelin antagonist considerably improves tumor perfusion and tumor

blood supply and improved tumor blood supply provides for a better tumor oxygenation and renders tumor tissue more sensitive to cancer treatments. This method is in particular suitable for increasing the susceptibility of a tumor tissue to radiation.

The invention also further provides a method for preparing an anti-cancer composition having reduced toxicity. This method comprises the steps of

- providing a suitable amount of an endothelin antagonist
- determining a suitable amount of anti-cancer composition in function of the amount of said endothelin antagonist, and
- preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

Reduction of the use of expensive anti-cancer compositions such as radiation or chemotherapeutics is not only cost-effective, but also allows reducing the toxicity of the applied anti-cancer compositions and the side-effects related thereto in the patient. This method is in particular suitable for preparing an anti-cancer composition comprising radiation, or chemotherapeutics or combinations thereof having reduced toxicity.

In addition, the invention provides a method for reducing the amount of radiation in radiotherapy, comprising prior to irradiating an individual in need of radiotherapy with a reduced amount of radiation, exposing said individual with a suitable amount of endothelin antagonist. The method is in particular characterised in that the reduction of the amount of radiation is dependent on the amount of endothelin antagonist.

Furthermore a method is also provided for reducing the amount of a chemotherapeutic used in chemotherapy, comprising prior to supplying an individual in need of chemotherapy with a reduced amount of chemotherapeutics, exposing said individual with a suitable amount of endothelin antagonist. The method is in particular characterised in that the reduction of the amount of chemotherapeutics is dependent on the amount of endothelin antagonist.

The invention also further provides a method for preparing an anti-cancer composition having improved delivery to a tumor site. A lack of efficacy in cancer therapy can originate from defects in delivery or access of an anti-cancer composition to a tumor.

According to the present invention, anti-cancer compositions can be prepared showing improved delivery to a tumor site by

- providing a suitable amount of an endothelin antagonist
- determining a suitable amount of anti-cancer composition in function of the amount of said endothelin antagonist,
- preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

Improved delivery allows the duration and the number of repetition of the conventional cancer therapy, which is cost-effective and advantageous from a patient's point of view as to reduce toxicity and side-effect problems. This method is in particular suitable for preparing an anti-cancer composition comprising chemotherapeutics, immunotherapeutics or vectors for use in gene-based therapies, or combinations thereof having improved delivery.

In particular, according to an embodiment, a method is provided for improving the delivery of a chemotherapeutic, comprising prior to supplying an individual in need of chemotherapy with a suitable amount of said chemotherapeutic, exposing said individual with a suitable amount of endothelin antagonist. The invention also relates to a method for improving the delivery of an immunotherapeutic, comprising prior to supplying an individual in need of immunotherapy with a suitable amount of said immunotherapeutic, exposing said individual with a suitable amount of endothelin antagonist. Further, the invention provides a method for improving the delivery of a vector used in gene-based cancer therapy, comprising prior to supplying an individual in need of said gene therapy with a suitable amount of said vector, exposing said individual with a suitable amount of endothelin antagonist.

In another embodiment, the invention provides a method for adjusting the amount of an anti-cancer composition supplied by a supplying device. Preferably, the invention provides a method for adjusting the amount of an anti-cancer composition supplied by a supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of an anti-cancer composition function of the amount of endothelin antagonist determined in step a),
c) adjusting said supplying device for supplying the amount of said anti-cancer composition radiation determined in step b), and

d) supplying the anti-cancer composition using said supplying device together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

The invention provides a method for adjusting the amount of radiation supplied by a radiation device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of radiation in function of the amount of endothelin antagonist determined in step a),
c) adjusting said radiation device for radiating the amount of radiation determined in step b), and
d) supplying radiation using said radiation device together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

The invention further provides a method for adjusting the amount of chemotherapeutic supplied by a chemotherapeutic supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of a chemotherapeutic in function of the amount of endothelin antagonist determined in step a),
c) providing the amount of said chemotherapeutic determined in step b) to said chemotherapeutic supplying device,
d) supplying said chemotherapeutic using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

A method is also provided for adjusting the amount of an immunotherapeutic supplied by an immunotherapeutic supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of immunotherapeutic in function of the amount of endothelin antagonist determined in step a),
c) providing the amount of said immunotherapeutic determined in step b) to said immunotherapeutic supplying device, and

d) supplying said immunotherapeutic using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

Furthermore the invention relates to a method for adjusting the amount of a vector used in gene based therapy supplied by a supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of vector in function of the amount of endothelin antagonist determined in step a),
c) providing the amount of said vector determined in step b) to said supplying device, and
d) supplying said vector using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

Diagnostic methods

In another aspect, the present invention relates to methods for diagnosing cancer and to methods for improving cancer diagnosis. In a preferred embodiment, the invention relates to a method for diagnosing cancer comprising the steps of

- exposing an individual in need of said cancer treatment to a suitable amount of an endothelin antagonist, and
- exposing said individual to a cancer diagnostic method.

In a particularly preferred embodiment, said individual is exposed to said endothelin antagonist preferably prior to exposing said individual to a diagnostic method. In a preferred embodiment, said individual is exposed to an endothelin antagonist between 10 minutes and 480 minutes, and more preferred between 15 minutes and 360 minutes, and even more preferred between 30 and 120 minutes prior to exposing said individual to a diagnostic method.

The term "improving cancer diagnosis", as used herein, refers to a *reduction of the amount* of the diagnostic compound which is applied during the diagnosis method, and/or to an *increase*

in efficacy of the diagnosis when applied at conventional doses or amounts, mainly as a result of a better delivery of the compound to the tumor site.

In another embodiment, the invention relates to a method for optimizing the supply of a diagnostic compound used in cancer diagnosis comprising the steps of

a) determining a suitable amount of an endothelin antagonist,
b) determining a suitable amount of said diagnostic compound,
c) exposing said tumor to the amount of endothelin antagonist determined in step a),
d) supplying said exposed tumor with the amount of said diagnostic compound, determined in step b) and
e) optionally reducing the amount of diagnostic compound determined in step b) by amending the amount of said endothelin antagonist determined in step a).

the amount of said diagnostic compound is determined in function of said amount of said endothelin antagonist.

In another preferred embodiment, the invention provides a method for reducing side-effects of a diagnostic compound used in cancer diagnosis, comprising the steps of:

a) determining a suitable amount of an endothelin antagonist,
b) reducing the amount of the a diagnostic compound in function of the amount of endothelin antagonist determined in step a),
c) exposing an individual in need of said treatment to the amount of endothelin antagonist determined in step a), and
d) exposing said individual to the reduced amount of said a diagnostic compound determined in step b).

A lack of efficacy in cancer diagnosis can originate from defects in access of a diagnostic compound to a tumor. By providing a patient with an endothelin antagonist prior to or together with the diagnostic compound, may significantly improve delivery of the compound to the tumor and may thus improve the efficacy of the diagnosis. The amount of diagnostic compound is determined in function of said amount of said endothelin antagonist. Diagnostics may reach the tumor in a more effective way, such that lower amounts of diagnostics need to be applied to obtain a same effect. In a preferred embodiment, the amount of diagnostic compound can be reduced with at least 10 %, and more preferred with at least 20% and even more preferred with at least 30 %. Improved delivery and reduction of

compound amounts are advantageous from a patient's point of view as to reduce toxicity and side-effect problems.

In another further embodiment, the invention relates to a method for increasing the susceptibility of a tumor tissue to a diagnostic compound. In such method the tumor tissue is exposed to a suitable amount of an endothelin antagonist. Simultaneously or subsequently, the exposed tumor tissue is provided with a suitable amount of a diagnostic compound. The increase in susceptibility of the tumor tissue depends in particular on the amount of endothelin antagonist exposed to the tissue.

The invention also provides a method for preparing a diagnostic composition having reduced toxicity, comprising the steps of

- providing a suitable amount of an endothelin antagonist
- determining a suitable amount of one or more diagnostic compounds in function of the amount of said endothelin antagonist, and
- preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of said diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

The invention also relates to a method for preparing a diagnostic composition having improved delivery to a tumor site by

- providing a suitable amount of an endothelin antagonist
- determining a suitable amount of one or more diagnostic compounds in function of the amount of said endothelin antagonist,
- preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of said diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

In the above-described methods, amounts of diagnostic compounds are combined with endothelin antagonists. This enables to reduce the amount of the diagnostic compounds used. Reducing the use of expensive diagnostic compounds is not only cost-effective, but also allows reducing the potential toxicity of the applied compounds and the side-effects related thereto in the patient.

Such preparation method enables to prepare a diagnostic composition having improved delivery to the tumor site and to increase the efficacy of the diagnostic method. In particular, according to another embodiment, a method is provided for improving the delivery of a diagnostic compound, comprising prior to supplying an individual in need of cancer diagnosis with a suitable amount of said diagnostic compound, exposing said individual with a suitable amount of endothelin antagonist. In addition, the invention provides a method for reducing the amount of diagnostic compound used in cancer diagnosis, comprising prior to supplying an individual in need of cancer diagnosis with a reduced amount of diagnostic compound, exposing said individual with a suitable amount of endothelin antagonist.

In another embodiment, the invention provides a method for adjusting the amount of a diagnostic compound supplied by a supplying device. The invention relates to a method for adjusting the amount of a diagnostic compound used in cancer diagnosis supplied by a supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of vector in function of the amount of endothelin antagonist determined in step a),
c) providing the amount of said diagnostic compound determined in step b) to said supplying device, and
d) supplying said diagnostic compound using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

According to the above-described methods, the endothelin antagonist may be administered orally, bucally, parenterally, sublingually by inhalation spray, rectally or topically. Preferably said antagonists are administered orally or parenterally to said individual.

Composition

In another aspect, the present invention relates to compositions for treating cancer and for improving cancer treatment. In particular, a composition is provided containing an endothelin antagonist and an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

The anti-cancer composition used in the composition is preferably selected from the group comprising chemotherapeutics, radiation, immunotherapeutics, vectors used in gene therapy or any combinations thereof. It is clear that more than one endothelin antagonist and more than one anti-cancer may be contained in said composition. The composition may also further comprise other vasomodulatory drugs.

In another aspect, the present invention relates to compositions for diagnosing cancer and for improving cancer diagnosis. In particular, a composition is provided containing an endothelin antagonist and a diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer diagnosis.

The first mentioned compound in the compositions is preferably an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof. In a preferred embodiment said first mentioned compound in the compositions is preferably an endothelin receptor antagonist. More preferably said endothelin antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

Endothelin receptor antagonists suitable for use in the present compositions may be selected from table 1, 2 and 3, mentioned above. However, it is clear that also other examples of endothelin antagonists, which are not provided in these tables, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

The use of an endothelin antagonist has the effect of considerably improving tumor perfusion and tumor blood supply. Moreover, classical cancer therapies can take advantage of a better tumor oxygenation and drug delivery, both partly dependent on the tumor blood supply. Therefore, the present composition is particularly suitable to be used in combination with classical cancer therapies, either simultaneously or sequentially and prior to classical cancer therapies.

In addition, diagnostic therapies can take advantage of a better tumor oxygenation and drug delivery, as well. Therefore, the present composition is also particularly suitable to be used in combination with diagnosis therapies, either simultaneously or sequentially and prior to the diagnosis therapies.

In a preferred embodiment, a composition is provided containing a specific ET-A endothelin antagonist and an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy. Specific endothelin-A antagonists suitable for use in the present compositions may be selected from table 1, mentioned above. However, it is clear that also other examples of specific ET-A endothelin antagonists, which are not provided in these tables, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

In an embodiment, a composition is provided containing BQ123 and radiation as a combined preparation. In an embodiment a composition is provided containing BQ123 and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing BQ123 and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing BQ123 and a vector used in gene therapy as a combined preparation.

In another embodiment, a composition is provided containing FR139317 and radiation as a combined preparation. In an embodiment a composition is provided containing FR139317 and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing FR139317 and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing FR139317 and a vector used in gene therapy as a combined preparation.

In yet another embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising PD156707, PD163140, RO611790, L749329, CGS27830, S-0139, TBC3711, TA-0201, ambrisentan (BSF208075), atrasentan (ABT327), darusentan (LU135252), endonentan (BMS207940) or sitaxsentan (TBC11251) and radiation as a combined preparation. In an embodiment a composition is provided containing a specific

ET-A endothelin antagonist selected from the group comprising PD156707, PD163140, RO611790, L749329, CGS27830, S-0139, TBC3711, TA-0201, ambrisentan (BSF208075), atrasentan (ABT327), darusentan (LU135252), endonentan (BMS207940) or sitaxsentan (TBC11251) and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising PD156707, PD163140, RO611790, L749329, CGS27830, S-0139, TBC3711, TA-0201, ambrisentan (BSF208075), atrasentan (ABT327), darusentan (LU135252), endonentan (BMS207940) or sitaxsentan (TBC11251) and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising P PD156707, PD163140, RO611790, L749329, CGS27830, S-0139, TBC3711, TA-0201, ambrisentan (BSF208075), atrasentan (ABT327), darusentan (LU135252), endonentan (BMS207940) or sitaxsentan (TBC11251) and a vector used in gene therapy as a combined preparation.

In another preferred embodiment, a composition is provided containing a non-specific ET-A/ET-B endothelin antagonist and an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy. Non-specific ET-A/ET-B endothelin antagonists suitable for use in the present compositions may be selected from table 3, mentioned above. However, it is clear that also other examples of non-specific ET-A/ET-B endothelin antagonists, which are not provided in these tables, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

In an embodiment, a composition is provided containing bosentan (RO462005) and radiation as a combined preparation. In an embodiment a composition is provided containing bosentan (RO462005) and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing bosentan (RO462005) and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing bosentan (RO462005) and a vector used in gene therapy as a combined preparation.

In an embodiment, a composition is provided containing feloprentan (BSF420627) and radiation as a combined preparation. In an embodiment a composition is provided containing

feloprentan (BSF420627) and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing feloprentan (BSF420627) and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing feloprentan (BSF420627) and a vector used in gene therapy as a combined preparation.

In an embodiment, a composition is provided containing tezosentan (RO610612) and radiation as a combined preparation. In an embodiment a composition is provided containing tezosentan (RO610612) and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing tezosentan (RO610612) and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing tezosentan (RO610612) and a vector used in gene therapy as a combined preparation.

In another embodiment, a composition is provided containing enrasentan (SB217742) and radiation as a combined preparation. In an embodiment a composition is provided containing enrasentan (SB217742) and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing enrasentan (SB217742) and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing enrasentan (SB217742) and a vector used in gene therapy as a combined preparation.

In yet another embodiment, a composition is provided containing a non-specific ET-A/ET-B endothelin antagonist selected from the group comprising J104132, PD156252, PD142893, PD145065, TAK044 or ATZ1993 and radiation as a combined preparation. In an embodiment a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising J104132, PD156252, PD142893, PD145065, TAK044 or ATZ1993 and a chemotherapeutic as a combined preparation. In an embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising J104132, PD156252, PD142893, PD145065, TAK044 or ATZ1993 and an immunotherapeutic as a combined preparation. In an embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising

J104132, PD156252, PD142893, PD145065, TAK044 or ATZ1993 and a vector used in gene therapy as a combined preparation

In another preferred embodiment, a composition is provided containing a specific ET-A endothelin antagonist and a diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer diagnosis. Specific endothelin-A antagonists suitable for use in the present compositions may be selected from table 1, mentioned above. However, it is clear that also other examples of specific ET-A endothelin antagonists, which are not provided in these tables, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

In an embodiment, a composition is provided containing BQ123 and a diagnostic compound as a combined preparation. In another embodiment, a composition is provided containing FR139317 and a diagnostic compound as a combined preparation. In yet another embodiment, a composition is provided containing a specific ET-A endothelin antagonist selected from the group comprising PD156707, PD163140, RO611790, L749329, CGS27830, S-0139, TBC3711, TA-0201, ambrisentan (BSF208075), atrasentan (ABT327), darusentan (LU135252), endonentan (BMS207940) or sitaxsentan (TBC11251) and a diagnostic compound as a combined preparation.

In yet another preferred embodiment, a composition is provided containing a non-specific ET-A/ET-B endothelin antagonist and a diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer diagnosis. Non-specific ET-A/ET-B endothelin antagonists suitable for use in the present compositions may be selected from table 3, mentioned above. However, it is clear that also other examples of non-specific ET-A/ET-B endothelin antagonists, which are not provided in these tables, but which are known and available in the art, as well as endothelin antagonists that are under development or that will be developed in the future may be suitable for use in the present invention as well.

In an embodiment, a composition is provided containing bosentan (RO462005) and a diagnostic compound as a combined preparation. In an embodiment, a composition is provided containing feloprentan (BSF420627) and a diagnostic compound as a combined

preparation. In an embodiment, a composition is provided containing tezosentan (RO610612) and a diagnostic compound as a combined preparation. In an embodiment, a composition is provided containing enrasentan (SB217742) and a diagnostic compound as a combined preparation.

In yet another embodiment, a composition is provided containing a non-specific ET-A/ET-B endothelin antagonist selected from the group comprising J104132, PD156252, PD142893, PD145065, TAK044 or ATZ1993 a diagnostic compound as a combined preparation.

The compositions according to the invention do not represent a mere aggregate of known agents, but a new combination with the surprising, valuable property that the side-effects, such as toxicity and a lack of selectivity, to be expected when administering the anti-cancer composition or a diagnostic compound are reduced and that synergetic effects can be obtained.

Both components in the compositions can be mixed in a single preparation or can be prepared separately. When prepared separately, both components of the composition can be used simultaneous or more preferred sequentially, whereby the endothelin antagonist is preferably applied before the conventional anti-cancer composition or the diagnostic compound. In a preferred embodiment, said endothelin antagonist is administered between 10 minutes and 480 minutes, and preferably between 15 minutes and 360 minutes, and even more preferred between 30 minutes and 120 minutes, prior to providing said individual with the anti-cancer composition or the diagnostic compound.

A composition containing an endothelin antagonist may be manufactured by, if necessary, by mixing an effective amount of the active ingredient with various pharmaceutical ingredients suitable for the final administration form, such as organic or inorganic carriers, excipients, binders, moistening agents, disintegrators, lubricants, and diluents and other additives suitable for parenteral administration, according to routine methods. When the composition containing an endothelin antagonist is to be used for injection, the active ingredient can be sterilized with a suitable carrier.

In a preferred embodiment, the endothelin antagonist of the invention is suitable for being administered orally or parenterally and can be prepared as oral solid dosage form, oral liquid dosage form or injection, by using organic or inorganic carriers, excipients and other additives suitable for oral or parenteral administration, according to routine methods.

Oral solid dosage forms may include tablet, powder, fine particle, granule, capsule, pill and sustained-release type. In such solid dosage forms, one or more active substances are mixed with at least one inactive diluent, for example lactose, mannitol, glucose, micro-fine cellulose, starch, cornstarch, polyvinylpyrrolidone and metasilicate aluminate magnesium. According to routine methods, the composition may satisfactorily contain additives other than inactive diluents, including for example binders such as hydroxypropyl cellulose and hydroxypropylmethyl cellulose (HPMC); lubricants such as magnesium stearate, polyethylene glycol, starch and talc; disintegrators such as fibrinogen calcium glycolate and cermellose calcium; stabilizers such as lactose; dissolution auxiliary agents such as glutamic acid or aspartic acid; plasticizers such as polyethylene glycol; and colorants such as titanium oxide, talc and yellow ferric oxide. If necessary, the resulting tablet or pill may satisfactorily be coated with sugar coating or films comprising substances solubilizable in stomach or intestine, such as sucrose, gelatin, agar, pectin, hydroxypropyl cellulose and hydroxypropylmethyl cellulose phthalate. The most preferable is an oral solid dosage form, which can be readily incorporated by patients by themselves and are convenient for storage and transfer.

Oral liquid dosage forms may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs and contains inactive diluents for general use, for example distilled water and ethanol. Other than inactive diluents, the composition may satisfactorily contain auxiliary agents such as moisturizers and suspending agents, sweeteners, flavor, fragrance, and preservatives.

Injections for intravenous, intra-muscular and subcutaneous injection may include sterile aqueous or non-aqueous solutions, suspensions and emulsions. The diluents for the aqueous solutions and suspensions include for example distilled water for injections and physiological saline. The diluents for the non-aqueous solutions and suspensions include for example propylene glycol, polyethylene glycol and vegetable oils such as olive oil, alcohols such as

ethanol and polysorbate 80. Such composition may additionally contain auxiliary agents such as preservatives, moisturizers, dispersants, stabilizers (for example, lactose), and dissolution auxiliary agents (for example, glutamic acid, aspartic acid). These are sterilized by filtration through bacteria trapping filters or blending with sterilizing agents or under irradiation. These may satisfactorily be used to produce sterile solid compositions, which are dissolved in sterile water or sterile solvents for injections prior to use, and are then used.

The dose of the endothelin antagonist as the effective component of the invention is appropriately determined, depending on each case, taking account of the patient's age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, type and extent of disease, the compound may usually be administered parenterally administered, in single or divided doses of 0.1 µg - 20 mg/kg/day.

Use

In another embodiment, the invention relates to the use of an endothelin antagonist for improving cancer therapy. In another embodiment, the invention relates to the use of an endothelin antagonist in the preparation of a medicament for improving cancer therapy.

In yet another embodiment, the invention relates to the use of an endothelin antagonist in cancer diagnosis and to the use of an endothelin antagonist for improving cancer diagnosis.

Preferably, the invention relates to the use of an endothelin antagonist wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof. Even more preferred the invention relates to the use of an endothelin antagonist wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

The following examples serve to more fully describe the manner of using the above-described invention, as well as to set forth the best modes contemplated for carrying out various aspects of the invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes.

Examples

Example 1 provides evidence for the expression of ET-1 in tumor cells and tumor vasculature. Example 2 illustrates the differential reactivity of tumor blood vessels to ET-1. Example 3 provides evidence of the effects of an endothelin antagonist on the myogenic tone of tumor blood vessels. Example 4 is an illustration of the increased tumor perfusion induced by an endothelin antagonist. Example 5 provides evidence for the improved sensitivity of tumors to radiation upon administrating of an endothelin antagonist. Example 6 provides evidence for the improved sensitivity of tumors to chemotherapy upon administrating of an endothelin antagonist. Materials and methods applied in the below-described examples comprise the following.

Mice

NMRI, C57BL/6J and C3H/He male mice (Elevage Janvier, Le Genest-St-Isle, France) were used in experiments with TLT (mouse hepatocarcinoma) (Taper et al. Cancer Res., 26: 143-148, 1966), LLc (mouse lung carcinoma) (Bertram & Janik, Cancer Lett., 11: 63-73, 1980), and FSA-II (mouse fibrosarcoma) (Volpe et al. Clin.Exp.Metastasis, 3: 281-294, 1985) syngeneic tumor cells, respectively. Anesthetized mice (ketamine/xylazine) received IM injections of 10^5-10^6 syngeneic tumor cells in the vicinity of the saphenous artery of the posterior right leg. The preexisting artery was progressively coopted as the tumor grew. The tumor diameters were tracked with an electronic caliper. When the tumor diameter reached 4.0 ± 0.5 mm, mice were sacrificed or randomly assigned to a treatment group. When indicated, they received an i.p. injection of the selective ET_A antagonist BQ123 (0.5, 1 and 2 mg/kg) (Sigma, Bornem, Belgium) or saline alone. Each procedure was approved by the local authorities according to national animal care regulations.

Immunoblottings and immunostainings

Immunoblottings were carried out, as previously described (Sonveaux et al. FASEB J., *16*: 1979-1981, 2002; Sonveau et al. Cancer Res., *63*: 1012-1019, 2003) with a sheep polyclonal antibody against ET-A (Abcam, Cambridge, UK) or ET-B (Alomone Labs, Jerusalem, Israel). For immunostainings, collected tissue samples were cryosliced and probed with a rabbit polyclonal antibody against ET-1. Endogenous peroxidase activity was inhibited by 0.3% H_2O_2 in PBS and Envision system (Dako, Glostrup, Denmark) was used for revelation.

Sections were finally counterstained with Mayer's hematoxylin. Quantitative image analyses of immunostained arteriole slices (5 arterioles per condition and 3 sections per animal) were performed with AnalySIS software (Soft Imaging System, Münster, Germany).

Myograph assays

Tumor arterioles and size-matched arterioles from healthy mice were dissected under a stereoscopic microscope and mounted on a 110P pressure myograph (DMT, Aarhus, Denmark). No macroscopic differences were detectable between the tumor and control arterioles (diameters of 284 ± 14 and 299 ± 17 µm, respectively; P>0.1). Changes in the outer diameters were tracked and measured with the Myoview software (DMT, Aarhus, Denmark). To evaluate the myogenic tone, isolated arterioles were left to recover for 45-60 min in no-flow condition (20 mm Hg, 37.5°C). Then, the pressure was increased by steps of 20 mm Hg (that were maintained for 15 min to allow arteriole response). For each pressure step, the active vessel diameter was determined in PSS buffer (NaCl 119 mM, KCl 4.7 mM, $NaHCO_3$ 25 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 1.17 mM, EDTA 0.026 mM, glucose 5.5 mM, $CaCl_2$ 1.6 mM); then the passive diameter of the same arteriole was measured in $CaCl_2$-deprived PSS supplemented with 10 μM sodium nitroprusside and 2 mM EGTA. In some experiments, the active diameter was determined after a 60-min preincubation in the calcium-containing PSS buffer with BQ123 (1 µM). For the establishment of the ET-1 dose-response curve, isolated arterioles were left to recover at physiological pressure for 45-60 min in no-flow condition in PSS medium (60 mm Hg, 37.5°C); additive doses of ET-1 (Sigma) were then delivered in the bathing medium. For each vessel used in this study, the ability of the vessels to contract upon application of a depolarizing KCl solution was verified at the end of the experiment and compared with a similar contraction performed at the very beginning of the experiment. If these two contractions differed by more than 10%, the experiment was disregarded.

Tumor blood flow monitoring

Tumor perfusion was measured with a Laser Doppler imager (LDI, Moor Instruments) and with Laser Doppler microprobes (Oxyflo, Oxford Optronix). Briefly, for the LDI measurements, mice were anaesthetized and fur was removed from the limbs using a depilatory cream. They were placed on a heating pad (37°C) to minimize variations in temperature. The average perfusions of the tumor-bearing leg and of the control leg were evaluated on the basis of colored histogram pixels. For the Oxyflo measurements, fiber-optic microprobes (Laser

Doppler + thermocouple) were introduced into the tumor. Back scattering measurements were used to validate the absence of movement artefacts. A 10 min-baseline of stable recordings was obtained before treatment administration through the catheterized tail vein; data were collected continuously at a sampling frequency of 20 Hz.

Electron paramagnetic resonance (EPR) oximetry Electron paramagnetic resonance (EPR) oximetry was used to track the changes in pO_2 induced by exposure to BQ123. This real-time, O_2-nonconsuming technique relies on the oxygen-dependent broadening of the EPR line width of a paramagnetic oxygen sensor pre-implanted in the tumor. Accordingly, 50 µl of a suspension (100 mg/ml) of the O_2-sensitive probe (charcoal wood powder, CX0670-1, EM Science, Gibbstown, NJ) were injected in the center of the tumor 24 h before X-ray irradiation, as previously described and validated (Jordan et al. Cancer Res., 62: 3555-3561, 2002; Sonveaux et al. FASEB J., 16: 1979-1981, 2002; Jordan et al. Int.J.Radiat.Oncol.Biol.Phys., 48: 565-570, 2000). The tumors of anaesthetized mice were placed in the center of the surface coil. EPR spectra were recorded using an EPR spectrometer (Magnettech, Germany) with a low frequency microwave bridge operating at 1.1 GHz and extended loop resonator. The measured pO_2 corresponds the mean value in a tumor volume of env. 10 mm^3 (i.e. the volume of charcoal dispersion as determined by histology).

Radiotherapy and tumor measurements

Anaesthetized TLT tumor-bearing mice were irradiated using the RT-250 device (Philips) for a dose delivery of 0.76 Gy/min. Mice were shielded with lead except for a 3-cm-diameter circular field where the tumor was centered. When indicated, mice received an IP injection of 1 mg/kg BQ123 or saline alone, 45-min before each dose delivery. Control and tumor-bearing legs were daily measured with an electronic caliper; this allowed determination of the tumor diameter. Mean regrowth delays refer to the doubling of the tumor diameters.

Chemotherapy and tumor measurements

TLT tumor-bearing mice received two doses of cyclophosphamide at day 0 and 1. When indicated, mice received an IP injection of 1 mg/kg BQ123 or saline alone, 45-min before the dose delivery. Control and tumor-bearing legs were daily measured with an electronic caliper;

this allowed determination of the tumor diameter. Mean regrowth delays refer to the doubling of the tumor diameters.

Statistical analyses

Data are reported as mean ± s.e.m.; Student's *t* test or one way ANOVA were used where appropriate. To determine synergism, we compared tumor doubling times using a non-parametric Mann-Whitney analysis; a generalized linear model with two-way ANOVA was also applied and led to similar results.

Example 1 Expression of ET-1 in tumor cells and in the tumor vasculature

First ET-1 production was characterized the in 3 syngeneic tumor models (TLT, LLc and FSA-II) obtained by IM injections of tumor cells. When reaching a diameter of 4.0 ± 0.5 mm, the tumors were resected and processed for immumostaining with ET-1 antibodies; mouse heart was used as control for antibody specificity (see Figure 1a). As shown in Figures 1b-d, the different tumors were found to produce large amounts of ET-1, that were detectable both inside the tumor cells and in the extracellular space. Very low amounts of ET-1 were found in the healthy surrounding muscular tissues (see Figures 1b-c) and in the necrotic core of the tumor (see Figure 1d). Importantly, the coopted tumor vasculature was strongly labelled, revealing a 13-fold increase in ET-1 in the vessel wall when compared to control size-matched arterioles (Figure 1e-f).

Example 2 Differential reactivity of tumor arterioles to ET-1

To evaluate the vasomodulatory effects of the increased ET-1 concentrations in tumor arterioles, TLT-coopted arterioles (see Figure 1g) and size-matched arterioles were isolated from healthy animals. These arterioles were mounted on a pressure myograph and after pressurization and equilibration, exposed to increasing doses of ET-1. In arterioles from healthy mice, low ET-1 concentrations (10^{-13} to 10^{-10} M) were ineffective whereas higher doses (10^{-9} to 10^{-7} M) dose-dependently constricted the vessels (Figure 2a). By contrast, tumor-coopted arterioles contracted with ET-1 concentrations as low as 10^{-11} M (Figure 2a) and reached a maximum at 10^{-8} M ET-1 (the max contraction was obtained at 10^{-7} M ET-1 in control vessels). Calculated EC_{50} were 0.40 nM and 2.01 nM ($P<0.05$, $n = 5$) in tumor and in size-matched healthy arterioles, respectively. Immunoblots performed on carefully microdissected arterioles (cleaned from surrounding tissues) also revealed that the density of

ET_A (e.g., the ET-1 receptor mediating vasoconstriction) was increased by 5-fold in coopted vessels *vs* healthy arterioles (Figure 2b). Interestingly, when using total lysates from tumors, we failed to detect ET_A receptor by immunoblotting suggesting a weaker density of this receptor in tumor cells than in vascular cells.

Example 3 Effects of an endothelin antagonist on the myogenic tone of tumor blood vessels

Tumor arterioles exhibit a myogenic tone sensitive to an ET_A antagonist. Since it was shown that tumor arterioles are chronically exposed to large amounts of ET-1 (see Fig. 1-example 1) and are (remain) hyperesponsive to ET-1 (see Fig. 2- example 2), it was examined whether intrinsic features of tumor arterioles were under the tonic control of this pathway. Accordingly, the presence in tumor arterioles of a differential myogenic tone (i.e. the ability of arterioles to contract when the intraluminal pressure increases) (Davis & Hill, Physiol Rev., *79*: 387-423, 1999) was examined. Myogenic vasoconstriction is thought to provide level of basal tone that enables arterioles to respond to neurohumoral stimuli with the appropriate change in vessels diameter.

Using a pressure myograph, the response of isolated tumor-coopted and control arterioles to step-by-step increases of the luminal pressure was examined. Accordingly, the changes in the active diameter (measured in calcium-containing medium) and in the passive diameter (determined in a medium devoid of calcium) were consecutively tracked for each arteriole. In healthy mice, no myogenic tone was observed: both the active and the passive diameters increased similarly when the pressure raised (Figure 3a). By contrast, tumor coopted arterioles were able to respond to the pressure increase by a vasoconstriction (detected as a lesser vasodilation) in calcium-containing, but not in calcium-deprived medium (Figure 3b), thereby acknowledging the existence of an intrinsic myogenic tone.

The implication of the ET-1/ET-A pathway in the myogenic tone of TLT tumor-coopted arterioles was further investigated by using the specific ET-A antagonist BQ123. The effect of the pre-incubation with BQ123 was dramatic since it completely abrogated the myogenic tone (Figure 3b) ($P<0.01$). In fact, the active diameter curve under ET_A inhibition (Fig. 3b, black triangles) was not different from the passive diameter curve (Fig. 3b, open circles). As a

control, we also demonstrated that ET_A inhibition left unaffected the active diameter of size-matched arterioles from healthy mice (Figure 3a).

This example illustrates that tumor arterioles exhibit a myogenic tone sensitive to an ET-A antagonist.

Example 4 Increased tumor perfusion induced by an endothelin antagonist

In this example it was investigated whether the BQ123-mediated abrogation of the myogenic tone (as elicited *ex vivo*) (see example 3) was correlated with an increased tumor perfusion *in vivo*. Accordingly, TLT tumor-bearing mice received a bolus i.p. injection of 1 mg/kg BQ123 or saline, and the perfusion of both the tumor-bearing and the control legs was monitored using a Laser Doppler Imaging system. In BQ123-treated mice, we consistently observed an important increase in the blood flow of the tumor-bearing leg (Figure 4a, upper panel, white circle) whereas the healthy opposite leg failed to show any significant changes in perfusion (Figure 4a, upper panel, white arrows). Importantly, isovolemic saline i.p. injection barely induced the basal perfusion in the tumor-bearing leg (see Figures 4a (lower panels) and 4b). We also used Laser Doppler needle probes directly introduced in the tumors to validate the results obtained with the imager. A similar increase in blood flow was observed after BQ123 administration (Figure 4C).

Using EPR oximetry, we also evaluated the BQ123-induced changes in tumor pO_2. Figure 4d shows that 60 min after administration of the ET_A antagonist (but not of the saline solution), the tumor oxygenation was significantly increased over basal level (+ 174 %; $P<0.01$, $n=7$). Of note, the measured pO_2 corresponds to the mean value in a tumor volume of ± 10 mm^3 (i.e. the volume of charcoal dispersion as determined by histology) and therefore does not preclude more dramatic variations in specific regions of the tumor.

This example thus illustrates that ET_A inhibition triggers a tumor-specific increase in blood flow and oxygenation.

Example 5 Improved sensitivity of tumors to radiation upon administrating of an endothelin antagonist in mice

This example illustrates that ET-A inhibition radiosensitizes tumors. The effect of ET_A inhibition on tumors treated with radtiotherapy was investigated. Accordingly, daily the tumor

diameters of mice receiving (or not) fractionated radiotherapy was determined and the effects of BQ123 administered 45 min before each radiation fraction (to have the maximum increase in pO_2 at the time of X-Ray exposure; see Figure 2) were evaluated. As shown in Figure 5 (a and b) and Table 4, when fractionated radiotherapy (5 x 2 Gy and 2 x 6 Gy) was combined to the BQ123 pretreatment, the overall benefit on tumor growth retardation was higher than the sum of the effects of the two treatments administered separately ($P<0.01$; n=8-11). Similar data were obtained with two other tumor models, namely LLc tumors and FSA-II (not shown); both tumor types overexpress ET-1 (see Figure 1c-d) and arterioles issued from the former were shown to also exhibit myogenic tone (not shown).

A dose-response curve was also generated using three different doses of BQ123 (0.5, 1 and 2 mg/kg) in combination with the 2 x 6 Gy protocol (Figure 5c). Although limitations in BQ123 availability precluded further exploration, it is noteworthy that with the higher dose (2 mg/kg), two mice (out of 6) were in remission 5 weeks after treatment (see Figure 5 legend). Of note, at each dose used in this study, BQ123 (two or five i.p. injections) only slightly restrained tumor growth by itself (see Figure 5 a-b and Table 4).

Example 6 Improved sensitivity of tumors to chemotherapy upon administrating of a endothelin antagonist in mice

This example illustrates that ET-A inhibition sensitizes tumors for chemotherapeutics. The effect of ET_A inhibition on tumors treated with the chemotherapeutic cyclophosphamide was investigated. Tumor diameters of mice receiving (or not) cyclophosphamide were determined daily and the effects of BQ123, administered 45 min before administration of the chemotherapeutic, were evaluated. Non-treated TLT tumors, tumors treated with cyclophosphamide and tumors treated with the ET-A antagonist alone exhibited a fast linear growth (see Figure 6). Importantly, when chemotherapy was combined to the BQ123 pre-treatment, retardation of tumor growth could be observed (Figure 6).

Discussion

The above given examples illustrate that basal production of ET-1 in tumors leads to the development of a myogenic tone which represents an important reserve for vasorelaxation and that the use of an ET-A antagonist can selectively increase tumor perfusion and the effectiveness of tumor radiotherapy and tumor chemotherapy *in vivo.*

The above-given examples show that isolated tumor arterioles mounted in a pressure myograph have the ability, contrary to size-matched healthy arterioles, to contract in response to a transluminal pressure increase. This myogenic tone was exquisitely dependent on the endothelin-1 (ET-1) pathway since it was completely abolished by the selective ET-A antagonist BQ123. This selectivity was further supported by the large increase in ET-1 abundance in tumors and the higher density of the ET-A receptors in tumor vessels. It was shown that administration of the ET-A antagonist led to a significant increase in tumor blood flow whereas the perfusion in control healthy tissue was not altered. Finally, we provided evidence that acute administration of the ET-A antagonist significantly stimulates tumor oxygenation, as determined by EPR oximetry, and increases the efficacy of low-dose, clinically-relevant fractionated radiotherapy. Thus, blocking the tumor-selective increase in the vascular ET-1/ET-A pathway unravels an important reserve of vasorelaxation which can be exploited to selectively increase tumor response to radiotherapy and chemotherapy.

The existence of a myogenic tone is almost impossible to quantitatively appreciate *in vivo* since it requires to track the changes in vascular resistance in response to an increase in transmural pressure. Here, a unique experimental model was used specifically dedicated to this purpose, e.g microdissected tumor vessels mounted in a myograph allowing step increases in pressure. This *ex vivo* mode of detection should not mask the *in vivo* importance of the myogenic tone in the autoregulation of blood flow by arterioles. Indeed, in normal vascular beds, spontaneous (oscillating) and pressure-induced variations in the muscular tone, known as vasomotion (Penget al. Circ.Res., *88*: 810-815, 2001) and myogenic tone (Davis and Hill Physiol Rev., *79*: 387-423, 1999; Hill et al. J.Appl.Physiol, *91*: 973-983, 2001), respectively, offer to the tissue circulation the intrinsic ability to maintain a relatively constant blood flow despite variations in blood pressure. In the case of tumors, such autoregulatory mechanisms are very likely to be needed to face the constant remodeling of tumor vascularization. Here, we have identified the implication of ET-1 as a trigger of the myogenic tone of tumor vessels. The signal transduction appears to transit through the smooth muscle-specific ET_A receptor (the abundance of which is increased by 5-fold in tumor arterioles) since BQ123 (a specific ET_A antagonist) completely abrogated the pressure-dependent development of vascular tone by tumor arterioles.

That the implication of the ET-1/ET_A pathway in the development of a myogenic tone is specific of the tumor vascular bed was further authenticated by the increase in blood flow observed after treatment with BQ123 in tumors but not in control tissue from the same mouse (see Figure 4). Interestingly, ET-1 has been shown to exert its vasoconstrictive effects when applied from the adventitial side of the vessels (38, 39), which in the case of ET-1-producing tumors is very likely to account for local (specific) effects. Moreover, we found an increased vascular ET_A expression in tumor arterioles which is also very likely to contribute to the observed myogenic tone. Exaggerated production of ET-1 has previously been reported to mediate similar increase in myogenic constriction in cerebral arteries exposed to oxidized LDL (40) and arterioles isolated from hypertensive rats (41). Together with our study, these reports indicate that pathological states such as atherosclerosis, hypertension and tumor growth can transform vessels devoid of myogenic tone in vessels prone to blood flow autoregulation. Of note, the phenotype shift can be considered as yet more dramatic for tumor vessels since we used vessels with an external diameter of 300 µm which is higher than the usually accepted diameter for resistance arterioles (<170 µm).

The observed *ex vivo* and *in vivo* vasodilating effects of BQ123 imply that functional smooth muscle cells are the target of this drug in tumor vessels. The presence of smooth muscle cells or mural cells on vessels is viewed as a hallmark of maturation (Jain Nat.Med., *9*: 685-693, 2003) and it is generally accepted that month/year old human tumors are more likely to recruit pericytes on a larger proportion of their vasculature (*vs* rapidly growing mouse tumors). This suggests that functional tumor vessels constitute a yet more attractive target for selective vasomodulatory treatments in humans. Although further studies are required to evaluate how our data can be extrapolated in the clinics, the tumor vessel selectivity of ET_A antagonist treatment, as identified in mouse, offers strong bases for the establishment of an adjuvant strategy aiming to increase the effectiveness of conventional anti-tumor treatments (by acting on tumor blood flow and oxygenation). Adequately scheduled combination of these latter approaches with anti-angiogenic drugs certainly represents a very attractive area of investigation for future cancer therapy development..

Evidence is provided that acute administration of BQ123 (i.e., i.p. injection 45 min before local tumor irradiation) exerts profound effects on the efficacy of low-dose radiotherapy (5 x 2 Gy and 2 x 6 Gy). Importantly, these effects were shown to be ET_A antagonist dose-dependent

and obtained using clinically relevant schemes of fractionated radiation administration; a complete remission was even obtained in mice treated with the highest BQ123 dose tested in this study. Although a small effect of BQ123 alone was observed in the absence of irradiation (probably attributable to the anti-proliferative effect of this drug) (Kurbel et al. Med.Hypotheses, 52: 329-333, 1999; Asham et al. Br.J.Cancer, 85: 1759-1763, 2001; Bagnato and Spinella. Trends Endocrinol.Metab, 14: 44-50, 2002), the combination of both irradiation and the ET_A antagonist led to a synergistic effects in both irradiation protocols, increasing the tumor regrowth delay by more than 50% over the additive effects (see Table 4).

The examples showed the implication of ET-1 as a trigger of the intrinsic tone of tumor vessels. A 5-fold increase in the smooth muscle-specific ET-A receptor and the specific blockade of the elicited myogenic tone by the ET-A-specific antagonist BQ123 was identified.

The implication of the ET-1/ET-A pathway in the development of a myogenic tone is specific of the tumor vascular bed as authenticated by the increase in blood flow observed after treatment with BQ123 in tumor vasculature but not in control vessels from the same mouse (see Figure 4a). Both the increased ET-1 abundance and vascular ET-A expression contribute to the observed myogenic tone in tumor arterioles.

It was further documented that the radiosensitizing effects of BQ123 arise from the increase in tumor oxygenation directly associated to the elevation in tumor blood flow (see Figure 4).

It was also shown that administration of BQ123, i.e. intra-peritoneal injection 45 min before the administration of a chemotherapeutic, exerts profound effects on the efficacy of the chemotherapeutic.

Table 4 Mean regrowth delays for TLT tumors as a function of ionizing radiation exposure(s) and BQ123 treatment(s). When indicated (+), BQ123 was delivered i.p. 45-min before each irradiation (2 or 6 Gy) or sham-irradiation (0 Gy). **P<0.01, *vs* additive effects of BQ123 and X-Ray administered separately.

BQ123 (1 mg/kg)	X-rays (# fraction x dose)	Tumor doubling time (days)	Mean regrowth delay (days)	Mice (n)
—	0	4.1 ± 0.7	—	11
—	5 x 2 Gy	8.5 ± 0.6	4.4 ± 0.8	11
+	5 x 0 Gy	4.6 ± 0.3	0.5 ± 0.7	11
+	5 x 2 Gy	13.6 ± 0.7	9.5 ± 0.8	11
—	2 x 6 Gy	13.2 ± 0.9	9.1 ± 0.9**	11
+	2 x 0 Gy	4.6 ± 0.4	0.5 ± 0.8	11
+	2 x 6 Gy	23.0 ± 1.4	18.9 ± 0.8**	8

In conclusion although derived from the host tissue, the tumor vasculature is under the influence of the tumor microenvironment and needs to adapt to the resistance to blood flow inherent to the dynamics of tumor growth. Such vascular remodeling can offer selective targets to pharmacologically modulate tumor perfusion and thereby improve the efficacy of conventional anti-cancer treatments. Radiotherapy and chemotherapy can, indeed, take advantage of a better tumor oxygenation and drug delivery, respectively, both partly dependent on the tumor blood supply.

Here, we showed that isolated tumor arterioles mounted in a pressure myograph have the ability, contrary to size-matched healthy arterioles, to contract in response to a transluminal pressure increase. This myogenic tone was exquisitely dependent on the endothelin-1 pathway since it was completely abolished by the selective ET_A antagonist BQ123. This selectivity was further supported by the large increase in endothelin-1 abundance in tumors and the higher density of the ET_A receptors in tumor vessels. We also documented by using laser doppler microprobes and imaging that administration of the ET_A antagonist led to a significant increase in tumor blood flow whereas the perfusion in control healthy tissue was not altered. Finally, we provided evidence that acute administration of the ET_A antagonist

could significantly stimulate tumor oxygenation, as determined by EPR oximetry, and increase the efficacy of low-dose, clinically-relevant fractionated radiotherapy.

Thus, blocking the tumor-selective increase in the vascular endothelin-1/ET_A pathway led us to unravel an important reserve of vasorelaxation that can be exploited to selectively increase tumor response to radiotherapy.

There is growing evidence that endothelin-1 production is increased in many tumor types where it plays crucial roles in proliferation and angiogenesis. In these studies, however, there was no mention of the powerful vasoconstrictive effects of ET-1 and its potential action on the contractile tone of tumor vessels. Indeed, although ET-1 can induce vasodilation through activation of the endothelial ETB receptors, a powerful vasoconstriction is obtained when ET-1 interacts with the ETA (and ETB) receptors located on smooth muscle cells. It was herein demonstrated that tumor production of ET-1 influences local vascular tone and blocking ET-1 signaling can have an impact on tumor blood flow and thereby be exploited to promote conventional anti-tumor therapy.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the present invention.

Claims

1. Method for treating cancer comprising the steps of
   - exposing an individual in need of said cancer treatment to a therapeutically effective amount of an endothelin antagonist, and
   - exposing said individual to a cancer therapy.

2. Method for optimizing the supply of an anti-cancer composition used in cancer therapy for inhibiting the growth of a tumor cell, comprising the steps of
   a) determining a suitable amount of an endothelin antagonist,
   b) determining a suitable amount of an anti-cancer composition,
   c) exposing said tumor to the amount of endothelin antagonist determined in step a),
   d) supplying said exposed tumor with the amount of said anti-cancer composition, determined in step b) and
   e) optionally reducing the amount of anti-cancer composition determined in step b) by amending the amount of said endothelin antagonist determined in step a).

3. Method according to claim 2, wherein said amount of said anti-cancer composition is determined in function of said amount of said endothelin antagonist.

4. Method for reducing side-effects of an anti-cancer composition used in cancer therapy, comprising the steps of:
   a) determining a suitable amount of an endothelin antagonist,
   b) reducing the amount of the anti-cancer composition in function of the amount of endothelin antagonist determined in step a),
   c) exposing an individual in need of said treatment to the amount of endothelin antagonist determined in step a), and
   d) exposing said individual to the reduced amount of said anti-cancer composition determined in step b).

5. Method according to any of claims 1 to 4, wherein said endothelin antagonist is orally or parenterally administered to said individual.

6. Method according to any of claims 1 to 5, wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof.

7. Method according to any of claims 1 to 6, wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

8. Method according to any of claims 1 to 7, wherein said anti-cancer composition is selected from the group comprising chemotherapeutics, radiation, immunotherapeutics, vectors used in gene therapy or any combinations thereof.

9. Method according to any of claims 1 to 8, wherein said cancer therapy comprises chemotherapy, radiotherapy, immunotherapy, cancer gene-based therapy and/or combinations thereof.

10. Method for preparing an anti-cancer composition having reduced toxicity comprising the steps of:

a) providing a suitable amount of an endothelin antagonist
b) determining a suitable amount of anti-cancer composition in function of the amount of said endothelin antagonist, and
c) preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

11. Method for adjusting the amount of an anti-cancer composition supplied by a supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of an anti-cancer composition function of the amount of endothelin antagonist determined in step a),
c) adjusting said supplying device for supplying the amount of said anti-cancer composition radiation determined in step b), and

d) supplying the anti-cancer composition using said supplying device together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

12. Method for diagnosing cancer comprising the steps of

- exposing an individual in need of said cancer treatment to a suitable amount of an endothelin antagonist, and
- exposing said individual to a cancer diagnostic method.

13. Method for optimizing the supply of a diagnostic compound used in cancer diagnosis comprising the steps of

a) determining a suitable amount of an endothelin antagonist,
b) determining a suitable amount of said diagnostic compound,
c) exposing said tumor to the amount of endothelin antagonist determined in step a),
d) supplying said exposed tumor with the amount of said diagnostic compound, determined in step b) and
e) optionally reducing the amount of diagnostic compound determined in step b) by amending the amount of said endothelin antagonist determined in step a).

14. Method according to claim 13, wherein said amount of said diagnostic compound is determined in function of said amount of said endothelin antagonist.

15. Method for reducing side-effects of a diagnostic compound used in cancer diagnosis, comprising the steps of:

a) determining a suitable amount of an endothelin antagonist,
b) reducing the amount of the a diagnostic compound in function of the amount of endothelin antagonist determined in step a),
c) exposing an individual in need of said treatment to the amount of endothelin antagonist determined in step a), and
d) exposing said individual to the reduced amount of said a diagnostic compound determined in step b).

16. Method for adjusting the amount of a diagnostic compound used in cancer diagnosis supplied by a supplying device, comprising the steps of

a) determining a suitable amount of endothelin antagonist
b) determining a suitable amount of vector in function of the amount of endothelin antagonist determined in step a),
c) providing the amount of said diagnostic compound determined in step b) to said supplying device, and
d) supplying said diagnostic compound using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

17. Composition containing an endothelin antagonist and an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

18. Composition containing an endothelin antagonist and a diagnostic compound as a combined preparation for simultaneous, separate or sequential use in cancer diagnosis.

19. Composition according to claim 17 or 18, wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof.

20. Composition according to any of claims claim 17 to 19, wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

21. Composition according to any of claims to 17 to 20, wherein said endothelin antagonist is suitable for being administered orally or parenterally.

22. Composition according to claim 17, wherein said anti-cancer composition is selected from the group comprising chemotherapeutics, radiation, immunotherapeutics, vectors used in gene therapy or any combinations thereof.

23. Use of an endothelin antagonist for improving cancer therapy.

24. Use of an endothelin antagonist in the preparation of a medicament for improving cancer therapy.

25. Use of an endothelin antagonist for improving cancer diagnosis.

26. Use according to any of claims 23 to 25, wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof.

27. Use according to any of claims claim 23 to 26, wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

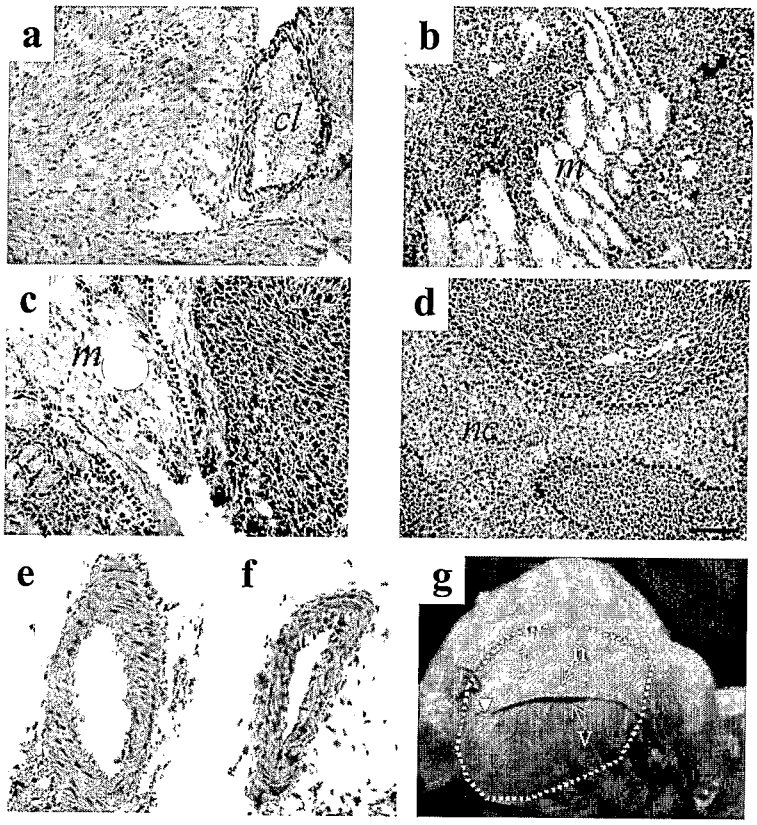

FIG. 1

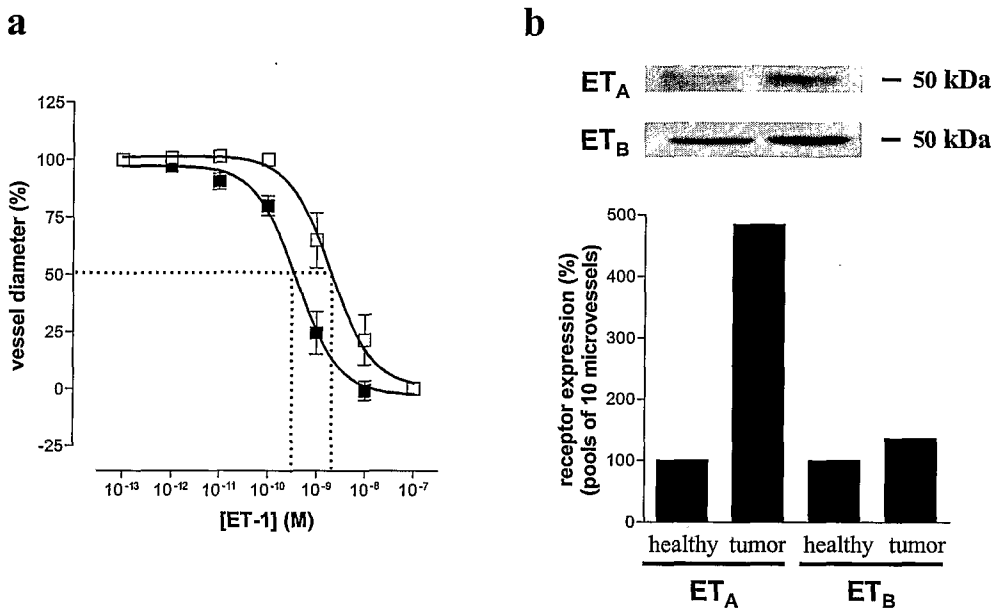
a
b
vessel diameter (%)
125
100
75
50
25
0
-25
10-13 10-12 10-11 10-10 10-9 10-8 10-7
[ET-1] (M)
ETA
ETB
50 kDa
50 kDa
receptor expression (%)
(pools of 10 microvessels)
500
400
300
200
100
0
healthy tumor healthy tumor
ETA
ETB

FIG. 2

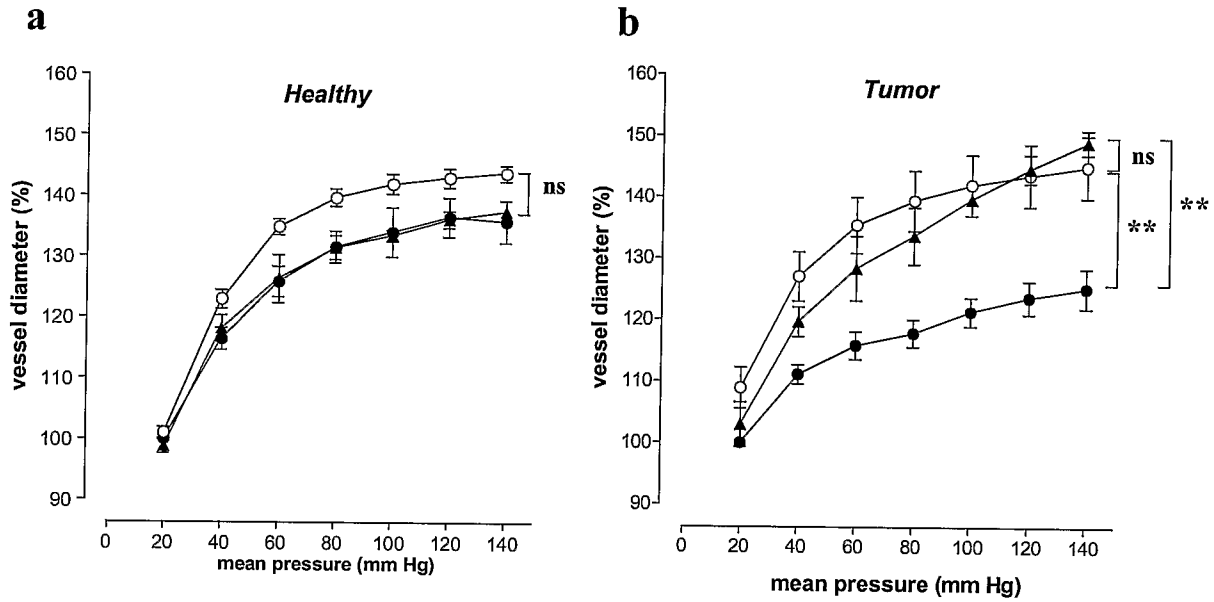
a
Healthy
vessel diameter (%)
160
150
140
130
120
110
100
90
0 20 40 60 80 100 120 140
mean pressure (mm Hg)
ns
b
Tumor
vessel diameter (%)
160
150
140
130
120
110
100
90
0 20 40 60 80 100 120 140
mean pressure (mm Hg)
ns
**
**

FIG. 3

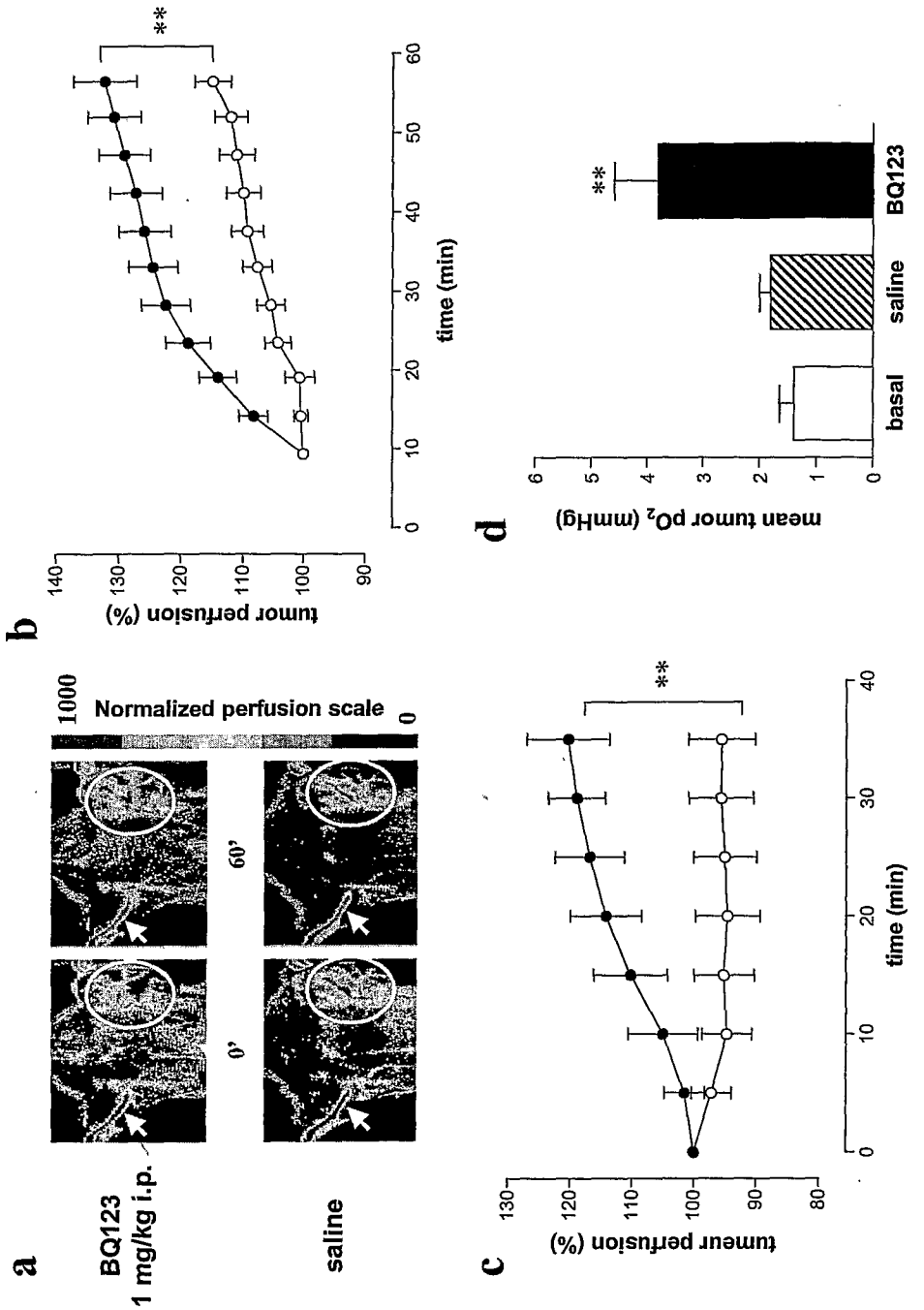
a
BQ123
1 mg/kg i.p.
saline
0'
60'
1000
Normalized perfusion scale
0
b
tumor perfusion (%)
time (min)
**
c
tumeur perfusion (%)
time (min)
**
d
mean tumor pO2 (mmHg)
basal
saline
BQ123
**

FIG. 4

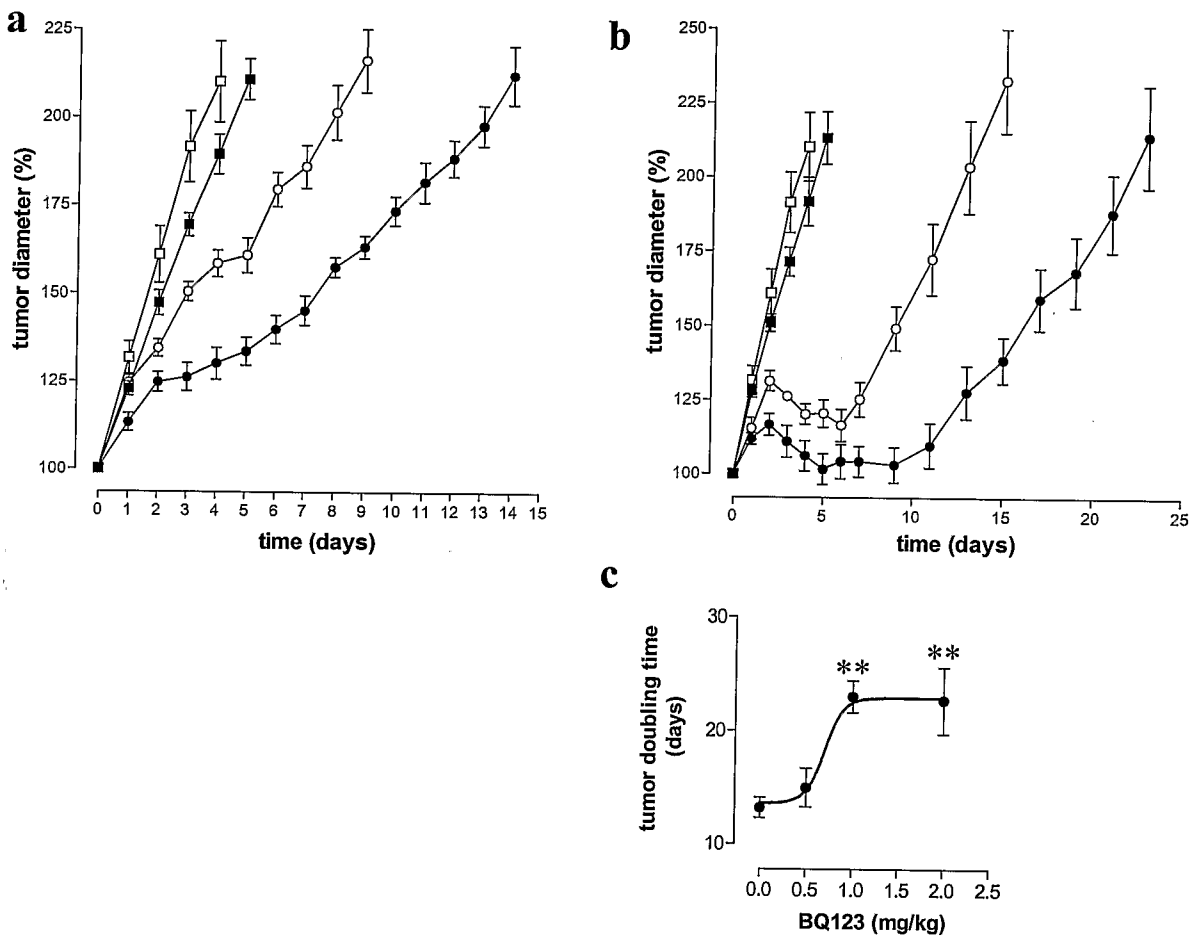
a
tumor diameter (%)
225
200
175
150
125
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
time (days)
b
tumor diameter (%)
250
225
200
175
150
125
100
0 5 10 15 20 25
time (days)
c
tumor doubling time (days)
30
20
10
**
**
0.0 0.5 1.0 1.5 2.0 2.5
BQ123 (mg/kg)

FIG. 5

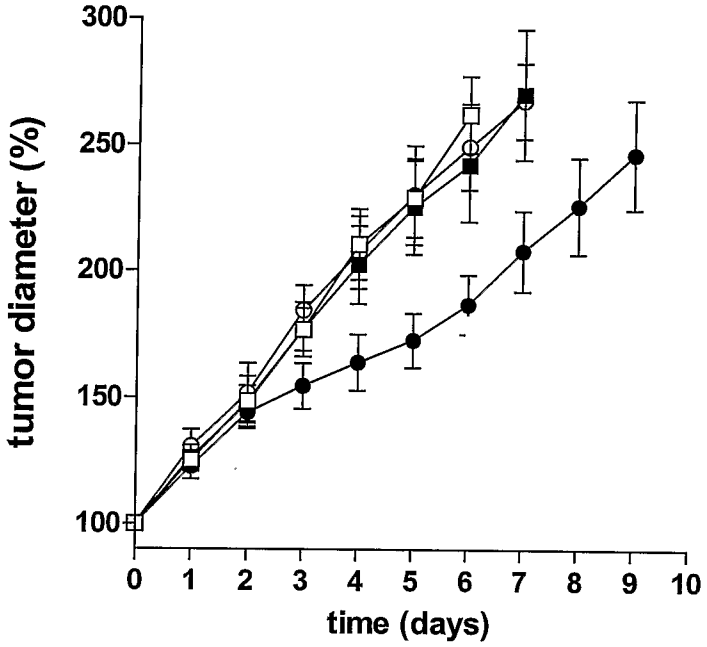

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P35/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2004/037235 A (TRUSTEES OF THE UNIVERSITY OF) 6 May 2004 (2004-05-06) claims 18,29,30,37,38	1-27
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X	WO 03/015820 A (KUSAKA MASAMI ; FURUYA SHUICHI (JP); TAKEDA CHEMICAL INDUSTRIES LTD (J) 27 February 2003 (2003-02-27) abstract	1-27
E	-& EP 1 424 080 A (TAKEDA CHEMICAL INDUSTRIES LTD) 2 June 2004 (2004-06-02) page 17, line 44 - page 19, line 56; claims 31-33	1-27

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document 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	Date of mailing of the international search report
6 September 2004	16/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Winger, R

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/004554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/085351 A (ABBOTT LAB) 31 October 2002 (2002-10-31) page 18, line 1 - line 26 page 19, line 28 - line 32	1-27
X	DEL BUFALO DONATELLA ET AL: "Endothelin-1 acts as a survival factor in ovarian carcinoma cells" CLINICAL SCIENCE (LONDON), vol. 103, no. Suppl. 48, August 2002 (2002-08), pages 302S-305S, XP001180799 ISSN: 0143-5221 figure 1	1-27
X	US 2002/055457 A1 (JANUS TODD J ET AL) 9 May 2002 (2002-05-09) paragraph '0129!; claims 1,5-7,10; examples	1-27
X	WO 00/36918 A (MARINE POLYMERS TECHNOLOGIES I) 29 June 2000 (2000-06-29) page 37, line 10 - page 39, line 24; claim 1	1-27
X	WO 02/49630 A (SQUIBB BRISTOL MYERS CO ; LEBWOHL DAVID E (US)) 27 June 2002 (2002-06-27) line 24 - page 7, line 32	17-22
X	EP 0 950 418 A (TAKEDA CHEMICAL INDUSTRIES LTD) 20 October 1999 (1999-10-20) claims	17-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/004554

Box 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. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-9, 11-16, 23 and 25 are directed to a method of treatment of the human/animal body and a diagnostic method practised on the human/animal body, respectively, the search has been carried out and based on the alleged effects of the compound.

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 III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP2004/004554

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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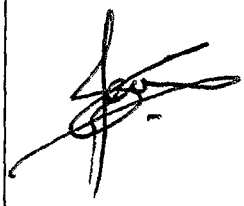
PCT REQUEST

Original (for SUBMISSION)

VIII-4-1	**Declaration: Inventorship (only for the purposes of the designation of the United States of America)** Declaration of Inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America:	I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought. This declaration is directed to the international application of which it forms a part (if filing declaration with application). I hereby declare that my residence, mailing address, and citizenship are as stated next to my name. I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications", by application number, country or Member of the World Trade Organization, day, month, and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.
VIII-4-1-1	Prior applications:	60/470,679, US, 15 May 2003 (15.05.2003)

PCT REQUEST

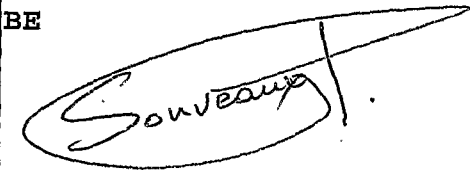
Original (for SUBMISSION)

		I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.
VIII-4-1-1-1	Name (LAST, First)	FERON, Olivier
VIII-4-1-1-2	Residence: (city and either US State, if applicable, or country)	Bruxelles, Belgium
VIII-4-1-1-3	Mailing address:	Van Severlaan 3 B-1970 Bruxelles Belgium Bruxelles Belgium
VIII-4-1-1-4	Citizenship:	BE
VIII-4-1-1-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1-1-6	Date: (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	28/05/2004

UCL-062-PCT

PCT REQUEST

Original (for **SUBMISSION**)

VIII-4-1-2-1	Name (LAST, First)	DESSY, Chantal
VIII-4-1-2-2	Residence: (city and either US State, if applicable, or country)	Bruxelles, Belgium
VIII-4-1-2-3	Mailing address:	Van Severlaan 3 B-1970 Bruxelles Belgium Bruxelles Belgium
VIII-4-1-2-4	Citizenship:	BE
VIII-4-1-2-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1-2-6	Date (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	28.05.04
VIII-4-1-3-1	Name (LAST, First)	SONVEAUX, Pierre
VIII-4-1-3-2	Residence: (city and either US State, if applicable, or country)	Louvain-la-Neuve, Belgium
VIII-4-1-3-3	Mailing address:	Sentier du Biéreau 13 B-1348 Louvain-la-Neuve Belgium Louvain-la-Neuve Belgium
VIII-4-1-3-4	Citizenship:	BE
VIII-4-1-3-5	Inventor's Signature: (if not contained in the request, or if declaration is corrected or added under Rule 26ter after the filing of the international application. The signature must be that of the inventor, not that of the agent)	
VIII-4-1-3-6	Date (of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26ter after the filing of the international application)	22.05.2004

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

RECEIVED
PCT 14 SEP 2004
WIPO PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/004554	29.04.2004	15.05.2003

International Patent Classification (IPC) or both national classification and IPC
A61K45/06, A61P35/00

Applicant
UNIVERSITE CATHOLIQUE DE LOUVAIN

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Winger, R

Telephone No. +49 89 2399-8129

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-9,11-16,23,25 (industrial applicability)

because:

☒ the said international application, or the said claims Nos. 1-9,11-16,23,25 (industrial applicability) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
☐ has not been furnished
☐ does not comply with the standard

the computer readable form
☐ has not been furnished
☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-27
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-27
Industrial applicability (IA)	Yes:	Claims	10,17-22,24,26,27
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Section III

1. Claims 1-9, 11-16, 23 and 25 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Section V

2. Prior Art: Reference is made to the following documents cited in the International Search Report

   D1: WO 2004/037235 A
   D2: WO 2004/035057 A
   D3: WO 03/015820 A
   D4: WO 02/085351 A
   D5: DEL BUFALO DONATELLA ET AL: "Endothelin-1 acts as a survival factor in ovarian carcinoma cells" CLINICAL SCIENCE (LONDON), vol. 103, no. Suppl. 48, August 2002 (2002-08), pages 302S-305S
   D6: US 2002/055457 A1
   D7: WO 00/36918 A
   D8: WO 02/49630 A
   D9: EP-A-0 950 418

2.1 Document D1 discloses a method of treating a solid tumor comprising an endothelin B antagonist in combination with an anticancer agent and/or radiation. Document D2 discloses a combination of an endothelin receptor antagonist and an EGFR TKI (anticancer agent) for the treatment of cancer. For the international phase documents D1 and D2 do not constitute prior art.

2.3 Document D3 discloses treating prostate cancer using a combination of a GnRH agonist and endothelin receptor antagonist.

2.4 Document D4 discloses endothelin receptor antagonists of formula I for the treatment of prostate cancer. The compounds may be administered in combination with anticancer drugs. In the examples the treatment with atrasentan followed by chemotherapy is described.

2.5 Document D5 discloses the combined treatment of carcinoma cells with taxol and endothelin antagonists.

2.6 Document D6 discloses a combination of (selective) ET-A endothelin antagonists for the treatment of metastatic growth. The combination with various other anticancer drugs is described. In the examples prostate cancer is treated with ABT-627 after leuprolide or goserelin administration.

2.7 Document D7 discloses an antitumor composition comprising at least one endothelin antagonist in combination with poly acetylglucosamin and additionally other antitumor agents.

2.8 Document D8 discloses the combination of entothelin antagonists with therapies used in the treatment of cancer.

2.9 Document D9 discloses combination of endothelin antagonists with NA-H exchange inhibitors such as cariporide (inhibitor of cell proliferation).

3. Novelty (Article 33(2) PCT):

3.1 Independent claims 1, 2, 4, 10, 11, 12, 13, 15, 16, 23, 24 and 25 relate to methods for treating, diagnosing and optimizing cancer therapy using a combination of an endothelin antagonist and a cancer therapy. However, as documents D3-D7 all disclosed such combinations for the treatment of cancer, the subject-matter of said claims and the dependent claims does not seem to be novel.

3.2 Independent claims 17 and 18 relate to compositions comprising an endothelin antagonist and an anti-cancer composition / diagnostic compound. The medical use of theses compositions, however, seems to be anticipated by documents D3-D9. Thus, the subject-matter of said claims and the dependent claims does not seem to be novel.

4. Inventive Step (Article 33(3) PCT):

As both the individual compounds and the combinations are known for the treatment of cancer, the selection of specific embodiments without any associated effect does not seem to be inventive either.

PATENT COOPERATION TREATY

PCT

REC'D 16 SEP 2005
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference UCL-062-PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/004554	International filing date *(day/month/year)* 29.04.2004	Priority date *(day/month/year)* 15.05.2003
International Patent Classification (IPC) or national classification and IPC A61K45/06, A61P35/00		
Applicant UNIVERSITE CATHOLIQUE DE LOUVAIN et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.03.2005	15.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Winger, R Telephone No. +49 89 2399-8129

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/004554

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-47 as originally filed

Claims, Numbers

1-17 received on 21.03.2005 with letter of 15.03.2005

Drawings, Sheets

1/6-6/6 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 3
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/004554

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 6-11,13-17 (industrial applicability)

because:

☒ the said international application, or the said claims Nos. 6-11,13-17 (industrial applicability) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-27
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-27
Industrial applicability (IA)	Yes:	Claims	1-5,12
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Section I

1. The amendments filed with the letter dated 15.03.2005 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendment concerned is claim 3 because in the application as filed only a dose per day was disclosed.

Re Section III

2. Claims 6-11 and 13-17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Section V

3. Prior Art: Reference is made to the following documents cited in the International Search Report
   - D1: WO 2004/037235 A
   - D2: WO 2004/035057 A
   - D3: WO 03/015820 A
   - D4: WO 02/085351 A
   - D5: DEL BUFALO DONATELLA ET AL: "Endothelin-1 acts as a survival factor in ovarian carcinoma cells" CLINICAL SCIENCE (LONDON), vol. 103, no. Suppl. 48, August 2002 (2002-08), pages 302S-305S
   - D6: US 2002/055457 A1
   - D7: WO 00/36918 A
   - D8: WO 02/49630 A
   - D9: EP-A-0 950 418

3.1 Document D1 discloses a method of treating a solid tumor comprising an endothelin B antagonist in combination with an anticancer agent and/or radiation. Document D2 discloses a combination of an endothelin receptor antagonist and an EGFR TKI (anticancer agent) for the treatment of cancer. For the international phase documents D1 and D2 do not constitute prior art.

3.2 Document D3 discloses treating prostate cancer using a combination of a GnRH agonist and endothelin receptor antagonist.

3.3 Document D4 discloses endothelin receptor antagonists of formula I for the treatment

of prostate cancer. The compounds may be administered in combination with anticancer drugs. In the examples the treatment with atrasentan followed by chemotherapy is described.

3.4 Document D5 discloses the combined treatment of carcinoma cells with taxol and endothelin antagonists.

3.5 Document D6 discloses a combination of (selective) ET-A endothelin antagonists for the treatment of metastatic growth. The combination with various other anticancer drugs is described. In the examples prostate cancer is treated with ABT-627 after leuprolide or goserelin administration.

3.6 Document D7 discloses an antitumor composition comprising at least one endothelin antagonist in combination with poly acetylglucosamin and additionally other antitumor agents.

3.7 Document D8 discloses the combination of entothelin antagonists with therapies used in the treatment of cancer.

3.8 Document D9 discloses combination of endothelin antagonists with NA-H exchange inhibitors such as cariporide (inhibitor of cell proliferation).

4. Novelty (Article 33(2) PCT):

Independent claims 1, 2, 6, 7, 8, 10, 12, 13, 14 relate to methods for treating, diagnosing and optimizing cancer therapy (defined via different mechanisms such as tumor perfusion or oxygenation) using an endothelin antagonist. However, as documents D3-D7 all disclose endothelin antagonist (alone or in combination) for the treatment of cancer, the subject-matter of claims 1-17 does not seem to be novel.

It is to be noted, that the presence of action described in this application, cannot be used to delimit the present claims from the state of the art. The end effect of the presently claimed invention is the treatment of cancer using the same compounds as claimed in the prior art. The mechanism of action is therefore merely a discovery of how the composition according to the prior art could work.

5. Inventive Step (Article 33(3) PCT):

As both the individual compounds and the combinations are known for the treatment of cancer, the selection of specific embodiments without any associated effect would not seem to be inventive either.

PCT/EP2004/004554

Amended set of claims

1. Use of an endothelin antagonist for the preparation of a medicament for increasing tumor perfusion.

2. Use of an endothelin antagonist for the preparation of a medicament for increasing tumor oxygenation.

3. Use of an endothelin according to claim 1 or 2, wherein the endothelin antagonist is used in a concentration comprised between 0.1µg to 20 mg/kg.

4. Use according to any of claims 1 to 3, wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof.

5. Use according to any of claims claim 1 to 4, wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

6. Method for increasing the blood flow to a tumor comprising the step of supplying a suitable amount of an endothelin antagonist to said tumor.

7. Method for increasing oxygenation of a tumor comprising the step of supplying a suitable amount of an endothelin antagonist to said tumor.

8. Method for optimizing the supply of an anti-cancer composition used in cancer therapy for inhibiting the growth of a tumor cell, comprising the steps of
   a. determining a suitable amount of an endothelin antagonist,
   b. determining a suitable amount of an anti-cancer composition,
   c. exposing said tumor to the amount of endothelin antagonist determined in step a),
   d. supplying said exposed tumor with the amount of said anti-cancer composition, determined in step b) and

e. optionally reducing the amount of anti-cancer composition determined in step b) by amending the amount of said endothelin antagonist determined in step a).

9. Method according to claim 8, wherein said amount of said anti-cancer composition is determined in function of said amount of said endothelin antagonist.

10. Method for optimizing the supply of a diagnostic compound used in cancer diagnosis comprising the steps of
    a. determining a suitable amount of an endothelin antagonist,
    b. determining a suitable amount of said diagnostic compound,
    c. exposing said tumor to the amount of endothelin antagonist determined in step a),
    d. supplying said exposed tumor with the amount of said diagnostic compound, determined in step b) and
    e. optionally reducing the amount of diagnostic compound determined in step b) by amending the amount of said endothelin antagonist determined in step a).

11. Method according to claim 10, wherein said amount of said diagnostic compound is determined in function of said amount of said endothelin antagonist.

12. Method for preparing an anti-cancer composition having reduced toxicity comprising the steps of:
    a. providing a suitable amount of an endothelin antagonist
    b. determining a suitable amount of anti-cancer composition in function of the amount of said endothelin antagonist, and
    c. preparing a composition containing said suitable amount of said endothelin antagonist and an optimal amount of an anti-cancer composition as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

13. Method for adjusting the amount of an anti-cancer composition supplied by a supplying device, comprising the steps of
    a. determining a suitable amount of endothelin antagonist
    b. determining a suitable amount of an anti-cancer composition function of the amount of endothelin antagonist determined in step a),
    c. adjusting said supplying device for supplying the amount of said anti-cancer composition radiation determined in step b), and
    d. supplying the anti-cancer composition using said supplying device together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

14. Method for adjusting the amount of a diagnostic compound used in cancer diagnosis supplied by a supplying device, comprising the steps of
    a. determining a suitable amount of endothelin antagonist
    b. determining a suitable amount of vector in function of the amount of endothelin antagonist determined in step a),
    c. providing the amount of said diagnostic compound determined in step b) to said supplying device, and
    d. supplying said diagnostic compound using said supplying device, together with or subsequent to supplying the suitable amount of said endothelin antagonist determined in step a).

15. Method according to any of claims 6 to 14, wherein said endothelin antagonist is an endothelin receptor antagonist, an endothelin converting enzyme inhibitor, an endothelin neutralizing agent, or any combinations thereof.

16. Method according to any of claims 6 to 15, wherein said endothelin receptor antagonist is a specific ET-A endothelin antagonist and/or a non-specific ET-A/ET-B endothelin antagonist.

17. Method according to any of claims 6 to 16, wherein said anti-cancer composition is selected from the group comprising chemotherapeutics, radiation, immunotherapeutics, vectors used in gene therapy or any combinations thereof.