USE OF EDG RECEPTOR BINDING AGENTS IN CANCER

VERWENDUNG VON EDG REZEPTOR BINDENDEN WIRKSTOFFEN FÜR DIE KREBSTHERAPIE

UTILISATION D’AGENTS DE LIAISON DU RECEPTEUR EDG DANS LE CANCER

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Description

[0001] The present invention relates to a use of a sphingosine-1-phosphate (S1P) receptor agonist, according to claim 1 in the treatment of lymphoproliferative or myeloproliferative disorders.

[0002] S1P receptor agonists are accelerating lymphocyte homing (LH) agents which elicit a lymphopenia resulting from a re-distribution, preferably reversible, of lymphocytes from circulation to secondary lymphatic tissue, without evoking a generalized immunosuppression. Naïve cells are sequestered; CD4 and CD8 T-cells and B-cells from the blood are stimulated to migrate into lymph nodes (LN) and Peyer’s patches (PP), and thus for example infiltration of cells into transplanted organs is inhibited.

[0003] S1P receptor agonists are sphingosine analogues.

[0004] Appropriate S1P receptor agonists are:

- Compounds as disclosed in EP627406A1, i.e. a compound of formula I

\[
\begin{align*}
\text{I} & \\
\text{R}_1 & \text{R}_6 \text{N} \text{CH}_2 \text{OR}_2 & \text{CH}_2 \text{OR}_3
\end{align*}
\]

wherein \( R_1 \) is a straight- or branched (C\(_{12-22}\)) carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR\(_6\), wherein R\(_6\) is H, alkyl, aralkyl, acyl or alkoxycarbonyl, and carbonyl, and/or
- which may have as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxyimino, hydroxy or carboxy; or \( R_1 \) is
- a phenylalkyl wherein alkyl is a straight- or branched (C\(_{6-20}\)) carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C\(_{1-30}\)) carbon chain wherein said phenylalkyl is substituted by
- a straight- or branched (C\(_{6-20}\)) carbon chain optionally substituted by halogen,
- a straight- or branched (C\(_{6-60}\)) alkoxy chain optionally substituted by halogen,
- a straight- or branched (C\(_{6-20}\)) alkenyloxy,
- phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
- cycloalkylalkyl substituted by C\(_{6-20}\) alkyl,
- heteroarylalkyl substituted by C\(_{6-20}\) alkyl,
- heterocyclic C\(_{6-20}\) alkyl or
- heterocyclic alkyl substituted by C\(_{2-20}\) alkyl,

and wherein the alkyl moiety may have

- in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR\(_6\), wherein R\(_6\) is as defined above, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, alkylthio, acylamino, alkoxycarbonyl, alkoxycarbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and each of R\(_2\), R\(_3\), R\(_4\) and R\(_5\), independently, is H, C\(_{1-4}\) alkyl or acyl

or a pharmaceutically acceptable salt thereof;

- Compounds as disclosed in WO02/18395, e.g. a compound of formula Iva
wherein $X_a$ is O, S, NA$_{1a}$ or a group -(CH$_2$)$_{na}$-, which group is unsubstituted or substituted by 1 to 4 halogen; $n_a$ is 1 or 2, $R_{1a}$ is H or (C$_{1-4}$)alkyl, which alkyl is unsubstituted or substituted by halogen; $R_{1a}$ is H, OH, (C$_{1-4}$)alkyl or O (C$_{1-4}$)alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; $R_{1b}$ is H, OH or (C$_{1-4}$)alkyl, wherein alkyl is unsubstituted or substituted by halogen; each $R_{2a}$ is independently selected from H or (C$_{1-4}$)alkyl, which alkyl is unsubstituted or substituted by halogen; $R_{3a}$ is H, OH, halogen or O(C$_{1-4}$)alkyl wherein alkyl is unsubstituted or substituted by halogen, or a pharmaceutically acceptable salt or hydrate thereof;

A preferred compound of formula I is 2-amino-2-tetradecyl-1,3-propanediol. A particularly preferred S1P receptor agonist of formula I is FTY720, i.e. 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol in free form or in a pharmaceutically acceptable salt form (referred to hereinafter as Compound A), e.g. the hydrochloride, as shown:

When the compounds of formulae I or IVa have one or more asymmetric centers in the molecule, the present invention is to be understood as embracing the various optical isomers, as well as racemates, diastereoisomers and mixtures thereof are embraced.

Examples of pharmaceutically acceptable salts of the compounds of the formulae I or IVa include salts with inorganic acids, such as hydrochloride, hydrobromide and sulfate, salts with organic acids, such as acetate, fumarate, maleate, benzoate, citrate, malate, methanesulfonate and benzenesulfonate salts, or, when appropriate, salts with metals such as sodium, potassium, calcium and aluminium, salts with amines, such as triethylamine and salts with dibasic amino acids, such as lysine. The compounds and salts of the methods of the present invention encompass hydrate and solvate forms.

The S1P receptor agonists have of the present invention, on the basis of observed activity, e.g. homing of lymphocytes, e.g. as described in EP627406A1 or USP 6,004,565, been found to be useful e.g. as immunosuppressant, e.g. in the treatment of acute allograft rejection. It has now been found that S1P receptor agonists have interesting properties which make them useful for cancer chemotherapy.

The present invention provides

A composition for enhancing the activity of a chemotherapeutic agent or for overcoming resistance to a chemotherapeutic agent in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a S1P receptor agonist, according to the invention or a pharmaceutically acceptable salt thereof, either concomitantly or sequentially with said chemotherapeutic agent.

A composition according to 1.8 wherein the chemotherapeutic agent is an inhibitor of signal transduction pathways directed either against host cells or processes involved in tumor formation and/or metastases formation or utilised by
tumour cells for proliferation, survival, differentiation or development of drug resistance.  
A composition as indicated above, wherein the S1P receptor agonist is administered intermittently.

In a series of further specific or alternative embodiments, the present invention also provides:

A composition for treating lymphoproliferative or myeloproliferative disorders, e.g. for treating tumor invasiveness or symptoms associated with such tumor growth in a subject in need thereof, comprising co-administering to said subject, e.g. concomitantly or in sequence, of a S1P receptor agonist, e.g. a S1P receptor agonist or a pharmaceutically acceptable salt thereof, and a second drug substance, said second drug substance being a chemotherapeutic agent, e.g. as indicated hereinafter.

By "lymphatic cancer" are meant e.g. tumors of blood and lymphatic system (e.g. Hodgkin's disease, Non-Hodgkin's lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, multiple myeloma and malignant plasma cell neoplasms, lymphoid leukemia, acute or chronic myeloid leukemia, acute or chronic lymphocytic leukemia, monosomy leukemia, other leukemias of specified cell type, leukemia of unspecified cell type, and other unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, for example diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma). Myeloid cancer includes e.g. acute or chronic myeloid leukaemia.

By the term "chemotherapeutic agent" is meant especially any chemotherapeutic agent other than the S1P receptor agonist. It includes but is not limited to,

i. an aromatase inhibitor,
ii. an antiestrogen, an anti-androgen (especially in the case of prostate cancer) or a gonadorelin agonist,
iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platun compound,
v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity, a further anti-angiogenic compound or a compound which induces cell differentiation processes,  
vi. a bradykinin 1 receptor or an angiotensin II antagonist,
vii. a cyclooxygenase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor (prevents heparan sulphate degradation), e.g. PI-88, a biological response modifier, preferably a lymphokine or interferons, e.g. interferon γ, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
viii. an inhibitor of Ras oncogenic isoforms, e.g. H-Ras, K-Ras or N-Ras, or a farnesyl transferase inhibitor, e.g. L-744,832 or DK8G557,
ix. a telomerase inhibitor, e.g. telomestatin,  
x. a protease inhibitor, a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, e.g. bengamide or a derivative thereof, or a pro tease inhibitor, e.g. PS-341, and/or  
xii) a mTOR inhibitor.

The term "aromatase inhibitor" as used herein relates to a compound which inhibits the estrogen production, i.e. the conversion of the substrates androstenedione and testosterone to estrone and estradiol; respectively. The term includes, steroids, especially atamestanee, exemestane and formestane and, in particular, non-steroids, especially aminoglutethimide, roglethimide, pyridoglutethimide, trilostane, testolactone, ketokonazole, vorozole, fadrozole, anastrozole and letrozole. Exemestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark ARIMIDEX™. Formestane can be administered, e.g., in the form as it is marketed, e.g. under the trademark LENTARON™. Fadrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark AFEMA™. Anastrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark ARIMIDEX™. Letrozole can be administered, e.g., in the form as it is marketed, e.g. under the trademark FEMARA™ or FEMAR™. Aminoglutethimide can be administered, e.g., in the form as it is marketed, e.g., under the trademark ORIMETEN™. A combination of the invention comprising a chemotherapeutic agent which is an aromatase inhibitor is particularly useful for the treatment of hormone receptor positive tumors, e.g. breast tumors.

The term "antiestrogen" as used herein relates to a compound which antagonizes the effect of estrogens at the estrogen receptor level. The term includes, tamoxifen, fulvestrant, raloxifene and raloxifene hydrochloride. Tamoxifen can be administered, e.g., in the form as it is marketed, e.g. under the trademark NOLVADEX™. Raloxifene hydrochloride can be administered, e.g., in the form as it is marketed, e.g. under the trademark EVISTA™. Fulvestrant can be formulated as disclosed in US 4,659,516 or it can be administered, e.g., in the form as it is marketed, e.g., under the trademark FASLODEX™. A combination of the invention comprising a chemotherapeutic agent which is an antiestrogen is particularly useful for the treatment of estrogen receptor positive tumors, e.g. breast tumors.

The term "anti-androgen" as used herein relates to any substance which is capable of inhibiting the biological effects of androgenic hormones and includes, bicalutamide (CASODEX™), which can be formulated, e.g. as disclosed in US 4,636,505.
The term "gonadorelin agonist" as used herein includes abarelix, goserelin and goserelin acetate. Goserelin is disclosed in US 4,100,274 and can be administered, e.g., in the form as it is marketed, e.g. under the trademark ZOLADEX™. Abarelix can be formulated, as disclosed in US 5,843,901.

The term "topoisomerase I inhibitor" as used herein includes, topotecan, irinotecan, 9-nitrocamptothecin and the macromolecular camptothecin conjugate PNU-166148 (compound A1 in WO99/17804). Irinotecan can be administered, e.g. in the form as it is marketed, e.g. under the trademark CAMPTOSAR™. Topotecan can be administered, e.g. in the form as it is marketed, e.g. under the trademark HYCAMTIN™.

The term "topoisomerase II inhibitor" as used herein includes the anthracyclines such as doxorubicin (including liposomal formulation, e.g. CAELYX™), daunorubicin, epirubicin, idarubicin and nemorubicin, the antirauquinoles mitoxantrone and losoxantrone, and the podophyllotoxines etoposide and teniposide. Etoposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark ETOPOPHOS™. Teniposide can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZAVEDOS™. Mitoxantrone can be administered, e.g. in the form as it is marketed, e.g. under the trademark NOVANTRON™.

The term "microtubule active agent" relates to microtubule stabilizing and microtubule destabilizing agents including, taxanes, e.g. paclitaxel and docetaxel, vinca alkaloids, e.g. vinblastine. especially vinblastine sulfate, vincristine especially vincristine sulfate, and vinorelbine, discodermolides and epothilones and derivatives thereof, e.g. epothilone B or a derivative thereof. Paclitaxel may be administered, e.g. in the form as it is marketed, e.g. under the trademark TAXOL™. Docetaxel can be administered, e.g. in the form as it is marketed, e.g. under the trademark TAXOTERE™. Vinblastine sulfate can be administered, e.g. in the form as it is marketed, e.g. under the trademark VINBLASTIN R.P.™. Vinristine sulfate can be administered, e.g. in the form as it is marketed, e.g. under the trademark FARMORUBICIN™. Idarubicin can be administered, e.g. in the form as it is marketed, e.g. under the trademark ZAVEDOS™. Mitoxantrone can be administered, e.g. in the form as it is marketed, e.g. under the trademark NOVANTRON™.

The term "alkylating agent" as used herein includes, busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan or nitrosourea (BCNU or Gliadel™). Cyclophosphamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark CYCLOSTIN™. Ifosfamide can be administered, e.g., in the form as it is marketed, e.g. under the trademark HOLOXAN™.

The term "antineoplastic antimetabolite" includes, 5-fluorouracil, capecitabine, cytarabine, fludarabine, thioguanine, methotrexate and edatrexate. Capecitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark XELODA™. Gemcitabine can be administered, e.g., in the form as it is marketed, e.g. under the trademark GEMZAR™.

The term "platin compound" as used herein includes, carboplatin, cisplatin and oxaliplatin. Carboplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark CARBOPLAT™. Oxaliplatin can be administered, e.g., in the form as it is marketed, e.g. under the trademark ELOXATIN™.

The term "compounds targeting/decreasing a protein or lipid kinase activity or further anti-angiogenic compounds" as used herein includes protein tyrosine kinase and/or serine and/or threonine kinase inhibitors or lipid kinase inhibitors, e.g. compounds targeting, decreasing or inhibiting the activity of the epidermal growth factor family of receptor tyrosine kinases (EGFR, ErbB2, ErbB3, ErbB4 as homo- or heterodimers), the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR), the platelet-derived growth factor-receptors (PDGFR), the fibroblast growth factor-receptors (FGFR), the insulin-like growth factor receptor 1 (IGF-1 R), the Trk receptor tyrosine kinase family, the Axl receptor tyrosine kinase family, the Ret receptor tyrosine kinase, the Kit/SCFR receptor tyrosine kinase, members of the c-Abl family and their gene-fusion products (e.g. BCR-Abl), members of the protein kinase C (PKC) and Raf family of serine/threonine kinases, members of the MEK, SRC, JAK, FAK, PDK or PI(3) kinase family, or of the PI(3)-kinase-related kinase family, and/or members of the cyclin-dependent kinase family (CDK) and anti-angiogenic compounds having another mechanism for their activity, e.g. unrelated to protein or lipid kinase inhibition.

Compounds which target, decrease or inhibit the activity of VEGFR are especially compounds, proteins or antibodies which inhibit the VEGF receptor tyrosine kinase, inhibit a VEGF receptor or bind to VEGF, and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 98/35958, e.g. 1-(4-chloroanilino)-4-(4-pyridyl)methyl)phthalazine or a pharmaceutically acceptable salt thereof, e.g. the succinate, in WO 00/27820, e.g. a N-aryl(thio) anthranilic acid amide derivative e.g. 2-[(4-pyridyl)methyl]amino-N-[3-methoxy-5-(trifluoromethyl)phenyl]benzamide or 2-[(1 -oxido-4-pyridyl)methyl]amino-N-[3-trifluoromethylphenyl]benzamide, or in WO 00/09495, WO 00/59509, WO 98/11223, WO 00/27819 and EP 0 769 947; those as described by M. Prewett et al in Cancer Research 59 (1999) 5209-5218, by F. Yuan et al in Proc. Natl. Acad. Sci. USA, vol. 93, pp. 14765-14770, Dec. 1996, by Z. Zhu et al in Cancer Res. 58, 1998, 3209-3214, and by J. Mordenti et al in Toxicologic Pathology, Vol. 27, no. 1, pp 14-21, 1999; in WO 00/37502 and WO 94/10202; Angiostatin™, described by M. S. O'Reilly et al, Cell 79, 1994, 315-328; Endostatin™, described by M. S. O'Reilly et al, Cell 88, 1997, 277-285; anthracilic acid amides; ZD4190; ZD6474; SU5416; SU6668; or anti-VEGF antibodies or anti-VEGF receptor antibodies, e.g. RhuMab.
EP 1 505 959 B1

[0026] By antibody is meant intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies formed from at least 2 intact antibodies, and antibodies fragments so long as they exhibit the desired biological activity.

[0027] Compounds which target, decrease or inhibit the activity of the epidermal growth factor receptor family are especially compounds, proteins or antibodies which inhibit members of the EGF receptor tyrosine kinase family, e.g. EGF receptor, ErbB2, ErbB3 and ErbB4 or bind to EGF or EGF related ligands, or which have a dual inhibiting effect on the ErbB and VEGF receptor kinase and are in particular those compounds, proteins or monoclonal antibodies generically and specifically disclosed in WO 97/02266, e.g. the compound of ex. 39, or in EP 0 564 409, WO 99/03854, EP 0520722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and, especially, WO 96/30347 (e.g. compound known as CP 358774), WO 96/33980 (e.g. compound ZD 1839) and WO 95/03283 (e.g. compound ZM105180) or PCT/EP02/08780; e.g. trastuzumab (Herceptin®), cetuximab, Iressa, OSI-1, OSI-1033, EKB-569, GW-2016, E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 or E7.6.3.

[0029] Compounds which target, decrease or inhibit the activity of PDGFR are especially compounds which inhibit the PDGF receptor, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib.

[0030] Compounds which target, decrease or inhibit the activity of c-Abi family members and their gene fusion products, e.g. a N-phenyl-2-pyrimidine-amine derivative, e.g. imatinib; PD180970; AG957; or NSC 680410.

[0031] Compounds which target, decrease or inhibit the activity of protein kinase C, Raf, MEK, SRC, JAK, FAK and PDK family members, or PI(3) kinase or PI(3) kinase-related family members, and/or members of the cyclin-dependent kinase family (CDK) are especially those staurosporine derivatives disclosed in EP 0 296110, e.g. midostaurin; examples of further compounds include e.g. UCN-01, safingol, BAY 43-9006, Bryostatin 1, Perifosine; UO126; limofosine; RO 318220 and RO 320432; GO 6976; Isis 3521; or LY333531/LY379196.

[0032] Further anti-angiogenic compounds are e.g. thalidomide (THALOMID) and TNP-470.

[0033] Compounds which target, decrease or inhibit the activity of a protein or lipid phosphatase are e.g. inhibitors of phosphatase 1, phosphatase 2A, PTEN or CDC25, e.g. okadaic acid or a derivative thereof.

[0034] Compounds which induce cell differentiation processes are e.g. retinoic acid, α, γ or δ-tocopherol or α-, γ- or δ-tocotrienol.

[0035] The term "cylooxygenase inhibitor" as used herein includes, to, e.g. celecoxib (Celebrex®), rofecoxib (Vioxx®), etoricoxib, valdecoxib or a 5-alkyl-2-arylaminophenolacetic acid, e.g. 5-methyl-2-(2'-chloro-6'-fluoroanilino)phenyl acetic acid.

[0036] The term "histone deacetylase inhibitor" as used herein includes, MS-27-275, SAHA, pyroxamide, FR-901228 or valproic acid.

[0037] The term "bisphosphonates" as used herein includes, etridronic, clodronic, tiludronic, pamidronic, alendronic, ibandronic, risedronic and zoledronic acid. "Etidronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark DIDERONEL™, "Clodronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONEFOS™, "Ibandronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark SKELED™, "Pamidronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark AREDIAD™, "Alendronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark FOSAMAX™, "Ibandronic acid" can be administered, e.g., in the form as it is marketed, e.g. under the trademark BONDRANAT™.

[0038] The term "matrix metalloproteinase inhibitor" as used herein includes, collagen peptidomimetic and nonpeptidomimetic inhibitors, tetracycline derivatives, e.g. hydroxamate peptidomimetic inhibitor batimastat and its orally bioavailable analogue marimastat, prinomastat, BMS-279251, BAY 12-9566, TAA211 or AAJ996.

[0039] The term "mTOR inhibitor" as used herein includes, rapamycin (sirolimus) or a derivative thereof. Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus. Suitable derivatives of rapamycin include e.g. compounds of formula A.
wherein

$R_{1aa}$ is CH$_3$ or C$_{3-6}$ alkynyl.

$R_{2aa}$ is H or -CH$_2$-CH$_2$-OH, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and

$X_{aa}$ is $=O$, (H,H) or (H,OH)

provided that $R_{2aa}$ is other than H when $X_{aa}$ is $=O$ and $R_{1aa}$ is CH$_3$.

or a prodrug thereof when $R_{2aa}$ is -CH$_2$-CH$_2$-OH, e.g. a physiologically hydrolysable ether thereof.

[0040] Compounds of formula A are disclosed e.g. in WO 94/09010, WO 95/16691, WO 96/41807, USP 5,362,718 or WO 99/15530. They may be prepared as disclosed or by analogy to the procedures described in these references.

[0041] Preferred rapamycin derivatives are 32-deoxorapamycin, 16-pent-2-ynyloxy-32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-rapamycin, 16-pent-2-ynyloxy-32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin and, more preferably, 40-O-(2-hydroxyethyl)-rapamycin. Further examples of rapamycin derivatives include e.g. CCI779 or 40-[(3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate)-rapamycin or a pharmaceutically acceptable salt thereof, as disclosed in USP 5,362,718, ABT578 or 40-(tetrazolyl)-rapamycin, particularly 40-epi-(tetrazolyl)-rapamycin, e.g. as disclosed in WO 99/15530, or rapalogs as disclosed e.g. in WO 98/02441 and WO01/14387, e.g. AP23573.

[0042] Comprised are likewise the pharmaceutically acceptable salts thereof, the corresponding racemates, diastereoisomers, enantiomers, tautomers as well as the corresponding crystal modifications of above disclosed compounds where present, e.g. solvates, hydrates and polymorphs, which are disclosed therein. The compounds used as active ingredients in the combinations of the invention can be prepared and administered as described in the cited documents, respectively. Also within the scope of this invention is the combination of more than two separate active ingredients as set forth above, i.e. a pharmaceutical combination within the scope of this invention could include three active ingredients or more. Further both the first agent and the co-agent are not the identical ingredient.

[0043] Utility of the S1P agonists, in treating solid tumors, may be demonstrated in animal test methods as well as in clinic, for example in accordance with the methods hereinafter described.

A. In Vitro Reference examples

A.1 Antitumor Activity

[0044] A mouse breast cancer cell line originally isolated from mammary carcinomas is used, e.g. JygMC(A). The cell number is adjusted to 5x10$^5$ for plating in fresh medium before the procedure. Cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12 h and then washed twice with PBS, followed by addition of fresh medium with 10% FCS and additionally incubated for another 12h. Thereafter the cells are incubated with fresh medium containing 2.5mM of thymidine without FCS for 12h. To release the cells from the block, the cells are washed twice with PBS and replated in fresh medium with 10% FCS. After synchronisation, the cells are incubated with or without various concentrations of a compound of formula I for 3, 6, 9, 12, 18 or 24h. The cells are harvested after treatment with 0.2% EDTA, fixed with ice-cold 70% ethanol solution, hydrolyzed with 250µg/ml of RNaseA (type I-A; Sigma Chem. Co.) at 37°C for 30 mn and stained with propidium iodide at 10µg/ml for 20 mn. After the incubation period, the number of cells is determined both by counting cells in a Coulter counter and by the SRB colorimetric assay. Under these conditions an S1P agonist, inhibits the proliferation of the tumor cells at concentrations ranging from 10$^{-12}$ to 10$^{-6}$ M.

A.2 S1P-Mediated HUVEC Tube Formation Assay

[0045] For the tube formation assay, HUVEC from passage 2-8 are used and are never greater than 70% confluent before harvesting. Cells are prepared for the assay by washing with Herpes Balanced Saline Solution (HBSS from
Clonetics) and then trypsinizing with Trypsin/EDTA (0.25 mg/ml, from Clonetics). After approximately 90% of the cells have lifted off the plate, an equal volume of Trypsin Neutralizing Solution (TNS from Clonetics) is added and the cells are collected into a conical tube containing at least 10 ml of EBM-2 (Clonetics) + 0.1% BSA (Sigma) media. Cells are centrifuged at 1000 rpm for 5 minutes and the supernatant is removed and replaced with 5 ml of fresh EBM-2 + 0.1% BSA. Cells are counted using a hemacytometer and the volume of the cell suspension is adjusted to achieve a concentration of 500,000 cells/ml. Conical tubes are prepared with test compounds at 100 nM, and pertussin toxin (PTx) at 10 ng/ml in each; then 1 ml of the cell suspension is added to each tube. Tubes are then incubated for ½ hour at 37 oC, 5% CO2. The migration assay is performed using Fluoro-Blok 24-Multiwell insert Plates coated with fibronectin (8 μm pore size, Falcon #351147) instead of the individual inserts in a 24-well plate. Cells and test compounds are prepared and pre-incubated as described above, then 100 μl is added to each appropriate well in the insert Plate. 300 μl of the EBM-2 + 2% charcoal-stripped media without S1P is added to the bottoms of the wells marked for no stimulation (-), and 300 μl of the media containing S1P (500 nM) is added to the bottoms of the wells marked for stimulation (+). The plate is then incubated for 4 hours at 37 oC, 5% CO2.

[0046] Calcein AM, 50 μg/vial, (Molecular Probes #C3100) is prepared by first adding 20 μl DMSO to the vial. Then 12.5 ml of HBSS (per plate) is warmed to 37 oC and 150 μl is added to the vial. The contents of the vial are then transferred back to the remaining HBSS to make the final concentration 4 μg/ml Calcein AM.

[0047] The Fluoro-Blok plate is removed from the incubator and the top insert plate is separated and “flicked” to remove excess media clinging to the inserts. The insert plate is then transferred to a fresh 24-well plate containing 500 μl/well of the 4 μg/ml Calcein AM. The plate is then incubated for 1½ hours at 37 oC, 5% CO2.

[0048] After incubation, the plate is read on a Cytocounter at an excitation of 485 nm and emission of 530 nm. The Fluoro-Blok coating in the inserts allows only the cells that have migrated to the bottom to be counted. Data are transferred to Excel for calculations, graphs are created using SigmaPlot, and SigmaStat is used for significance tests (t-test). (Figure 7).

[0049] Tube formation is quantitated by counting the number of branching points (two independent cords connecting) in 3 independent fields at 4X magnification. The results are reported as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Branching Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>8 ± 5</td>
</tr>
<tr>
<td>S1P</td>
<td>42 ± 13</td>
</tr>
<tr>
<td>FTY720-Phosphate</td>
<td>48 ± 15</td>
</tr>
<tr>
<td>FTY720-Phosphate + S1P</td>
<td>14 ± 7</td>
</tr>
<tr>
<td>Compound C-Phosphate</td>
<td>44 ± 16</td>
</tr>
<tr>
<td>Compound C-Phosphate + S1P</td>
<td>18 ± 16</td>
</tr>
</tbody>
</table>

[0050] These results demonstrate the unique ability of FTY720-Phosphate or Compound C-Phosphate to act as an agonist of angiogenesis on its own, but then surprisingly, as an antagonist of S1P-mediated angiogenesis. Compound C-Phosphate is preferably the racemate or the R-enantiomer. PTx is used as a control to inhibit Gia (EDG-1) mediated activity.

B. In Vivo Reference examples

B.1 Antitumor Activity

[0051] Antitumor activity is expressed as T/C% (mean increase in tumor volumes of treated animals divided by the mean increase of tumor volumes of control animals multiplied by 100).

[0052] Aliquots of cancer cells (1x10^7), e.g. human A375 melanoma cells, are transplanted into BALB/c nu/nu mice. When the tumors have reached ca. 10x10 mm in size, the animals are assigned randomly to four subgroups and the treatment with a compound of formula I is initiated. Animals are sacrificed after 2 week treatment, at which times tumors and tissues are harvested and prepared for morphological and molecular analysis. The size of the tumors is determined with a caliper. In this assay, an S1P agonist, slows tumor growth when administered at a dose of from 0.5 to 5 mg/kg vs saline control.

B.2 Combination with a VEGF-R protein tyrosin kinase inhibitor

[0053] Nude mice transplanted with human MDA-MB-435 breast tumors are treated for 2 weeks with a VEGF-R protein tyrosin kinase inhibitor, e.g. 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine succinate, at a dose of 100 mg/kg p.o.
5x/week, a S1P receptor agonist, at a dose of 2.5 mg/kg i.v. 5x/week, or a combination of both. Antitumor is expressed as T/C% as indicated above.

Good antitumor responses are also obtained when nude mice are transplanted with human A375 melanoma cells and treated in a similar way with the same combination: the combined treatment results in a T/C% 15 whereas treatment with each agent alone results in a T/C% 35 and 44, respectively.

B.3 Antiangiogenic Activity

Porous chambers containing (i) sphingosine-1-phosphate (5 μM/chamber) or (ii) human VEGF (1 μg/chamber) in 0.5 ml of 0.8% w/v agar (containing heparin, 20 U/ml) are implanted subcutaneously in the flank of mice. S1P or VEGF induces the growth of vascularized tissue around the chamber. This response is dose-dependent and can be quantified by measuring the weight and blood content of the tissue. Mice are treated once a day orally with Compound A (0.3, 3, 30 or 50 mg/kg) or orally or intravenously with vehicle (5% glucose, 10 ml/kg), starting 4-6 hours before implantation of the chambers and continuing for 4 days. The animals are sacrificed for measurement of the vascularized tissues 24 h after the last dose. The weight and blood content of the vascularized tissues around the chamber is determined.

Animals treated with Compound A show reduced weight and/or blood content of the vascularized tissues compared to animals treated with vehicle alone.

C. Clinical Trial

C.1 Investigation of clinical benefit of a S1P receptor agonist, e.g. a compound of formula I, II or III, e.g. Compound A.

20 patients with progressing, advanced-stage solid tumors, resistant or refractory to standard therapies, to receive said compound at a dosage as determined by a dose escalating study. The general clinical state of the patient is investigated weekly by physical and laboratory examination. Changes in tumor and metastases burden are assessed every 2 months by radiological examination. Initially patients receive treatment for 2 months. Thereafter, they remain on treatment for as long as their disease does not progress and the drug is satisfactorily tolerated.

Main variables for evaluation: Safety (adverse events), standard serum biochemistry and haematology, tumor dimensions by computerised tomographic (CT) scan or magnetic resonance imaging (MRI).

C.2 Combined Treatment

Suitable clinical studies are, for example, open label non-randomized, dose escalation studies in patients with advanced solid tumors. Such studies prove in particular the synergism of the active ingredients of the combination of the invention. The beneficial effects on proliferative diseases can be determined directly through the results of these studies or by changes in the study design which are known as such to a person skilled in the art. Such studies are, in particular, suitable to compare the effects of a monotherapy using the active ingredients and a combination of the invention. Preferably, the dose of agent (a) is escalated until the Maximum Tolerated Dosage is reached, and the co-agent (b) is administered with a fixed dose. Alternatively, the agent (a) is administered in a fixed dose and the dose of co-agent (b) is escalated. Each patient receives doses of the agent (a) either daily or intermittently. The efficacy of the treatment can be determined in such studies, e.g., after 12, 18 or 24 weeks by radiologic evaluation of the tumors every 6 weeks.

Alternatively, a placebo-controlled, double blind study can be used in order to prove the benefits of the combination of the invention mentioned herein.

Daily dosages required in practicing the composition of the present invention when a S1P receptor agonist alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 100 mg as a single dose or in divided doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 50 mg p.o. The S1P receptor agonist may be administered by any conventional route, for example enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.1 to 30 mg, usually 0.25 to 30 mg S1P receptor agonist, together with one or more pharmaceutically acceptable diluents or carriers therefore. In order to inhibit angiogenesis it is important to select a sufficiently high dose of the S1P receptor agonist, as low concentrations of S1P receptor agonists promote angiogenesis. A suitable dose for providing an antiangiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

The combination of the invention can also be applied in combination with surgical intervention, mild prolonged
whole body hyperthermia and/or irradiation therapy.

[0063] The administration of a pharmaceutical combination of the invention results in a beneficial effect, e.g. a synergistic therapeutic effect, e.g. with regard to slowing down, arresting or reversing the neoplasm formation, metastases spread or growth or a longer duration of tumor response or inhibition of angiogenesis; it may also result in other beneficial effects, e.g. less side-effects, an improved quality of life or a decreased mortality and morbidity, compared to a monotherapy applying only one of the pharmaceutically active ingredients used in the combination of the invention, in particular in the treatment of a tumor that is refractory to other chemotherapeutics known as anti-cancer agents.

[0064] A further benefit is that lower doses of the active ingredients of the combination of the invention can be used, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used in order to diminish the incidence of side-effects, while controlling the growth of neoplasm formation. This is in accordance with the desire and requirements of the patients to be treated. According to one embodiment of the invention, a preferred pharmaceutical combination comprises

a) a compound of formula I, IVa, e.g. Compound A, and
b) as a co-agent, one or more compounds as indicated in paragraphs (ii), (iii), (iv), (v), (vii) or (xi) above, e.g. carboplatin, cisplatinum, paclitaxel, docetaxel, gemcitabine, doxorubicin, a compound targeting, decreasing or inhibiting the activity of the vascular endothelial growth factor family of receptor tyrosine kinases (VEGFR) or the platelet-derived growth factor-receptors (PDGFR), a bisphosphonate or a mTOR inhibitor.

[0065] A further embodiment of the invention relates to the use of S1P receptor agonist (a) in combination with a chemotherapeutic agent (b) in the treatment of a lymphatic or myeloid cancer, e.g. as disclosed above. The combination may comprise as a further co-agent b) e.g. busulfan, cytarabine, 6-thioguanine, fludarabine, hydroxyurea, procarbazine, bleomycin or methotrexate. Topoisomerase II inhibitors e.g. daunorubicin or, particularly, compounds which target, decrease or inhibit the activity of PDGFR or of c-Abl family members and their gene fusion products, e.g. imatinib, are preferred as co-agent (b), e.g. for use in the treatment of a lymphatic cancer.

[0066] The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

[0067] It is one objective of this invention to provide a pharmaceutical composition comprising a quantity, which is preferably therapeutically effective against a proliferative malignant disease comprising a combination of the invention. In this composition, the first agent a) and co-agent (b) can be administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms. The unit dosage form may also be a fixed combination.

[0068] The pharmaceutical compositions according to the invention can be prepared in a manner known per se and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans, comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents, especially suitable for enteral or parenteral application.

[0069] Suitable pharmaceutical compositions contain, for example, from about 0.1 % to about 99.9 %, preferably from about 1 % to about 60 %, of the active ingredient(s). Pharmaceutical preparations for the combination therapy for enteral or parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known per se, for example by means of conventional mixing, granulating, sugar-coating, dissolving or lyophilizing processes. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount can be reached, by administration of a plurality of dosage units.

[0070] In particular, a therapeutically effective amount of each of the combination partner of the combination of the invention may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the delay of progression or treatment of a proliferative malignant disease according to the invention may comprise (i) administration of the first agent a) In free or pharmaceutically acceptable salt form and (ii) administration of a co-agent b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners of the combination of the invention may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term administering also encompasses the use of a pro-drug of a combination partner that convert in vivo to the combination partner as such. The instant invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.
The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, the severity of the condition being treated. Thus, the dosage regimen of the combination of the invention is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A physician, clinician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the single active ingredients required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of the active ingredients within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the active ingredients' availability to target sites.

Daily dosages for the first agent or component (a) will, of course, vary depending on a variety of factors, for example the compound chosen, the particular condition to be treated and the desired effect. In general, however, satisfactory results are achieved on administration of a S1P receptor agonist, e.g. Compound A, at daily dosage rates of the order of ca. 0.1 to 100 mg as a single dose or in divided doses. The S1P receptor agonist may be administered by any conventional route, in particular enterally, e.g. orally.

Fradrozole may be administered orally to a human in a dosage range varying from about 0.5 to about 10 mg/day, preferably from about 1 to about 2.5 mg/day. Exemestane may be administered orally to a human in a dosage range varying from about 5 to about 200 mg/day, preferably from about 10 to about 25 mg/day, or parenterally from about 50 to 500 mg/day, preferably from about 100 to about 250 mg/day. If the drug shall be administered in a separate pharmaceutical composition, it can be administered in the form disclosed in GB 2,177,700. Formestane may be administered parenterally to a human in a dosage range varying from about 100 to 500 mg/day, preferably from about 250 to about 300 mg/day. Anastrozole may be administered orally to a human in a dosage range varying from about 0.25 to 20 mg/day, preferably from about 0.5 to about 2.5 mg/day. Aminoglutethimide may be administered to a human in a dosage range varying from about 200 to 500 mg/day.

Tamoxifen citrate may be administered to a human in a dosage range varying from about 10 to about 40 mg/day. Vinblistine may be administered to a human in a dosage range varying from about 1.5 to 10 mg/m²/day. Vincristine sulfate may be administered parenterally to a human in a dosage range varying from about 0.025 to 0.05 mg/kg body weight · week. Vinorelbine may be administered to a human in a dosage range varying from about 10 to about 50 mg/m²/day.

Etoposide phosphate may be administered to a human in a dosage range varying from about 25 to 115 mg/m²/day, e.g. 56.8 or 113.6 mg/m²/day.

Teniposide may be administered to a human in a dosage range varying from about 75 to 150 mg every about two weeks. Doxorubicin may be administered to a human in a dosage range varying from about 10 to 100 mg/m²/day, e.g. 25 or 50 mg/m²/day. Epirubicin may be administered to a human in a dosage range varying from about 10 to 200 mg/m²/day. Idarubicin may be administered to a human in a dosage range varying from about 0.5 to 50 mg/m²/day. Mitoxantrone may be administered to a human in a dosage range varying from about 2.5 to 25 mg/m²/day.

Paclitaxel may be administered to a human in a dosage range varying from about 50 to 300 mg/m²/day. Docetaxel may be administered to a human in a dosage range varying from about 25 to about 100 mg/m²/day.

Cyclophosphamide may be administered to a human in a dosage range varying from about 50 to 1500 mg/m²/day. Meplhalan may be administered to a human in a dosage range varying from about 0.5 to 10 mg/m²/day.

5-Fluorouracil may be administered to a human in a dosage range varying from about 50 to 1000 mg/m²/day, e.g. 500 mg/m²/day. Capecitabine may be administered to a human in a dosage range varying from about 10 to 1000 mg/m²/day. Gemcitabine hydrochloride may be administered to a human in a dosage range varying from about 1000 mg/m²/week. Methotrexate may be administered to a human in a dosage range varying from about 5 to 500 mg/m²/day.

Topotecan may be administered to a human in a dosage range varying from about 1 to 5 mg/m²/day. Irinotecan may be administered to a human in a dosage range varying from about 50 to 350 mg/m²/day.

Carboplatin may be administered to a human in a dosage range varying from about 200 to 400 mg/m² about every four weeks. Cisplatin may be administered to a human in a dosage range varying from about 25 to 75 mg/m² every two weeks. Oxaliplatin may be administered to a human in a dosage range varying from about 50 to 85 mg/m² every two weeks.

Imatinib may be administered to a human in a dosage in the range of about 2.5 to 850 mg/day, more preferably 5 to 600 mg/day and most preferably 20 to 300 mg/day.

Alendronic acid may be administered to a human in a dosage range varying from about 5 to 10 mg/day. Clodronic acid may be administered to a human e.g. in a dosage range varying from about 750 to 1500 mg/day. Etridonic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day. Ibandronic acid may be administered to a human in a dosage range varying from about 750 to 1500 mg/day. Etridonic acid may be administered to a human in a dosage range varying from about 20 to 30 mg/day. Pamidronic acid may be administered to a human in a dosage range varying from about 20 to 500 mg/day.
administered to a human in a dosage range varying from about 15 to 90 mg every three to four weeks. Tiludronic acid may be administered to a human in a dosage range varying from about 200 to 400 mg/day.

[0086] Trastuzumab may be administered to a human in a dosage range varying from about 1 to 4 mg/m²/week.

[0087] Bicalutamide may be administered to a human in a dosage range varying from about 25 to 50 mg/m²/day.

[0088] 1-(4-chloroanilino)-4-(4-pyridylmethyl)phthalazine or salt thereof, e.g. succinate, may be administered to a human in a dosage range of about 50 to 1500, more preferably about 100 to 750, and most preferably 250 to 500, mg/day.

[0089] Rapamycin or a derivative thereof, e.g. 40-O-(2-hydroxyethyl))-rapamycin, may be administered in a dosage range varying from about 0.1 to 25 mg.

[0090] Formulation Example: soft capsules

| Compound of formula I, e.g. Compound A, HCl | 30 mg |
| Polyethylene glycol 300 | 300 mg |
| Polysorbate 80 | 20 mg |
| **Total** | **350 mg** |

[0091] The S1P receptor agonists, of the invention are well tolerated at dosages required for use in accordance with the present invention. For example, the acute LD<sub>50</sub> for Compound A is > 10 mg/kg p.o. in rats and monkeys.

[0092] As described above, high concentrations of S1P receptor agonists (2 μM or greater, e.g. 2-5 μM or around 5 μM) exhibit anti-angiogenic effects, and S1 P receptor agonists can inhibit VEGF-induced angiogenesis. In contrast, low concentrations (0.1 - 1 μM, e.g. 0.1 - 0.5 μM or 0.5 - 1 μM) of S1P agonists have an enhancing effect on angiogenesis and are able to potentiate VEGF-mediated angiogenesis. Thus, S1P agonists may have biphasic effects in angiogenesis.

[0093] S1P agonists suitable for promoting angiogenesis include those defined above in relation to the treatment of cancer, e.g. S1P agonists according to formulae I or IVa, or pharmaceutically acceptable salts or esters thereof. Preferably the S1P agonist is Compound A-phosphate. The S1P agonist in combination with a chemotherapeutic agent and may be used in combination with one or more further agents which promote angiogenesis, e.g. VEGF.

[0094] In order to promote angiogenesis it is important to select a sufficiently low dose of the S1P receptor agonist, as high concentrations of S1P receptor agonists inhibit angiogenesis. A suitable dose for providing a pro-angiogenic effect when a S1P agonist is administered to a patient may be selected by concentration- and dose-escalating studies as described at A, B, and C above.

**Description of the Figures**

[0095]

**Figure 1**
shows that Compound A-phosphate strongly promotes capillary-like network formation in a bell-shape dose-dependent manner showing maximal activity around 0.5 μM.

**Figure 2**
shows that both Compound A-phosphate and Compound A at 0.5 - 1 μM do not attenuate VEGF-mediated remodelling but rather cooperate with polypeptide growth factor.

**Figure 3**
Shows that Compound A-phosphate as well as S1 P-stimulated tube formation is practically completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of α<sub>i/o</sub>-type. This may be interpreted as a possible involvement of EDG-1 (S1P₁) receptor-mediated signaling events in Compound A-phosphate-stimulated bioresponses.

**Figure 4**
Shows that sphingosine at 1 μM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an inhibitory effect on the VEGF-induced tube formation. In this respect, sphingosine behaves different from Compound A. The data indicate that the balance between sphingosine and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of sphingosine and Compound A (2 - 5 μM) inhibits VEGF-triggered tube formation.
Figure 5
Shows that the treatment of HUVEC with Compound A-phosphate at 0.5 μM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes.

Figure 6
It was tested whether Compound A, Compound A-phosphate, sphingosine or S1P also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity as shown in Figure 6. Compound A and Compound A-phosphate may slightly enhance the VEGF-but not TNF-α-induced tissue factor.

[0096] The following abbreviations are used:

- BSA: bovine serum albumine
- ECGS: endothelial cell growth factor set
- S: sphingosine
- JNK1/2: c-jun-N-terminal kinase1/2
- TF equivalents: tissue factor equivalents
- EGR-1/NFAT: early growth response protein 1/nuclear factor of activated T-cells
- F1 P: Compound A-phosphate (FTY720-phosphate)

[0097] Utility of the S1P receptor agonists in the promotion of angiogenesis may be demonstrated for example in accordance with the methods described hereinafter.

D. Cell culture and Materials

[0098] Human umbilical vein endothelial cells (HUVEC) are cultured at 37°C and 5% CO₂ in medium M199 supplemented with 20% SCS (HyClone, Logan, UT), 1U/ml heparin, 50 μg/ml ECGS, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells are used for experiments up to passage number 5. Short-starved HUVEC are obtained by starving with 1 % SCS-containing M199 for 5 h. Recombinant human VEGF165 is obtained from PromoCell (Heidelberg, Germany). Phospho-specific ERK1/2, p38 kinase polyclonal antibodies, nonphospho ERK1/2 antibodies and LumiGLO chemiluminescent reagent are from New England BioLabs (Beverly, MA), polyclonal IκB antibodies from Santa Cruz Biotechnology (Santa Cruz, Calif.). Peroxidase-conjugated donkey anti-rabbit immunoglobulin G (IgG) and sheep anti-mouse IgG are purchased from Amersham LIFE SCIENCE (Amersham Place, England). Immobilon-P transfer membranes are products of Millipore (Bedford, MA). S is obtained from Sigma Chemical Co.; S1P is from Biomol. Compound A-phosphate stock solution is prepared by the following protocol. Compound A-phosphate is dissolved in methanol tracing with concentrated HCl (0.5 mg Compound A-phosphate in 50 μl of methanol plus 2 μl of HCl). Solvent from the resulting solution is evaporated under vacuum and the residue obtained is redissolved (variant 1) in 0.1 % of defatted BSA solution in sterile deionized water (500 μl) or (variant 2) in 0.5 % Triton X-100 In deionized water. The resulting stock solutions (2.5 mM) are sonicated and stored at 4°C.

Clotting Assay

[0099] Cells are seeded in 6-well plates at 80-90% confluency arid grown overnight. Cells are scraped from the plates and analyzed for tissue factor activity according to the method as described in Claus, M., J. Biol. Chem. 271,17629-17634 (1996). Mechtcheriakova, D., Blood 93,3811-3823 (1999). Briefly, after induction for 4 hours with VEGF (1.5 nM), TNF-α (100 U/ml), S (0.5-2 μM), S1P (0.5-2 μM), Compound A (0.5-2 μM), and Compound A-phosphate (0.5-2 μM), cells are washed twice and then scraped in 1 ml clotting buffer (12 mM sodium acetate, 7 mM diethylbarbiturate and 130 mM sodium chloride; pH 7.4). 50 μl of resuspended cells are mixed with 50 μl of citrated plasma, and clotting times are determined after recalcification with 50 μl of 20 mM CaCl₂ solution at 37°C. TF-equivalents are determined by using a standard curve obtained from rabbit brain thromboplastin.

E. Western Blot Analysis

[0100] After various treatments, the cells are washed twice with cold PBS, lysed in 100 μl of Laemmli buffer, scraped and heated for 5 min at 95°C. Total cell lysates are separated by SDS-PAGE and transferred to Immobilon-P membrane. The membrane is blocked for 30 minutes with PBS containing 0.1% Tween-20 and 3% skim milk and incubated for 1
hour at RT with a primary antibody diluted in blocking buffer. The membrane obtained is washed three times for 5 minutes with PBS containing 0.1% Tween-20 and incubated with peroxidase-conjugated secondary antibody for 1 hour at RT. After a washing step, the membrane is incubated for 1 minute with ECL reagent and exposed to film as required. For reprobing with another antibody, the membrane is washed twice in PBS, stripped for 30 min at 55°C with stripping buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol) and washed three times for 5 minutes with PBS at RT. The membrane is stored wet wrapped in SaranWrap at 4°C after each immunodetection.

**In vitro angiogenesis assay on Matrigel**

**[0101]** Morphogenesis of endothelial cells into capillary-like structures on Growth Factor Reduced Matrigel Matrix (BD Bioscience) is performed according to the manufacture procedure. Briefly, HUVEC are trypsinized, resuspended in serum-free M199 medium containing soybean trypsin inhibitor (1 mg/ml, Sigma). After centrifugation cells are resuspended in serum-free medium at a density 0.5 x 10^5 cells/ml, and cell suspension is seeded in 96-well cell culture plates (Costar, Coming Incorporated) precoated with 50 μl of Matrigel in the absence or presence of various stimuli: VEGF at 1.5 nM, S1P at 0.1-2 μM, S at 0.5-2 μM, Compound A at 0.5-2 μM, and Compound A-phosphate at 0.1-2 μM. Eight hours later, cells on Matrigel are fixed with 3 % formaldehyde in PBS and kept at 4°C. Results are quantitated from images made with a Nikon Diaphot microscope equipped with a cooled CCD camera (Kappa GmbH, Gleichen, Germany) by direct counting of branching points on two microscopic fields from each well done in duplicates.

**F. Compound A-phosphate-induces morphogenesis of endothelial cells in in vitro tube formation assay on Matrigel and a possibly involvement of G_i-mediated signaling pathway(s)**

**[0102]** The effect of Compound A and Compound A-phosphate on morphogenic differentiation of endothelial cells is determined using an in vitro angiogenesis assay on Matrigel. Endothelial cell morphogenesis is a complex process that requires cell-extracellular matrix interactions, followed by matrix remodelling, stimulated migration, cell-cell interactions, and perivascular proteolysis. As shown in Figure 1, Compound A-phosphate strongly may promote capillary-like network formation In a bell-shape dose-dependent manner showing maximal activity around 0.5 μM. The number of branching points per microscopic field, which reflects the induction potency of the stimulus, is comparable for Compound A-phosphate and S1P, and may exceed significantly the VEGF-triggered effects. Compound A itself at 0.5 - 1 μM has a weak, in comparison to Compound A-phosphate, but consistent enhancing effect. Both Compound A-phosphate and Compound A at 0.5 -1 μM does not attenuate VEGF-mediated remodelling but rather cooperates with polypeptide growth factor (see e.g. Figure 2). Furthermore, Compound A-phosphate- as well as S1P-stimulated tube formation is completely inhibited by pertussis toxin (PTX, 50 ng/ml), an inhibitor of heterotrimeric G proteins of α_i/o-type. This may be interpreted as a possible involvement of EDG-1 (S1P_1) receptor-mediated signaling events in Compound A-phosphate-stimulated bioreponses (see e.g. Figure 3), S at 1 μM, which itself seems to be less potent than S1P, attenuates the ability of both S1P and Compound A-phosphate to induce capillary-like structures, without having an Inhibitory effect on the VEGF-induced tube formation (see e.g. Figure 4). In this respect, S behaves differently from Compound A. The data indicate that the balance between S and S1P seems to be critically important for endothelial cell activation/angiogenesis most likely via the EDG receptor family. Importantly, high concentrations of S and Compound A (2 - 5 μM) inhibited VEGF-triggered tube formation. That data suggest biphasic dose-dependent effects of Compound A and Compound A-phosphate on angiogenesis in vitro.

**G. Activation of ERK1/2 MAP kinases by Compound A-phosphate**

**[0103]** Signal transduction via MAP kinases plays a key role in a variety of endothelial cell functions. Treatment of HUVEC with Compound A-phosphate at 0.5 μM may result in transient activation of ERK1/2 with a peak of phosphorylation/activation at 10 minutes and returning to baseline by 20 minutes (see e.g. Figure 5). No activation of p38 kinase and JNK1/2 by Compound A-phosphate is detectable in HUVEC. Furthermore, Compound A-phosphate may trigger ERK1/2 activation in a dose-dependent manner, showing stronger activity at 2 μM. This is in contrast to the results from the tube formation assay, where Compound A-phosphate at 2 μM may be less potent than at 0.5 μM. Neither Compound A nor S are able to induce MAP kinase activation in endothelial cells in a kinetics ranging from 5 minute to 60 minute treatment. To estimate the possible role of inflammatory/NFκB-dependent program in Compound A-phosphate-stimulated bioreponses of endothelial cells, the membranes are reprobed with anti-iκB antibodies. iκB levels are not affected by Compound A-phosphate treatment. Moreover the treatment of endothelial cells with Compound A-phosphate may fail to induce E-Selectin expression as a NFκB-dependent secondary responsive gene. Thus, the data strongly indicate that Compound A-phosphate signaling does not involve NFκB activation - the main cascade in the acute inflammatory response in endothelial cells.
H. Compound A and Compound A-phosphate do not induce tissue factor expression on endothelial cells

[0104] An important characteristic feature of both classical inflammatory stimulus TNF-α and the main angiogenic growth factor VEGF on endothelial cells is their potency to upregulate tissue factor. Compound A, Compound A-phosphate, S or S1P are tested whether they also do induce tissue factor on HUVEC. The data found demonstrate that none of these compounds alone or in combinations may elevate tissue factor activity (see e.g. Figure 6). Compound A and Compound A-phosphate may slightly enhance the VEGF- but not TNF-α-induced tissue factor. The data obtained together indicate that Compound A, Compound A-phosphate, S and S1P mechanistically work distinctly to angiogenic VEGF and inflammatory TNF-α.

I. Binding affinity of S1P receptor agonists to individual human S1P receptors may be determined in following assays:

Transient transfection of human S1P receptors into HEK293 cells

[0105] EDG receptors and Gi proteins are cloned, and equal amounts of 4 cDNAs for the EDG receptor, Giα, Giβ and Giγ are mixed and used to transfect monolayers of HEK293 cells using the calcium phosphate precipitate method (M. Wigler et al., Cell. 1977;11:223 and DS. Im et al., Mol. Pharmacol. 2000;57:753). Briefly, a DNA mixture containing 25 μg of DNA and 0.25 M CaCl₂ is added to HEPES-buffered 2 mM Na₂HPO₄. Subconfluent monolayers of HEK293 cells are poisoned with 25 mM chloroquine, and the DNA precipitate is then applied to the cells. After 4 h, the monolayers are washed with phosphate-buffered saline and re-fed media (90% 1:1 Dulbecco’s modified essential media (DMEM): F-12 + 10% fetal bovine serum). The cells are harvested 48-72 h after addition of the DNA by scraping in HME buffer (in mM: 20 HEPES, 5 MgCl₂, 1 EDTA, pH 7.4) containing 10% sucrose on ice, and disrupted using a Dounce homogenizer. After centrifugation at 800 x g, the supernatant is diluted with HME without sucrose and centrifuged at 100,000 x g for 1 h. The resulting pellet is rehomogenized and centrifuged a second hour at 100,000 x g. This crude membrane pellet is resuspended in HME with sucrose, aliquoted, and snap-frozen by Immersion in liquid nitrogen. The membranes are stored at 70°C. Protein concentration is determined spectroscopically by Bradford protein assay.

GTPγS binding assay using S1P receptor/HEK293 membrane preparations

[0106] GTPγS binding experiments are performed as described by DS. Im et al., Mol. Pharmacol. 2000;57:753. Ligand-mediated GTPγS binding to G-proteins is measured in GTP binding buffer (in mM: 50 HEPES, 100 NaCl, 10 MgCl₂, pH 7.5) using 25 μg of a membrane preparation from transiently transfected HEK293 cells. Ligand is added to membranes in the presence of 10 μM GDP and 0.1 nM [35S]GTPγS (1200 Ci/mmol) and incubated at 30°C for 30 min. Bound GTPγS is separated from unbound using the Brandel harvester (Gaithersburg, MD) and counted with a liquid scintillation counter.

Claims

1. Use of a S1P receptor agonist of formula I

wherein R₁ is a straight- or branched (C₁₂-22)carbon chain

- which may have in the chain a bond or a hetero atom selected from a double bond, a triple bond, O, S, NR₆,
  wherein R₆ is H, alkyl, aralkyl, acyl or alkoxy carbonyl, and carbonyl, and/or

- which may have as a substituent alkoxy, alkenyloxy, alkynlyloxy, aralkyloxy, acyl, alkylamino, alkylthio,
  acylamino, alkoxy carbonyl, alkoxy carbonylamino, acyloxy, alkyl carbamoyl, nitro, halogen, amino, hydroxy-
  imino, hydroxy or carboxy; or
R₁ is
- a phenylalkyl wherein alkyl is a straight- or branched (C₆-₂₀) carbon chain; or
- a phenylalkyl wherein alkyl is a straight- or branched (C₁-₃₀) carbon chain wherein said phenylalkyl is substituted by
  - a straight- or branched (C₆-₂₀) carbon chain optionally substituted by halogen,
  - a straight- or branched (C₆-₂₀) alkoxy chain optionally substituted by halogen,
  - phenylalkoxy, halophenylalkoxy, phenylalkoxyalkyl, phenoxyalkoxy or phenoxyalkyl,
  - cycloalkylalkyl substituted by C₆-₂₀ alkyl,
  - heteroarylalkyl substituted by C₆-₂₀ alkyl,
  - heterocyclic C₆-₂₀ alkyl or
  - heterocyclic alkyl substituted by C₂-₂₀ alkyl,
  - in the carbon chain, a bond or a heteroatom selected from a double bond, a triple bond, O, S, sulfinyl, sulfonyl, or NR₆, wherein R₆ is as defined above, and
- as a substituent alkoxy, alkenyloxy, alkynyloxy, aralkyloxy, acyl, alkylamino, acylamino, alkoxy carbonyl, alkoxy carbonylamino, acyloxy, alkylcarbamoyl, nitro, halogen, amino, hydroxy or carboxy, and each of R₂, R₃, R₄ and R₅, independently, is H, C₁-₄ alkyl or acyl,
or a pharmaceutically acceptable salt thereof, or
a compound of formula IVa

wherein Xₐ is O, S, NRᵣ, or a group -(CH₂)ᵦ⁺, which group is unsubstituted or substituted by 1 to 4 halogen; ρₐ is 1 or 2, R₁ₐ is H or (C₁-₄) alkyl, which alkyl is unsubstituted or substituted by halogen; R₁ₐ is H, OH, (C₁-₄) alkyl or O(C₁-₄) alkyl wherein alkyl is unsubstituted or substituted by 1 to 3 halogen; R₁ₐ is H, OH or (C₁-₄) alkyl, wherein alkyl is unsubstituted or substituted by halogen; each R₂ₐ is independently selected from H or (C₁-₄) alkyl, which alkyl is unsubstituted or substituted by halogen; R₃ₐ is H, OH, halogen or O(C₁-₄) alkyl wherein alkyl is unsubstituted or substituted by halogen, or a pharmaceutically acceptable salt or hydrate thereof,
for the preparation of a medicament for preventing or treating lymphoproliferative or myeloproliferative disorders, wherein the medicament is administered with a chemotherapeutic agent.

2. Use according to claim 1 wherein the lymphoproliferative disorder is tumors of blood or lymphatic system.

3. Use according to claim 1 wherein the myeloproliferative disorder is myeloid cancer.

4. Use according to any one of the preceding claims wherein the medicament is administered intermittently.

5. Use according to any one of the preceding claims, wherein the S₁P receptor agonist is 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol or a compound of formula IVa wherein R₂ₐ is H, R₃ₐ is OH, Xₐ is O and each of R₁ₐ and R₁ₐ is OH (called FTY720-phosphate), or a pharmaceutically acceptable salt thereof.

6. Use according to claim 5 wherein the S₁P receptor agonist is 2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol, hydrochloride.
7. Use according to any one of the preceding claims, wherein the medicament is administered concomitantly or in sequence with the chemotherapeutic agent.

8. Use according to any one of the preceding claims, wherein the chemotherapeutic agent is selected from

   i. an aromatase inhibitor,
   ii. an antiestrogen, an anti-androgen or a gonadorelin agonist,
   iii. a topoisomerase I inhibitor or a topoisomerase II inhibitor,
   iv. a microtubule active agent, an alkylating agent, an antineoplastic antimetabolite or a platin compound,
   v. a compound targeting/decreasing a protein or lipid kinase activity or a protein or lipid phosphatase activity,
   vi. a further anti-angiogenic compound or a compound which induces cell differentiation processes,
   vii. a bradykinin 1 receptor or an angiotensin II antagonist,
   viii. an aromatase inhibitor, a bisphosphonate, a histone deacetylase inhibitor, a heparanase inhibitor, a biological response modifier, an ubiquitination inhibitor, or an inhibitor which blocks anti-apoptotic pathways,
   ix. an inhibitor of Ras oncogenic isoforms,
   x. a telomerase inhibitor,
   xi) a matrix metalloproteinase inhibitor, a methionine aminopeptidase inhibitor, or a proteosome inhibitor, and/or
   xii) a mTOR inhibitor.

9. Use according to claim 8 wherein the chemotherapeutic agent is selected from busulfan, cytarabine, 6-thioguanine, fludarabine, hydroxyurea, procarbazine, bleomycin, methotrexate, a topoisomerase II inhibitor, or a compound which targets, decreases or inhibits the activity of the platelet-derived growth factor-receptors (PDGFR) or of c-Abl family member and their gene fusion products, and wherein the use is for treating or preventing tumors of blood or lymphatic system.

**Patentansprüche**

1. Verwendung eines S1P Rezeptoragonisten der Formel I

```
CH2OR3
R1 R2 N—CH2OR2
```

worin R₈ für eine geradkettige oder verzweigte (C₁₂⁻C₂₂)-Kohlenstoffkette steht,

- die in der Kette eine Bindung oder ein Heteroatom aufweisen kann, das ausgewählt ist aus einer Doppelbindung, einer Dreifachbindung, O, S, NR₆, worin R₆ für H, Alkyl, Aralkyl, Acyl oder Alkoxycarbonyl steht, und Carbonyl, und/oder
- die als einen Substituenten Alkoxy, Alkenyloxy, Alkinyloxy, Aralkyloxy, Acyl, Alkylarnino, Alkylthio, Acylarnino, Alkoxyacidon, Alkoxycarbonylamino, Acyloxy, Alkylcarbamoyl, Nitro, Halogen, Amino, Hydroxyimino, Hydroxy oder Carboxy enthalten kann, oder

worin R₁ steht für

- ein Phenylalkyl, worin Alkyl eine geradkettige oder verzweigte (C₆⁻C₂₀)-Kohlenstoffkette ist, oder
- ein Phenylalkyl, worin Alkyl eine geradkettige oder verzweigte (C₁⁻C₃₀)-Kohlenstoffkette ist und worin Phenylalkyl substituiert ist durch
  - eine geradkettige oder verzweigte (C₆⁻C₂₀)-Kohlenstoffkette, die optional substituiert ist mit Halogen,
  - eine geradkettige oder verzweigte (C₆⁻C₂₀)-Alkoxykette, die optional substituiert ist mit Halogen,
  - ein geradkettiges oder verzweigtes (C₆⁻C₂₀)-Alkenyloxy,
  - Phenylalkoxy, Halogenphenylalkoxy, Phenylalkoxyalkyl, Phenoxyalkoxy oder Phenoxyalkyl,
  - Cycloalkylalkyl, das mit (C₆⁻C₂₀)-Alkyl substituiert ist,
  - Heteroarylalkyl, das mit (C₆⁻C₂₀)-Alkyl substituiert ist,
- Heterocycl-(C<sub>6</sub>-C<sub>20</sub>)-alkyl oder
- Heterocyclylalkyl, das mit (C<sub>2</sub>-C<sub>20</sub>)-Alkyl substituiert ist, und worin
der Alkylteil
- in der Kohlenstoffkette eine Bindung oder ein Heteroatom aufweisen kann, das ausgewählt ist aus einer
  Doppelbindung, einer Dreifachbindung, O, S, Sulfanyl, Sulfonyl oder NR<sub>6</sub>, worin R<sub>6</sub> wie oben definiert ist, und
- als einen Substituenten Alkoxy, Alkenyloxy, Alkyloxy, Aryl, Alkylamin, Alkylthio, Acylamin,
Alkoxy carbonyl, Alkoxy carbonylamin, Acyloxy, Alkyl carbamoyl, Nitro, Halogen, Amino, Hydroximino, Hydroxy
oder Carboxy enthalten kann, und
R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> und R<sub>5</sub> jeweils unabhängig für H, (C<sub>1</sub>-C<sub>4</sub>)-Alkyl oder Acyl stehen,
odeming ein pharmazeutisch akzeptables Salz hiervon, oder
-eine Verbindung der Formel IVa

\[
\text{IVa}
\]

worin X<sub>a</sub> für O, S, NR<sub>1s</sub> oder für -(CH<sub>2</sub>)<sub>n</sub>- steht, das unsubstituiert oder substituiert ist mit 1 bis 4 Halogenen, n<sub>a</sub> für
1 oder 2 steht, R<sub>1a</sub> für H oder (C<sub>1</sub>-C<sub>4</sub>)-Alkyl steht, worin Alkyl unsubstituiert oder substituiert ist mit Halogen, R<sub>1a</sub>
für H, OH, (C<sub>1</sub>-C<sub>4</sub>)-Alkyl oder O-(C<sub>1</sub>-C<sub>4</sub>)-Alkyl steht, worin Alkyl unsubstituiert oder substituiert ist mit 1 bis 3 Halogenen,
R<sub>1s</sub> für H, OH oder (C<sub>1</sub>-C<sub>4</sub>)-Alkyl steht, worin Alkyl unsubstituiert oder substituiert ist mit Halogen, R<sub>2a</sub> jeweils
unabhängig ausgewählt ist aus H oder (C<sub>1</sub>-C<sub>4</sub>)-Alkyl, worin Alkyl unsubstituiert oder substituiert mit Halogen, R<sub>3a</sub>
für H, OH, Halogen oder O-(C<sub>1</sub>-C<sub>4</sub>)-Alkyl steht, worin Alkyl unsubstituiert oder substituiert ist durch Halogen,
odeming ein pharmazeutisch akzeptables Salz oder Hydrat hiervon,
zur Herstellung eines Arzneimittels für die Prävention oder Behandlung lymphoproliferativer oder myeloproliferativer
Störungen, worin das Arzneimittel mit einem Chemotherapeutikum verabreicht wird.

2. Verwendung nach Anspruch 1, worin die lymphoproliferative Störung ein Tumor des Blut- oder Lymphsystems ist.

3. Verwendung nach Anspruch 1, worin die myeloproliferative Störung ein Myeloidkrebs ist.

4. Verwendung nach einem der vorhergehenden Ansprüche, worin das Arzneimittel intermittierend verabreicht wird.

5. Verwendung nach einem der vorhergehenden Ansprüche, worin der S1P Rezeptoragonist 2-Amino-2-[2-(4-octyl-
  phenyl)-ethyl]-propan-1,3-diol oder eine Verbindung der Formel IVa ist, worin R<sub>2a</sub> für H steht, R<sub>3a</sub> für OH steht, X<sub>a</sub>
für O steht und R<sub>1a</sub> und R<sub>1b</sub> jeweils für OH stehen (was als FTY720-Phosphat bezeichnet wird), oder ein pharma-
zeutisch akzeptables Salz hiervon.

6. Verwendung nach Anspruch 5, worin der S1P Rezeptoragonist 2-Amino-2-[2-(4-octylphenyl)-ethyl]-propan-1,3-diol-
hydrochlorid ist.

7. Verwendung nach einem der vorhergehenden Ansprüche, worin das Arzneimittel gleichzeitig oder in Sequenz mit
dem Chemotherapeutikum verabreicht wird.

8. Verwendung nach einem der vorhergehenden Ansprüche, worin das Chemotherapeutikum ausgewählt ist aus
i. einem Aromataseinhibitor,
ii. einem Antiöstrogen, einem Antiandrogen oder einem Gonadorelinagonisten,
iii. einem Topoisomerase-I-Inhibitor oder einem Topoisomerase-II-Inhibitor,
iv. einem mikrotubularen Wirkstoff, einem Alkylierungsmittel, einem antineoplastischen Antimetaboliten oder einer Platinkomplexverbindung,
v. einer Verbindung mit einer Ansteuerung/Verringerung einer Protein- oder Lipidkinaseaktivität, oder einer Protein- oder Lipidphosphatasaktivität, einer weiteren antiangiogenen Verbindung oder einer Verbindung, die Zeldifferenzierungsprozesse induziert,
vi. einem Bradykinin-1-Rezeptor oder einem Angiotensin-II-Antagonisten,
vii. einem Cyclooxygenaseinhibitor, einem Bisphosphonat, einem Histondeacetylaseinhibitor, einem Heparanaseinhibitor, einem Modifikator für eine biologische Antwort, einem Ubiquitininisitor oder einem Inhibitor, der antiapoptotische Wege blockiert,
viii. einem Inhibitor von Ras onkogenen Isoformen,
ix. einem Telomeraseinhibitor,
x. einem Proteaseinhibitor, einem Matrixmetalloproteinaseinhibitor, einem Methioninaminopeptidaseinhibitor oder einem Proteosominhibitor, und/oder
xi. einem mTOR Inhibitor.


Revendications

1. Utilisation d’un agoniste du récepteur S1P de formule 1

\[
\text{R}_1 \text{R}_2 \text{R}_3 \text{N} - \text{CH}_2 \text{O} \text{R}_2
\]

dans laquelle \( \text{R}_1 \) est une chaîne carbonée en C_{12} à C_{22}, linéaire ou ramifiée,

- pouvant avoir dans la chaîne une liaison ou un hétéroatome choisis parmi une liaison double, une liaison triple, O, S, NR_6, où \( \text{R}_6 \) représente H, un groupe alkyle, un groupe aralkyle, un groupe acyle ou un groupe alcoxycarbonyle, et un groupe carbonyle, et/ou
- pouvant avoir comme substituant un groupe alcoxy, un groupe alcényloxy, un groupe alcynyloxy, un groupe aralkyloxy, un groupe acyle, un groupe alkylaminogruppe, un groupe alkoxygruppe, un groupe acylaminogruppe, un groupe alcoxycarbonylaminogruppe, un groupe acyloxygruppe, un groupe alkylcarbamoyle, un groupe nitro, un atome d’halogène, un groupe amino, un groupe hydroxyiminogruppe, un groupe hydroxy ou un groupe carboxy ; ou
- \( \text{R}_1 \) est
  - un groupe phénylalkyle où le groupe alkyle est une chaîne carbonée en C_6 à C_{20}, linéaire ou ramifiée ; ou
  - un groupe phénylalkyle où le groupe alkyle est une chaîne carbonée en C_1 à C_{30}, linéaire ou ramifiée, dans laquelle ledit groupe phénylalkyle est substitué par
    - une chaîne carbonée en C_6 à C_{20}, linéaire ou ramifiée, éventuellement substituée par un atome d’halogène,
    - une chaîne alcoxy en C_6 à C_{20}, linéaire ou ramifiée, éventuellement substituée par un atome d’halogène,
    - un groupe alcényloxy en C_6 à C_{20}, linéaire ou ramifiée,
    - un groupe phénylacétoxy, un groupe haloxyphénylalcoxy, un groupe phénylacétoxyalkyle, un groupe phénynoxyalcoxy ou un groupe phénynoxyalkyle,
    - un groupe cycloalkylalcoxy substitué par un groupe alkyle en C_6 à C_{20},
    - un groupe hétéroarylacétoxy substitué par un groupe alkyle en C_6 à C_{20},
- un groupe alkyle en C₆ à C₂₀ hétérocyclique ou
- un groupe alkyle hétérocyclique substitué par un groupe alkyle en C₂ à C₂₀,

et dans laquelle
le fragment alkyle peut avoir
- dans la chaîne carbonée, une liaison ou un hétéroatome choisis parmi une liaison double, une liaison triple, O, S, un groupe sulfényle, un groupe sulfonyle, ou NR₆, où R₆ est tel que défini ci-dessus, et
- comme substituant, un groupe alcôxy, un groupe alcényloxy, un groupe alcoxycarbonyle, un groupe acylamino, un groupe alcylthio, un groupe alcoxycarbamoyle, un groupe nitro, un atome d’halogène, un groupe amino, un groupe hydroxy ou un groupe carboxy, et

R₂, R₃, R₄ et R₅ représentent chacun indépendamment H, un groupe alkyle en C₁ à C₄ ou un groupe acyle en C₁ à C₄,

ou d’un sel de celui-ci acceptable au niveau pharmaceutique, ou
d’un composé de formule IVa

![Chemical structure](image)

dans laquelle X₄ représente O, S, NR₁a ou un groupe -(CH₂)ₙa-, lequel groupe est soit non substitué soit substitué par 1 à 4 atomes d’halogène ; nₐ est égal à 1 ou 2, R₁ₐ représente H ou un groupe alkyle en C₁ à C₄, lequel groupe alkyle est soit non substitué soit substitué par un atome d’halogène ; R₁₃ représente H, OH, un groupe alkyle en C₁ à C₄ ou un groupe O-(alkyle en C₁ à C₄) où le groupe alkyle est soit non substitué soit substitué par 1 à 3 atomes d’halogène ; R₁₅ représente H, OH, un groupe alkyle en C₁ à C₄, où le groupe alkyle est soit non substitué soit substitué par un atome d’halogène ; chaque R₂ₐ est indépendamment choisi parmi H ou un groupe alkyle en C₁ à C₄, lequel groupe alkyle est soit non substitué soit substitué par un atome d’halogène ; R₃ₐ représente H, OH, un atome d’halogène ou un groupe O-(alkyle en C₁ à C₄) où le groupe alkyle est soit non substitué soit substitué par un atome d’halogène,

ou d’un sel ou hydrate de celui-ci acceptable au niveau pharmaceutique,
pour la préparation d’un médicament destiné à prévenir ou à traiter des troubles lymphoprolifératifs ou myéloprolifératifs, dans laquelle le médicament est administré avec un agent chimiothérapeutique.

2. Utilisation selon la revendication 1, dans laquelle le trouble lymphoprolifératif est constitué par une tumeur du système sanguin ou lymphatique.

3. Utilisation selon la revendication 1, dans laquelle le trouble myéloprolifératif est un cancer myéloïde.

4. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle le médicament est administré de manière intermittente.

5. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle l’agoniste du récepteur S₁P est le 2-amino-2′-[2-(4-octylphényl)éthyl]propane-1,3-diol ou un composé de formule IVa dans laquelle R₂ₐ représente H, R₃ₐ représente OH, X₄ représente O et R₁₃ et R₁₅ représentent chacun OH (appelé FTY720-phosphate), ou un
sel de ceux-ci acceptable au niveau pharmaceutique.

6. Utilisation selon la revendication 5, dans laquelle l’agoniste du récepteur S1P est le chlorhydrate de 2-amino-2-[2-(4-octylphényl)éthyl]propane-1,3-diol.


8. Utilisation selon l’une quelconque des revendications précédentes, dans laquelle l’agent chimiothérapeutique est choisi parmi

   i. un inhibiteur de l’aromatase,
   ii. un anti-oestrogène, un anti-androgène ou un agoniste de la gonadoréline,
   iii. un inhibiteur de la topoisoénérase I ou un inhibiteur de la topoisoénérase II,
   iv. un agent actif au niveau des microtubules, un agent alkyllant, un antimétabolite antinéoplasique ou un composé de platine,
   v. un composé ciblant/réduisant l’activité d’une protéine- ou lipide-kinase ou l’activité d’une protéine- ou lipide-phosphatase, un composé étant en outre antiangiogénique ou un composé qui induit des processus de différenciation cellulaire,
   vi. un récepteur de la bradykinine 1 ou un antagoniste de l’angiotensine II,
   vii. un inhibiteur de la cyclooxygénase, un bisphosphonate, un inhibiteur de l’histone-désacétylase, un inhibiteur de l’héparanase, un modificateur de la réponse biologique, un inhibiteur d’ubiquitination, ou un inhibiteur qui bloque les voies anti-apoptotiques,
   viii. un inhibiteur des isoformes oncogènes de Ras,
   ix. un inhibiteur de la télosémérase,
   x. un inhibiteur de protéase, un inhibiteur de la métalloprotéinase matricielle, un inhibiteur de la méthionine-aminopeptidase, ou un inhibiteur du protéosome, et/ou
   xi. un inhibiteur de la mTOR.

9. Utilisation selon la revendication 8, dans laquelle l’agent chimiothérapeutique est choisi parmi le busulfane, la cytarabine, la 6-thioguanine, la fludarabine, l’hydroxyurée, la procarbazine, la bléomycine, le méthotrexate, un inhibiteur de la topoisoénérase II, ou un composé qui cible, réduit ou inhibe l’activité des récepteurs du facteur de croissance dérivé des plaquettes (PDGFR) ou d’un membre de la famille c-Abl et de leurs produits de fusion génique, et dans laquelle l’utilisation vise à traiter ou à prévenir les tumeurs du système sanguin ou lymphatique.
Fig. 3

5 branching points

<table>
<thead>
<tr>
<th>Condition</th>
<th>Branching Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
</tr>
<tr>
<td>VEGF</td>
<td>26</td>
</tr>
<tr>
<td>SIP 0.5</td>
<td>36</td>
</tr>
<tr>
<td>FIP 0.1</td>
<td>35</td>
</tr>
<tr>
<td>FIP 0.5</td>
<td>54</td>
</tr>
<tr>
<td>FIP 1</td>
<td>39</td>
</tr>
<tr>
<td>FIP 2</td>
<td>21</td>
</tr>
</tbody>
</table>
Figure 2

![Bar chart showing branching points/fields with and without VEGF treatment.](chart.png)
Figure 3

The bar chart shows the branching points per microscopic field for different conditions:
- Control: 13 untreated, 5 PTX
- S1P: 70 untreated, 3 PTX
- F1P: 54 untreated, 3 PTX
Fig 4

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>FTY720</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>+VEGF</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>+F1P</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>+S1P</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

Branching points/microscopic field

untreated +VEGF +F1P +S1P
**Fig. 5**

<table>
<thead>
<tr>
<th></th>
<th>F1P, 0.5 μM</th>
<th>VEGF, 1 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>time, min</td>
<td>- + + + + +</td>
<td>- - - - - +</td>
</tr>
</tbody>
</table>

- ERK1/2-Phosphorylated
- IκB
- Sp1

**Fig. 6**

Tissue factor activity

- unstimul
- S1P
- F1P
- S
- FTY720
- S+S1P
- FTY720+F1P
- VEGF
- VEGF+S1P
- VEGF+F1P
- VEGF+S
- VEGF+FTY720

Tissue factor equivalents, μM

- 0
- 100
- 200
- 300
- 400
- 500
- 600
Fig. 7

** t-test P≤0.01

% of Control (Di Water + 500 nM S1P)

- Di Water
- Comp C 100nM
- PTx 10ng/ml

500 nM S1P
REFERENCES CITED IN THE DESCRIPTION

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