(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 April 2012 (26.04.2012)

PCT

(10) International Publication Number
WO 2012/052757 A1

(51) International Patent Classification:
G01N 33/574 (2006.01)

(21) International Application Number:
PCT/GB2011/052021

(22) International Filing Date:
19 October 2011 (19.10.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/394,941 20 October 2010 (20.10.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2012/052757 A1

(54) Title: TUMOUR PHENOTYPE PATIENT SELECTION METHOD

(57) Abstract: The present invention relates to a method for stratifying cancer patients according to the cellular phenotype of the tumour based and in particular, according to the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour. This patient stratification permits identification of patients whose tumours are more likely to be responsive to treatment with anti-cancer drugs, such as Vascular Endothelial Growth Factors (VEGF) signalling inhibitors. This invention therefore provides personalised healthcare opportunities in the cancer arena.

TUMOUR PHENOTYPE PATIENT SELECTION METHOD

Field of the Invention

The present invention is based on identifying a link between tumour phenotype and susceptibility to treatment with anti-cancer drugs, such as Vascular Endothelial Growth Factors (VEGF) signalling inhibitors. The inventors have found that tumours can generally be grouped into those that possess a tumour-vessel phenotype or a stromal-vessel phenotype; furthermore, that those with a tumour-vessel phenotype are particularly susceptible to efficacious treatment with Vascular Endothelial Growth Factors (VEGF) signalling inhibitors. The present invention therefore opens up the possibility to stratifying cancer patients according to the cellular phenotype of the tumour they possess and identify those patients likely to respond favourably to treatment with a Vascular Endothelial Growth Factors (VEGF) signalling inhibitor. This therefore provides opportunities, methods and tools for selecting patients for treatment with a Vascular Endothelial Growth Factors (VEGF) signalling inhibitor, based on whether the tumour to be treated possess a tumour-vessel phenotype or a stromal-vessel phenotype.

Introduction

Inhibition of angiogenesis with agents such as Vascular Endothelial Growth Factors (VEGF) signalling inhibitors was predicted to give pan-tumour efficacy. Although these agents, exemplified by bevacizumab, cediranib, sunitinib, sorafenib and IMC1211b give efficacy in preclinical models (reviewed in Ivy *et al.*, Nat Rev Clin Oncol 6:569-579, 2009; Grothey and Galanis Nat Rev Clin Oncol 6:507-517, 2009; Sullivan and Brekken mAbs 2:165-175, 2010), in the clinical setting, they have only been effective as single agents in a restricted set of human diseases such as renal cell cancer and glioblastoma (Escuda *et al.*, Lancet 370:2103-2111, 2007; Friedman *et al.*, JCO 27:4733-4740, 2009; Motzer *et al.*, JCO 27:3584-3590, 2009; Bachelor *et al.*, JCO 28:2817-2823, 2010). These agents can also add to the benefit of chemotherapy in combination in colorectal and lung cancer (Sandler *et al.*, New Engl J Med 355:2543-2550, 2006; Hurwitz *et al.*, New Engl J Med 350:2335-2342, 2004). However, even in these diseases, where the drugs are less effective as single agent, there are indications that responses can be achieved in a sub-population of patients. The ability to select disease segments and even individual tumours where VEGF agents are likely to give benefit is pivotal to maximising the successful use of these agents in the clinical setting. Therefore it

is important that we understand the key features of a tumour that determine the response to VEGF targeted therapy to be able to implement patient selection strategies.

Within human tumours, stromal fibroblast-like cells can be observed as single cells distributed through the body or mass of the tumour, as pericytes supporting vessels within the tumour and as fibrotic or desmoplastic multicellular structures surrounding nests of tumour cells (Sugimoto *et al.*, Cancer Biol Ther, 5(12):1640-1646, 2006). This class of stromal fibroblast-like cells are extremely adaptive and show heterogeneous phenotypes. They can constitute significant proportions of the tumour, in particular certain human tumours such as pancreatic, breast, colorectal, and prostate (for review see Nyberg *et al.*, Front Biosci 13:6537, 2008; and Pietras *et al.*, Plos Med 5:e19, 10.1371/journal.pmed.0050019, 2008. Emerging data also suggest that the presence of stromal fibroblast-like cells in the tumour can be prognostic, or identify high risk populations of patients. In colorectal cancer (CRC), the presence of high levels of alpha SMA positive cells in the tumour indicate a poor outcome and high likelihood of recurrance (Tsujino *et al.*, CCR, 13:2082, 2007). Moreover stromal infiltrate and stroma gene expression predicts clinical outcome in breast cancer (Howell *et al.*, Breast Cancer Research 11(Suppl 3):S16, 2009; Van den Eynden *et al.*, Histopathology 51:440-451, 2007; Finak *et al.*, 14:518, 2008). In pre-clinical models co-implantation of stromal fibroblast-like cells or recruitment to tumours results in an increase in tumour growth (E.g. see - Anderberg *et al.*, Cancer Res 69:369, 2009; Camps *et al.*, PNAS 87:75, 1990; Erez *et al.*, Cancer Cell 17:135, 2010; Bhowmick *et al.*, Science 303:848, 2004; Bhowmick *et al.*, Nature 432:332, 2004).

It has been shown that the common targets of VEGF signalling inhibitors, VEGFR-2 and VEGFR-3 are localised to, and up-regulated on, tumour vasculature (Smith *et al.*, Clinical Cancer Research 16: 3548, 2010). Therefore the primary clinical mechanism of action of this class of agents is through the targeting of tumour vasculature. In order to understand the discrepancy between the *in vivo* and clinical efficacy of VEGF signalling inhibitors, the inventors used a CD31-αSMA immunofluorescent assay to assess both the gross morphology of the tumour relative to stroma and the distribution of the vasculature between these two compartments in *in vivo* and primary human tumours. Two phenotypes were identified and described as tumour-vessel (vessels within tumour) and stromal-vessel (vessels within stroma); the former was associated primarily with xenografts (95%) and tumour types responding to VEGFi as monotherapy [renal cell cancer (80%), glioblastoma (96%) and hepatocellular carcinoma (93%)], whereas the latter phenotype was rare in

xenografts (5%) but common in tumour types insensitive to VEGFi as a single agent [colorectal (88%), prostate (80%) and lung cancer (91%)]. The inventors also demonstrated *in vivo* that the two phenotypes elicited different tumour responses to the VEGFR-2 inhibitor DC-101 and showed that a sub-population of CRC tumours, which exhibit the tumour-vessel phenotype, are more likely to predict patients that respond to bevacizumab.

An approach based on the screening of patient tumour samples for the two vessel phenotypes should predict whether a tumour type (e.g. renal or colorectal tumours) or even the individual tumours within a tumour type (individual colorectal tumours) are likely to show a RECIST response to a VEGF signalling inhibitor, and hence an increased likelihood of progression free survival.

Amongst other things, the present invention provides methods of screening and preselecting patients for anti-VEGF therapy, based on the tumour phenotype.

Detailed description.

According to a first aspect of the invention there is provided a method for stratifying cancer patients comprising determining the cellular phenotype of the tumour and stratifying the patients into at least two groups according to the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour. Examples of patient tumours that can be stratified in this manner are: renal cell cancer, glioblastoma, ovarian cancer, liver cancer, thyroid cancer, head and neck cancer, colorectal cancer, lung cancer, prostate cancer, pancreatic cancer, breast cancer and skin cancer.

Typically the tumour can be stratified into two distinct phenotypes, a tumour-vessel and a stromal-vessel phenotype. Thus, in one embodiment, one of the stratified groups consists of patients whose tumours possess a tumour-vessel phenotype and in another embodiment, one of the stratified groups are patients whose tumour exhibit a stromal-vessel phenotype.

According to a further aspect of the invention there is provided a method for stratifying a cancer patient comprising determining the phenotype of the patient's tumour and stratifying the patient into one of two groups according to the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour.

As used herein, a tumour is defined as having a tumour-vessel phenotype if the predominant morphology (>50%, such as >60%, >70%, >80% or >90% of the tumour mass) is that of a sheet of malignant/cancerous cells (>2mm^2) with tumour vessels embedded within

the malignant/cancerous regions such that the tumour vessels lie adjacent to malignant/cancerous cells. As used herein, a tumour is defined as having a stromal-vessel phenotype if the predominant morphology (>50%, such as >60%, >70%, >80% or >90% of the tumour mass) is small islands/nests of malignant/cancerous cells (<2mm^2) separated by stroma. Importantly, vessels are localised to the stroma surrounding the malignant/cancerous regions but are either absent or rarely embedded within the malignant/cancerous regions. Stromal and tumour-vessel phenotypes are shown and illustrated in Fig.1.

Accordingly, this allows the patient(s) to be classified or stratified into two distinct groups depending on whether their tumour exhibits a tumour-vessel phenotype or a stromal-vessel phenotype.

There are many different ways of determining the cellular phenotype of a tumour sample. For example, the cellular phenotype of the tumour can be determined using a tissue or cell staining technique on a tumour sample taken from the patient. Typically this involves treating the tumour sample with one or more staining agents capable of detecting or facilitating the visualisation of the presence of one or more of the following cells types: tumour vessels (primarily blood vessels comprising endothelial cells either embedded within the tumour or present in tumour associated stroma), tumour (malignant/cancerous cells) and stromal cells (fibroblast-like cells and immune cells associated with the tumour).

The tumour sample may, for example, be a biopsy sample or surgical resection sample.

In a particular embodiment, extraction of the sample from the patient is part of the method of the invention, in another embodiment the tumour sample has previously been isolated from the patient and the methods of the invention proceed from the point where the sample is made available.

According to particular embodiments of the invention, the cellular phenotype of the tumour is determined by histological staining, immunohistochemistry or *in situ* hybridisation.

Histological (or tinctorial) staining is a well-established traditional technique whereby the tumour sample (e.g. a tissue slice mounted on a microscope slide) is contacted with one or more stains capable of binding to particular cellular components so as to facilitate discrimination of these different cellular components visually using a microscope, or using algorithms generated with imaging software such as Definiens Image Analysis Software, Definiens Tissue Studio, Aperio Genie Pro or Image J.

Any suitable histological stain capable of highlighting or "showing up" the cellular compartments and vessels, in particular the tumour vessels, tumour cells and stromal cells can be employed in the methods of the invention. Particular stains that may be employed (the generic histological stain name is given first, common preparations are given in brackets) include: Haematoxylin (Ehrlich's, Delafield's, Mayer's, Harris's, Cole's, Carazzi's, Gill's) and Eosin (Eosin Y, Ethyl eosin, Eosin B) (H+E), Haematoxylins (Heidenhain's, Loyez, Verhoeff's, PTAH, Molybdenum), Trichrome stains for connective tissue (Van Gieson, Masson, MSB), elastic tissue fibre stains (Verhoeff's, Orcein, Weigert's resorcin-fuchsin and aldehyde fuchsin method), reticular fiber stains (Gordon and Sweets' and Gomori's) and immunoflurescence counterstains, DAPI, TOR3, Hoescht and PI. Suitable stains and how to use them are well known in the art (see e.g. Bancroft and Gamble, Theory and Practice of Histological Techniques, 5th Edition).

The stains listed above can generally be classified as: Haematoxylin, Eosin, Trichrome stain for connective tissue, elastic tissue fibre stain, reticular fibre stain and immunofluorescence counterstain.

The cellular phenotype of the tumour can also be determined using immunohistochemistry (IHC) using one or more antibodies capable of binding to a marker protein present in, on or associated with a vessel, tumour cells or stromal cells.

IHC is a widely used technique for identifying cellular or tissue constituents (marker proteins/antigens) by means of antigen/antibody interactions, the site of antibody being identified either by direct labelling of the antibody or by use of a secondary labelling method.

"Label" or "labelling" as used herein, refers to a composition capable of producing a detectable signal indicative of the presence of the target moiety e.g. marker protein or mRNA) in an assay sample. Suitable labels include radioisotopes, nucleotide chromophores, enzymes, substrates, fluorescent molecules, chemiluminescent moieties, magnetic particles, bioluminescent moieties, and the like. As such, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means.

Two general methods of IHC are available; direct and indirect assays. According to the first assay, binding of antibody to the target antigen is determined directly. This direct assay uses a labelled reagent, such as a fluorescent tag or an enzyme-labelled primary antibody, which can be visualized without further antibody interaction. In a typical indirect

assay, unconjugated primary antibody binds to the antigen and then a labelled secondary antibody binds to the primary antibody. Where the secondary antibody is conjugated to an enzymatic label, a chromagenic or fluorogenic substrate is added to provide visualization of the antigen. Signal amplification occurs because several secondary antibodies may react with different epitopes on the primary antibody.

The primary and/or secondary antibody used for immunohistochemistry will typically be labelled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories:

(a) Radioisotopes, such as S^{35}, C^{14}, I^{125}, H^{3}, and I^{131}. The antibody can be labelled with the radioisotope using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen *et al.*, Ed. Wiley-Interscience, New York, New York, Pubs. (1991) for example and radioactivity can be measured using scintillation counting.

(b) Colloidal gold particles.

(c) Fluorescent labels including, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, Lissamine, umbelliferone, phycocrytherin, phycocyanin, or commercially available fluorophores such as SPECTRUM ORANGE® and SPECTRUM GREEN® and/or derivatives of any one or more of the above. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescence can be quantified using a fluorimeter.

(d) Various enzyme-substrate labels are available and U.S. Pat. No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate that can be measured using various techniques. For example, the enzyme may catalyze a colour change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, [beta]-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating

enzymes to antibodies are described in O'Sullivan *et al.*, Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in Methods in Enzym. (ed J. Langone & H. Van Vunakis), Academic press, New York, 73:147-166 (1981).

Examples of enzyme-substrate combinations include, for example:
(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor [e.g.,orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB)];
(ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and
(iii) [beta]-D-galactosidase ([beta]-D-Gal) with a chromogenic substrate (e.g. p-nitrophenyl-[beta]-D-galactosidase) or fluorogenic substrate (e.g., 4-methylumbelliferyl-[beta]-D-galactosidase).

According to a particular embodiment, the marker protein is present in, on or associated with a *vessel* and is selected from the group consisting of: CD34, vWF, CD31, VEGFR-2, VEGFR-3, NRP1, Tie-2, CD105 (Endoglin), ALK1, DLL4, Ang2, podoplanin, alpha-SMA, integrin alpha5beta1, PDGFR-beta, NG2 and VE-Cadherin. Whereby a vessel is defined as a tube comprising endothelial cells through which blood circulates.

According to another particular embodiment, the marker protein is present in, on or associated with *tumour cells* and the marker protein is one or several cytokeratins. Cytokeratins are the intermediate filament proteins of epithelial cells.

According to another particular embodiment, the marker protein is present in, on or associated with *stromal cells* and is selected from the group consisting of: alpha or gamma-SMA, paladin 4Ig, podoplanin, endosialin (TEM1/CD248), stromelysin, cadherin 2, cadherin 11, integrin alpha11, FAP, FSP, PDGFR-beta, CD68, CD11b, neutrophil elastase, CD3, CD4, CD8, FOXP3, CD56, CD57, P4H, vimentin, desmin, collagen, tenascin, fibronectin, and laminin, MMP-3 and MMP-9.

The marker protein(s) can be detected using techniques well known in the art, e.g. using monoclonal or polyclonal antibodies that detect these target proteins.

In the examples herein, the inventors have used CD31 and α-SMA antibodies to visualize vessels and fibroblast-like stromal cells, respectively in order to determine the tumour-vessel and stromal-vessel phenotypes of tumours.

As used herein, the terms “antibody” and “antibodies”, also known as immunoglobulins, refers to a polypeptide or group of polypeptides that are comprised of at

least one binding domain that is formed from the folding of polypeptide chains having three-dimensional binding spaces with internal surface shapes and charge distributions complementary to the features of an antigenic determinant of an antigen, and encompasses monoclonal antibodies (including full-length monoclonal antibodies), polyclonal antibodies, multispecific antibodies formed from at least two different epitope binding fragments (e.g., bispecific antibodies), human antibodies, humanized antibodies, camelised antibodies, chimeric antibodies, single-chain Fvs (scFv), single-chain antibodies, single domain antibodies, domain antibodies, Fab fragments, F(ab')2 fragments, antibody fragments that exhibit the desired biological activity (e.g. the antigen binding portion), disulfide-linked Fvs (dsFv), and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intrabodies, and epitope-binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, i.e., molecules that contain at least one antigen-binding site.

Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a target (marker protein) polypeptide or immunogenic fragment thereof can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

Polyclonal antibodies can be raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent (reactive group), e.g., activated ester (conjugation through cysteine or lysine residues), glutaraldehyde, succinic anhydride,

$SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups. Conjugates also can be made in recombinant cell culture as fusion proteins.

Typically animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining an appropriate concentration of antigen or conjugate with adjuvant and injecting the solution at multiple sites. One month later, the animals are boosted with ⅕ to 1/10 the original amount of antigen or conjugate in adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. In addition, aggregating agents such as alum are suitably used to enhance the immune response.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma (Kohler *et al.*, *Nature,* 256:495 (1975); Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981), recombinant, and phage display technologies, or a combination thereof. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous or isolated antibodies, e.g., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site or multiple antigenic sites in the case of multi-specific engineered antibodies. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against the same determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In the hybridoma method, mice or other appropriate host animals, such as hamster, are immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the antigen used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent or fusion partner, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press,

1986)). In certain embodiments, the selected myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the un-fused parental cells.

Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumours in an animal e.g., by i.p. injection of the cells into mice.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, affinity tags, hydroxylapatite chromatography, gel electrophoresis, dialysis, etc.

Once a labelled antibody has bound a tumour marker, the complex may be visualized or detected in a variety of ways, with the particular manner of detection being chosen based on the particular detectable label, where representative detection means include, e.g., scintillation counting, autoradiography, measurement of paramagnetism, fluorescence measurement, light absorption measurement, measurement of light scattering and the like.

As noted before, the cellular phenotype of the tumour can be determined visually using a microscope or computer assisted image analyser. Computer assisted image analysis is performed using algorithms generated with imaging software such as Definiens Image Analysis Software, Definiens Tissue Studio, Aperio Genie Pro or Image J. There are numerous computer assisted image analysers and imaging software packages available.

The cellular phenotype of the tumour can also be determined using *in situ* hybridization (ISH) techniques, such as silver *in situ* hybridization (SISH), chromogenic *in situ* hybridization (CISH) and fluorescence *in situ* hybridization (FISH), collectively referred to as ISH. ISH is distinct from IHC, in that ISH employs one or more nucleic acid probe(s) capable of binding to a transcript encoding a marker protein whereas IHC detects proteins.

In situ hybridization, as the name suggests, is a method of localizing and detecting specific mRNA sequences in morphologically preserved tissues sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest.

The basic principles for *in situ* hybridization are the same as normal hybridisation wherein a labelled nucleic acid probe is allowed to bind to matching nucleic acid in a test

sample, except with ISH the probe is utilized to detect specific nucleotide sequences within cells and tissues. The sensitivity of the technique is such that threshold levels of detection are in the region of 10-20 copies of mRNA per cell.

In particular embodiments, the nucleic acid probe selected is capable of hybridising to the transcript of a marker protein present in, on or associated with (i) *a vessel*, wherein the marker protein is selected from the group consisting of: CD34, vWF, CD31, VEGFR-2, VEGFR-3, NRP1, Tie-2, CD105 (Endoglin), ALK1, DLL4, Ang2, podoplanin, alpha-SMA, integrin alpha5beta1, PDGFR-beta, NG2 and VE Cadherin. (ii) *tumour cells*, wherein the marker protein is a cytokeratin; or (iii) *stromal cells*, wherein the marker protein is selected from the group consisting of: alpha or gamma-SMA, paladin 4Ig, podoplanin, endosialin (TEM1/CD248), stromelysin, cadherin 2, cadherin 11, integrin alpha11, FAP, FSP, PDGFR-beta, CD68, CD11b, neutrophil elastase, CD3, CD4, CD8, FOXP3, CD56, CD57, P4H, vimentin, desmin, collagen, tenascin, fibronectin, and laminin, MMP-3 and MMP-9.

The nucleic acid and amino acid sequences of the markers recited above are known and available in various publicly available databases and scientific publications. It is well within the capabilities of a person skilled in the art to prepare and use one or more nucleic acid probe(s) capable of binding to a transcript encoding a marker protein.

As before, the cellular phenotype of the tumour can be determined visually using a microscope or computer assisted image analyser.

The inventors have found that human tumours for specific tissue origin that display a particular tumour phenotype respond differentially to treatment with a pharmaceutical drug.

Thus, according to another aspect of the invention the patient stratification methods of the invention permit identification of patients suitable for treatment with a pharmaceutical drug.

In one embodiment, the patient stratification permits identification of patients for treatment with a drug targeting tumour vasculature (agents that inhibit the growth of new functional tumour vessels and/or promote the regression or disruption of existing tumour vasculature). In particular, the drug targeting tumour vasculature is an inhibitor of VEGF signalling pathway.

There are many examples of compounds or drugs that inhibit VEGF signalling pathway. Compounds that inhibit VEGF signalling pathway typically block binding of VEGF ligand to its receptor. Percy *et al.*, (Nature Reviews Clinical Oncology, 6:569-579, 2009) provides an overview of small-molecule inhibitors of VEGFR signalling. Particular examples

are identified therein in Table 1, which includes: AEE788 (Novartis), axitinib (Pfizer), motesanib (Amgen), cediranib (AstraZeneca), vandetanib (AstraZeneca), sorafenib (Bayer), telatinib (Bayer), BIBF 1120 (Boehringer), brivanib alaninate (Bristol Myers Squibb), dovitinib lactate (Chiron), CP-547, 632 (Pfizer), pazopanib (Glaxo), OSI 930 (OSI), vatalanib (Novartis), semaxinib (Sugen/ Pfizer), SU6668 (Sugen/Pfizer) and sunitinib (Sugen/Pfizer). Wedge and Jürgensmeier ("Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitors for the Treatment of Cancer". In *Tumour Angiogenesis: Basic Mechanisms and Cancer Therapy* Edited by: Marme D, Fusenig N. 2008, Chapter 23:395-424) also review the various VEGFR tyrosine kinase inhibitors in clinical development (see e.g. Table 23.1 therein). Any of these compounds could be used in the invention disclosed herein. Large molecule (e.g. monoclonal antibody) agents that inhibit VEGF signalling pathway can also be used, such as bevacizumab, ramicirumab, aflibercept and 33C3.

In particular embodiments, the inhibitor of VEGF signalling pathway is a compound or drug selected from the group consisting of: AEE788 (Novartis), axitinib (Pfizer), motesanib (Amgen), cediranib (AstraZeneca), vandetanib (AstraZeneca), sorafenib (Bayer), telatinib (Bayer), BIBF-1120 (Boehringer), brivanib alaninate (Bristol Myers Squibb), dovitinib lactate (Chiron), CP-547, 632 (Pfizer), pazopanib (Glaxo), OSI-930 (OSI), vatalanib (Novartis), semaxinib (Sugen), SU6668 (Pfizer), sunitinib (Pfizer),CEP-7055 (Cephalon), E7080 (Esai), SU14813 (Pfizer), KRN-951 (Kirin), ABT-869 (Abbott), BMS 582664 (Bristol Myers Squibb), CHR265 (Chiron), ZK-304709 (Bayer), bevacizumab, ramicirumab, aflibercept and 33C3, or a bispecific molecule targeting more than one pathway, e.g. an inhibitor of both Ang-2 and VEGFA.

The chemical names of certain of these agents are as follows: AEE788 - (6-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine); axitinib - (*N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl]sulfanyl]benzamide); motesanib - (*N*-(3,3-Dimethyl-2,3-dihydro-1*H*-indol-6-yl)-2-[(pyridin-4-ylmethyl)amino]pyridine-3-carboxamide); cediranib - (4-[(4-fluoro-2-methyl-1*H*-indol-5-yl)oxy]-6-methoxy-7-[3-(pyrrolidin-1-yl)propoxy]quinazoline); vandetanib - (*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine); sorafenib - (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino] phenoxy]-*N*-methyl-pyridine-2-carboxamide); telatinib - (9,13-Dihydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-2-azabicyclo[16.3.1]docosa-4,6,10,18,21-pentaene-3,20,22-trione, 9-carbamate); BIBF1120 - (methyl (3Z)-3-{[(4-{methyl[(4-methylpiperazin-1-

yl)acetyl]amino}phenyl)amino](phenyl)methylidene}-2-oxo-2,3-dihydro-1H-indole-6-carboxylate); Brivanib alaninate - ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Dovitinib lactate - (4-Amino-5-fluoro-3-[6-(4-methyl-1-piperazinyl)-1H-benzimidazol-2-yl]-2(1H)-quinolinone 2-hydroxypropanoate hydrate); CP-547, 632 - (3-(4-Bromo-2,6-difluoro-benzyloxy)-5-[3-(4-pyrrolidin-1-yl-butyl)-ureido]-isothiazole-4-carboxylic acid amide); pazopanib - 5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide); OSI-930 - (N-(4-trifluoromethoxyphenyl) 3-[(quinolin-4-ylmethyl)amino]thiophene-2-carboxamide}); vatalanib - (*N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin- 1-amine); semaxinib - (3*Z*)-3-[(3,5-dimethyl-1*H*-pyrrol-2-yl)methylidene]-1,3-dihydro-2*H*-indol-2-one); sunitinib - (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide); CEP-7055 - (N,N-dimethylglycine 3-{5,6,7,13-tetrahydro-9-[(1-methyle-thoxy)methyl]-5-oxo-12H-indeno(2,1-a)pyrrolo(3,4-c)carbazol-12-yl}propyl ester); E7080 - (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxy-quinoline-6-carboxamide); SU14813 - (5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-N-[(2S)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide maleate); KRN-951 - (N-{2-Chloro-4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N′-(5-methyl-3-isoxazolyl) urea hydrochloride monohydrate); and, ABT-869 - (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N1-(2-fluoro-5-methylphenyl) urea).

The methods of the invention can also be employed to select patients suitable for treatment with a drug that targets other angiogenic pathways that are important for tumour growth such as the Ang-2-Tie-2, DLL4-Notch, a5b1-FN, Alk-1-BMP pathways.

According to an aspect of the invention there is provided an e*x vivo* method for determining whether a patient suffering from cancer is a likely to be responsive to pharmaceutical treatment with an inhibitor of VEGF signalling pathway said method comprising the steps of: (a) obtaining a tumour containing sample previously collected from said patient; (b) determining whether the tumour displays or exhibits a tumour-vessel phenotype or a stromal-vessel phenotype, wherein if said tumour displays or exhibits a tumour-vessel phenotype said patient is likely to respond favourably to treatment with the inhibitor of VEGF signalling pathway whereas if the tumour exhibit or display the stromal-vessel phenotype they are unlikely to respond favourably to treatment with the inhibitor of VEGF signalling pathway.

According to an aspect of the invention there is provided a method for selecting a treatment for a patient suffering from cancer, the method comprising (a) determining whether the patient's tumour exhibits a tumour-vessel or stromal-vessel phenotype; and, (b) selecting a treatment for the patient according to the particular cellular phenotype identified in step (a)

According to an aspect of the invention there is provided a method for selecting a patient for treatment with a drug that targets tumour vasculature, wherein the method comprises: a) determining the whether the patient's tumour exhibits a tumour-vessel or stromal-vessel phenotype; and b) selecting said patient for treatment with a vascular targeting agent if the tumour exhibits a tumour-vessel phenotype or selecting said patient for treatment with a stromal targeting agent e.g. an agent inhibiting the following receptors PDGFR, Alk-5, FGFR kinases, the αvβ6 integrin, or an antibody drug conjugate targeting tumour fibroblasts, in combination with a vascular targeting agent if the tumour exhibits the stromal phenotype.

Stromal targeting agent that target tumour modified fibroblasts are exemplified by agents that inhibit the TGF-β signalling pathway such as small molecule agents like LY550410 (5-bromo-thiophene-2-sulfonic acid 2,4-dichlorobenzoylamide sodium salt) and SB-505124 (2-(5-benzo[1,3]dioxol-5-yl-2-*tert*-butyl-3himidazol-4-yl)-6-methylpyridine hydrochloride), or large molecule agents such as the antibodies metelimumab and fresolimumab (GC-1008). Inhibitors of the PDGFR signalling pathway are exemplified by small molecule agents such as imatinib (4-[(4-methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), crenolanib (1-(2-{5-[(3-Methyloxetan-3-yl)methoxy]-1*H*-benzimidazol-1-yl}quinolin-8-yl)piperidin-4-amine) and TKI258 (4-amino-5-fluor-3-[5-(4-metylpiperazin-1-yl)-1H-benzimidazol-2-yl]quinolin-2(1H)-one), or large molecule agents. Inhibitors of CXCR4 signalling is exemplified the peptide AMD3100 (1,1′-[1,4-Phenylenebis(methylene)]bis [1,4,8,11-tetraazacyclotetradecane]). Inhibitors of FGFR signalling are specifically exemplified by AZD4547 (N-[3-[2-(3,5-dimethoxyphenyl)ethyl]-1H-pyrazol-5-yl]-4-[(3S,5R)-3,5-dimethylpiperazin-1-yl]benzamide), and non-specific inhibitors such as dovitinib (TKI258; 4-amino-5-fluoro-3-[5-(4-methylpiperazin-1-yl)-1H-benzimidazol-2-yl]quinolin-2(1H)-one) that target FGFR through broad selectivity. Modulators of IL-6-Jak signalling exemplified by the small molecules 5-chloro-N2-[(1S)-1-(5-fluoro-2-pyrimidinyl)ethyl]-N4-(5-methyl-1H-pyrazol-3-yl)-2,4-pyrimidinediamine) and ruxolitinib ((3*R*)-3-cyclopentyl-3-[4-(7*H*-pyrrolo[2,3-d]pyrimidin-4-yl)pyrazol-1-yl]propanenitrile).

Thus, in particular embodiments the stromal targeting agent is selected from the group consisting of: LY550410 (5-bromo-thiophene-2-sulfonic acid 2,4-dichlorobenzoylamide sodium salt), SB-505124 (2-(5-benzo[1,3]dioxol-5-yl-2-*tert*-butyl-3Himidazol-4-yl)-6-methylpyridine hydrochloride), metelimumab, GC-1008, imatinib (4-[(4-methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), Crenolanib(1-(2-{5-[(3-Methyloxetan-3-yl)methoxy]-1*H*-benzimidazol-1-yl}quinolin-8-yl)piperidin-4-amine), TKI258 (4-amino-5-fluor-3-[5-(4-metylpiperazin-1-yl)-1H-benzimidazol-2-yl]quinolin-2(1H)-one), the peptide AMD3100 (1,1′-[1,4-Phenylenebis(methylene)]bis [1,4,8,11-tetraazacyclotetradecane]), N-[3-[2-(3,5-dimethoxyphenyl)ethyl]-1H-pyrazol-5-yl]-4-[(3S,5R)-3,5-dimethylpiperazin-1-yl]benzamide, TKI258, 5-Chloro-N2-[(1S)-1-(5-fluoro-2-pyrimidinyl)ethyl]-N4-(5-methyl-1H-pyrazol-3-yl)-2,4-pyrimidinediamine and ruxolitinib ((3*R*)-3-cyclopentyl-3-[4-(7*H*-pyrrolo[2,3-d]pyrimidin-4-yl)pyrazol-1-yl]propanenitrile).

In one embodiment the tumour phenotype is determined using a method as described herein. Such method may include extraction of the tumour sample from the patient or may be conducted on a tumour sample previously extracted from the patient.
Stromal agents in the context of this invention include agents that target the fibroblast population in the tumour. Vascular Targeting Agents are agents that induce rapid endothelial cell death resulting in vessel capitulation, reduction in tumour perfusion and driving necrosis within the tumour mass.

In particular embodiments the drug that targets tumour vasculature is a VEGF signalling pathway inhibitor, a Tie-2-Ang-2 signalling pathway inhibitor, a Alk-1 signalling pathway inhibitor, or a DLL4 signalling pathway inhibitor.

In a particular embodiment the drug that targets tumour vasculature is a VEGF signalling pathway inhibitor. Particularly suitable drugs (or compounds) are selected from the group consisting of: AEE788, axitinib, motesanib, cediranib, vandetanib, sorafenib, telatinib, BIBF-1120, brivanib alaninate, dovitinib lactate, CP-547, 632, pazopanib, OSI-930, vatalanib, semaxinib, SU6668, sunitinib, CEP-7055, E7080, SU14813, Telatinib, KRN-951, ABT-869, BMS 582664, CHR265 and ZK-304709. Vandetanib and cediranib are particularly suitable compounds for use in the present invention.

Agents that target Ang-2 signalling include: AMG-386 and CVX-060. An agent that targets Alk-1 signalling Ang-2 is the antibody PF-03446962.

According to a further aspect of the invention there is provided a method for selecting a patient for treatment with a drug that targets tumour vasculature, wherein the method comprises: a) determining the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour in a tumour tissue sample previously taken from the patient so as to determine whether or not the tumour exhibits a tumour-vessel or stromal-vessel phenotype; and b) selecting said patient for treatment with a vascular targeting agent if the tumour exhibits a tumour-vessel phenotype or c) selecting said patient for treatment with a stromal targeting agent in combination with a vascular targeting agent if the tumour exhibits the stromal phenotype.

According to a further aspect of the invention there is provided a method for selecting a patient for treatment with a VEGF signalling pathway inhibitor comprising: a) determining the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour in a tumour tissue sample from the patient so as to determine whether or not the tumour exhibits a tumour-vessel or stromal-vessel phenotype; and, b) selecting said patient for treatment with an VEGF signalling pathway inhibitor if the tumour exhibits the tumour-vessel phenotype. The tumour sample may, for example, be a sectioned sample or biopsy sample. In a particular embodiment the sample has been previously taken from the patient so that the method is not specifically practised on the human body.

According to a further aspect of the invention there is provided a method for selecting a patient for combination treatment with a VEGF signalling pathway inhibitor and a stromal targeting agent comprising: a) determining the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour in a tumour tissue sample from the patient so as to determine whether or not the tumour exhibits a tumour-vessel or stromal-vessel phenotype; and (b) selecting said patient for treatment with a stromal targeting agent in combination with a VEGF signalling pathway inhibitor if the tumour exhibits the stromal-vessel phenotype. In a particular embodiment the sample has been previously taken from the patient so that the method is not specifically practised on the human body.

In particular embodiments, following selection of the patients for treatment with a VEGF signalling pathway inhibitor or another vascular targeting agent, the patient is subsequently treated by administration of an effective amount of said VEGF signalling pathway inhibitor or other vascular targeting agent, as appropriate.

According to a further aspect of the invention there is provided a method for treating a patient suffering from cancer comprising:

(i) extracting a tumour tissue sample from the patient

(ii) determining whether the tumour exhibits a tumour-vessel or stromal-vessel phenotype; and

(iii)if the tumour exhibits a tumour-vessel phenotype administering to said patient an effective amount of an inhibitor of VEGF signalling pathway.

According to a further aspect of the invention there is provided the use of a compound or drug capable of inhibiting VEGF signalling pathway in the preparation of a medicament for treating an individual with cancer, whose cancer cells have been determined to exhibit the tumour-vessel phenotype. The medicament can be a pharmaceutical composition.

According to a further aspect of the invention there is provided the use of an inhibitor of VEGF signalling pathway to treat tumours exhibiting the tumour -vessel phenotype.

In particular embodiments, the inhibitor of VEGF signalling pathway for a method or use as contemplated herein is a compound or drug selected from the group consisting of: AEE788, axitinib, motesanib, cediranib, vandetanib, sorafenib, telatinib, BIBF-1120, brivanib alaninate, dovitinib lactate, CP-547, 632, pazopanib, OSI-930, vatalanib, semaxinib, SU6668, sunitinib, CEP-7055, E7080, SU14813, Telatinib, KRN-951, ABT-869, BMS 582664, CHR265 and ZK-304709.

The compound or drug may be administered in an effective amount to a subject in need of such treatment. As such, the compounds/drugs described herein may be useful for the treatment of cancer and other proliferative disorders. Administration of the compounds/drugs, in the form of a therapeutic agent, may be carried out using oral, enteral, parenteral or topical administration, including, for example, intravenous, oral, transdermal or any other mode of administration.As used herein "subject" or "patient" refers to an animal or mammal including, but not limited to, human, dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rabbit, rat, mouse, etc.

As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve an unwanted condition or disease of a patient. The methods herein for use contemplate prophylactic use as well as curative use in therapy of an existing condition.

The terms "therapeutically effective" or "effective", as used herein, may be used interchangeably and refer to an amount of a therapeutic composition embodiments of the present invention. For example, a therapeutically effective amount of a composition comprising anti-VEGFR therapy is a predetermined amount calculated to achieve the desired effect, i.e., to effectively inhibit VEGF receptor signalling in an individual to whom the composition is administered.

The medicament or pharmaceutical composition can take the form of a solution, suspension, emulsion, tablet, pill, pellet, capsule, capsule containing liquids, powder, sustained-release formulation, suppository, emulsion, aerosol, spray, suspension, or any other form suitable for use.

Administration may be topical, i.e., substance is applied directly where its action is desired, enteral or oral, i.e., substance is given via the digestive tract, parenteral, i.e., substance is given by other routes than the digestive tract such as by injection.

In a particular embodiment, the active compound and optionally another therapeutic or prophylactic agent are formulated in accordance with routine procedures as pharmaceutical compositions adapted for intravenous administration to human beings. Typically, the active compound for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally include a local anaesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule. Where the active compound is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the active compound is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Compositions for oral delivery can be in the form of tablets, lozenges, cachets, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can contain one or more optional agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavouring agents such as peppermint, oil of wintergreen, or cherry; colouring agents; and preserving agents, to provide a pharmaceutically palatable preparation. A time delay material such as glycerol monostearate or glycerol stearate can also be used. Oral compositions can include standard

vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. Such vehicles are of pharmaceutical grade in particular embodiments.

Compositions for use in accordance with the present invention can be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, the VEGF signalling inhibitor compound and optionally another therapeutic or prophylactic agent and their physiologically acceptable salts and solvates can be formulated into pharmaceutical compositions for administration by inhalation or insufflation (either through the mouth or the nose) or oral, parenteral or mucosol (such as buccal, vaginal, rectal, sublingual) administration. In one embodiment, local or systemic parenteral administration is used.

For oral administration, the compositions can take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

In some embodiments, compounds or salts disclosed herein or can be administered as a pharmaceutical composition in which the pharmaceutical composition comprises between 0.1-1mg, 1-10 mg, 10-50mg, 50-100mg, 100-500mg, or 500mg to 5g of said IAP antagonist compound or salt.

The invention will be further described by the following non-limiting examples and figures wherein Figure 1 shows photographs of tumour samples possessing tumour-vessel phenotype and stromal-vessel phenotype as well as a computer generated diagrams illustrating

the phenotypes. A. Vasculature embedded within tumour can be seen. B. Islands/nests of tumour surrounded by stroma and vessels found in stroma but rarely embedded within tumour can be seen.

Figure 2 shows that the two phenotypes define monotherapy or combination therapy disease types for VEGF signalling inhibitors. The y axis represents % ratio of phenotype.

Figure 3 shows growth curves for DC101 treated Calu-6 and Calu-3 xenograft models showing that the two phenotypes determine response to VEGF inhibition *in vivo*.

Figure 4 shows a graph of the ratio of the three RECIST outcome categories for tumour-vessel and stromal-vessel phenotypes. The y-axis showing the ratio of RECIST response classifications.

All documents identified herein, and in particular the subject matter disclosed therein, are incorporated by reference.

Examples

Materials and Methods

In vivo tumour models

In vivo tumours model tissue was generated for baseline stromal/tumour – vessel analysis as follows. Tumour xenograft tissue was derived from experiments conducted with licences issued under the UK Animals (Scientific Procedures) Act 1986 and after local ethical review and approval. Cell lines were maintained in the recommended growth medium and implanted subcutaneously into the left flank of immuno-compromised mice, nude, scid or scid-bg. Tumours were grown to approximately 1 cm^3 volume then collected and fixed in formalin for 24 h before being embedded in paraffin. See Table 1 for details of the preparation of each model used in this analysis.

Table 1

Cell line	Source	Cell inoculum per mouse (x 10^6)	50% matrigel	Mouse strain	Mouse sex	Culture medium	FCS	Glutamine	Other	CO2
A2780	ECACC	1	-	nude?	female	RPMI 1640	10%	1%	-	7.5%
A375	ECACC	10	-	nude	female	DMEM	5%	1%	-	7.5%
A431	ECACC	fragment	-	nude	female	DMEM	10%	1%	-	7.5%
A549a	ATCC	5	Y	nude	female	DMEM	10%	1%	-	7.5%
BT474c1	Dr. Albavell, Barcelona	10	-	nude	female	DMEM	10%	-	10% M1, 1mM OAA	7.5%
C6	ATCC	0.1	-	nude	female	199	10%	1%	1% NEAA	7.5%
Calu-6	ATCC	1	-	nude	female	EMEM	10%	1%	1% NEAA, 1% NaPyr	7.5%
Colo205	ECACC	5	Y	nude	female	RPMI 1640	10%	1%	-	7.5%
CWR 22Rv1	ATCC	10	Y	nude	female	RPMI 1640	10%	1%	10% M1	7.5%
DU145	ATCC	10	Y	nude	female	EMEM	10%	1%	1% NEAA	7.5%
FaDu	ATCC	5	-	nude	female	MEM	10%	1%	1% NEAA	7.5%
HCT116	ATCC	10	-	nude	female	DMEM	10%	1%	-	7.5%
HL-60	Dept Immunology, Birmingham university	10	-	Scid-bg	male	RPMI 1640	10%	-	-	5%
HPAC	GHP	10	-	nude	female	DMEM	10%	1%	-	7.5%
HT1080	ECACC	5	-	nude	female	MEM	10%	1%	1% NEAA	7.5%
HT29	ECACC	5	-	nude	male	EMEM	10%	1%	1% NEAA	7.5%
HX147	Dr. Duchesne, ICRF, Sutton	fragment	-	nude	female					?
LoVo	ECACC	10	-	nude	female	DMEM	10%	1%	-	5%
MCF7	ICRF, London	5	Y	scid	male	DMEM	10%	1%	-	7.5%

MDA MB 231	ATCC	Fragment	-	nude	female	DMEM	10%	1%	-	7.5%
MES-SA	ECACC	10	-	scid	female	McCoys	10%	1%	-	7.5%
NCI H1975	ATCC	10	-	nude	female	DMEM	10%	1%	-	7.5%
NCI H460	ATCC	5	-	nude	female	RPMI 1640	10%	1%	10% M1	7.5%
NCI H526	ATCC	10	Y	nude	female	RPMI 1640	10%	1%	10% M1	7.5%
PC-3	ATCC	1	Y	nude	female	Iscoves	10%	1%	-	7.5%
PC-9	AZ Japan	10	Y	scid	female	DMEM	10%	1%	-	7.5%
SW620	ECACC	1	Y	nude	female	L-15	10%	1%	-	-
U118MG	ATCC	10	Y	nude	female	DMEM	10%	1%	10% M1	7.5%
U87MG	ATCC	1	-	nude	female	DMEM	10%	1%	1% NEAA, 1% NaPyr	7.5%
ZR-75-1	M. Lippman, NCI	1	Y	nude	female	DMEM	10%	1%	-	7.5%

In vivo DC101 tumour growth studies

Calu-6 and Calu-3 xenografts, established in nude and SCID female mice, respectively were each randomised across two groups, both containing 12 animals. Once tumours reached a mean volume of ~2cm^3 (Calu-6) and ~3cm^3 (Calu-3), mice were then intraperitoneally injected twice weekly with either 15mg/kg of DC101 (Cell Essentials Inc.) or isotype control antibody for 25 (Calu-6) and 41 (Calu-3) days. DC101 is a VEGF receptor-2–specific (flk-1) monoclonal neutralizing antibody. Tumours in each group were excised and split in half; one half was snap frozen in liquid nitrogen and stored at -80°C until required, the other half was fixed in formalin for 24 hours.

Human tissues

Formalin-fixed paraffin-embedded human primary cancer resection samples and tumour microarrays (TMA) were sourced under approved legal contract from three commercial tissue suppliers, Asterand, Cytomyx and TriStar Technology Group and a hospital tissue bank, Wales Cancer Bank. Appropriate consents, licensing and ethical approval was obtained for this research. The suitability of each specimen for immunohistochemical analyses was determined by pathology assessment of tissue morphology and preservation (H&E) and the general extent of antigen preservation (CD31 and p-Tyr immunostains).

Stromal phenotyping assay

An immunofluorescent assay was developed to detect vessels (using an antibody to vessel endothelial cell marker, CD31) and stromal fibroblasts (using an antibody to stromal fibroblast marker, alpha-smooth muscle actin (αSMA) and tumour cells (DAPI counter stain). Custom rabbit polyclonal antibody raised to C-terminus peptide of mouse CD31 (CHG-CD31-P1, AstraZeneca) and mouse monoclonal antibody to α-smooth muscle actin (αSMA; 1A4, Sigma) were used in the development of this assay. Immunofluorescence was performed on dewaxed and rehydrated FFPE sections. Antigen retrieval was carried out as above in pH 9 retrieval buffer. After blocking in 20% horse serum, sections were incubated for 1 h in a combination of 1:20 CHG-CD31-P1 and 1:1000 1A4 diluted in 20% horse serum. Donkey anti-rabbit IgG conjugated to Alexa Fluor 488 (A21206, Molecular Probes) and donkey anti-mouse IgG Alexa Fluor 555 (A31570, Molecular Probes) combined 1:800 in serum were added for 30 min. Sections were

counterstained with ProLong Gold anti-fade reagent with 4',6-diamidino-2-phenylindole (DAPI; P36931, Molecular Probes), fluorescent images were scanned and captured using a MIRAX scan (Carl Zeiss) .

Example 1. Classification of tumour xenografts based on distribution of tumour stroma and vasculature

In order to characterise a range of tumour xenograft models based on the distribution of tumour and stroma and location of the vasculature between these two compartments, 42 histologically distinct human tumour xenograft models formatted onto a tissue microarray (TMA), were analysed using the immunofluorescence CD31-αSMA assay. After morphological assessment, two basic phenotypes were identified and described as follows (Figure 1):

A) **tumour-vessel (TV) phenotype:** predominant (>60% of tumour mass) morphology is that of a sheet of malignant/cancerous cell (>2mm^{2,} as detected with DAPI) with vasculature (CD31 positive) embedded within tumour.

B) **stromal-vessel (SV) phenotype:** predominant (>60% of tumour mass) morphology is that of smaller islands/nests of malignant/cancerous cells (<2mm^2 as detected with DAPI) separated by stroma (αSMA positive) with vessels (CD31 positive) localised to the intervening stroma but either absent or rarely embedded within tumour.

These two phenotypes are exemplified in Figure 1. The tumour-vessel phenotype was common to the majority of models analysed [(40/42 (95%)] whereas the stromal-vessel phenotype was rare [(2/42 (5%], Calu-3 and NCIN87).

This demonstrates that the preclinical models of human disease display two distinct phenotypes based on the distribution of CD31 positive vessels relative to αSMA positive fibroblast like cells. The majority of xenografts display a tumour-vessel phenotype

Example 2. Tumour- and stromal-vessel phenotypes define disease types that are either responsive or insensitive to VEGF signalling inhibitor monotherapy

The relationship between both phenotypes and tumour types broadly classified as sensitive or insensitive to VEGFR inhibitor monotherapy as a result of published clinical trial outcome data was determined. Pre-treatment baseline tumour samples

formatted onto arrays were analysed for CD31-αSMA and scored for either phenotype whereby a tumour sample was classified as either TV or SV based on the predominant phenotype of the two (>60% cut off). The tumour-vessel phenotype was associated with tumour types classified as responding to VEGF signalling inhibitors as single agent renal cell cancer [RCC, 80% (40/50)], glioblastoma [GBM, 96% (51/53)],hepatocellular carcinoma [HCC, 93% (28/30)], thyroid cancer [ThC, 97% (35/36)] and ovarian cancer [OvC, 69% (25/36)], whereas tumour types insensitive to these agents as monotherapy, head and neck squmaous cell carcinoma [HNSCC, 86% (42/49)], colorectal [CRC, 88% (23/26)], prostate [PC, 81% (39/48)] and lung cancer [(NSCLC, 82% (50/61)] were characterised by the stromal-vessel phenotype. Figure 2 shows the ratio of the two phenotypes for RCC, GBM, HCC, CRC, PC and NSCLC.

Diseases commonly classified as responsive to VEGF inhibition as a monotherapy exhibit the tumour-vessel phenotype. In contrast those disease where VEGF inhibitors are used as combination therapy, and show limited single agent activity (defined as activity in a small percentage of patients e.g. <15%) exhibit a stromal-vessel phenotype.

Example 3. Concordance between the phenotype classifications of TMA cores and matched donor tumour tissue

A TMA tissue core represents a small area of a larger donor specimen, this combined with erroneous sampling may mean that the core does not always represent the bulk of the tumour and therefore the predominant phenotype To assess the concordance between the phenotypes of TMA cores and matched donor tumour tissue we scored 20 donor tumours and their matched TMA cores (10 CRC and 10 NSCLC) for the two phenotypes. A 90% concordance was observed between donors and TMA cores (Table 2).

Table 2 concordance between the phenotype classifications of TMA cores and matched donor tumour tissue.

CRC	Donor	Core (TMA)
20272B2	2	2
23015A2	2	2
30727B2	1	1
41727B1	2	1
43621A2	2	2

44092A2	2	2
50003A1	2	2
50036A1	2	2
66034A2	2	2

LUNG	Donor	Core (TMA)
69774A1	2	2
69784A1	2	2
P352	2	2
P722	2	2
P869	2	2
P888	2	2
P890	1	1
P891	1	1
P946	2	2
P981	2	2

1 = tumour vessel phenotype

2 = stromal-vessel phenotype

This data indicates that for CRC and NSCLC there is high concordance between the phenotype classifications of smaller TMA cores and larger matched donor tumour tissue and that TMAs can be used to score CRC and NSCLC for the two phenotypes with approximately 90% accuracy. This data also demonstrates that the phenotyping approach may also be applicable to biopsy samples close in size to TMA cores.

Example 4. The effect of VEGFR-2 inhibitor, DC101 on tumour growth is determined by tumour- and stromal-vessel phenotypes in vivo.

To evaluate the hypothesis that the two phenotypes predict differential responses to VEGF signalling inhibitors we measured the effects of VEGFR-2 inhibitor, DC-101 (15mg/kg) on tumour growth inhibition in Calu-6 (tumour-vessel phenotype) and Calu-3 (stromal-vessel phenotype) lung tumour xenograft models. Figure 3 shows growth curves for DC101 treated Calu-6 and Calu-3 xenograft models. From 3 days dosing with DC-101,

the growth rate of the Calu-6 model was significantly reduced compared to control Calu-6 tumours. Conversely, after an immediate growth inhibitory response with DC-101, the rate of Calu-3 tumour growth returned to that of the control arm after 3 days and was maintained for the duration of the regime.

These data support the premise that the tumour-vessel phenotype is more likely to predict a response to VEGFR targeted monotherapy than the stromal-vessel phenotype.

Example 5. Association of stromal phenotypes with tumour response to FOLFIRI/Avastin™ (bevacizumab) in human CRC (RECIST).

The predictive utility of the two phenotypes was analysed further in pre-treatment stage IV CRC samples taken from patients with RECIST outcome information to receiving first line FOLFIRI/Avastin™ treatment. CRC samples (n=23, representing 5 non- and 4 complete-responders and 14 stable disease) formatted on to TMAs, were analysed for CD31-αSMA and scored for either phenotype. Figure 4 shows a graph of the ratio of the three RECIST outcome categories for tumour-vessel and stromal-vessel phenotypes. In line with our previous data, the majority of CRC specimens exhibited the stromal-vessel phenotype [19/23 (83%)], with the remaining four characterised by the tumour-vessel phenotype [4/23 (17%)]. Proportionally the tumour-vessel phenotype was associated more strongly with a complete response to FOLFIRI/Avastin™ [2/4 (50%)] compared to the stromal-vessel phenotype [2/19 (11%)].

These data suggest that the sub-population of CRC tumours, which exhibit the tumour-vessel phenotype, are more likely to predict patients that respond to Avastin™ in combination with the FOLFIRI chemotherapy drug combination.

Claims

1. A method for selecting a treatment for a patient suffering from cancer, the method comprising:

   (a) determining whether the patient's tumour exhibits a tumour-vessel or stromal-vessel phenotype; and,

   (b) selecting a treatment for the patient according to the particular cellular phenotype identified in step (a).

2. The method according to claim 1, wherein the patient's tumour phenotype is determined by determining the presence of stroma and/or location of tumour vessels within the tumour or stromal compartments of a tumour in a tumour tissue sample
3. The method according to claim 1 or 2, wherein if the patient's tumour exhibits a tumour-vessel phenotype the patient is selected for treatment with a vascular targeting agent.
4. The method according to claim 1 or 2, wherein if the patient's tumour exhibits a stromal-vessel phenotype the patient is selected for treatment with a stromal targeting agent in combination with a vascular targeting agent.
5. The method according to any of the preceding claims, wherein the cellular phenotype of the tumour is determined using one or more agents capable of detecting the presence of one or more of the following cells types; tumour vessels, tumour cells and stromal cells.
6. The method according to any of the preceding claims, wherein the cellular phenotype of the tumour is determined using a tissue or cell staining technique, immunohistochemistry or *in situ* hybridisation
7. The method according to claim 5 or 6, wherein the cellular phenotype of the tumour is determined using histological staining using one or more stains selected from: a Haematoxylin, an Eosin, a Trichrome stain for connective tissue, an elastic tissue fibre stain, a reticular fibre stain and an immunofluorescence counterstain.
8. The method according to claim 5 or 6, wherein the cellular phenotype of the tumour is determined using immunohistochemistry using an antibody capable of

binding to a marker protein present in, on or associated with a vessel, tumour cells or stromal cells.

9. The method according to claim 8, wherein the marker protein is either (i) present in, on or associated with a vessel and is selected from the group consisting of: CD34, vWF, CD31, VEGFR-2, VEGFR-3, NRP1, Tie-2, CD105 (Endoglin), ALK1, DLL4, Ang2, podoplanin, alpha-SMA, integrin alpha5beta1, PDGFR-beta, NG2 and VE Cadherin; (ii) present in, on or associated with tumour cells and the marker protein is a cytokeratin; or (iii) present in, on or associated with stromal cells and is selected from the group consisting of: alpha or gamma-SMA, paladin 4Ig, podoplanin, endosialin (TEM1/CD248), stromelysin, cadherin 2, cadherin 11, integrin alpha11, FAP, FSP, PDGFR-beta, CD68, CD11b, neutrophil elastase, CD3, CD4, CD8, FOXP3, CD56, CD57, P4H, vimentin, desmin, collagen, tenascin, fibronectin, and laminin, MMP-3 and MMP-9.
10. The method according to claim 5 or 6, wherein the cellular phenotype of the tumour is determined using *in situ* hybridisation with a nucleic acid probe capable of binding to a transcript encoding a marker protein present in, on or associated with (i) a vessel, wherein the marker protein is selected from the group consisting of: CD34, vWF, CD31, VEGFR-2, VEGFR-3, NRP1, Tie-2, CD105 (Endoglin), ALK1, DLL4, Ang2, podoplanin, alpha-SMA, integrin alpha5beta1, PDGFR-beta, NG2 and VE Cadherin; (ii) tumour cells, wherein the marker protein is a cytokeratin; or (iii) stromal cells, wherein the marker protein is selected from the group consisting of: alpha or gamma-SMA, paladin 4Ig, podoplanin, endosialin (TEM1/CD248), stromelysin, cadherin 2, cadherin 11, integrin alpha11, FAP, FSP, PDGFR-beta, CD68, CD11b, neutrophil elastase, CD3, CD4, CD8, FOXP3, CD56, CD57, P4H, vimentin, desmin, collagen, tenascin, fibronectin, and laminin, MMP-3 and MMP-9.
11. The method according to any of claims 1-5, wherein the cellular phenotype of the tumour is determined visually using a microscope or computer assisted image analyser.
12. The method according to claim 3 or 4, wherein the vascular targeting agent is an inhibitor of VEGF signalling pathway, Tie-2-Ang-2 signalling pathway, Alk-1 signalling pathway, or DLL4 signalling pathway.
13. The method according to claim 4, wherein the stromal targeting agent is selected from the group consisting of: LY550410 (5-bromo-thiophene-2-sulfonic acid 2,4-

dichlorobenzoylamide sodium salt), SB-505124 (2-(5-benzo[1,3]dioxol-5-yl-2-*tert*-butyl-3Himidazol-4-yl)-6-methylpyridine hydrochloride), metelimumab, GC-1008, imatinib (4-[(4-methylpiperazin-1-yl)methyl]-*N*-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), crenolanib(1-(2-{5-[(3-Methyloxetan-3-yl)methoxy]-1*H*-benzimidazol-1-yl}quinolin-8-yl)piperidin-4-amine), TKI258 (4-amino-5-fluor-3-[5-(4-metylpiperazin-1-yl)-1H-benzimidazol-2-yl]quinolin-2(1H)-one), the peptide AMD3100 (1,1′-[1,4-Phenylenebis(methylene)]bis [1,4,8,11-tetraazacyclotetradecane]), N-[3-[2-(3,5-dimethoxyphenyl)ethyl]-1H-pyrazol-5-yl]-4-[(3S,5R)-3,5-dimethylpiperazin-1-yl]benzamide, TKI258, 5-Chloro-N2-[(1S)-1-(5-fluoro-2-pyrimidinyl)ethyl]-N4-(5-methyl-1H-pyrazol-3-yl)-2,4-pyrimidinediamine and ruxolitinib ((3*R*)-3-cyclopentyl-3-[4-(7*H*-pyrrolo[2,3-d]pyrimidin-4-yl)pyrazol-1-yl]propanenitrile).

14. The method according to claim 12, wherein the VEGF signalling pathway inhibitor is selected from the group consisting of: small molecule chemical antagonists - AEE788 (6-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine), axitinib (*N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl]sulfanyl]benzamide), motesanib (*N*-(3,3-Dimethyl-2,3-dihydro-1*H*-indol-6-yl)-2-[(pyridin-4-ylmethyl)amino]pyridine-3-carboxamide), cediranib (4-[(4-fluoro-2-methyl-1*H*-indol-5-yl)oxy]-6-methoxy-7-[3-(pyrrolidin-1-yl)propoxy]quinazoline), vandetanib (*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine), sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), telatinib (9,13-Dihydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-2-azabicyclo[16.3.1]docosa-4,6,10,18,21-pentaene-3,20,22-trione, 9-carbamate), BIBF1120 (methyl (3Z)-3-{[(4-{methyl[(4-methylpiperazin-1-yl)acetyl]amino}phenyl)amino](phenyl)methylidene}-2-oxo-2,3-dihydro-1H-indole-6-carboxylate), brivanib alaninate: ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate), CP-547, 632 (3-(4-Bromo-2,6-difluoro-benzyloxy)-5-[3-(4-pyrrolidin-1-yl-butyl)-ureido]-isothiazole-4-carboxylic acid amide), pazopanib: 5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide), OSI-930 (N-(4-trifluoromethoxyphenyl) 3-[(quinolin-4-ylmethyl)amino]thiophene-2-carboxamide}), vatalanib (*N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin- 1-amine), semaxinib (3*Z*)-3-[(3,5-dimethyl-1*H*-

pyrrol-2-yl)methylidene]-1,3-dihydro-2*H*-indol-2-one), sunitinib (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide), CEP-7055 (N,N-dimethylglycine 3-{5,6,7,13-tetrahydro-9-[(1-methyle-thoxy)methyl]-5-oxo-12H-indeno(2,1-a)pyrrolo(3,4-c)carbazol-12-yl}propyl ester), E7080 (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxy-quinoline-6-carboxamide), SU14813 (5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-N-[(2**S**)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide maleate), KRN-951 (N-{2-Chloro-4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N′-(5-methyl-3-isoxazolyl) urea hydrochloride monohydrate) and ABT-869 (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N1-(2-fluoro-5-methylphenyl) urea); and protein based antagonists - bevacizumab, ramicirumab, aflibercept and 33C3.

15. The method according to claim 3 or 4, wherein the patient is subsequently treated by administration of an effective amount of a VEGF signalling pathway inhibitor or another vascular targeting agent.

16. The method according to claim 15, wherein the VEGF signalling pathway inhibitor is selected from: small molecule chemical antagonists - AEE788 (6-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine), axitinib (*N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl]sulfanyl]benzamide), motesanib (*N*-(3,3-Dimethyl-2,3-dihydro-1*H*-indol-6-yl)-2-[(pyridin-4-ylmethyl)amino]pyridine-3-carboxamide), cediranib (4-[(4-fluoro-2-methyl-1*H*-indol-5-yl)oxy]-6-methoxy-7-[3-(pyrrolidin-1-yl)propoxy]quinazoline), vandetanib (*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine), sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), telatinib (9,13-Dihydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-2-azabicyclo[16.3.1]docosa-4,6,10,18,21-pentaene-3,20,22-trione, 9-carbamate), BIBF1120 (methyl (3Z)-3-{[(4-{methyl[(4-methylpiperazin-1-yl)acetyl]amino}phenyl)amino](phenyl)methylidene}-2-oxo-2,3-dihydro-1H-indole-6-carboxylate), brivanib alaninate: ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate), CP-547, 632 (3-(4-Bromo-2,6-difluoro-benzyloxy)-5-[3-(4-pyrrolidin-1-yl-butyl)-ureido]-isothiazole-4-carboxylic acid amide), pazopanib: 5-

[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide), OSI-930 (N-(4-trifluoromethoxyphenyl) 3-[(quinolin-4-ylmethyl)amino]thiophene-2-carboxamide}), vatalanib (*N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin- 1-amine), semaxinib (3*Z*)-3-[(3,5-dimethyl-1*H*-pyrrol-2-yl)methylidene]-1,3-dihydro-2*H*-indol-2-one), sunitinib (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide), CEP-7055 (N,N-dimethylglycine 3-{5,6,7,13-tetrahydro-9-[(1-methyle-thoxy)methyl]-5-oxo-12H-indeno(2,1-a)pyrrolo(3,4-c)carbazol-12-yl}propyl ester), E7080 (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxy-quinoline-6-carboxamide), SU14813 (5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-N-[(2**S**)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide maleate), KRN-951 (N-{2-Chloro-4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N′-(5-methyl-3-isoxazolyl) urea hydrochloride monohydrate) and ABT-869 (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N1-(2-fluoro-5-methylphenyl) urea); and protein based antagonists - bevacizumab, ramicirumab, aflibercept and 33C3.

17. An e*x vivo* method for determining whether a patient suffering from cancer is a likely to be responsive to pharmaceutical treatment with an inhibitor of VEGF signalling pathway said method comprising the steps of: (a) obtaining a tumour containing sample previously collected from said patient; (b) determining whether the tumour possesses a tumour-vessel phenotype or a stromal-vessel phenotype, wherein if said tumour possesses a tumour-vessel phenotype said patient is likely to respond favourably to treatment with the inhibitor of VEGF signalling pathway whereas if the tumour possesses a stromal-vessel phenotype they are unlikely to respond favourably to treatment with the inhibitor of VEGF signalling pathway.

18. Use of a compound capable of inhibiting VEGF signalling pathway in the preparation of a medicament for treating an individual with cancer, whose cancer cells have been determined to exhibit the tumour-vessel phenotype.

19. Use according to claim 18, wherein the compound is selected from: small molecule chemical antagonists - AEE788 (6-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine), axitinib (*N*-Methyl-2-[[3-[(*E*)-2-pyridin-2-ylethenyl]-1*H*-indazol-6-yl]sulfanyl]benzamide), motesanib (*N*-(3,3-Dimethyl-2,3-dihydro-1*H*-indol-6-yl)-2-[(pyridin-4-

ylmethyl)amino]pyridine-3-carboxamide), cediranib (4-[(4-fluoro-2-methyl-1*H*-indol-5-yl)oxy]-6-methoxy-7-[3-(pyrrolidin-1-yl)propoxy]quinazoline), vandetanib (*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine), sorafenib (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide), telatinib (9,13-Dihydroxy-8,14,19-trimethoxy-4,10,12,16-tetramethyl-2-azabicyclo[16.3.1]docosa-4,6,10,18,21-pentaene-3,20,22-trione, 9-carbamate), BIBF1120 (methyl (3Z)-3-{[(4-{methyl[(4-methylpiperazin-1-yl)acetyl]amino}phenyl)amino](phenyl)methylidene}-2-oxo-2,3-dihydro-1H-indole-6-carboxylate), brivanib alaninate: ((S)-((R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate), CP-547, 632 (3-(4-Bromo-2,6-difluoro-benzyloxy)-5-[3-(4-pyrrolidin-1-yl-butyl)-ureido]-isothiazole-4-carboxylic acid amide), pazopanib: 5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide), OSI-930 (N-(4-trifluoromethoxyphenyl) 3-[(quinolin-4-ylmethyl)amino]thiophene-2-carboxamide}), vatalanib (*N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin- 1-amine), semaxinib (3*Z*)-3-[(3,5-dimethyl-1*H*-pyrrol-2-yl)methylidene]-1,3-dihydro-2*H*-indol-2-one), sunitinib (N-(2-diethylaminoethyl)-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide), CEP-7055 (N,N-dimethylglycine 3-{5,6,7,13-tetrahydro-9-[(1-methyle-thoxy)methyl]-5-oxo-12H-indeno(2,1-a)pyrrolo(3,4-c)carbazol-12-yl}propyl ester), E7080 (4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxy-quinoline-6-carboxamide), SU14813 (5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-N-[(2**S**)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide maleate), KRN-951 (N-{2-Chloro-4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N′-(5-methyl-3-isoxazolyl) urea hydrochloride monohydrate) and ABT-869 (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N1-(2-fluoro-5-methylphenyl) urea); and protein based antagonists - bevacizumab, ramicirumab, aflibercept and 33C3.

20. A method according to any of claims 1-17 or a use according to claim 18 or 19, wherein the cancer is selected from: renal cell cancer, glioblastoma, ovarian cancer, liver cancer, thyroid cancer, head and neck cancer, colorectal cancer, lung cancer, prostate cancer, pancreatic cancer, breast cancer and skin cancer.

Fig. 1

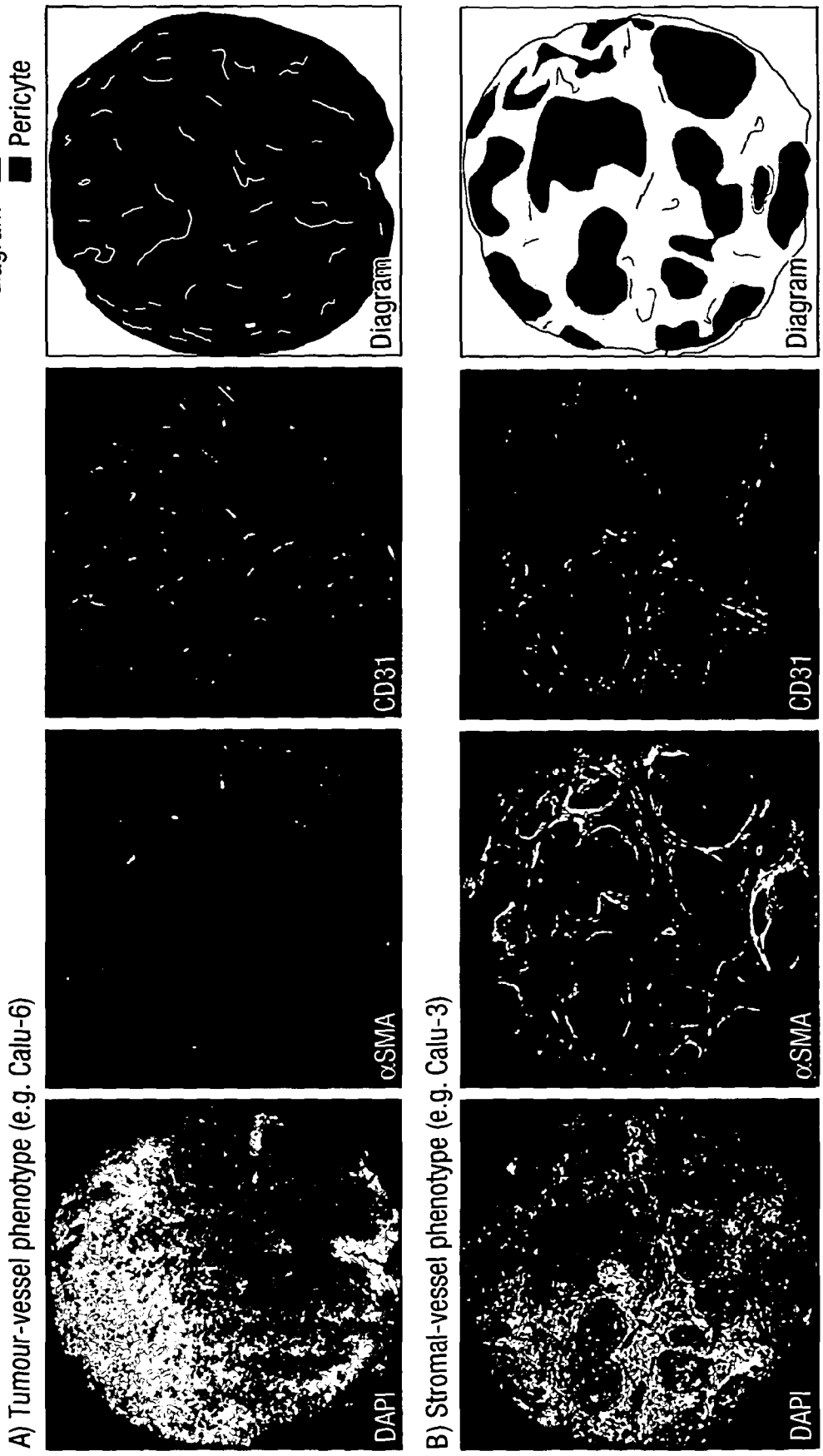
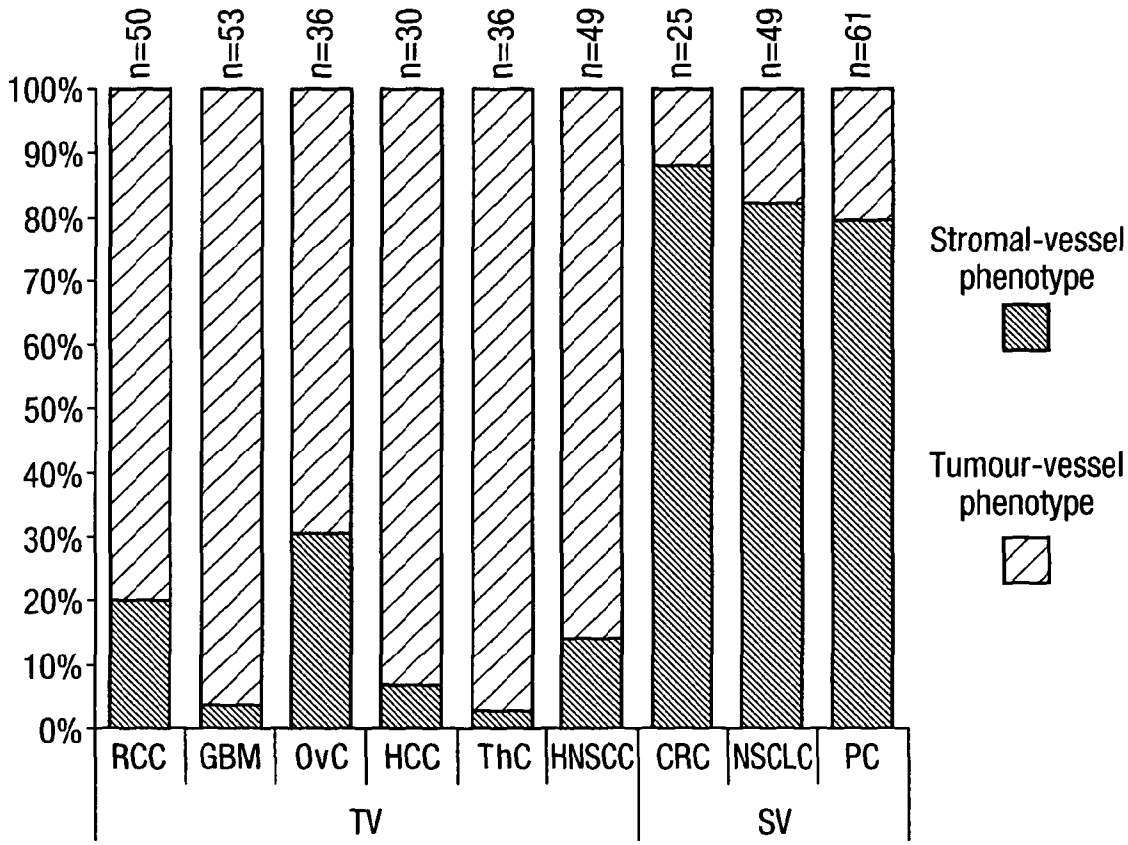

A) Tumour-vessel phenotype (e.g. Calu-6)
B) Stromal-vessel phenotype (e.g. Calu-3)
DAPI
αSMA
CD31
Diagram
Key to diagram
Tumour
Stroma
Vessel
Pericyte

Fig. 2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n=53
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n=30
n=36
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n=25
n=49
n=61
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90%
80%
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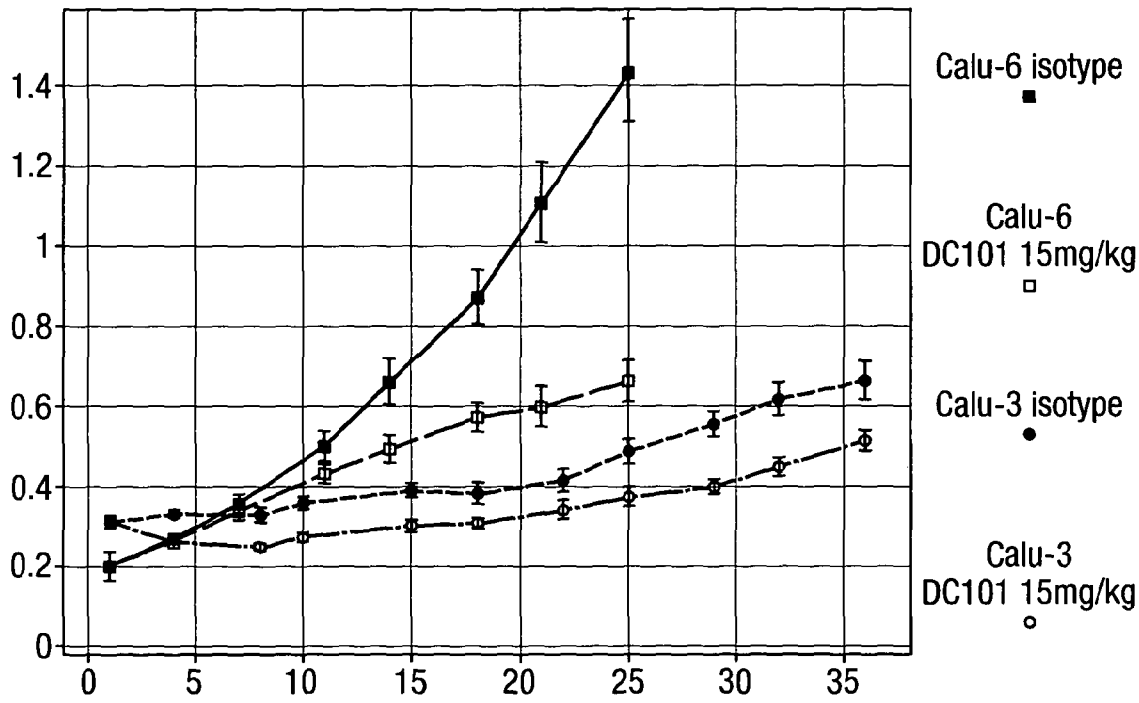
60%
50%
40%
30%
20%
10%
0%
RCC
GBM
OvC
HCC
ThC
HNSCC
CRC
NSCLC
PC
TV
SV
Stromal-vessel phenotype
Tumour-vessel phenotype

Fig. 3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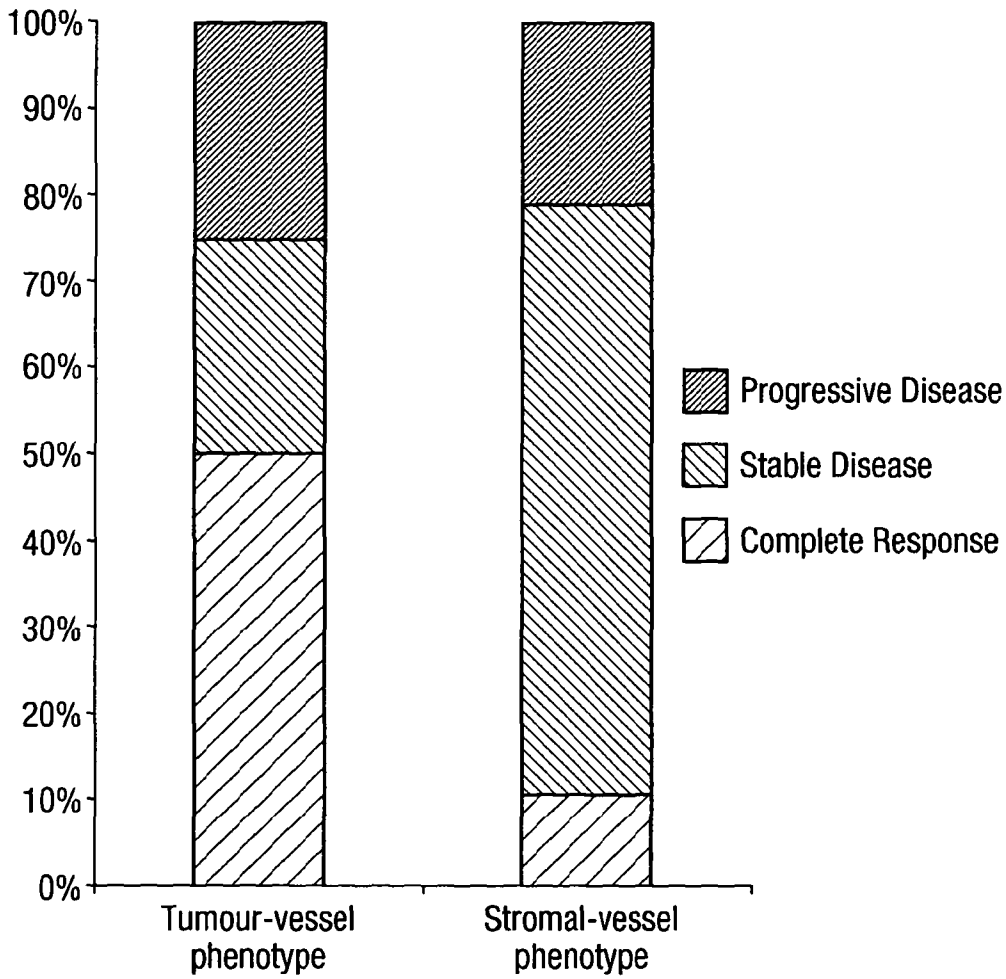
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
Calu-6 isotype
Calu-6
DC101 15mg/kg
Calu-3 isotype
Calu-3
DC101 15mg/kg

Fig. 4


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Tumour-vessel phenotype
Stromal-vessel phenotype
Progressive Disease
Stable Disease
Complete Response

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/052021

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FRIEDMAN, H. S. ET AL: "Bevacizumab alone and in combination with irinotecan in recurrent gliobastoma", JOURNAL OF CLINICAL ONCOLOGY, vol. 27, no. 28, 1 October 2009 (2009-10-01), pages 4733-4740, XP002665469, cited in the application	18-20
A	page 4733 - page 4734	1-17
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 December 2011	02/01/2012

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/052021

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SENNINO B ET AL: "Cellular source and amount of vascular endothelial growth factor and platelet-derived growth factor in tumors determine response to angiogenesis inhibitors", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 69, no. 10, 15 May 2009 (2009-05-15), pages 4527-4536, XP009154715, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-08-3779	18-20
A	the whole document	1-17

X	LEE SUNYOUNG: "What tumor vessels can tell us", PIGMENT CELL & MELANOMA RESEARCH, WILEY INTERSCIENCE, UNITED STATES, DENMARK, vol. 23, no. 3, 1 June 2010 (2010-06-01), pages 309-311, XP009154708, ISSN: 1755-1471	18-20
A	the whole document	1-17
