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(54) **TREATMENT OF ER-NEGATIVE BREAST CANCER WITH AN PDGF-CC INHIBITOR AND AN ANTI ESTROGEN**

BEHANDLUNG VON ER-NEGATIVEM BRUSTKREBS MIT EINEM PDGF-CC-INHIBITOR UND EINEM ANTIÖSTROGEN

TRAITEMENT D'UN CANCER DU SEIN NÉGATIF POUR ER À L'AIDE D'UN INHIBITEUR DU PDGF-CC ET D'UN ANTI- STROGÈNE

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Description

Field of invention

[0001] The present invention relates to the field of kits-of-parts for use in treatment of cancer, and in particular to the field of kits-of-parts for use in treatment of ER-negative breast cancer.

Background of invention

[0002] In the year 2012, the global incidence of breast cancer alone was 1.7 million new cases. Breast cancer can be subdivided into 5 clinically relevant subtypes: normal-like, luminal A, luminal B, HER2 and basal-like breast cancer.

[0003] The molecular subtype of breast cancer impacts on the recurrence rate and median time to recurrence. Out of all breast cancer patients, women carrying basal-like tumors have the highest recurrence rate (34% vs 20% for all other subtypes) and the shortest median time to recurrence (2.6 years vs 5 years for all other subtypes). Thus, the prognosis for women carrying basal-like breast cancers is the worst among all subtypes and the only therapeutic option offered today is high-dose chemotherapy; a treatment regimen that is accompanied by severe side effects. Endocrine therapy which is associated with mild side effects compared to high-dose chemotherapy is not effective against basal-like tumors.

[0004] Women diagnosed with breast cancers characterized by the absence of expression of the estrogen receptor (ER-negative breast cancer) are not treated with anti-hormonal agents, because such therapies have proven not effective for ER-negative breast cancers. For example a meta-analysis conducted by Early Breast Cancer Trialists' Collaborative Group (EBCTCG) published in the Lancet 2011 (doi: 10.1093/annonc/mds194) concludes that in ER-negative disease, tamoxifen had little or no effect on breast cancer recurrence or mortality. Similarly, several other studies have shown that adjuvant treatment with anti-estrogens is not effective in treatment of triple-negative breast cancer (see e.g. Foulkes et al. (doi: 10.1056/NEJMra1001389); Joensuu et al. (doi: 10.1093/annonc/mds194); Baselga et al. (doi: 10.1200/JCO.2012.46.2408); Clifford et al. (doi: 10.1634/theoncologist.2011-S1-01); Liedtke et al., (doi: 10.1200/JCO.2007.14.4147)).

[0005] Pedersen et al., International Journal of Oncology, vol. 45, 2013, p. 2167-2175 describes that sorafenib and nilotinib resensitize tamoxifen resistant breast cancer cells to tamoxifen treatment via estrogen receptor α.

[0006] Weng et al., Molecular Cancer Therapeutics, vol. 7, no. 4, 2008, p. 800-808 describes sensitizing estrogen receptor-negative breast cancer cells to tamoxifen with OSU-03012.

[0007] Mundhenke et al., Journal of Clinical Oncology, vol. 26, 2008, p. 1 describes the effects of tamoxifen and imatinib on the radiosensitivity of breast carcinoma cells.

[0008] WO2013/160359 discloses anti-PDGF-C antibodies as well as applications of such antibodies, for example for use in treatment of cancer.

[0009] Kaygusuz-Atagunduz et al., J Cancer Research and Therapeutics, vol. 10, issue 4, 2014, p. 1107-1108 is a case report disclosing newly diagnosed breast cancer in a patient receiving imatinib mesylate.

[0010] Scandlyn et al., British Journal of Cancer, vol 99., 2008, p. 1056-1063 discloses a new role for tamoxifen in oestrogen receptor-negative breast cancer when it is combined with epigallocatechin gallate.

Summary of invention

[0011] Accordingly, there is a great need for improved treatment of ER-negative breast cancer. Any references in the description to methods of treatment refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human (or animal) body by therapy (or for diagnosis).

[0012] Interestingly, the present invention discloses that ER-negative breast cancers can be converted into ER-positive breast cancers, such as to a breast cancer of luminal-like phenotype by treatment with anti-PDGF-CC antibodies. ER-positive breast cancers, including luminal-like breast cancers can be treated with anti-estrogen treatment. On this basis the invention discloses that surprisingly, ER-negative breast cancers can be treated with anti-estrogen treatment, if the treatment is combined with treatment with anti-PDGF-CC antibodies. Said treatment may for example be an adjuvant treatment, for example a treatment aiming at reducing the risk of relapse of a breast cancer after removal of the primary tumor by surgery.

[0013] Accordingly, the present invention provides kits-of-parts comprising an anti-PDGF-CC antibody and an anti-estrogen for use in the treatment of ER-negative breast cancer in an individual in need thereof.

[0014] The invention also provides kit-of-parts comprising an inhibitor of PDGF-R, wherein said inhibitor of PDGF-R is imatinib and an anti-estrogen for use in the treatment of ER-negative breast cancer in an individual in need thereof.

[0015] The disclosure also provides methods for treatment of ER-negative breast cancer in an individual in need thereof, said method comprising administering an anti-PDGF-CC antibody and an anti-estrogen to said individual either simultaneously or sequentially in any order, thereby treating the ER-negative breast cancer.

[0016] The disclosure also provides methods for treatment of ER-negative breast cancer in an individual in need thereof, said method comprising administering an inhibitor of PDGF-R and an anti-estrogen to said individual either simultaneously or sequentially in any order, thereby treating the ER-negative breast cancer.

[0017] The disclosure also provides methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment, said method comprising administering an anti-PDGF-CC to an individual suffering from ER-negative breast, thereby sensitizing said ER-negative breast cancer to anti-estrogen treatment.

[0018] The disclosure also provides methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment, said method comprising administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast, thereby sensitizing said ER-negative breast cancer to anti-estrogen treatment.

[0019] The disclosure also provides methods of converting an ER-negative breast cancer to an ER-positive breast cancer, said method comprising administering an anti-PDGF-CC to an individual suffering from ER-negative breast, thereby converting said ER-negative breast cancer to an ER-positive breast cancer.

[0020] The disclosure also provides methods of reducing the risk of relapse of an ER-negative breast cancer in an individual having suffered from ER-negative breast cancer, wherein said breast cancer in said individual has been treated by surgery, said method comprising

a. Sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

[0021] The disclosure also provides methods of reducing the risk of relapse of an ER-negative breast cancer in an individual having suffered from ER-negative breast cancer, wherein said breast cancer in said individual has been treated by surgery, said method comprising

a. sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby reducing the risk of relapse of said ER-negative breast cancer.

[0022] The invention also provides kits-of-parts comprising an anti-PDGF-CC antibody and an anti-estrogen for use in above-mentioned methods.

Description of drawings

[0023]

Figure 1 shows expression of PDGF-CC. Panel A and B shows expression in normal breast tissue. Panels C to F shows expression in breast tumors. Panel G shows expression correlated to clinicopathological parameters. Panel H shows months survival in patients having moderate to high expression of PDGF-C compared to PDGF-C negative patients. Panel I shows expression of PDGFR.

Figure 2. Panel A shows PDGF-CC, PDGFRα and PDGFRβ expression in tumors of MMTV-PyMT mice. Panel B shows tumor volume of mammary tumors of MMTV-PyMT mice. Panel C-D shows tumor latency and survival. Panel E-F shows tumor stage and necrosis. Panel G shows pulmonary metastases. Panel H shows tumor volume after transplantation. Panel I shows tumor volume after injection.

Figure 3. Panel A shows Masson tri-chrome staining of tumor sections. Panel B shows that MMTV-PyMT; *Pdgf-c*$^{lacz/lacz}$ mice were severely hemorrhagic. Panel C shows immunostaining for HIF-1α. Panel D shows expression of VEGF-A as determined by quantitative PCR analysis. Panels E-F show tumor volume and blood vessel density in SCID mice bearing orthotopically implanted MDA-MB-231 tumors and treated with A3B6 antibody or with control antibody.

Figure 4. Panel A and B show expression of Foxa1; lacZ/lacZ is equivalent of PDGFC - /-. Panel C shows that expression of *Foxa1* is highly correlated with a non-basal-like molecular subtype based on transcriptional profiles of breast tumors collected within The Cancer Genome Atlas project. Panel D shows expression of *Foxa1* as a specific feature of tumors of the luminal subtype. Panel E shows immunostaining of a cohort of human breast tumor specimens for Foxa1. Panel F shows expression of PDGF-CC in breast tumor cell lines. Panel G shows that expression of Foxa1 is inversely correlated to expression of PDGF-CC.

Figure 5. Panel A shows expression of FoxA1 and ERα in tumor protein lysates. Panel B shows expression of stanniocalcin (STC)-1, hepatocyte growth factor (HGF) and insulin growth factor binding protein 3 (IGFBP3) as determined by quantitative PCR. Panels C to E shows expression of the luminal-like subtype markers FoxA1, ERα and GATA3, respectively after stimulation with STC-1, HGF and/or IGFBP3. Panel F shows sensitivity to tamoxifen-induced growth arrest. Panel G shows that conditioned medium from stromal fibroblasts can be substituted for the three paracrine PDGF-CC-induced factors. Panel H shows immunostaining of tumors from MMTV-PyMT mice for STC-1, HGF and IGFBP3.

Figure 6. Panel A shows tumor growth of wt tumors of tamoxifen-treated mice and untreated mice. Panel B shows tumor growth of tumors from Pdgfc-deficient mice upon treatment with tamoxifen. Panel C shows tumor growth of fully established MDA-MB-231 tumors after treatment with tamoxifen. Panel D shows tumor growth of fully established MDA-MB-231 tumors after combined treatment with anti-PDGF-CC antibody A3B6 and tamoxifen. Panel E shows expression of ERα after treatment with monoclonal anti-PDGF-CC antibody A3B6. Panel F shows ERα expression in MDA-MB-231 tumors following blockade of signaling by PDGF-CC. Panel G shows a theoretical model of a paracrine signaling network in breast tumor microenvironment.

Figure 7. Tumor growth in mice treated with anti-PDGF-CC antibody, Letrozole, a combination of anti-PDGF-CC antibody and Letrozole, and control.

Figure 8. Tumor growth in mice treated with Imatinib, Tamoxifen, a combination of Imatinib and Tamoxifen, control.

Detailed description of the invention

Definitions

[0024] The term "antibody" as used herein is a polypeptide or protein capable of recognizing and binding an antigen comprising at least one antigen binding site. Said antigen binding site preferably comprises at least one CDR. The antibody may be a naturally occurring antibody, a fragment of a naturally occurring antibody or a synthetic antibody.

[0025] The term "antigen" as used herein refers to a molecule comprising at least one epitope. The antigen may for example be a polypeptide, polysaccharide, protein, lipoprotein or glycoprotein.

[0026] The term "Basal-like breast cancer" as used herein refers to a breast cancer of a triple-negative phenotype, i.e. said cancer does not express estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER)-2 at detectable levels. Furthermore, basal-type breast cancer typically does not express FoxA1. Basal-like breast cancer is associated with high grade, poor prognosis, and younger patient age.

[0027] The term "epitope" as used herein refers to a determinant capable of specific binding to an antibody. Within the present invention the epitope may be comprised within PDGF-C or PDGF-CC. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Epitopes may be conformational or nonconformational, wherein binding to the former but not the latter is lost in the presence of denaturing solvents. Epitopes may be continous or discontinuous, wherein a discontinous epitope is a conformational epitope on a protein antigen which is formed from at least two separate regions in the primary sequence of the protein.

[0028] The term "ER-negative breast cancer" refers to a breast cancer lacking expression of the estrogen receptor. A breast cancer is considered an ER-negative breast cancer, when =<10% of the tumor cells of said breast cancer express estrogen receptor at levels detectable by immunohistochemistry. Preferably, an ER-negative breast cancer is a breast cancer, where <1 % of the tumor cells of said breast cancer express estrogen receptor at levels detectable by immunohistochemistry.

[0029] The term "ER-positive breast cancer" refers to a breast cancer expressing the estrogen receptor. A breast cancer is considered an ER-positive breast cancer, when >10% of the tumor cells of said breast cancer express estrogen receptor at levels detectable by immunohistochemistry.

[0030] The term "luminal-like breast cancer" refers to a breast cancer which is responsive to anti-estrogen therapy. In particular, a "luminal-like breast cancer" expresses the estrogen receptor (ER). A "lumical-like breast cancer" according to the present invention may be true luminal breast cancer, such as a luminal A or luminal B breast cancer.

[0031] The term "naturally occurring antibody" as used herein refers to heterotetrameric glycoproteins capable of recognizing and binding an antigen and comprising two identical heavy (H) chains and two identical light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region (abbreviated herein as C_H). Each light chain comprises a light chain variable region (abbreviated herein as V_L) and a light chain constant region (abbreviated herein as C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed

with regions that are more conserved, termed framework regions (FRs). Antibodies may comprise several identical heterotetramers.

[0032] The term "treatment" as used herein may refer to any kind of treatment. The treatment may be a curative treatment, it may also be an ameliorating treatment and/or a treatment reducing the effects of the cancer. The treatment may also be a treatment which delays progression of the cancer, for example the treatment may reduce the growth of cancer, reduce metastasis or in other ways delay cancer progression. The treatment may also be a treatment to reduce the risk of relapse.

Method of treatment

[0033] The present invention provides kits-of-parts for use in methods for treatment of ER-negative breast cancers in an individual in need thereof. The methods comprise administering an anti-PDGF-CC antibody and an anti-estrogen to an individual suffering from ER-negative breast cancer either simultaneously or sequentially in any order, thereby treating the ER-negative breast cancer.

[0034] The present disclosure also provides kit-of-parts for use treatment of ER-negative breast cancers in an individual in need thereof, the methods comprising administering an inhibitor of PDGF-R and an anti-estrogen to an individual suffering from ER-negative breast cancer either simultaneously or sequentially in any order, thereby treating the ER-negative breast cancer.

[0035] The method of treatment according to the invention may be combined with one or more conventional methods for treatment of ER-negative breast cancer. Thus, the methods of the disclosure may comprise a combination of treatment with an anti-PDGF-CC antibody and an anti-estrogen combined with one or more additional methods.. The methods of the invention may also comprise a combination of treatment with an inhibitor of PDGF-R and an anti-estrogen combined with one or more additional methods. For example, said ER-negative breast cancer may be treated by a method selected from the group consisting of surgery, irradiation and chemotherapy. In particular the individual to be treated with the methods of the invention may be an individual suffering from ER-negative breast cancer, wherein said individual has already been subjected to treatment of said breast cancer by surgery. Thus, the individual to be treated may be an individual who has suffered from ER-negative breast cancer, wherein the primary tumor has been removed by surgery. In such cases, the treatment of the present invention can frequently be considered an adjuvant therapy, which reduces the risk of relapse. In particular, the treatment may be a treatment to reduce the risk of relapse within 5 years from onset of the treatment. For example, the treatment may be treatment to prevent relapse within 5 years from the onset of treatment. Preferably, the treatment with anti-PDGF-CC antibodies and anti-estrogen is initiated at the latest 1 months after surgery, for example at the latest one week after surgery. Treatment may be initiated earlier, for example even prior to surgery. Similarly, the treatment with inhibitors of PDGF-R and anti-estrogen is initiated at the latest 1 month after surgery, for example at the latest one week after surgery. Treatment may be initiated earlier, for example even prior to surgery.

[0036] It is also comprised within the present invention that the individual to be treated with anti-PDGF-CC antibodies and anti-estrogen, or the individual to be treated with inhibitors of PDGF-R and anti-estrogen have not been subjected to surgery. This may be because the particular breast cancer is an inoperable breast cancer, a breast cancer less suitable for removal by surgery or because the individual has not yet undergone surgery. Such treatment may for example be a neoadjuvant treatment.

[0037] The anti-PDGF-CC antibody may be any antibody capable of binding PDGF-CC, for example any of the antibodies described herein below in the section "anti-PDGF-CC antibody". The anti-estrogen may be any compound having an anti-estrogen effect, for example any of the compounds described herein below in the section anti-estrogen.

[0038] The disclosure also provides methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment. ER-negative breast cancers are not responsive to anti-estrogen treatment (see e.g. Early Breast Cancer Trialists' Collaborative Group (EBCTCG) doi: 10.1016/S0140-6736(11)60993-8), but the invention interestingly discloses that ER-negative breast cancer can be sensitized to treatment with anti-estrogen by treatment with anti-PDGF-CC antibodies.

[0039] Thus, the disclosure provides methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment, said methods comprising administering an anti-PDGF-CC to an individual suffering from ER-negative breast cancer, thereby sensitizing said ER-negative breast cancer to anti-estrogen treatment.

[0040] The disclosure also provides methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment, said methods comprising administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer, thereby sensitizing said ER-negative breast cancer to anti-estrogen treatment.

[0041] In particular, the disclosure may provide methods for sensitizing an ER-negative breast cancer to anti-estrogen treatment in an individual,

wherein said individual has suffered from ER-negative breast cancer, and wherein said breast cancer in said individual has been treated by surgery,

wherein following anti-estrogen treatment in said individual, no relapse is observed or the risk of relapse is significantly reduced.

[0042] A breast cancer sensitized to anti-estrogen treatment may thus be treated with anti-estrogen. The methods may thus comprise a step of administering an anti-estrogen to the individual suffering from ER-negative breast cancer, wherein said anti-PDGF-CC antibody and said anti-estrogen may be administered simultaneously or sequentially in any order.

[0043] Thus, the disclosure also provides methods of treatment of ER-negative breast cancer in an individual in need thereof, said method comprising

a. Sensitizing an ER-negative breast cancer to treatment with anti-estrogen by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby treating said ER-negative breast cancer.

[0044] The disclosure also provides methods of treatment of ER-negative breast cancer in an individual in need thereof, said method comprising

a. Sensitizing an ER-negative breast cancer to treatment with anti-estrogen by administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby treating said ER-negative breast cancer.

[0045] The disclosure also provides methods of treatment of ER-negative breast cancer in an individual, wherein said individual has suffered from ER-negative breast cancer, and wherein said breast cancer in said individual has been treated by surgery, said method comprising

a. Sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby reducing the risk of relapse of said ER-negative breast cancer.

[0046] The disclosure also provides methods of treatment of ER-negative breast cancer in an individual, wherein said individual has suffered from ER-negative breast cancer, and wherein said breast cancer in said individual has been treated by surgery, said method comprising

a. Sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby reducing the risk of relapse of said ER-negative breast cancer.

[0047] The disclosure also provides methods of treatment of ER-negative breast cancer in an individual in need thereof, said method comprising

a. Sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer;
b. Treatment of said ER-negative breast cancer by surgery
c. Sensitizing remaining ER-negative breast cancer to treatment with anti-estrogen by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer
d. administering an anti-estrogen to said individual

thereby reducing the risk of relapse of said ER-negative breast cancer.

[0048] The disclosure also provides methods of treatment of ER-negative breast cancer in an individual in need thereof, said method comprising

a. Sensitizing the ER-negative breast cancer to treatment with anti-estrogen by administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer;
b. Treatment of said ER-negative breast cancer by surgery

c. Sensitizing remaining ER-negative breast cancer to treatment with anti-estrogen by administering an inhibitor of PDGF-R to an individual suffering from ER-negative breast cancer
d. administering an anti-estrogen to said individual

thereby reducing the risk of relapse of said ER-negative breast cancer.

[0049] The invention also provides methods of converting an ER-negative breast cancer to an ER-positive breast cancer. Such methods comprise administering an anti-PDGF-CC to an individual suffering from ER-negative breast cancer, thereby converting said ER-negative breast cancer to an ER-positive breast cancer.

[0050] The invention also provides methods of converting an ER-negative breast cancer to a luminal-like breast cancer. Such methods comprise administering an anti-PDGF-CC to an individual suffering from ER-negative breast cancer, thereby converting said ER-negative breast cancer to a luminal-like breast cancer.

[0051] A breast cancer is considered to be a luminal-like breast cancer, when said cancer is expressing the estrogen receptor (ER) at detectable levels. It is preferred that at least 1%, such as at least 10% of the breast cancer cells of said breast cancer are expressing ER at detectable levels.

[0052] In some embodiments the method may comprise an additional step of testing whether the breast cancer has been converted to a luminal-like breast cancer and/or to an ER-positive breast cancer. Said test may be performed subsequent to administration of said anti-PDGF-CC antibody and may in general comprise the steps of:

a) obtaining a sample from said breast cancer
b) testing expression of estrogen receptor (ER) in said sample
c) wherein detectable expression of ER in said sample is indicative of that said breast cancer has been converted to a luminal-like breast cancer or an ER-positive breast cancer.

[0053] Said test may be any test useful for determining whether a breast cancer expresses ER. In one embodiment the test is an immunohistochemical test, for example a test, wherein step b) involves staining the sample obtained in step a) with the aid of antibodies recognizing ER, and followed by detection of ER expression e.g. by microscopy. If a larger percentage of cells express ER than in the initial ER-negative tumor (e.g. if more than 1% of the tumor cells express ER) then ER may be considered expressed. Preferably, if at least 10% of tumor cells of said sample expressed ER, then the breast cancer is considered ER -positive.

[0054] Thus, the invention provides kits-of-parts for use in methods of treatment of ER-negative breast cancer in an individual in need thereof, said method comprising

a. converting an ER-negative breast cancer to a luminal-like breast cancer by administering an anti-PDGF-CC antibody to an individual suffering from ER-negative breast cancer;
b. administering an anti-estrogen to said individual

thereby treating said ER-negative breast cancer.

[0055] The methods may comprise the steps of administering an anti-PDGF-CC antibody and an anti-estrogen. Said anti-PDGF-CC antibody and said anti-estrogen may be administered simultaneously or sequentially in any order.

[0056] The methods may in alternative comprise the steps of administering an inhibitor of PDGF-R and an anti-estrogen. Said inhibitor of PDGF-R and said anti-estrogen may be administered simultaneously or sequentially in any order.

[0057] The invention also provides kits-of-parts comprising

a) an anti-PDGF-CC antibody and an anti-estrogen, or
b) an inhibitor of PDGF-R and an anti-estrogen

for treatment of ER-negative breast cancer in an individual, wherein said individual has suffered from ER-negative breast cancer, and wherein said breast cancer in said individual has been treated by surgery, and wherein said treatment reduces the risk of relapse.

[0058] In some embodiments of the invention said anti-PDGF-CC antibody or said inhibitor of PDGF-R is administered to said individual simultaneously with administration of said anti-estrogen.

[0059] In some embodiments it may however be preferred that the anti-PDGF-CC antibody or the inhibitor of PDGF-R is administered to said individual prior to administration of said anti-estrogen. Such an order of administration may ensure that the ER-negative breast cancer is sensitized to anti-estrogen prior to administration of said anti-estrogen.

[0060] In some embodiments of the invention the anti-PDGF-CC antibody or the inhibitor of PDGF-R is administered more than once, for example it may be administered at least twice, such as at least 3 times, for example in the range of 1 to 20 times, such as in the range of 2 to 10 times.

[0061] In one embodiment, the anti-PDGF-CC antibody or the inhibitor of PDGF-R is administered at least twice to

an individual suffering from ER-negative breast cancer, wherein one or more administrations are prior to treatment by surgery, and one or more additional administrations are administered post treatment by surgery. The administration(s) after surgery may be simultaneous with anti-estrogen treatment.

[0062] Similarly, anti-estrogen may be administered more than once. Many anti-estrogens are administered over an extended period of time, for example once daily, twice daily or even more frequently for an extended period of time. Anti-estrogens may also be administered less frequently, e.g. in the range of 1 to 6 times per week, or for example in the range of 1 to 4 times per months. Thus, administration of an anti-estrogen may be very frequent over an extended period of time, for example for at least 1 month, such as for at least 6 months, for example for at least 1 year, such as for several years. For example the anti-estrogen treatment may be once daily for at least 1 year, for example of in the range 1 to 10 years, such as in the range of 4 to 6 years, such as for 5 years. In such embodiments, the first administration of anti-estrogen may be simultaneous with at least one administration of anti-PDGF-CC antibodies or inhibitors of PDGF-R, whereas subsequent administrations may be performed individually. It is also possible that each administration of anti-PDGF-CC antibody or inhibitors of PDGF-R is performed simultaneously with an administration of anti-estrogen, but that anti-estrogens in addition are administered separately.

[0063] In one embodiment of the invention the anti-PDGF-CC antibody or the inhibitor of PDGF-R is administered at least once prior to the first administration of anti-estrogen. Thus, the first dosage of said anti-estrogen may be administered in the range of 1 hours to several weeks after the first administration of said anti-PDGF-CC antibody or of said inhibitor of PDGF-R.

[0064] Typically, the anti-estrogen is administered for a longer time than the anti-PDGF-CC antibody or the inhibitor of PDGF-R. Thus, the anti-PDGF-CC antibody or the inhibitor of PDGF-R may for example be administered at the onset of treatment, whereas the anti-estrogen typically may be administered continuously for a longer time period. Thus, as described above, the anti-PDGF-CC antibody or the inhibitor of PDGF-R may for example be administered in the range of 1 to 5 times, whereas the anti-estrogen typically may be administered continuously for in the range of 1 to 10 years, such as in the range of 4 to 6 years, such as for 5 years. The last administration of anti-estrogen is preferably given later than the last administration of anti-PDGF-CC antibody or inhibitor of PDGF-R.

[0065] The route of administration may be chosen according to the particular anti-PDGF-CC antibody, inhibitor of PDGF-R and the anti-estrogen. Frequently, the anti-PDGF-CC antibody or the inhibitor of PDGF-R is administered parenterally. Inhibitors of PDGF-R can be administered parenterally, for example as intravenous formulation, and also enetrally, for example orally in the form of tablets. Methods and useful formulations for parenteral administration are described below in the section "Pharmaceutical formulation". The anti-estrogen may be administered by any useful route, which may be chosen according to the particular anti-estrogen used. Frequently, the anti-estrogen is administered orally. Methods and useful formulations for oral administration are described below in the section "Pharmaceutical formulation".

[0066] The individual to be treated may be any individual suffering from ER-negative breast cancer. Frequently, the individual will be a human being, for example a male or a female human being. Preferably, the individual is a female human being.

[0067] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist as described in the section below "Anti-estrogen".

[0068] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist selected from the group consisting of tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY1 17018, fulvestrant and toremifene.

[0069] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an estrogen antagonist, wherein the estrogen antagonist is tamoxifen.

[0070] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an aromatase inhibitor as described in the section below "Anti-estrogen".

[0071] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an aromatase inhibitor selected from the group consisting of exemestane, formestane, aminoglutethimide, vorozole, fadrozole, anastrozole and letrozole.

[0072] In some embodiments the methods of the present disclosure comprise administration of an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is letrozole.

[0073] In some embodiments the methods of the present disclosure comprise administration of an inhibitor of PDGF-R and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist as described in the section below "Anti-estrogen".

[0074] In some embodiments the methods of the present disclosure comprise administration of an inhibitor of PDGF-R and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist selected from the group consisting of tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY1 17018, fulvestrant and toremifene.

[0075] In some embodiments the methods of the present disclosure comprise administration of an inhibitor of PDGF-

R and an anti-estrogen, wherein the anti-estrogen is tamoxifen.

Kit-of-parts

[0076] The invention provides a kit-of-parts comprising an anti-PDGF-CC antibody and an anti-estrogen. The invention also provides a kit-of-parts comprising an inhibitor of PDGF-R and an anti-estrogen. The kit-of-part may in particular be for the treatment of ER-negative breast cancer in an individual in need thereof. Thus, the kit-of-parts may be prepared for use in any of the methods of treatment, described herein above in the section "Method of treatment".

[0077] The kit-of-parts may be provided as separate units, i.e. one or more units comprising an anti-PDGF-CC antibody and one or more units comprising an anti-estrogen, wherein the units are separately provided. Alternatively, the kit-of-parts may comprise one or more units comprising an inhibitor of PDGF-R and one or more units comprising an anti-estrogen, wherein the units are separately provided.

[0078] Thus, the kit-of-parts may be prepared for sequential administration, wherein each part of the kit-of-part are provided and administered separately. Thus, the anti-PDGF-CC antibody and the anti-estrogen may be prepared for sequential administration. Also, the inhibitor of PDGF-R and the anti-estrogen may be prepared for sequential administration. It is comprised within the invention that said kit-of-part is prepared for administration according to any of the methods described above in the section "Method of treatment". In particular, the kit-of-parts may be prepared for treatment of ER-negative breast cancer, wherein said treatment comprises the steps of

a. administration of the anti-PDGF-CC antibody to an individual in need thereof;
b. subsequent administration of the anti-estrogen.

[0079] Alternatively, the kit-of-parts may be prepared for treatment of ER-negative breast cancer, wherein said treatment comprises the steps of

a. administration of the inhibitor of PDGF-R to an individual in need thereof;
b. subsequent administration of the anti-estrogen.

[0080] Frequently, the anti-PDGF-CC antibody or the inhibitor of PDGF-R is prepared for parenteral administration for a limited number of times. E.g. the anti-PDGF-CC antibody or the inhibitor of PDGF-R may be prepared for parenteral administration as described herein above in the section "Method of treatment". The inhibitor of PDGF-R is also frequently prepared for oral administration, for example in the form of tablets. In contrast the anti-estrogen may be prepared by administration by any means. Thus, for example the anti-estrogen may be prepared for frequent oral administration as described herein above in the section "Method of treatment".

[0081] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist as described in the section below "Anti-estrogen".

[0082] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist selected from the group consisting of tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY1 17018, fulvestrant and toremifene.

[0083] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is tamoxifen.

[0084] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an aromatase inhibitor as described in the section below "Anti-estrogen".

[0085] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is an aromatase inhibitor selected from the group consisting of exemestane, formestane, aminoglutethimide, vorozole, fadrozole, anastrozole and letrozole.

[0086] In some embodiments the kit-of-parts of the present disclosure comprises an anti-PDGF-CC antibody and an anti-estrogen, wherein the anti-estrogen is letrozole

[0087] In some embodiments the kit-of-parts of the present disclosure comprises an inhibitor of PDGF-R and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist as described in the section below "Anti-estrogen".

[0088] In some embodiments the kit-of-parts of the present disclosure comprises an inhibitor of PDGF-R and an anti-estrogen, wherein the anti-estrogen is an estrogen antagonist selected from the group consisting of tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY1 17018, fulvestrant and toremifene.

[0089] In some embodiments the kit-of-parts of the present disclosure comprises an inhibitor of PDGF-R and an anti-estrogen, wherein the anti-estrogen is tamoxifen.

Anti-PDGF-CC antibody

[0090] The present invention relates to a kit-of-part comprising an anti-PDGF-CC antibody as well as to methods of treatment employing an anti-PDGF-CC antibody. Said anti-PDGF-CC antibody may be any antibody capable of binding PDGF-CC, in particular it may be an antibody specifically binding PDGF-CC. PDGF-CC is described in detail in the section "PDGF-CC" herein below. Since PDGF-CC is a dimer of PDGF-C, and accordingly, the anti-PDGF-CC antibody may specifically bind both PDGF-CC and PDGF-C.

[0091] The present invention relates also to a kit-of-part comprising an inhibitor of PDGF-R as well as to methods of treatment employing an inhibitor of PDGF-R. However, use of an anti-PDGF-CC antibody is preferred as the antibody targets with great specificity PDGF-CC, but not other members of the PDGF family and therefore side effects are minimal. Inhibitors of PDGF-R are also effective in blocking the PDGF-R signaling pathways, but they act non-specifically on all the PDGF-R and may so result in undesired effects. However, such undesired effects are minimized when the inhibitor of PDGF-R is an antibody against PDGF-R.

[0092] The anti-PDGF-CC antibodies may bind to any PDGF-CC. However, in general it is preferred that the anti-PDGF-CC antibodies to be used are capable of binding PDGF-CC of the individual to be treated. Accordingly, in embodiments of the invention where the individual is a human being, then it is preferred that the anti-PDGF-CC antibodies are capable of binding human PDGF-CC. The sequence of human PDGF-C is provided as SEQ ID NO:1 herein.

[0093] The anti-PDGF-CC antibody according to the present invention may be any polypeptide or protein capable of recognizing and binding PDGF-CC. By the term "specifically binding" is meant binding with at least 10 times higher affinity to PDGF-CC than to a non-specific antigen (e.g. BSA). Typically, the antibody binds with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, for example about 10^{-10} M or less, when measured as apparent affinities based on IC_{50} values.

[0094] In one embodiment the anti-PDGF-CC antibody specifically binds PDGF-CC and optionally also PDGF-C, but not any other PDGF.

[0095] In one embodiment said anti-PDGF-CC antibody is a naturally occurring antibody or a functional homologue thereof. A naturally occurring antibody is a heterotetrameric glycoproteins capable of recognizing and binding an antigen comprising two identical heavy (H) chains and two identical light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises or preferably consists of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region (abbreviated herein as C_H). Each light chain comprises or preferably consists a light chain variable region (abbreviated herein as V_L) and a light chain constant region (abbreviated herein as C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each V_H and V_L comprises and preferably consists of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0096] Naturally occurring antibodies according to the invention may consist of one heterotetramer or they may comprise several identical heterotetramers. Thus, the naturally occurring antibody according to the invention may for example be selected from the group consisting of IgG, IgM, IgA, IgD and IgE. The subunit structures and three-dimensional configurations of these different classes of immunoglobulins are well known. In a preferred embodiment of the invention the antibody is IgG, e.g. IgG-1, IgG-2, IgG-3 and IgG-4.

[0097] Naturally occurring antibodies according to the invention may be antibodies of a particular species, for example the antibody may be a murine, a rat, a rabbit, a goat, a sheep, a chicken, a donkey, a camelid or a human antibody. The antibody according to the invention may however also be a hybrid between antibodies from several species, for example the antibody may be a chimeric antibody, such as a humanized antibody.

[0098] It is not always desirable to use non-human antibodies for human therapy, accordingly the anti-PDGF-CC antibody according to the invention may be a human antibody or a humanized antibody, e.g. a naturally occurring human antibody.

[0099] The anti-PDGF-CC antibody according to the invention may be a human immunoglobulin or a humanized immunoglobulin, e.g. a naturally occurring human immunoglobulin.

[0100] A human antibody as used herein is an antibody, which is obtained from a system using human immunoglobulin sequences. Human antibodies may for example be antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom. Human antibodies may also be isolated from a host cell transformed to express the antibody, e.g., from a transfectoma. Human antibodies may also be isolated from a recombinant, combinatorial human antibody library.

[0101] Human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis or *in vivo* somatic mutagenesis and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0102] A human antibody is preferably at least 90%, more preferably at least 95%, even more preferably at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by a wild type human immunoglobulin gene.

[0103] Said transgenic of transchromosomal animal may contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy (μ and/or γ) and κ light chain immunoglobulin sequences. Furthermore, the animal may contain one or more mutations that inactivate the endogenous heavy and light chain loci. Examples of such animals are described in Lonberg, N. et al. (1994) Nature 368 (6474):856-859 and WO 02/43478.

[0104] The anti-PDGF-CC antibody according to the invention may be a chimeric antibody, i.e. an antibody comprising regions derived from different species. The chimeric antibody may for example comprise variable regions from one species of animal and constant regions from another species of animal. For example, a chimeric antibody can be an antibody having variable regions which derive from a mouse monoclonal antibody and constant regions which are human. Such antibodies may also be referred to as humanized antibodies.

[0105] Thus, the anti-PDGF-CC antibody according to the invention may also be a humanized antibody, which is encoded partly by sequences obtained from human germline immunoglobulin sequences and partly from other sequences. Said other sequences are preferably germline immunoglobulines from other species, more preferably from other mammalian species. In particular a humanized antibody may be an antibody in which the antigen binding site is derived from an immunoglobulin from a non-human species, preferably from a non-human mammal, e.g. from a mouse or a rat, whereas some or all of the remaining immunoglobulin-derived parts of the molecule are derived from a human immunoglobulin. The antigen binding site from said non-human species may for example consist of a complete V_L or V_H or both or one or more CDRs grafted onto appropriate human framework regions in V_L or V_H or both. Thus, in a humanized antibody, the CDRs can be from a mouse or rat monoclonal antibody and the other regions of the antibody are of human origin.

[0106] The anti-PDGF-CC antibody according to the invention may be a monoclonal antibody, such as a naturally occurring monoclonal antibody or it may be polyclonal antibodies, such as naturally occurring polyclonal antibodies.

[0107] The anti-PDGF-CC antibody may be any protein or polypeptide containing an antigen binding site, such as a single polypeptide, a protein or a glycoprotein. Preferably, the antigen binding site comprises at least one CDR, or more preferably a variable region.

[0108] Thus the antigen binding site may comprise a V_H and/or V_L. In an antibody, the V_H and V_L together may contain the antigen binding site, however, either one of the V_H or the V_L may comprise an antigen binding site.

[0109] The anti-PDGF-CC antibody may for example be an antigen binding fragment of antibody, preferably an antigen binding fragment of a naturally occurring antibody, a heterospecific antibody, a single chain antibody or a recombinant antibody.

[0110] An anti-PDGF-CC antibody according to the invention may comprise one or more antigen binding sites. Naturally occurring heterotetrameric antibodies comprises two antigen binding sites.

[0111] As mentioned herein above, the anti-PDGF-CC antibodies to be used with the invention are capable of recognizing and binding PDGF-CC. Thus, in general the anti-PDGF-CC antibodies specifically bind one or more epitopes on PDGF-CC. In embodiments of the invention wherein the antibody is a monoclonal antibody, then the antibody generally binds one epitope on PDGF-CC.

[0112] Said epitope(s) may be positioned in any useful part of PDGF-CC. However, in a preferred embodiment of the invention, the antibodies are inhibitory antibodies, i.e. the antibodies are capable of inhibiting PDGF-CC activity. In particular it may be preferred that the antibodies are capable of inhibiting binding of PDGF-CC to the PDGFRα homodimer and/or to the PDGFRα/β heterodimer. The anti-PDGF-CC antibodies may also be capable of inhibiting activation of the PDGFRα homodimer and/or of the PDGFRα/β heterodimer. Activation of PDGFRα homodimer and/or the PDGFRα/β heterodimer may for example be determined by determining the kinase activity of PDGFRα homodimer and/or to the PDGFRα/β heterodimer.

[0113] In one embodiment it is preferred that the anti-PDGF-CC antibody is capable of inhibiting proteolytic processing of PDGF-CC.

[0114] In one embodiment of the invention said anti-PDGF-CC antibody may be any of the antibodies described in US patent application no. 62/357,536, the priority of which is claimed by international patent application WO2018/005904. Thus, the anti-PDGF-CC may include the entire antibody, a fragment or substantially homologous fragment of the monoclonal antibodies (mAbs) A3B6, 11F5, 19C7 and 12F5, of the chimeric antibody chA3B6 or of the humanized antibody huA3B6 described in US application 62/357,536, the priority of which is claimed by international patent application WO2018/005904. Fragments may include one or a portion of the variable light and heavy chain sequences or CDR regions of A3B6, chA3B6, huA3B6, 10 11F5, 12F5 and 19C7 as described in US application 62/357,536, the priority of which is claimed by international patent application WO2018/005904. The anti-PDGF-CC antibody may in a preferred embodiment be a humanized antibody, in particular the antibody huA3B6 described in US application 62/357,536, the priority of which is claimed by international patent application WO2018/005904.

[0115] In one embodiment, the anti-PDGF-CC antibody may bind one or more epitopes within the PDGF-CC core

active domain, which is provided at residues 230-345 of the full-length sequence, which is provided as SEQ ID NO.: 1.

[0116] Thus, in one embodiment of the invention it is preferred that the anti-PDGF-CC antibody binds an epitope positioned in the region of PDGF-CC, which includes the cleavage site. In human PDGF-C the cleavage site is positioned at amino acids 231 to 234 of SEQ ID NO:1. Accordingly, it is preferred that the anti-PDGF-CC antibody is capable of binding an epitope comprising at least part of an amino acid selected from the group consisting of amino acids 231, 232, 233 and 234 of SEQ ID NO:1. In particular, the anti-PDGF-CC antibody may be capable of binding an epitope comprising at least one of amino acids 231, 232, 233 and 234 of SEQ ID NO:1. In other embodiments the anti-PDGF-CC antibody may be capable of binding an epitope immediately adjacent to the cleavage site thereby inhibiting proteolytic processing of PDGF-C. Thus, in one embodiment the anti-PDGF-CC antibody is capable of binding an epitope positioned within amino acids 230 to 250 of SEQ ID NO:1.

[0117] In one embodiment of the invention the anti-PDGF-CC antibody binds a PDGF-C epitope described in WO2005/087812. For example the anti-PDGF-CC antibody may bind an epitope comprised of amino acids 231 to 274 of SEQ ID NO:1.

[0118] In one embodiment of the invention the anti-PDGF-CC antibody binds a PDGF-C epitope described in WO2007/124308. For example the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 228 to 238 of SEQ ID NO:1.

[0119] In one embodiment of the invention the anti-PDGF-CC antibody binds a PDGF-C epitope described in WO2013/160359. For example the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 308 to 322 of SEQ ID NO:1.

[0120] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 242 to 254 of SEQ ID NO:1.

[0121] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 288 to 308 of SEQ ID NO:1.

[0122] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 325 to 345 of SEQ ID NO:1.

[0123] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 256 to 274 of SEQ ID NO:1.

[0124] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 256 to 264 of SEQ ID NO:1.

[0125] In one embodiment of the invention the anti-PDGF-CC antibody may bind an epitope positioned within, comprising or consisting of amino acids 256 to 260 of SEQ ID NO:1.

PDGF-CC

[0126] Platelet-derived growth factors (PDGFs) are growth factors important for normal tissue growth and maintenance. PDGF-C is secreted from cells as a latent dimer, PDGF-CC. PDGF-CC signals through the PDGFR-α, in particular through the PDGFRα homodimer and/or to the PDGFRα/β heterodimer. Tissue plasminogen activator (tPA) is a secreted serine protease with highly restricted substrate specificity, and tPA cleaves and activates latent dimeric PDGF-CC.

[0127] In preferred embodiments of the invention PDGF-CC is human PDGF-CC. The sequence of human PDGF-C is provided herein as SEQ ID NO:1 and human PDGF-CC is a dimer of two polypeptides of SEQ ID NO:1.

[0128] PDGF-CC may however also be a functional homologue of human PDGF-C, for example a dimer of polypeptides, which each share at least 70%, such as at least 80%, for example at least 85%, such as at least 90%, for example at least 95% sequence identity with SEQ ID NO:1.

[0129] PDGF-CC may thus also be PDGF-CC of other mammals.

Inhibitors of PDGF-R

[0130] The present disclosure relates to a kit-of-part comprising an inhibitor of platelet-derived growth factor receptor (PDGF-R) as well as methods of treatment employing an inhibitor of PDGF-R. The inventors have found that inhibition of the PDGF receptor results in sensitization of ER-negative breast tumor to the action of endocrine therapy.

[0131] PDGF-Rs are cell surface tyrosine kinase receptors for members of the platelet-derived growth factor (PDGF) family. There are two forms of the PDGF-R, alpha (UniProt accession number P16234; SEQ ID NO:8) and beta (UniProt accession number P09619; SEQ ID NO:9) each encoded by a different gene. Depending on which growth factor is bound, PDGF-R may homo- or heterodimerize. The extracellular region of the receptor consists of five immunoglobulin-like domains while the intracellular part is a tyrosine kinase domain. The PDGFs bind the tyrosine kinase domain of PDGF-R alpha or beta and so cause the receptor to dimerize. The different PDGFs interact with different receptor dimers. Dimerization is a prerequisite for the activation of the kinase, which will phosphorylate some critical residues of the receptor itself as well as of the receptor substrates. The phosphorylated residue of the receptor is located in proximity

to usually three specific binding sites for signal transduction molecules, in the extracellular region. The signal transduction molecules may be equipped with different enzymatic activities, or may act as adaptor molecules, which in some but not all cases are found in complexes with subunits that carry a catalytic activity. Upon interaction with the activated receptor, the catalytic activities become up-regulated. The main downstream mediators of the PDGF-R signaling appear to be Ras/mitogen-activated protein kinase (MAPK), PI-3 kinase and phospholipase-γ (PLCγ) pathways. In addition, reactive oxygen species (ROS)-dependent STAT3 activation has been established to be a key downstream mediator of PDGF-R signaling in vascular smooth muscle cells.

[0132] Expression of both receptors and each of the four PDGFs is under independent control, which gives the PDGF/PDGF-R system a high flexibility. Different cell types vary greatly in the ratio of PDGF isoforms and PDGF-Rs expressed.

[0133] The inventors have found that by using inhibitors of PDGF-R, a result similar to that obtained by using anti-PDGF-CC antibodies is obtained, however the mechanism behind the treatment is different.

[0134] The inhibitor of PDGF-R, or a variant thereof, according to the present disclosure may be any compound capable of interacting with the PDGF-R and blocking its tyrosine kinase activity. For example, the inhibitor of PDGF-R may bind and specifically occupy the tyrosine kinase site of PDGF-R.

[0135] Throughout the present disclosure, the term "PDGF-R" refers to both PDGF-R alpha (PDGF-Rα) and PDGF-R beta (PDGF-Rβ) as well as variants thereof, for example the naturally occurring isoforms of PDGF-Rα and PDGF-Rβ.

[0136] In some embodiments, the inhibitor of PDGF-R is capable of inhibiting the tyrosine kinase activity of PDGF-Rα and PDGF-Rβ.

[0137] In some embodiments, the inhibitor of PDGF-R is capable of inhibiting the tyrosine kinase activity of PDGF-Rα.

[0138] In some embodiments, the inhibitor of PDGF-R is capable of inhibiting the tyrosine kinase activity of PDGF-Rβ.

[0139] In some embodiments, the inhibitor of PDGF-R is a tyrosine-kinase inhibitor.

[0140] Several inhibitors of PDGF-R are known, for example imatinib, nilotinib, axitinib sunitinib, dasitinib, sorafenib, SU6668, pazopanib, lenvatinib, cabozantinib and nintedanib.

[0141] Preferably, in some embodiments of the present disclosure the inhibitor of PDGF-R is imatinib.

[0142] In other embodiments of the present disclosure, the inhibitor of PDGF-R is an antibody against PDGF-R. Some antibodies are in fact capable of interacting with PDGF-Rs and block or neutralize their activity; in particular they can block or neutralize signaling departing from the PDGF-Rs.

[0143] The anti-PDGF-R antibodies may bind to any PDGF-R. However, in general it is preferred that the anti-PDGF-R antibodies to be used are capable of binding PDGF-R of the individual to be treated. Accordingly, in embodiments where the individual is a human being, then it is preferred that the anti-PDGF-R antibodies are capable of binding human PDGF-R, for example human PDGF-Rα and/or human PDGF-Rβ.

[0144] The anti-PDGF-R antibody according to the present disclosure may be any polypeptide or protein capable of recognizing and binding PDGF-R. By the term "specifically binding" is meant binding with at least 10 times higher affinity to PDGF-R, PDGF-Rα and/or PDGF-Rβ, than to a non-specific antigen (e.g. BSA). Typically, the antibody binds with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, for example about 10^{-10} M or less, when measured as apparent affinities based on IC_{50} values. Naturally occurring antibodies according to the disclosure may consist of one heterotetramer or they may comprise several identical heterotetramers. Thus, the naturally occurring antibody according to the disclosure may for example be selected from the group consisting of IgG, IgM, IgA, IgD and IgE. The subunit structures and three-dimensional configurations of these different classes of immunoglobulins are well known.

[0145] In one embodiment said anti-PDGF-R antibody is a naturally occurring antibody or a functional homologue thereof, as defined in the section "Anti-PDGF-CC antibody".

[0146] As described in the above section "Anti-PDGF-CC antibody", naturally occurring antibodies according to the invention may be antibodies of a particular species. However the antibodies may also be a hybrid between antibodies from several species, for example the antibody may be a chimeric antibody, such as a humanized antibody.

[0147] It is not always desirable to use non-human antibodies for human therapy, accordingly the anti-PDGF-R antibody according to the disclosure may be a human antibody or a humanized antibody, e.g. a naturally occurring human antibody, as described in the above section "Anti-PDGF-CC antibody".

[0148] The anti-PDGF-R antibody according to the disclosure may be a human immunoglobulin or a humanized immunoglobulin, e.g. a naturally occurring human immunoglobulin.

[0149] The anti-PDGF-R antibody according to the disclosure may be a monoclonal antibody, such as a naturally occurring monoclonal antibody or it may be polyclonal antibodies, such as naturally occurring polyclonal antibodies, as described in the above section "Anti-PDGF-CC antibody".

[0150] The anti-PDGF-R antibody may be any protein or polypeptide containing an antigen binding site, such as a single polypeptide, a protein or a glycoprotein. Preferably, the antigen binding site comprises at least one CDR, or more preferably a variable region.

[0151] As mentioned herein above, the anti-PDGF-R antibodies to be used are capable of recognizing and binding

PDGF-Rα and/or PDGF-Rβ. Thus, in general the anti-PDGF-R antibodies specifically bind one or more epitopes on PDGF-Rα and/or PDGF-Rβ. In embodiments wherein the antibody is a monoclonal antibody, then the antibody generally binds one epitope on PDGF-Rα and/or PDGF-Rβ.

[0152] Polyclonal antibodies that bind one or more epitope on PDGF-Rα and/or PDGF-Rβ can also be used.

[0153] In some embodiments, the inhibitor of PDGF-R is an antibody that targets both PDGF-Rα and PDGF-Rβ. The antibody may also be specific for PDGF-Rα. Alternatively, the antibody may be specific for PDGF-Rβ.

Anti-estrogen

[0154] The present invention relates to a kit-of-part comprising an anti-estrogen as well as to methods of treatment employing an anti-estrogen. Said anti-estrogen may be any compound capable of reducing the production or utilization of estrogen.

[0155] In general anti-estrogens can be divided into two different subclasses, namely compounds capable of reducing or inhibiting production of estrogens and compounds capable of reducing and/or inhibiting the activity of estrogen. The latter group includes compounds capable of preventing or reducing signaling mediated by estrogen receptors.

[0156] Thus, in one embodiment of the invention the anti-estrogen is an aromatase inhibitor. Aromatase inhibitors work by blocking or reducing the synthesis of estrogen in a mammal and thereby lowering the level of estrogen. Examples of aromatase inhibitors include but are not limited to exemestane, anastrozole, letrozole, aminoglutethimide, testolactone, vorozole, formestane and fadrozole.

[0157] In another embodiment the anti-estrogen is an estrogen antagonist. Examples of estrogen antagonists include but are not limited to tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY1 17018, fulvestrant, arzoxifene, lasofozone and toremifene.

[0158] The estrogen antagonist may be a compound which both is an antagonist, but also a partial agonist of estrogen. An example of such an anti-estrogen is tamoxifen.

[0159] The estrogen antagonist may also be a full antagonist of estrogen. An example of such an anti-estrogen is fulvestrant.

[0160] A preferred anti-estrogen to be used with the present invention is tamoxifen. Tamoxifen is a triphenylalkylene derivative that binds to the estrogen receptor (ER). The therapeutic mechanisms of tamoxifen are complex, but the primary effect of tamoxifen is exerted via estrogen receptors. In addition to tamoxifen its active metabolites N-desmethyltamoxifen and endoxifen (4-hydroxy-N-desmethyl-tamoxifen) may be used as anti-estrogens with the invention.

[0161] The anti-estrogen to be used with the present invention may thus be a triphenylalkylene derivative, such as tamoxifen or structurally similar compounds including clomiphene, 4- hydroxylated, the N-dealkylated and the 4-hydroxy-N-dealkylated analogs of clomiphene, tamoxifen, pyrrolidinotamoxifen, toremifene, fixed ring tamoxifen, fispemifene, as well as all other molecules with substantially similar structures. Also both cis and trans isomer of the aforementioned may be employed.

[0162] The anti-estrogen may also be a selective estrogen receptor modulator (SERM). SERMs of the invention include, without limitation, triphenylalkylenes, which include: 2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine (tamoxifen) and other compounds described in U.S. Patent No. 4,536,516,; 4'-hydroxy-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl- ethanamine (4'-hydroxytamoxifen) and other compounds described in U.S. Patent No. 4,623,660, as well as the dealkylated variant 4'- hydroxy-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N-monomethyl-ethanamine (N-desmethy1-4'-hydroxytamoxifen also known as endoxifen); fixed ring tamoxifen and its 4'-hydroxyl, N-desmethyl, N-desethyl, 4'-hydroxy-N-desmethyl and 4'-hydroxy-N-desethyl fonns; 1-[4'-(dimethylaminoethoxy)pheny1]-1-(3'-hydroxypheny1)-2-phenylbut-1-ene (droloxifene) and other compounds described in U.S. Patent No. 5,047,431 as well as their 4'-hydroxy, N-desethyl and 4'-hydroxy-N-desethyl fonns; 2-[p-[4-chloro-1,2-dipheny1-1-butenyl]phenoxy]-N,N-dimethylethylamine (toremifene) and other compounds described in U.S. Patent Nos. 4,696,949, 5,491,173 and 4,996,225, as well as 4'- hydroxytoremifene, N-dcsmethyl-toremifene and N-desmethy1-4'-hydroxytoremifene; 1-(2-(4-(1-(4-iodo-pheny1)-2-phenyl-but-1-eny1)-phenoxy)-ethyl)-pyrrolidinone (idoxifene) and other compounds described in U.S. Patent No. 4,839,155; as well as 4-hydroxypyrrolidinotamoxifen; 2-(2-{4-[(1Z)- 4- chloro-1,2-diphenylbut-1-en-I-yl]phenoxyl ethoxy)ethan-I-ol (fispemifene) and other compounds described in U.S. Patent No. 7,504,530, as well as 4'-hydroxyfispemifene; clomiphene and both its isomers; and compounds described in U.S. Patent Nos. 4,696,949 and 5,491,173 and 6,576,645, as well as (E) 4'- hydroxyclomiphene, (E) N-desethyl-clomiphene and (E) N-desethy1-4'-hydroxyclomiphene.

[0163] SERMS to be used with the invention also include, without limitation, benzothiphene derivatives such as: [6-hydroxy-2-(4-hydroxypheny1)-benzothiophen-3-y1]-[4-[2- (1-piperidinyl)ethoxy)pheny1]-methanone (raloxifene) and other compounds described in U.S. Patent Nos. 4,418,068 and 5,393,763; LY353381; and LY335563 and other compounds described in WO 98/45286, WO 98/45287 and WO 98/45288; benzopyran derivatives such as: (+)-7- pivaloyloxy-3-(4'pivaloyloxypheny1)-4-methy1-2-(4"-(2"piperidinoethoxy)pheny1)- 2H- benzopyran (EM 800 /SCH 57050) and other compounds described in WO 96/26201; (2S)-3-(4-hydroxypheny1)-4-methy1-2-[4-[2-(1-piperidypethoxy]phenyl]-2H-

chromen- 7-ol (EM 652); naphthalene derivatives such as: Cis-6-pheny1-544-(2-pyrrolidin- 1-yl- ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalen-2-ol (lasofoxifene/CP 336,156) and other compounds described in U.S. Patent No. 5,552,412; 3,4-dihydro-2-(p-methoxypheny1)- 1-naphthyl-p-[2-(1-pyrrolidinypethoxy]phenyl ketone (trioxifene/LY133314) and other compounds described in U.S. Patent No. 4,230,862; and 1-(4-Substituted alkoxy)benzyl)naphthalene compounds such as those described in U.S. Patent No. 6,509,356; chromans such as 3,4- trans-2,2-dimethy1-3-pheny1-4-[4-(2-(2-(pyrrolidin-1-ypethoxy)phenyl]-7-methoxychroman (levormeloxifene) and other compounds described in WO 97/25034, WO 97/25035, WO 97/25037 and WO 97/25038; and 1-(2-((4-(-methoxy-2,2, dimethy1-3-phenyl-chroman-4-y1)-phenoxy)-ethyl)-pyrrolidine (centchroman) and other compounds described in U.S. Patent No. 3,822,287.

[0164] Other SERMs of the invention include, without limitation, the compounds described in U.S. Patent Nos. 6,387,920, 6,743,815, 6,750,213, 6,869,969, 6,927,224, 7,045,540, 7,138,426, 7,151,196, and 7,157,604.

[0165] Further non-limiting anti-estrogens to be used with the invention include: 6a-chloro-16a- methyl-pregn-4-ene-3,20-dione (clometherone); 6-chloro-17-hydroxypregna-1,4,6-triene-3,20-dione (delmadinone); 1-[2-[4-[1-(4-methoxypheny1)-2-nitro-2-phenylethenyl]phenoxy]ethy1]-pyrrolidine (nitromifene/CN-55,945-27); and 1-[2- [p-(3,4-Dihydro-6-methoxy-2-pheny1-1-naphthyl)phenoxy]ethyl]pyrrolidine (nafoxidene).

[0166] Further non-limiting anti-estrogens to be used with the invention include indoles such as those disclosed in J. Med. Chem., 33:2635-2640 (1990), J. Med. Chem., 30:131-136 (1987), CA 02889770 2015-04-24 WO 2014/070523 WO 93/10741, WO 95/17383, WO 93/23374 and U.S. Patent Nos. 6,503,938 and 6,069,153.

[0167] Further non-limiting anti-estrogens to be used with the invention include 2-[3-(1- cyano-1- methyl-ethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl]-2-methyl-propanenitrile (anastrozole) and other compounds described in EP 0296749; 6-Methylenandrosta-1,4- diene-3,17-dione (exemestane) and other compounds described in U.S. Patent No. 4,808,616; 4-[(4-cyanopheny1)-(1,2,4-triazol- 1- y1)methylThenzonitrile (letrozole) and other compounds described in U.S. Patent No. 5,473,078; 1-[4'-dimethylaminoethoxy)pheny1]- 1-(3'- hydroxypheny1)-2-phenylbut-1-ene (droloxifene) and other compounds described in U.S. Patent 5,047,431; 2a,3a-Epithio-5a-androstan- 1713-01 (epitiostanol); 2a,3a-Epitio-5a-androstane-1713-yl-I-methoxycyclopentyloxy (mepitiostane); 4-[(2Z,4Z)-4-(4-hydroxyphenyphexa-2,4-dien-3-yl]phenol (cycladiene) and other compounds described in U.S. Pat. Nos. 2,464,203 and 2,465,505; CI-680 described in Unlisted Drugs, 28(10): 169(0) (1976); CI-628 described in Unlisted Drugs, 26(7): 106(1) (1974); 13- ethy1-17a-ethyn1-1713-hydroxygona-4,9,1-trien-3-one (R2323); diphenol hydrochrysene and erythyro-MEA both described in Geynet, et al., Gynecol. Invest. 3(1):2-29 (1972); 1-[1-chloro-2,2-bis(4-methoxyphenyl)etheny1]-4-methoxy-benzene (chlorotrianisene) described in Merck Index, 10th ed., #2149; 144-(2-Diethylaminoethoxy)pheny11-1- pheny1-2-(p-anisyl)ethanol (ethamoxytriphetol) described in Merck Index, 10th ed., #3668; and 2-p-Chloropheny1-14p-(2-diethylaminoethoxy)pheny1]-1-p-tolylethanol (triparanol) and other compounds described in U.S. Patent No. 2,914,562. [0057] Still other antiestrogens of the invention include, without limitation: (2e)-3-(4- ((1e)-1,2-diphenylbut-1-enyl)phenyl)acrylic acid (GW5638), GW7604 and other compounds described in Wilson et al., Endocrinology, 138(9):3901-3911 (1997) and WO 95/10513; 144-(2-diethylaminoethoxy)pheny1]-2-(4-methoxypheny1)-1-phenyl- ethanol (MER-25), N,N-diethy1-244-(5-methoxy-2-phenyl-3H-inden-1- y1)phenoxylethanamine hydrochloride (U-11.555A), 1-[2-[4-(6-methoxy-2-pheny1- 3,4- dihydronaphthalen-1-yl)phenoxy]ethyllpyrrolidine hydrochloride (U-11, 100A), ICI- 46,669, 2-[4-[(Z)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-dimethyl-ethanamine; 2- CA 02889770 2015-04-24 WO 2014/070523 hydroxypropane-1,2,3-tricarboxylic acid (ICI-46,474) and other compounds described in Terenius etal., Gynec. Invest., 3:96-107 (1972); 2-Hydroxy-6-naphthalenepropionic acid (allenolic acid); [4-[(4-acetyloxypheny1)-cyclohexylidene-methyl]phenyliacetate (cyclofenyl/ICI-48213); [6-hydroxy-2-(4-hydroxyphenyl)benzothiophen-3-y1]-[4- [2-(1- piperidypethoxy]phenylimethanone (keoxifene); 4-[(Z)-1-[4-(2- dimethylaminoethoxy)pheny1]-2-(4-propan-2-ylphenyl)but-1-enyl]phenol (DP-TAT- 59/miproxifene); (1RS,2RS)-4,4'-diacetoxy-5,5'-difluoro-(1-ethy1-2- methylene)di-m- phenylenediacetate (acefluranol); 6-hydroxy-2-(p-hydroxypheny1)-benzo(b)thien- 3- y1[2-(1-pyrrolidiny1)-ethoxyphenyl]ketone (LY-117018); and [6-hydroxy-2-(4- hydroxy-phenyl)benzo(b)thien-3-y1]-[4-(2-(1-piperidiny1)-ethoxy)phenyl]methanone (LY-156758). [0058] Still other antiestrogens of the invention include, without limitation: non- steroidal estrogen receptor ligands such as those described in U.S. Patent Nos. 5,681,835, 5,877,219, 6,207,716, 6,340,774 and 6,599,921; steroid derivatives such as those described in U.S. Patent No. 4,659,516; 7a-11-aminoalkyl- estratrienes such as those described in WO 98/07740; 11-f3-halogen-7a-substituted estratrienes such as those described in WO 99/33855; 17a-alky1-173-oxy-estratrienes such as those described in U.S. Patent Application No. 10/305,418; 2-phenyl-144-(2-aminoethoxy)-benzyll-indoles such as those described in U.S. Patent No. 7.132,417; 4-fluoroalky1-2h-benzopryans such as those described in U.S. Patent No. 6,844,336; (4-(2- (2-aza-bicyclo[2.2. 1]hept-2-y1)-ethoxy)-pheny1)-(6-hydroxy-2-(4-hydroxy- pheny1)-benzo[b]thiop hen-3-y1)-methanone and other benzothiophenes described in WO 95/10513 and U.S. Patent No. 4,133,814; 2-pheny1-1- [4-(2-aminoethoxy)-benzyl]-indoles such as those described in U.S. Patent No. 5,998,402; 3-[4-(2- Phenyl-Indole-I- ylmethyl) Phenyl]-Acrylamides and other compounds described in U.S. Patent No. 5,985,910; 2-phenyl-I -[4-(amino-1-yl-alk-1-yny1)- benzyl]-1H- indo1-5-ols and other compounds described in U.S. Patent Nos. 5,780,497 and 5,880,137; steroids such as those described in U.S. Patent Nos. 6,455,517, 6,548,491, 6,747,018 and 7,041,839; Di-(3'-hydroxypheny1)-alkane compounds CA 02889770 2015-04-24 WO 2014/070523 such as those described in U.S. Patent No. 4,094,994; phenol derivatives such as those described in U.S. patent No. 4,751,240; 2,3-

diary1-2H-1-benzopyran analogs such as those described in Saeed et al., J. Med. Chem., 33:3210-3216 (1990) and Sharma et al., J. Med. Chem. 33:3216-3229 (1990); and benzofuran and triarylfuran analogs such as those described in Durani et al., J. Med. Chem., 32:1700-1707 (1989).

[0168] The anti-estrogen to be used with the invention may also be a pharmaceutically acceptable salt, ester, or prodrug of any of the aforementioned anti-estrogens.

[0169] Pharmaceutically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

[0170] The anti-estrogens of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent in an effective amount.

[0171] Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, p-toluenesulphonic acids, and arylsulphonic, for example.

ER-negative breast cancer

[0172] The anti-PDGF-CC antibody, the kit-of-parts and the methods of the invention are useful for treatment of ER-negative breast cancer.

[0173] The ER-negative breast cancer may be any breast cancer characterized by lack of expression of the estrogen receptor (ER). As used herein a breast cancer is considered an ER-negative breast cancer, when =<10% of the tumor cells of said breast cancer express estrogen receptor at levels detectable by immunohistochemistry. In some embodiments of the invention, the ER-negative breast cancer is a breast cancer, where <1% of the tumor cells of said breast cancer express estrogen receptor at levels detectable by immunohistochemistry Immunohistochemistry may preferably be a test involving staining of a sample from a breast cancer with the aid of antibodies recognizing ER, and followed by detection of ER expression in the cells of said sample, e.g. by microscopy.

[0174] In one embodiment of the invention the ER-negative breast cancer is a breast cancer wherein <1% tumor nuclei are positive for ER expression as recommended by American Society of Clinical Oncology as described by Hammond et al., 2010.

[0175] According to the present invention, ER expression may be determined by any useful means, preferably by any useful immunohistochemical method. Preferably, ER expression may be determined as described by Hammond et al., 2010.

[0176] In one embodiment of the invention the ER-negative breast cancer also is a Progesterone receptor negative (PR-negative) breast cancer. Thus, the breast cancer be to treated may in particular be an ER-negative and PR-negative breast cancer. Said PR-negative breast cancer may be a breast cancer where =<10% of the tumor cells of said breast cancer express the progesterone receptor (PR) at levels detectable by immunohistochemistry. In some embodiments of the invention, the PR-negative breast cancer is a breast cancer, where <2% of the tumor cells of said breast cancer express PR at levels detectable by immunohistochemistry. In some embodiments of the invention, the PR-negative breast cancer is a breast cancer, where <1% of the tumor cells of said breast cancer express PR at levels detectable by immunohistochemistry. PR expression may be determined by any useful means, preferably by any useful immunohistochemical method. Preferably, PR expression may be determined as described by Hammond et al., 2010.

[0177] The ER-negative breast cancer may in some embodiments express the human epidermal growth factor receptor 2 (HER-2).

[0178] In another embodiment of the invention the ER-negative breast cancer is a triple-negative breast cancer, i.e. said cancer is ER-negative, PR-negative and human epidermal growth factor receptor (HER)-2 negative. The terms ER-negative and PR-negative are explained above. A HER-2 negative breast cancer may be a breast cancer which expresses no detectable HER-2. The test for HER-2 expression may be performed by any useful method, for example by an immunohistochemical method or by FISH. Preferably, a HER-2 negative breast cancer is HER-2 negative when determined as recommended in Wolff et al., 2013.

[0179] In one embodiment of the invention the ER-negative breast cancer is a basal-like breast cancer. In one embodiment said basal-like breast cancer may be a triple-negative breast cancer.

[0180] A basal-like breast cancer may also be an ER-negative breast cancer, which expresses one or more high-molecular weight/basal cytokeratins, for example selected from the group consisting of CK5/6, CK14 and CK17.

[0181] The basal-like breast cancer may also be an ER-negative and HER-2 negative breast cancer, which expresses CK5/& and/or epidermal growth factor receptor.

[0182] The basal-like breast cancer may also be a triple-negative breast cancer expressing CK5/6 and/or EGFR.

[0183] Furthermore, basal-type breast cancer typically does not express FoxA1. Basal-like breast cancer is associated

with high grade, poor prognosis, and younger patient age.

[0184] In particular, the ER-negative breast cancer may be a breast cancer, which at the time of first diagnosis was an ER-negative breast cancer. Thus, the ER-negative breast cancer may be characterized as ER-negative from the onset, rather than as a breast cancer having lost ER expression as a result of treatment. It may be preferred that the ER-negative breast cancer is a breast cancer, wherein the primary tumor is ER-negative.

Pharmaceutical formulation

[0185] Whilst it is possible for the anti-PDGF-CC antibodies and the anti-estrogens of the present invention to be administered as the raw compounds, it is preferred to present them in the form of a pharmaceutical formulation. The pharmaceutical formulations may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 2005, Lippincott, Williams & Wilkins.

[0186] The compounds to be used with the present invention may be formulated for parenteral administration and may be presented in any suitable form, for example in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers, optionally with an added preservative. In particular it is foreseen that the anti-PDGF-CC antibodies are formulated for parenteral administration, however, also anti-estrogens may be formulated for parenteral administration.

[0187] For parenteral administration the formulations may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water. The formulations can for example be presented in unit-dose or multi-dose sealed containers, such as ampoules, vials, pre-filled syringes, infusion bags, or can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0188] Examples of oily or non-aqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils, and injectable organic esters, and may contain formulatory agents such as preserving, wetting, emulsifying or suspending, stabilizing and/or dispersing agents.

[0189] The formulations for injection will typically contain from about 0.5 to about 25% by weight of the active ingredient in solution for example of the anti-PDGF-CC antibody. For example, the dosage may be in the range of 1 to 100 mg anti-PDGF-CC antibody per kg body weight, such as in the range of 1 to 20 mg of anti-PDGF-CC antibody per kg body weight, for example in the range of 5 to 15 mg of anti-PDGF-CC antibody per kg body weight.

[0190] The compounds of the present invention may also be formulated in a wide variety of formulations for oral administration. This may in particular be the case for the anti-estrogens. Solid form preparations may include powders, tablets, drops, capsules, cachets, lozenges, and dispersible granules. Other forms suitable for oral administration may include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, tooth-paste, gel dentrifrice, chewing gum, or solid form preparations which are intended to be converted shortly before use to liquid form preparations, such as solutions, suspensions, and emulsions.

[0191] In powders, the carrier is a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding capacity in suitable proportions and compacted in the shape and size desired. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like.

[0192] Drops according to the present invention may comprise sterile or non-sterile aqueous or oil solutions or suspensions, and may be prepared by dissolving the active ingredient in a suitable aqueous solution, optionally including a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

[0193] Emulsions may be prepared in solutions in aqueous propylene glycol solutions or may contain emulsifying agents such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

[0194] Any dosage of anti-estrogen used as required for efficacy, as recommended by the manufacturer, can be used. Appropriate dosages for anti-estrogens are known in the art. Thus, conventional dosages used for treatment of luminal-like breast cancers can be used for treatment of the basal-like breast cancers according to the invention. For example, anti-estrogens may be prepared form administration in a dosage range between 0.01 to 10 mg/kg of body weight per day (preferably 0.05 to 1.0 mg/kg), with 20 to 40 mg per day being preferred for a person of average body weight when orally administered, or in a dosage range between 0.003 to 3.0 mg/kg of body weight per day (preferably 0.015 to 0.3

mg/ml), with 1.5 mg per day, especially 3.0 mg per day, in two equally divided doses being preferred for a person of average body weight when parentally administered.

Sequence listing

[0195]

SEQ ID NO:1	Amino acid sequence of PDGF-CC from Homo sapiens
SEQ ID NO:2	MMTV-PyMT primer
SEQ ID NO:3	MMTV-PyMT primer
SEQ ID NO:4	PDGF-C wild-type primer
SEQ ID NO:5	PDGF-C wild-type primer
SEQ ID NO:6	PDGF-C mutant primer
SEQ ID NO:7	PDGF-C mutant primer
SEQ ID NO:8	PDGF-R alpha
SEQ ID NO:9	PDGF-R beta

SEQ ID NO:1 platelet-derived growth factor C precursor Homo sapiens

MSLFGLLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERIIT
VSTNGSIHSPRFPHTYPRNTVLVWRLVAVEENVWIQLTFDERFGLEDPEDDICKYDFV
EVEEPSDGTILGRWCGSGTVPGKQISKGNQIRIRFVSDEYFPSEPGFCIHYNIVMPQFT
EAVSPSVLPPSALPLDLLNNAITAFSTLEDLIRYLEPERWQL
DLEDLYRPTWQLLGKAFVFGRKSRVVDLNLLTEEVRLYSCTPRNFSVSIRE
ELKRTDTIFWPGCLLVKRCGGNCACCLHNCNECQCVPSKVTKKYHEVLQLRPKTGVR
GLHKSLTDVALEHHEECDCVCRGSTGG

Examples

[0196] The invention is further illustrated by the following examples, which should not be construed as being limiting for the invention.

Example 1. Anti-PDGF-CC antibody sensitizes tumor to estrogen therapy

Patient cohort and the definition of breast tumor subtypes

[0197] Tissues from 890 patients with primary invasive breast cancer, diagnosed at the Institute of Surgical Pathology, USZ, between 1965 and 2004 (median July 1999), were analyzed. All patients enrolled voluntarily under Institutional Review Board-approved protocols and sample donors gave written informed consent. The ethics committee SPUK surgical-anesthetic-pathology at university hospital of Zürich, Switzerland, approved this study with reference number: *StV 12-2005.* For all these patients follow up data from the cantonal cancer registry were available; patients without follow up information were not considered (Theurillat et al, Int J Cancer, 2007). Additionally, 69 normal tissues and 152 in situ lesions (DCIS, LN) were analyzed. Molecular cancer subtypes were defined from detected ER, HER2 and CK5/6 by IHC. Luminal type: ER-positive cases, that were HER2- negative; HER2-type: Her2-positive cases; Basal like: CK5/6-positive, ER-negative and HER2-negative. Cases negative for all markers were designated NIL-type.

Tissue Microarray construction

[0198] Formalin fixed paraffin embedded material of a representative variety of normal and malignant human tissues

and tumor cell lines were compiled and assembled on a single block, as described (Kristiansen et al, Br J Cancer 2008).

Immunohistochemistry on human tumor tissue array

[0199] To assess PDGF-CC expression in various tumor types, we used a rabbit polyclonal antibody against PDGF-CC, 615, on a commercially available tumor tissue array (Chemicon) according to the instruction of the manufacturer. For the breast tumor cohort and to confirm the results gained from the polyclonal antibody 615, the mab anti-PDGF-CC antibody, A3B6 (2 μg/ml) was used on an automated Ventana platform (protocol CC1m for pre-treatment, UView HRP detection system). The epitope of the A3B6 antibody is amino acids 256 to 260 of SEQ ID NO:1 within the sequence of amino acids 256 to 274 of SEQ ID NO:1. Specific immuno-reactivity was fully blocked by an excess of active PDGF-CC. The basal cell marker cytokeratin CK5/6 (clone cocktail D5/16B4, 1:25, Dako, Denmark), HER2 (clone 10A7, 1:50, Novocastra, UK), EGFR (clone 3C6, pre-diluted, Ventana, Tucson, USA) and Ki-67 (clone Mib-1, 1:20, Dako, Danmark) were processed in parallel.

Mouse tissue preparation, histology and immunostaining

[0200] Upon completion of the treatment, mice were anesthetized with 2.5% Avertin (12,5 mg/kg body weight; Sigma-Aldrich) and 300 μL of blood were collected by heart puncture and immediately mixed with RNAlater solution (Life Technologies) and stored at-20 °C. Mice were heart-perfused with PBS followed by 4% paraformaldehyde (for transplanted FVB/N mice only).

[0201] For paraffin embedding, organs were post-fixed in 4% paraformaldehyde for 2 h before proceeding to embedding. Paraffin-embedded sections were deparaffinized and rehydrated followed by antigen retrieval in high pH buffer (pH 6; DAKO) in a pressure cooker (ERα) or in 95 °C water-bath for 20 minutes (PR, STC1, IGFBP3 and HGF). Peroxidase activity was quenched with 3% H_2O_2 in methanol for 10 minutes at room temperature, followed by washes with 0.1% BSA in PBS.

[0202] ERα staining required subsequent steps in M.O.M. blocking (Mouse on Mouse basic kit, Vectorlabs), CAS-block (Life Technologies) and M.O.M. diluent. The primary antibody against estrogen receptor ERα (1:200, clone 1D5; DAKO) was incubated in M.O.M. diluent.

[0203] CAS-block was used for the blocking and incubation of primary antibodies against STC1 (1:200; SC-30183, Santa Cruz), IGFBP3 (1:200; SC-9028, Santa Cruz) and HGF (1:200; ab83760, Abcam). Primary incubation was performed overnight at 4 °C in a humidified chamber.

[0204] After washing, appropriate secondary biotinlylated antibodies and the ABC peroxidase system were used (ABC Elite standard kit, Vector Laboratories) with DAB as the colorimetric substrate (Vector Laboratories).

[0205] For cryopreservation, primary tumor, lungs, liver and brain were kept in 30% sucrose at 4 °C overnight, followed by embedding in Optimal Cutting Temperature (OCT) medium (HistoLab). Frozen sections were fixed in ice-cold acetone, followed by blocking using Serum Free Protein Block (DAKO) for > 90 minutes at room temperature. Primary antibodies directed against PDGFRα (PE-conjugated, 1:200; 12-1401, eBioscience) and PDGFRβ (1:200; 3169S, Cell Signaling) were incubated overnight at 4 °C in a humidified chamber. Appropriate Alexa488- flourochrome-conjugated secondary antibody (Life Technologies) was used and sections were finally mounted using 4',6- diamidino-2-phenylindole (DAPI)-containing mounting media (Vector Laboratories).

[0206] For RNA isolation and preparation, primary tumor, liver, lungs and brain were snap-frozen in liquid N_2 and stored at -80 °C.

Mice

[0207] All animal experiments were approved by the Ethical Committee for Animal Experiments (Stockholm Norra djurförsöksetiska nämnd, application N96/11, and Lund, application M142/13). FVB/N-Tg(MMTV-PyVT)$^{634Mul/J}$ transgenic mice have been described previously (Guy et al Molecular and Cellular Biology 1992) and were purchased from The Jackson Laboratory. The presence of the MMTV-PyMT transgene and the generation of heterozygous and homozygous knock-out PDGF-C offspring were verified by genotyping. DNA was prepared from either ear or tail biopsies according to a common tissue lysis, nucleic acid extraction and purification protocol. PCR products were run on a 1,5% agarose gel. MMTV-PyMT primer pair (5' to 3'):

GGAAGCAAGTACTTC ACAAGGG [SEQ ID NO:2] and
GGAAAGTCACTAGGAGCAGGG [SEQ ID NO:3]. PDGF-C wild-type pair:

AGCTGACAT TTGATGAGAGAT [SEQ ID NO:4] and
AGTAGGTGAAATAAGAGGTGAACA [SEQ ID NO:5]. PDGF-C mutant pair: CTC

ATGTTCTCGTGACTCTGA [SEQ ID NO:6] and TAGCTAGTCGATACCGTCGA [SEQ ID NO:7].

[0208] Tumor size of the ten different glands were measured at 12 weeks of age using a caliper. Tumor volume was calculated as length $\times$ width2 $\times$ π/6. Mice of different ages where anesthetized with Avertin (Sigma Aldrich, St Louis, MO) and then euthanized by heart perfusion with Hank's balanced salt solution (HBSS) followed by 4% Paraformaldehyde (PFA). The left cervical and thoracic mammary glands where excised and subjected to overnight fixation in 4% PFA before embedding in paraffin. For frozen sectioning the tumor tissue was subjected to 30% sucrose before embedding in OCT.

Tumor piece transplantation into mammary fat pad

[0209] 3 weeks old FVB/N (common background strain for both MMTV-PyMT and PDGF-C mice) female mice were anesthetized and maintained under Isolfluorane during the surgical procedure. A 4 mm incision under the nipple of the right abdominal mammary gland created a pocket where a 2x2 mm tumor piece (kept on ice, either from MMTV-PyMT or MMTV-PyMT;PDGF-C tumors) was inserted. Suturing was performed with 6-O Ethilon polyamide filament (Ethicon). Pain-killer and anti-inflammatory Rimadyl (5 mg/kg body weight; Orion Pharma Animal Health) was injected i.p. at the end of the surgical procedure and for the following two days. For the therapeutic trial, Tamoxifen (2 mg/mL) was diluted in corn oil (vehicle) and administered via oral gavage daily.

[0210] Mouse mammary cell lines MMTV... MMTV/PDGF-C-/-... (established in our laboratory) were orthotopically injected into the 4th inguinal mammary gland on FVB/N mice. Furthermore, small tumor pieces (2mm^3) of MMTV-PyMT and MMTV-PDGF-CC-/- tumors were directly orthotopically transplanted under anesthesia. The mice were subjected to Rimadyl immediately after the surgery and the following two days for analgetics. The tumors were measured twice a week and mice were euthanized as described above.

Xenograft establishment

[0211] 2 $\times$ 10^6 Human MDA-MB-231 cells were inoculated subcutaneously in immunodeficient mice. Tumor growth was monitored and measured once a week with a caliper in live sedated animals. Anti-PDGF-CC (A3B6) antibody or IgG2a control were delivered *via* i.p. injection twice a week (300 mg/kg per week) starting from the day of tumor establishment. When tumors were palpable (longest diameter > 3 mm), mice were randomized and treated with Tamoxifen (3 mg/kg) or vehicle (corn oil) via oral gavage daily.

Statistics

[0212] All statistics were calculated using SPSS V17 (SPSS, Chicago, USA). Spearman rank correlation was used to determine the associations of PDGF-CC expression with clinico-pathological parameters. Kaplan Meier analysis (with log rank test) and the Cox regression model were used for univariate or multivariate analyses. The statistics of the mouse experiments was evaluated using two-tailed independent student t-test with P$\leq$ 0.05 considered significant.

Cell culture

[0213] Murine MMTV-PyMT or MMTV-PyMT;PDGF-C$^{-/-}$ cells and human MDA-MB-231 cells were maintained in culture in DMEM Glutamax (Invitrogen), supplemented with 1% Penicillin/Streptomycin, 10% Fetal Bovine Serum (FBS) and glutamate.

In vitro stimulation

[0214] 3 $\times$ 10^6 MMTV-PyMT;PDGF-C$^{-/-}$ cells were seeded in culture medium. After 24 hours, the cells were starved for 24 hours in DMEM Glutamax (Invitrogen), supplemented with 1% bovine serum albumin (BSA; Sigma Aldrich). The cells were stimulated with rmSTC1 (400 ng/mL; BioVendor), rmHGF (30 ng/mL;), rhIGFBP3 (250 ng/mL; R&D) or combinations of these factors in starvation medium for 48 hours. The cell line CAF2 (Kojima et al, PNAS, 2010) was used to produce CAF-conditioned medium. A monolayer of CAF2 cells was incubated for 48 hours in starvation medium. This conditioned medium was spun down (1500 $\times$ g) to remove cells and used for downstream experiments.

Quantitative reverse-transcription PCR

[0215] *In* vitro-grown cells were washed twice with ice-cold PBS. RNA was isolated using RNAeasy MiniKit (Qiagen). cDNA was prepared using iScript cDNA Synthesis Kit (Bio Rad). KAPA SYBR FAST qPCR Kit Master Mix (KAPA

Biosystems) was used for quantitative real-time PCR. The mRNA expression was normalized to the housekeeping gene L19. For FOXA1, EGFR and ESR1 QuantiTect Primer assay (Qiagen) primers were used. L19 primer pair (5' to 3'): GGTGACCTGGATGAGAAGGA and TTCAGCTTGTGGATGTGCTC. GATA3 primer pair (5' to 3'): CAATGCCTGCGGACTCTACC and GGTGGTGGTCTCGACAGTTCG.

Western blot

[0216] *In* vitro-grown cells were washed twice with ice-cold PBS and lysed 30 minutes on ice with 50 μl lysis buffer (20 mM Tris-HCl pH 7,5, 150 mM NaCl, 5 mM EDTA, 0.5 % sodium deoxycholate, 0.5 % Triton X). The lysate was spun down at 12000 × g and the pellet discarded. The protein concentration was determined by absorption spectroscopy. The protein suspension was mixed with 5× loading buffer, denaturated at 96 °C for 2 minutes and separated by SDS-PAGE on a 10% acrylamide gel. The proteins were transferred to an ethanol-activated PDCV membrane. The membrane was blocked 1 hour with 5% milk powder in PBST (0.05% Tween-20 in PBS) and incubated O/N at 4 °C with anti-Estrogen Receptor alpha antibody (ERα 1:200; SC-542, Santa Cruz) in blocking buffer. After washing, anti-Rabbit-HSP was applied 1:5000 in blocking buffer and incubated for 2 hours at RT. The membrane was washed and developed with SuperSignalR West Pico Chemoluminescent Substrate (Thermo Scientific). Luminescence signal was measured with an CCD camera (FluorChem E, Cell Biosciences).

In vitro 4-hydroxytamoxifen treatment

[0217] 15000 cells (either MMTV-PyMT or MMTV-PyMT;PDGF-$C^{-/-}$) were seeded in 96-well plates. After 24 hours incubation in growth medium followed by 24 hours starvation, the cells were stimulated either with CAF-conditioned medium or recombinant factors, as described before.

[0218] The cells were treated with increasing concentration of 4-hydroxytamoxifen (0-5 μM; Sigma Aldrich) in the respective stimulation medium at day 4 and 6 post-seeding. The cell proliferation reagent WST-1 (Roche) was used for the viability assay at day 7.

Tumor grade assessment

[0219] Tumor tissue from MMTV-PyMT, MMTV-PyMT;PDGF-$C^{+/-}$ and MMTV-PyMT;PDGF-$C^{-/-}$ mice (n = 5 mice/genotype) was classified into different degrees of progression by quantifying the area of transformed glands occupied by each stage. Progression follows from normal fat tissue to a "precancerous stage" characterized by premalignant hyperplasia and adenoma (with the retention of some normal ductal and acinar mammary gland morphology), to a more epithelial cell-dense "early carcinoma" with stromal invasion, and finally to an invasive, very dense, high-mitotic index "late-stage carcinoma". Tumors were evaluated for the proportion of mammary fat tissue, hyperplastic tissue, adenoma, early carcinoma and late carcinoma. PDGF-C specific necrosis was described by a pathologist and scored blindly in the samples.

ERa assessment

[0220] MDA-MB-231 human xenograft tumor tissue was immunostained and nuclear ERα positivity was evaluated at the end of the therapeutic trials. The region of interested was restricted to the tumor mass, without including the surrounding fat tissue. Both single-cell and foci quantification (n >3 cells/focus) was performed.

Quantification of metastases

[0221] The left lung lobes of MMTV-PyMT were embedded in paraffin upon tissue fixation. The metastatic burden was assessed by serial sectioning of the entire lung/liver lobe. Following hematoxylin and eosin staining on every 25th section, the number of metastatic foci (>8 cells in diameter) was determined in >15 sections per mouse and > 5 mice per group.

Results

[0222] *PDGF-CC is an independent prognostic factor for poor survival in breast cancer* In order to investigate the expression pattern of PDGF-CC in human breast, we performed immunostaining of a tissue microarray containing 890 tumor specimens, as well as normal breast tissue. The expression of PDGF-CC in normal breast tissue was limited to myoepithelial/basal cells and endothelial cells in capillaries, whereas most luminal cells were found to be negative for PDGF-CC expression (Fig. 1a-b). In breast tumors, PDGF-CC was expressed by malignant cells, intra-tumoral capillaries and stromal fibroblasts (Fig. 1b-e). Notably, PDGF-CC immunoreactivity was most conspicuous in the stroma directly

adjacent to the malignant epithelium (Fig. 1f). Next, the staining intensity for PDGF-CC was graded independently for the epithelial and stromal compartment and correlated to clinico-pathological parameters (Fig. 1g). Stromal immunoreactivity for PDGF-CC was not correlated to patient outcome. In sharp contrast, moderate to high expression of PDGF-CC (score of 2+ and 3+) was found to be a highly significant prognostic factor for poor survival in univariate Cox regression and Kaplan-Meier analysis (RR 1.52, 95%CI 1.16-1.99, p=0.003; Fig. 1h). Importantly, multivariate analysis adjusting for established clinical risk factors, such as age at diagnosis, stage, grade and lymph node status, among others, demonstrated that epithelial expression of PDGF-CC served as an independent prognostic factor for poor survival (RR 1.48, 95%CI 1.04-2.13, p=0.03). Interestingly, the two receptors of the PDGF family, *i.e.* PDGFRα and PDGFRβ, were both exclusively expressed by stromal fibroblasts, indicating that malignant cells engage in paracrine communication with mesenchymal cells of the tumor microenvironment (Fig. 1i).

[0223] *PDGF-CC is functionally important for the growth of experimental breast cancer* Next, in order to investigate the functional aspects of PDGF-CC expression in the context of mammary gland tumorigenesis, we generated a genetically engineered mouse model of breast cancer based on the widely studied and clinically relevant MMTV-PyMT mouse intercrossed with mice deficient for *Pdgfc* (*Pdgfc*$^{lacz/lacz}$). Visualization of PDGF-CC, PDGFRα and PDGFRβ expression in tumors of MMTV-PyMT mice demonstrated faithful recapitulation of the expression pattern in human breast cancers and established the existence of a paracrine circuitry between malignant cells and stromal fibroblasts (Fig. 2a). Strikingly, genetic deficiency for *Pdgfc* severely impacted on the growth of mammary tumors of MMTV-PyMT mice (Fig. 2b). Whereas control mice presented with tumors of an average size of 220 $\pm$ xx mm^3, deficiency for a single, or both, copies of the gene encoding *Pdgfc* reduced the average tumor size to 98 $\pm$ xx mm^3 and 95 $\pm$ xx mm^3, respectively (Fig. 2b). In addition to reducing the overall tumor burden, genetic deficiency for *Pdgfc* was associated with a significantly longer tumor latency, as well as prolonged survival of MMTV-PyMT mice (Fig. 2c-d). Furthermore, tumors from age-matched mice lacking the gene encoding PDGFC were of lower stage, compared to tumors from control mice, and incorporated substantial areas of necrosis (Fig. 2e-f). Accordingly, 14-weeks old tumor-bearing mice presented with 26.3% fewer pulmonary metastases in the absence of signaling by PDGF-CC (Fig. 2g). However, this was most likely due to the delayed onset of disease, as a cohort of 12-weeks old wt mice displayed a similar metastatic burden as the 2 weeks older mice lacking *Pdgfc* (Fig. 2g).

[0224] To ascertain that the delayed tumor development in *Pdgfc*-deficient mice was not due to developmental defects, we transplanted fragments of tumors from MMTV-PyMT; *Pdgfc*$^{+/+}$ and MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice orthotopically into the mammary fat pad of young wt mice. Consistent with our findings in the transgenic setting, transplanted Pdgfc-deficient tumors displayed a dramatically hampered growth, compared to Pdgfc-proficient tumors (Fig. 2h). Furthermore, whereas cell lines isolated from wt MMTV-PyMT mice readily gave rise to exponentially growing tumors following orthotopic transplantation into the mammary fat pad of wt or Pdgfc-deficient mice, two independently isolated cell lines from tumors of MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice were unable to establish as palpable tumors (Fig. 2i and data not shown).

Deficiency for Pdgfc results in a blunted fibrotic and angiogenic response in the tumor microenvironment

[0225] Histological analysis revealed considerable differences in the architecture of tumors from MMTV-PyMT; *Pdgfc*$^{+/+}$ and MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice. Masson tri-chrome staining of tumor sections demonstrated a severely reduced deposition of intratumoral collagen in the matrix of Pdgfc-deficient tumors, consistent with the notion that PDGF-CC acts to recruit and/or activate stromal fibroblasts in the breast tumor microenvironment (Fig. 3a). In addition, MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice were severely hemorrhagic (Fig. 3b) and exhibited significantly more hypoxia, as evidenced by immunostaining for HIF-1α (Fig. 3c). Accordingly, quantitative PCR analysis revealed a 65% lower expression of VEGF-A in the absence of PDGF-CC (Fig. 3d). In order to investigate whether pharmacological targeting of PDGF-CC as a mono-therapy impacted on breast tumor growth or angiogenesis, we treated SCID mice bearing orthotopically implanted MDA-MB-231 tumors (basal-like subtype) twice-weekly with the PDGF-CC antibody, A3B6 or with control antibody. Pharmacological blockade of PDGF-CC signaling marginally, albeit statistically significantly, impaired tumor growth and angiogenesis following 4 weeks of treatment (Fig. 3e-f). Taken together, we have demonstrated that deficiency for *Pdgfc* results in a blunted fibrotic and angiogenic response in the tumor microenvironment.

Expression of PDGF-CC in breast tumors is associated with the basal-like molecular subtype.

[0226] In order to elucidate the molecular significance of Pdgfc-deficiency, we performed transcriptional analysis on tumors derived from MMTV-PyMT; *Pdgfc*$^{+/+}$ and MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice using a quantitative PCR array designed to analyze the expression of genes of importance for breast tumor development and progression. The analysis revealed that the most differentially regulated gene was *Foxa1,* which was found to be expressed on average 8.9-fold higher in whole tumor lysates from Pdgfc-deficient mice compared to wt mice (Fig. 4a). The expression of *Foxa1* was also found to be dramatically elevated in tumor cell lines isolated from MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice (Fig. 4b). Human breast cancers may be classified into different molecular subtypes, including ER-positive breast cancers, such as normal-

like, luminal A, luminal B, HER2$^+$ and ER-negative breast cancers, for example basal-like tumors. Analysis of transcriptional profiles of breast tumors collected within The Cancer Genome Atlas project revealed that expression of *Foxa1* is highly correlated with a non-basal-like molecular subtype (Fig. 4c), confirming previous studies (ref). Indeed, mining of transcriptional data from a panel of 50 breast tumor cell lines revealed expression of *Foxa1* as a specific feature of tumors of the luminal subtype (Fig. 4d). Immunostaining of a cohort of human breast tumor specimens confirmed the association between Foxa1 and the luminal subtype, as identified using hormone receptor (estrogen receptor-α (ER) and progesterone receptor (PR)) expression as a proxy (Fig. 4e). Given the fact that Foxa1 was found to be upregulated in tumor lysates in the absence of PDGF-CC, we investigated the correlation between Foxa1 and PDGF-CC in breast cancer. Firstly, expression of PDGF-CC was exclusively observed in breast tumor cell lines of basal-like subtype, but not in cells of luminal subtype origin (Fig. 4f). Secondly, in the panel of 50 breast tumor cell lines, Foxa1 and PDGF-CC expression were found to be inversely correlated (Fig. 4g). Thirdly, the association of PDGF-CC expression with the basal-like subtype of breast cancer was further established by analysis of expression of basal-like markers (cytokeratin 5/6) in a cohort of 890 human breast tumors by immunostaining. Strikingly, PDGF-CC was highly significantly associated with cytokeratin 5/6 expression, whereas low or absent expression of PDGF-CC denoted tumors of the luminal subtype expressing Foxa1, ER and PR.

A paracrine signaling circuit in stromal fibroblasts established by PDGF-CC determines molecular subtype of breast tumor cells.

[0227] In accordance with the transcriptional analysis, the luminal subtype markers FoxA1 and ERα protein was found to be more abundant in tumor protein lysates from Pdgfc-deficient mice (Fig. 5a). In order to elucidate the mechanism whereby paracrine signaling by epithelium-derived PDGF-CC elicited specification of basal-like features of breast tumors, we stimulated the immortalized breast cancer-associated fibroblast cell line CAF2 with PDGF-CC. Following global gene expression analysis, we focused on genes encoding secreted proteins and validated 3 of these, stanniocalcin (STC)-1, hepatocyte growth factor (HGF) and insulin growth factor binding protein 3 (IGFBP3), by quantitative PCR to ensure induction by PDGF-CC (Fig. 5b). Next, we assessed whether stimulation of primary breast cancer cells isolated from tumors of MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice with STC-1, HGF and IGFBP3 rescued the basal-like phenotype of tumor cells from wt MMTV-PyMT mice using expression of the luminal-like subtype markers FoxA1, ERα and GATA3. Indeed, while each factor alone had varying effect, the concerted action of STC-1, HGF and IGFBP3 substantially suppressed the luminal-like features of *Pdgfc*-deficient mammary carcinoma cells (Fig. 5c-e). Importantly, the altered expression of ERα held functional significance, as pre-treatment of luminal breast cancer cells with STC-1, HGF and IGFBP3 reduced their sensitivity to tamoxifen-induced growth arrest (Fig. 5f). In addition, conditioned medium from stromal fibroblasts could be substituted for the three paracrine PDGF-CC-induced factors (Fig. 5g). The presence of STC-1, HGF and IGFBP3 in the tumor stroma of tumors from MMTV-PyMT mice was confirmed by immunostaining (Fig. 5h).

Genetic or pharmacological targeting of PDGF-CC sensitizes basal-like breast tumors to hormone therapy.

[0228] The clinically most important distinguishing feature of luminal subtype breast tumors is the expression of ERα, which confers sensitivity to hormone therapy, such as tamoxifen. We next set out to investigate whether targeting of PDGF-CC would convey sensitivity to hormone therapy to previously impervious ER-negative breast tumors of basal-like subtype. Non-transgenic mice bearing orthopically transplanted tumors from MMTV-PyMT; *Pdgfc*$^{+/+}$ or MMTV-PyMT; *Pdgfc*$^{lacz/lacz}$ mice were treated daily with tamoxifen starting from x weeks of age. As expected, the wt tumors of tamoxifen-treated mice continued to grow at a similar rate as tumors from untreated mice (Fig. 6a). In sharp contrast, and in agreement with them being ER-positive (Fig. 5a), tumors from *Pdgfc*-deficient mice were severely growth-retarded upon treatment with tamoxifen (Fig. 6b). At the end of the trial, tumors from tamoxifen-treated mice devoid of paracrine PDGF-CC signaling had a reduced volume, whereas untreated mice presented with tumors that had grown, evidently revealing functional sensitization of ERα-signaling within breast tumors by the *Pdgfc*-deficient tumor microenvironment. Histologically, tumors from tamoxifen-treated mice displayed. Finally, to conclusively demonstrate the therapeutic utility of agents targeting PDGF-CC to sensitize breast tumors of the basal-like subtype to the action of tamoxifen, we implanted MDA-MB-231 cells orthotopically into SCID mice. Treatment with tamoxifen together with a control antibody was unable to influence the growth of fully established MDA-MB-231 tumors (Fig. 6c). Strikingly, combined administration of tamoxifen and the PDGF-CC antibody A3B6 led to significant growth retardation of MDA-MB-231 tumors (Fig. 6d). Indeed, the basal-like subtype tumor that originally did not express meaningful levels of ERα, substantially upregulated expression of ERα upon treatment with monoclonal anti-PDGF-CC antibody A3B6, corroborating the role of paracrine signaling by PDGF-CC in establishing the absence of ERα in basal-like subtype breast tumors (Fig. 6e). Interestingly, the upregulation of ERα expression in MDA-MB-231 tumors following blockade of signaling by PDGF-CC was not uniform, but rather occurred in differentiated nests of malignant cells (Fig. 6f).

[0229] Without being bound by theory it is a paracrine signalling network is suggested manifested in the breast tumor

microenvironment, in which epithelium-derived PDGF-CC orchestrates specification of the basal-like subtype through interactions with cancer-associated fibroblasts that express STC-1, HGF and IGFBP3 (Fig. 6g). Importantly, blockade of PDGF-CC, either by genetic or pharmacologic means, effected a sensitization of previously impervious basal-like subtype breast tumors to the action of hormone therapy by tamoxifen (Fig. 6g).

Example 2. Anti-PDGF-CC and aromatase inhibitor

[0230] For therapeutic studies, 2 × 106 Human MDA-MB-231 cells were inoculated orthotopically in the 4th mammary fat pad in SCID mice. Tumor growth was monitored and measured once a week with a caliper in live sedated animals. Mice had been randomly assigned before tumor inoculation to receive treatment with anti-PDGF-C (mouse monoclonal antibody clone A3B6) antibody or IgG2a isotype control antibody (Bio X Cell), which were delivered via i.p. injection twice a week (300 μg per week) starting from the day of tumor establishment. For therapeutic trials involving endocrine therapy, when a tumor was palpable (longest diameter >3 mm), mice were alternately assigned into the treatment groups in which mice were treated with letrozole (1 mg/dose via oral gavage daily, Sigma), dissolved in a vehicle of ethanol and corn oil (Sigma) by heating to 55 °C, or with vehicle alone. All therapeutic administrations were open-label.

[0231] The A3B6 + letrozole group is statistically significantly smaller than all other groups ($p<0.01$ vs control, $p<0.05$ vs A3B6, $p<0.001$ vs letrozole, Student's unpaired, 2-sided t-test assuming equal variance); no other differences are statistically significant (Figure 7).

[0232] Conclusion: neutralization of PDGF-CC sensitizes previously impervious basal-like/triple-negative breast tumors to the action of endocrine therapy in the form of aromatase inhibitors.

Example 3. PDGF-R inhibitor and estrogen antagonist

[0233] For therapeutic studies, 2 × 106 Human MDA-MB-231 cells were inoculated orthotopically in the 4th mammary fat pad in SCID mice. Tumor growth was monitored and measured once a week with a caliper in live sedated animals. Mice had been randomly assigned before tumor inoculation to receive treatment with the PFGF-R inhibitor imatinib or to placebo, which were delivered via i.p. injection twice a week (300 μg per week) starting from the day of tumor establishment. For therapeutic trials involving endocrine therapy, when a tumor was palpable (longest diameter >3 mm), mice were alternately assigned into the treatment groups in which mice were treated with tamoxifen (1 mg/dose via oral gavage daily, Sigma), dissolved in a vehicle of ethanol and corn oil (Sigma) by heating to 55 °C, or with vehicle alone. All therapeutic administrations were open-label.

[0234] The imatinib + tamoxifen group is statistically significantly smaller than all other groups ($p<0.01$ vs control, $p<0.05$ vs A3B6, $p<0.01$ vs letrozole, Student's unpaired, 2-sided t-test assuming equal variance); no other differences are statistically significant (Figure 8).

[0235] Conclusion: inhibition of the PDGFR tyrosine kinase sensitizes previously impervious basal-like/triple-negative breast tumors to the action of endocrine therapy in the form of tamoxifen.

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[0236]

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Claims

1. A kit-of-parts comprising:

    a. an anti-PDGF-CC antibody and an anti-estrogen, or
    b. an inhibitor of PDGF-R and an anti-estrogen, wherein said inhibitor of PDGF-R is imatinib,

    for use in the treatment of ER-negative breast cancer in an individual in need thereof.

2. The kit-of-parts for use according to any one of the preceding claims for use in the treatment of ER-negative breast cancer, wherein said treatment comprises the steps of

    a. administration of the anti-PDGF-CC antibody or the inhibitor of PDGF-R to an individual in need thereof;
    b. subsequent administration of the anti-estrogen.

3. The-kit-of-parts for use according to any one of claims 1 and 2, wherein the individual has suffered from ER-negative breast cancer, and wherein said breast cancer in said individual has been treated by surgery, and wherein said treatment reduces the risk of relapse.

4. The kit-of-parts for use according to any one of claims 2 and 3, wherein the first administration of anti-PDGF-CC antibody or inhibitor of PDGF-R to said individual is prior to the first administration of said anti-estrogen.

5. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-PDGF-CC antibody is a monoclonal antibody specifically binding PDGF-CC.

6. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-PDGF-CC antibody is capable of binding an epitope positioned in, comprising or consisting of:

    a. aa 230 to 250 of SEQ ID NO:1
    b. aa 228 to 238 of SEQ ID NO:1
    c. aa 308 to 322 of SEQ ID NO:1
    d. aa 242 to 254 of SEQ ID NO:1
    e. aa 288 to 308 of SEQ ID NO:1
    f. aa 325 to 345 of SEQ ID NO:1

g. aa 256 to 274 of SEQ ID NO:1;
h. aa 256 to 264 of SEQ ID NO:1; and/or
i. aa 256 to 260 of SEQ ID NO:1

7. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-PDGF-CC antibody is capable of binding a epitope positioned within amino acids 230 to 250 of SEQ ID NO:1.

8. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-estrogen is an estrogen antagonist.

9. The kit-of-parts for use according to any one of the preceding claims, wherein the estrogen antagonist is selected from the group consisting of tamoxifen, raloxifene, 4-hydroxytramoxifen, trioxifene, keoxifene, afimoxifene, LY117018, fulvestrant and toremifene

10. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-estrogen is an aromatase inhibitor.

11. The kit-of-parts for use according to any one of the preceding claims, wherein the anti-estrogen is selected from the group consisting of exemestane, formestane, aminoglutethimide, vorozole, fadrozole, anastrozole and letrozole.

12. The kit-of-parts for use according to any one of the preceding claims, wherein the ER-negative breast cancer is a triple negative breast cancer.

13. The kit-of-parts for use according to any one of the preceding claims, wherein the ER-negative breast cancer is a basal-like breast cancer.

14. The kit-of-parts for use according to any one of the preceding claims, wherein the ER-negative breast cancer is a breast cancer wherein the primary tumor was ER-negative.

Patentansprüche

1. Teilesatz, umfassend:

a. einen Anti-PDGF-CC-Antikörper und ein Antiöstrogen, oder
b. einen Inhibitor von PDGF-R und ein Antiöstrogen, wobei der Inhibitor von PDGF-R Imatinib ist,

zur Verwendung bei der Behandlung von ER-negativem Brustkrebs bei einem Individuum, das dessen bedarf.

2. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche zur Verwendung bei der Behandlung von ER-negativem Brustkrebs, wobei die Behandlung die Schritte umfasst:

a. Verabreichung des Anti-PDGF-CC-Antikörpers oder des Inhibitors von PDGF-R an ein Individuum, das dessen bedarf;
b. anschließende Verabreichung des Antiöstrogens.

3. Teilesatz zur Verwendung nach einem der Ansprüche 1 und 2, wobei das Individuum an ER-negativem Brustkrebs leidet und wobei der Brustkrebs bei dem Individuum chirurgisch behandelt worden ist und wobei die Behandlung das Rückfallrisiko reduziert.

4. Teilesatz zur Verwendung nach einem der Ansprüche 2 und 3, wobei die erste Verabreichung des Anti-PDGF-CC-Antikörpers oder Inhibitors von PDGF-R an das Individuum vor der ersten Verabreichung des Antiöstrogens erfolgt.

5. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der Anti-PDGF-CC-Antikörper ein monoklonaler Antikörper ist, der spezifisch PDGF-CC bindet.

6. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der Anti-PDGF-CC-Antikörper in der Lage ist, ein Epitop zu binden, das positioniert ist in, umfassend oder bestehend aus:

a. Aminosäure 230 bis 250 von SEQ ID NO:1
b. Aminosäure 228 bis 238 von SEQ ID NO:1
c. Aminosäure 308 bis 322 von SEQ ID NO:1
d. Aminosäure 242 bis 254 von SEQ ID NO:1
e. Aminosäure 288 bis 308 von SEQ ID NO:1
f. Aminosäure 325 bis 345 von SEQ ID NO:1
g. Aminosäure 256 bis 274 von SEQ ID NO: 1;
h. Aminosäure 256 bis 264 von SEQ ID NO: 1; und/oder
i. Aminosäure 256 bis 260 von SEQ ID NO:1.

7. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der Anti-PDGF-CC-Antikörper in der Lage ist, ein Epitop zu binden, das innerhalb der Aminosäuren 230 bis 250 von SEQ ID NO: 1 positioniert ist.

8. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei das Antiöstrogen ein Östrogenantagonist ist.

9. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der Östrogenantagonist ausgewählt ist aus der Gruppe bestehend aus Tamoxifen, Raloxifen, 4-Hydroxytramoxifen, Trioxifen, Keoxifen, Afimoxifen, LY117018, Fulvestrant und Toremifen.

10. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei das Antiöstrogen ein Aromatase-Inhibitor ist.

11. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei das Antiöstrogen ausgewählt ist aus der Gruppe bestehend aus Exemestan, Formestan, Aminoglutethimid, Vorozol, Fadrozol, Anastrozol und Letrozol.

12. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der ER-negative Brustkrebs ein dreifach negativer Brustkrebs ist.

13. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der ER-negative Brustkrebs ein basalartiger Brustkrebs ist.

14. Teilesatz zur Verwendung nach einem der vorhergehenden Ansprüche, wobei der ER-negative Brustkrebs ein Brustkrebs ist, bei dem der Primärtumor ER-negativ war.

Revendications

1. Kit de pièces comprenant :

a. un anticorps anti-PDGF-CC et un anti-œstrogène, ou
b. un inhibiteur du PDGF-R et un anti-œstrogène, ledit inhibiteur de PDGF-R étant l'imatinib,

pour une utilisation dans le traitement du cancer du sein négatif pour ER chez un individu en ayant besoin.

2. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes pour une utilisation dans le traitement du cancer du sein négatif pour ER, dans lequel ledit traitement comprend les étapes

a. d'administration de l'anticorps anti-PDGF-CC ou de l'inhibiteur du PDGF-R à un individu en ayant besoin ;
b. d'administration ultérieure de l'anti-œstrogène.

3. Kit de pièces à utiliser selon l'une quelconque des revendications 1 et 2, dans lequel l'individu a souffert d'un cancer du sein négatif pour ER, et dans lequel ledit cancer du sein chez ledit individu a été traité par chirurgie, et dans lequel ledit le traitement réduit le risque de rechute.

4. Kit de pièces à utiliser selon l'une quelconque des revendications 2 et 3, dans lequel la première administration d'anticorps anti-PDGF-CC ou d'inhibiteur de PDGF-R audit individu est antérieure à la première administration dudit anti-oestrogène.

5. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anticorps anti-PDGF-CC est un anticorps monoclonal se liant spécifiquement à PDGF-CC.

6. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anticorps anti-PDGF-CC est capable de se lier à un épitope positionné dans, comprenant ou constitué de :

a. aa 230 à 250 de SEQ ID NO:1
b. aa 228 à 238 de SEQ ID NO:1
c. aa 308 à 322 de SEQ ID NO:1
d. aa 242 à 254 de SEQ ID NO:1
e. aa 288 à 308 de SEQ ID NO:1
f. aa 325 à 345 de SEQ ID NO:1
g. aa 256 à 274 de SEQ ID NO:1
h. aa 256 à 264 de SEQ ID NO:1 ; et/ou
i. aa 256 à 260 de SEQ ID NO:1.

7. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anticorps anti-PDGF-CC est capable de se lier à un épitope positionné dans les acides aminés 230 à 250 de SEQ ID NO:1.

8. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anti-œstrogène est un antagoniste des œstrogènes.

9. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'antagoniste des œstrogènes est choisi dans le groupe constitué du tamoxifène, du raloxifène, du 4-hydroxytramoxifène, du trioxifène, du kéoxifène, de l'afimoxifène, du LY117018, du fulvestrant et du torémifène.

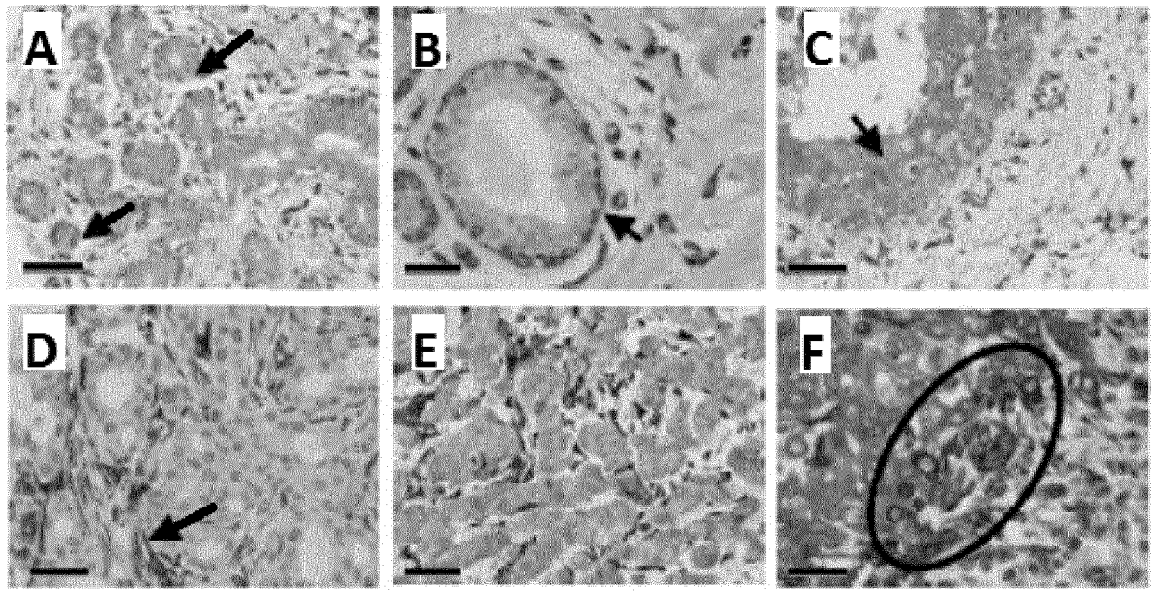
10. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anti-œstrogène est un inhibiteur d'aromatase.

11. Kit de pièces à utiliser selon l'une quelconque des revendications précédentes, dans lequel l'anti-œstrogène est choisi dans le groupe constitué de l'exémestane, du formestane, de l'aminoglutéthimide, du vorozole, du fadrozole, de l'anastrozole et du létrozole.

12. Kit de pièces destiné à être utilisé selon l'une quelconque des revendications précédentes, dans lequel le cancer du sein négatif pour ER est un cancer du sein triple négatif.

13. Kit de pièces destiné à être utilisé selon l'une quelconque des revendications précédentes, dans lequel le cancer du sein négatif pour ER est un cancer du sein de type basal.

14. Kit de pièces destiné à être utilisé selon l'une quelconque des revendications précédentes, dans lequel le cancer du sein négatif pour ER est un cancer du sein dans lequel la tumeur primaire était négative pour ER.



G

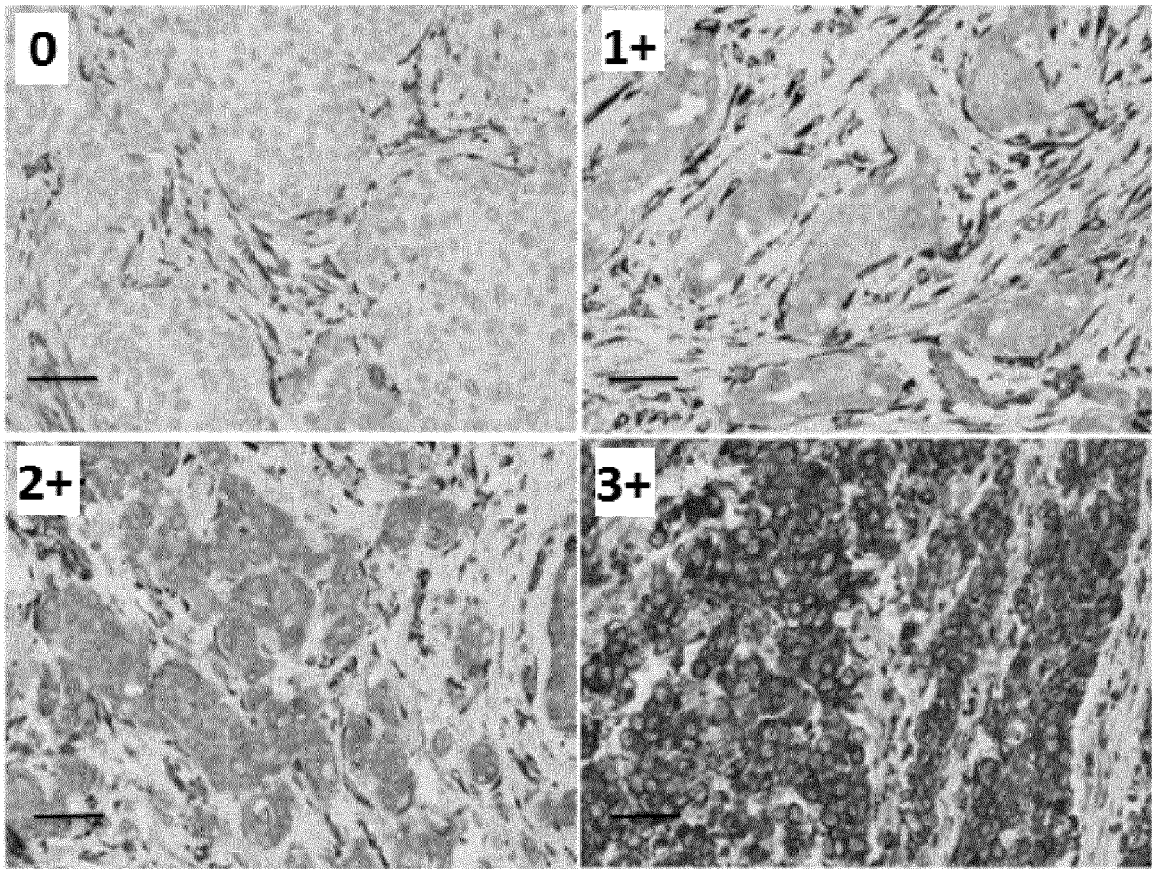
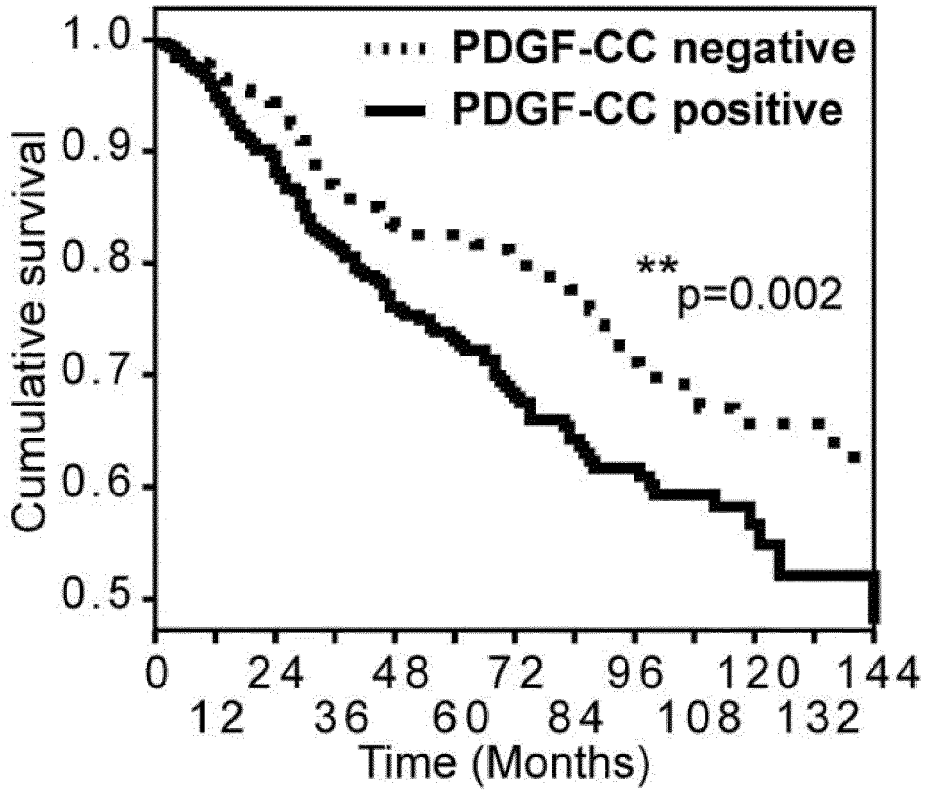


Fig. 1

H


PDGF-CC negative
PDGF-CC positive
**p=0.002
Cumulative survival
1.0
0.9
0.8
0.7
0.6
0.5
0
12
24
36
48
60
72
84
96
108
120
132
144
Time (Months)

I

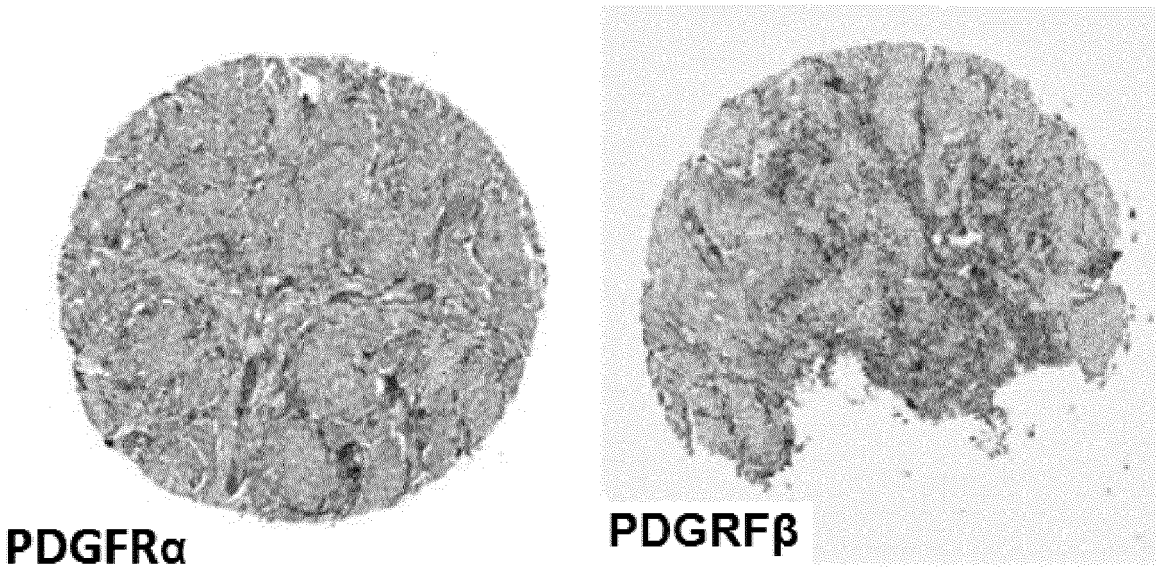
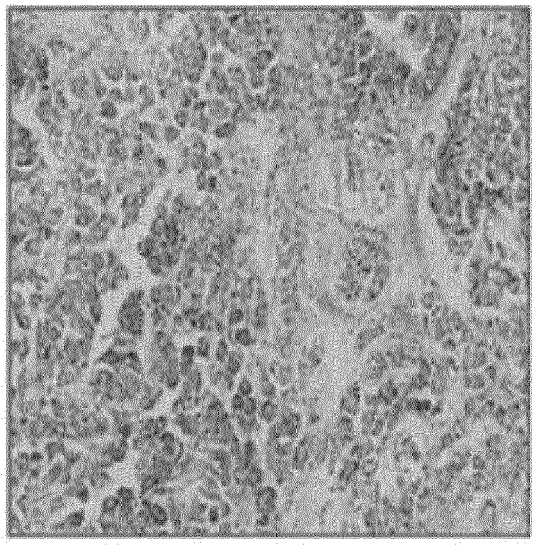
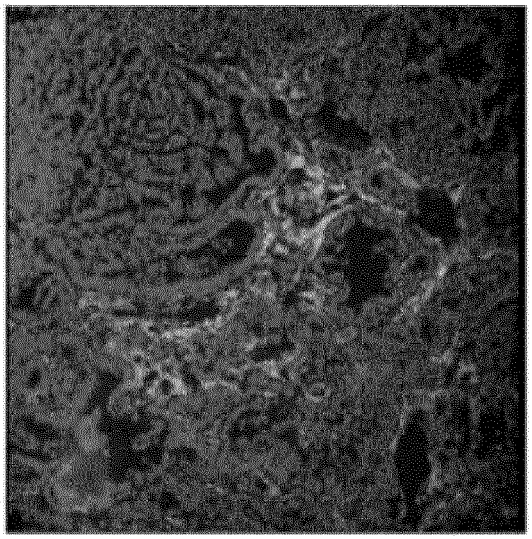

PDGFRα
PDGRFβ

Fig. 1 cont'

A



PDGF-CC



PDGRFα
PDGRFβ

B

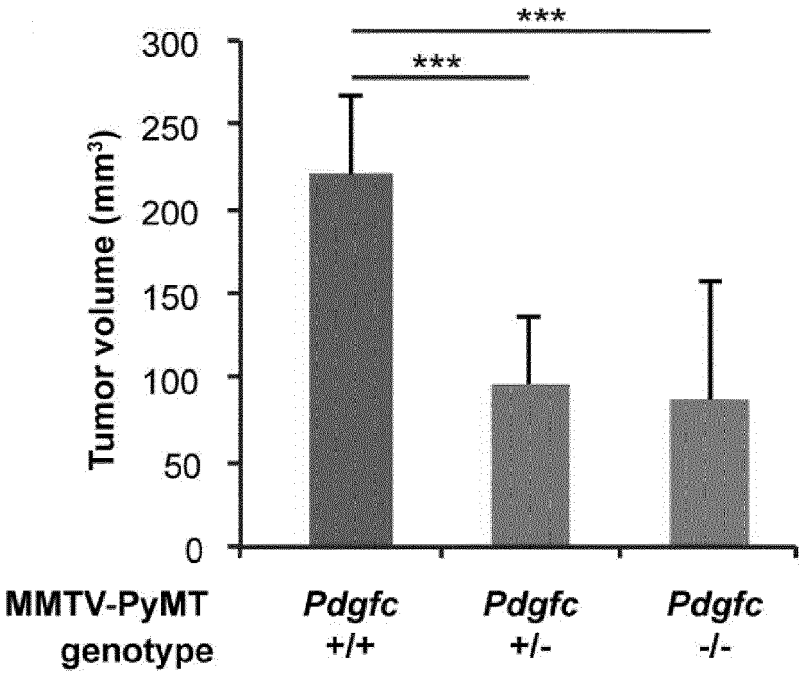
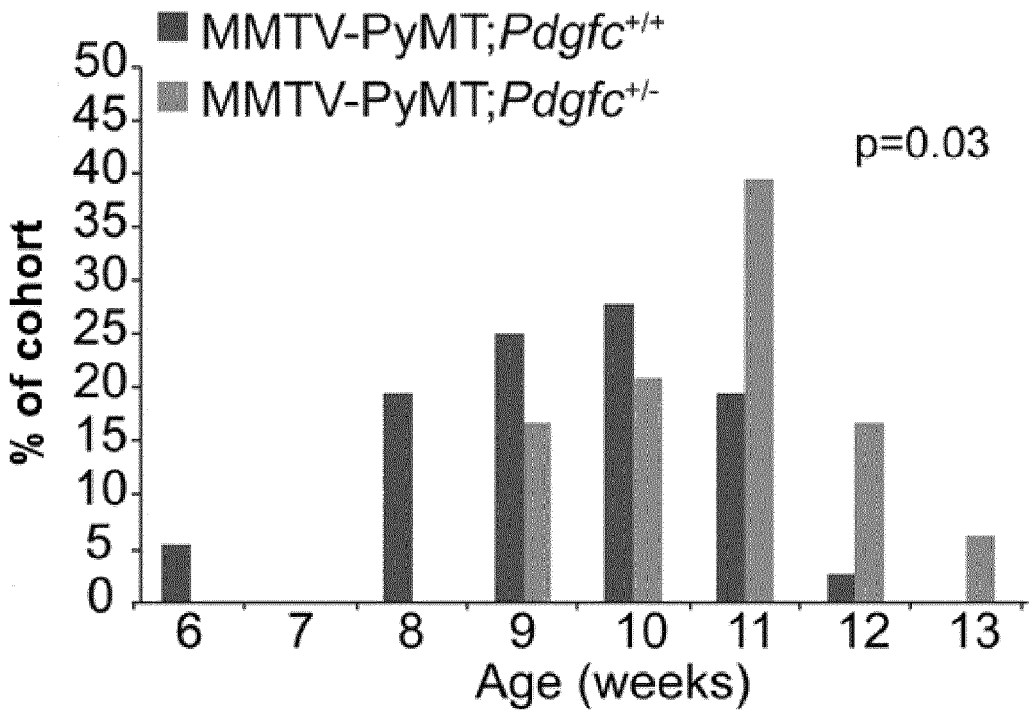


Fig. 2

C


MMTV-PyMT;Pdgfc+/+
MMTV-PyMT;Pdgfc+/-
p=0.03
% of cohort
50
45
40
35
30
25
20
15
10
5
0
6
7
8
9
10
11
12
13
Age (weeks)

D

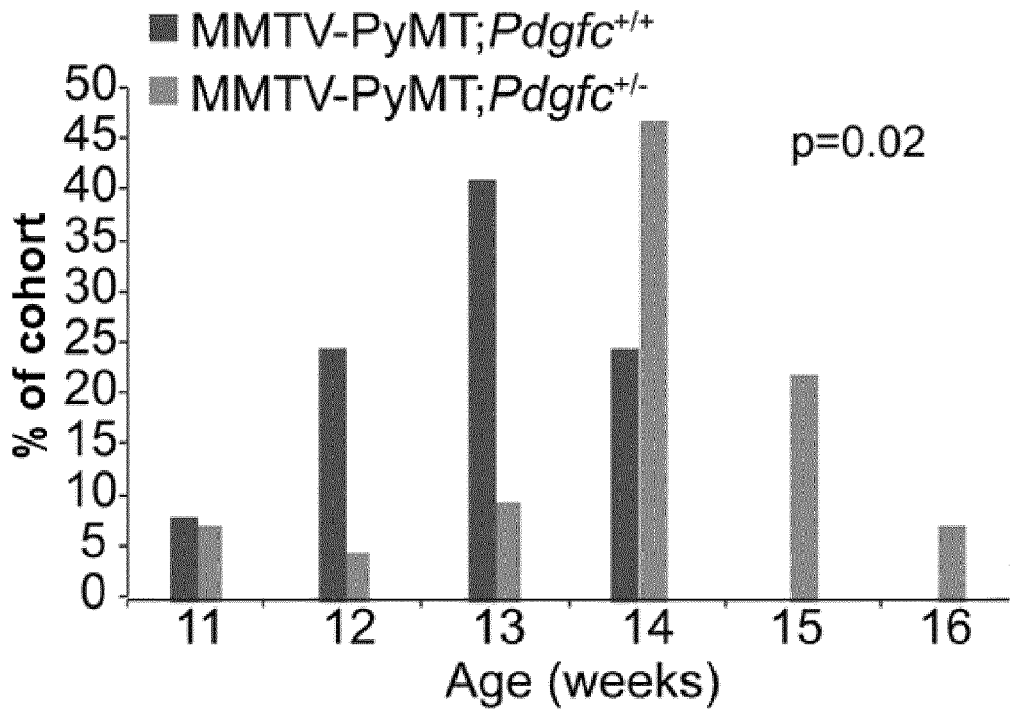

MMTV-PyMT;Pdgfc+/+
MMTV-PyMT;Pdgfc+/-
p=0.02
% of cohort
50
45
40
35
30
25
20
15
10
5
0
11
12
13
14
15
16
Age (weeks)

Fig. 2 cont'

E

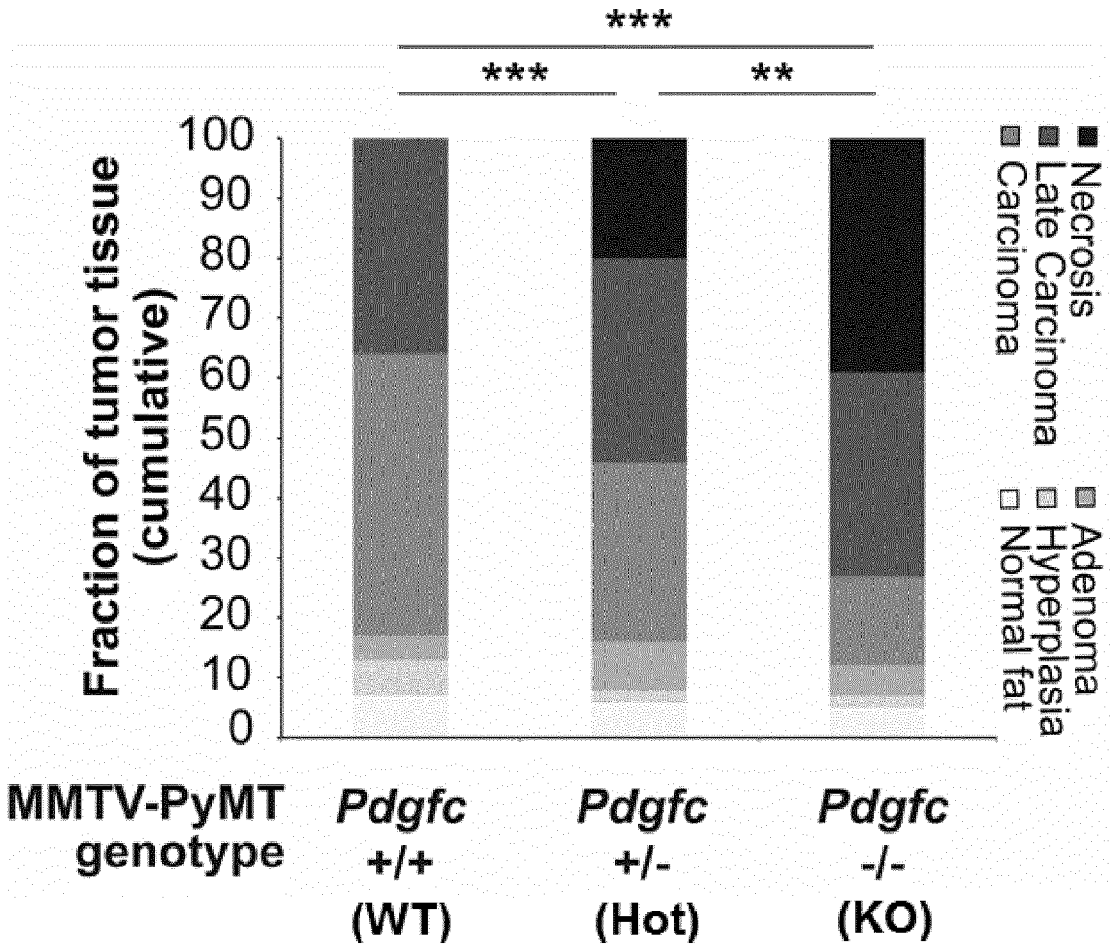

***
***
**
Fraction of tumor tissue (cumulative)
100
90
80
70
60
50
40
30
20
10
0
Necrosis
Late Carcinoma
Carcinoma
Adenoma
Hyperplasia
Normal fat
MMTV-PyMT genotype
Pdgfc +/+ (WT)
Pdgfc +/- (Hot)
Pdgfc -/- (KO)

Fig. 2 cont'

F

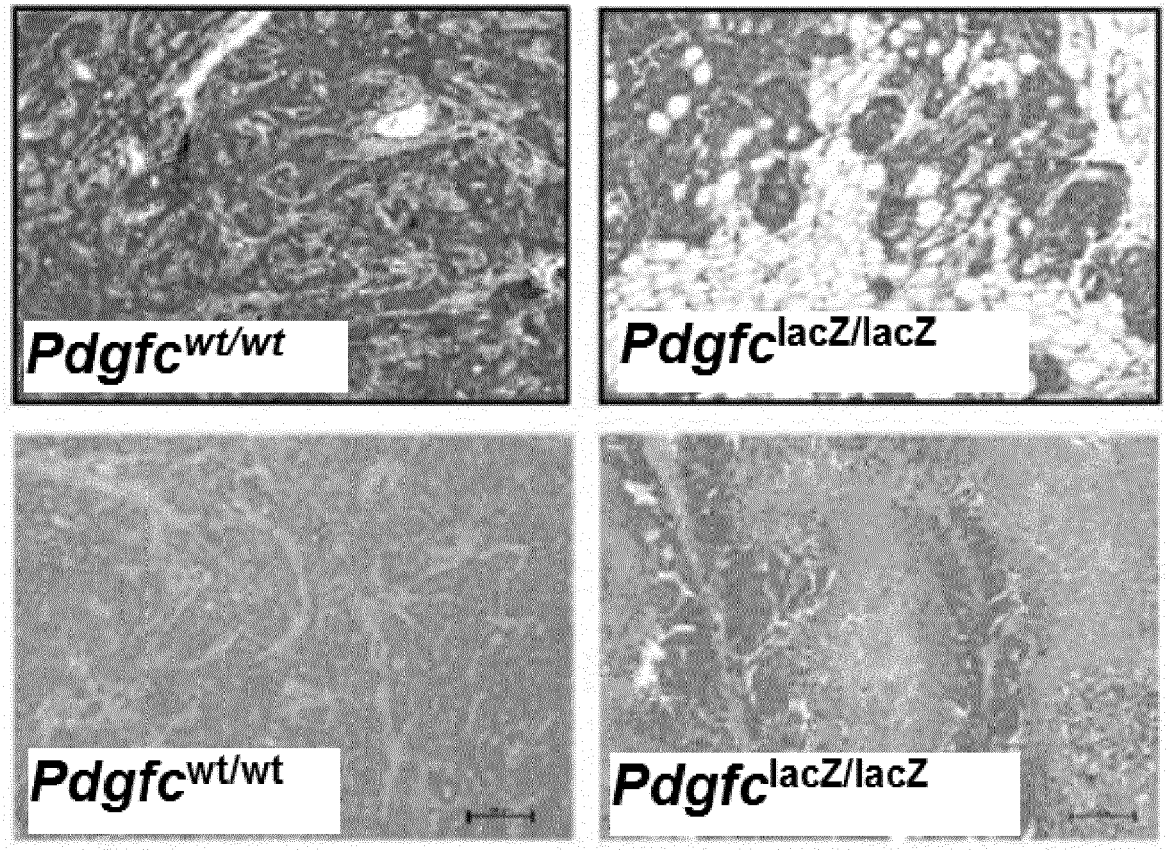
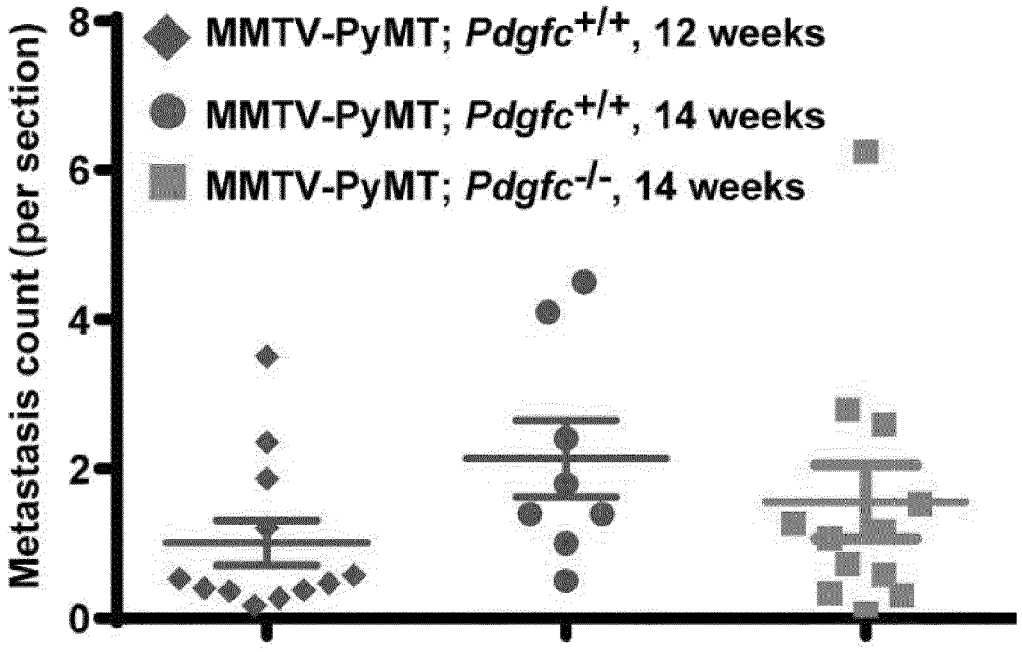


Fig. 2 cont'

G


MMTV-PyMT; Pdgfc+/+, 12 weeks
MMTV-PyMT; Pdgfc+/+, 14 weeks
MMTV-PyMT; Pdgfc-/-, 14 weeks
Metastasis count (per section)
0
2
4
6
8

H

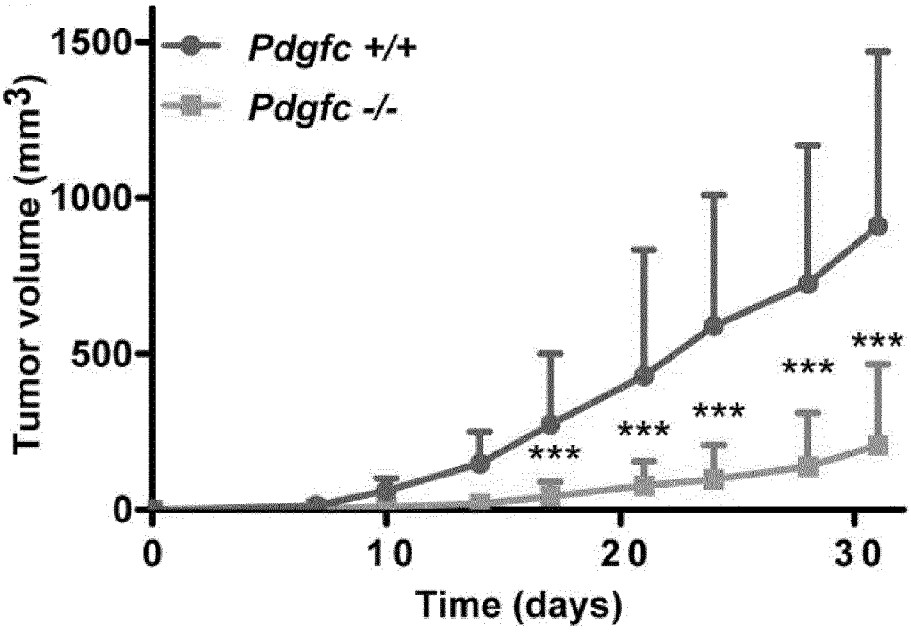

Pdgfc +/+
Pdgfc -/-
Tumor volume (mm3)
0
500
1000
1500
0
10
20
30
Time (days)
***
***
***
***
***

Fig. 2 cont'

I

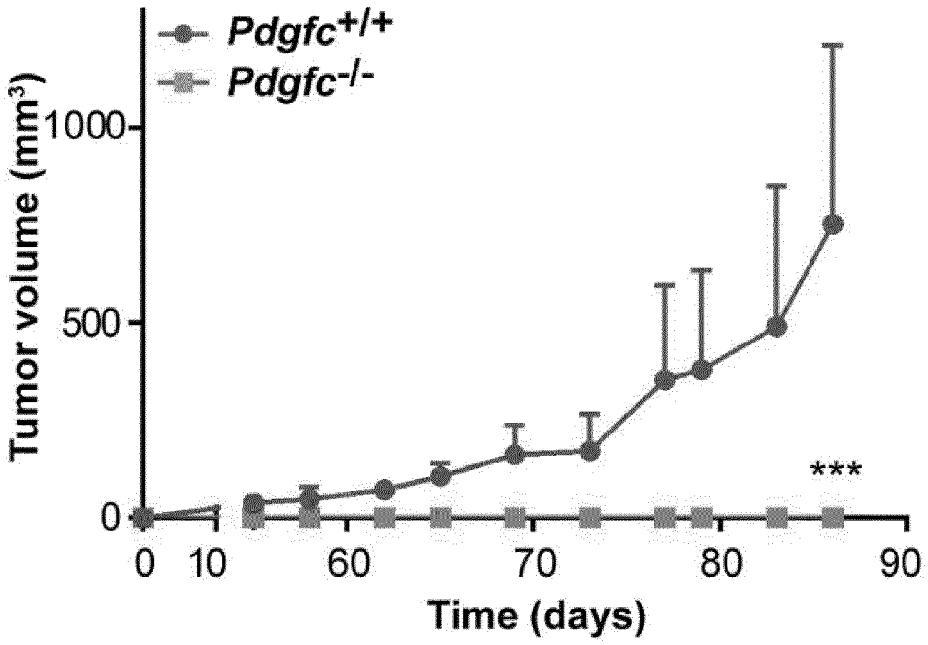
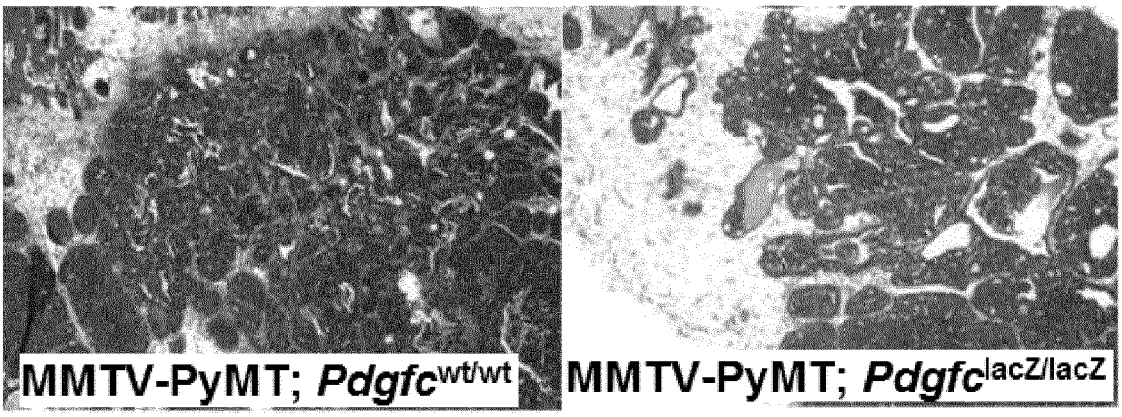

Pdgfc+/+
Pdgfc-/-
Tumor volume (mm3)
1000
500
0
***
0
10
60
70
80
90
Time (days)

Fig. 2 cont'

A


MMTV-PyMT; Pdgfc wt/wt
MMTV-PyMT; Pdgfc lacZ/lacZ

B

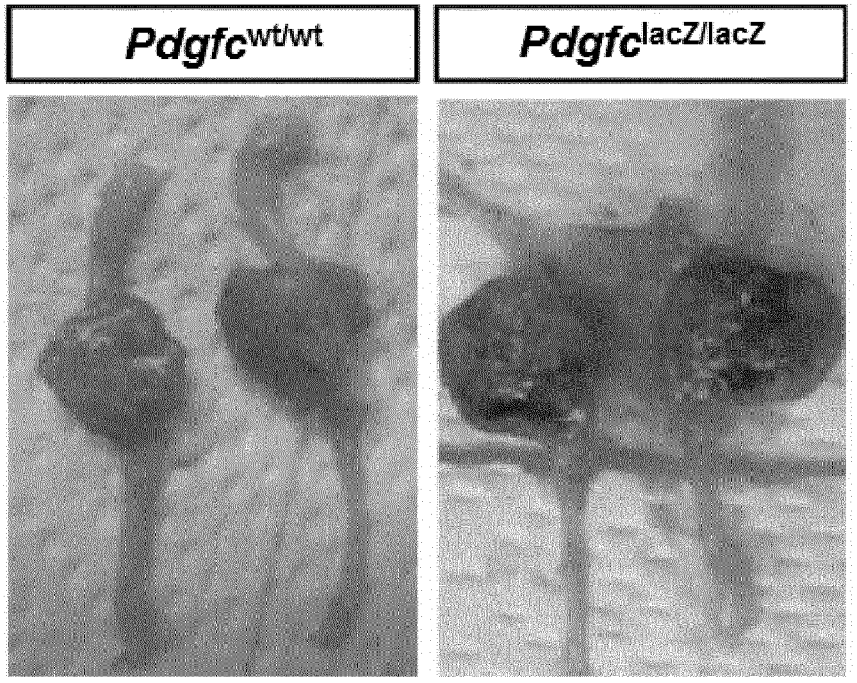
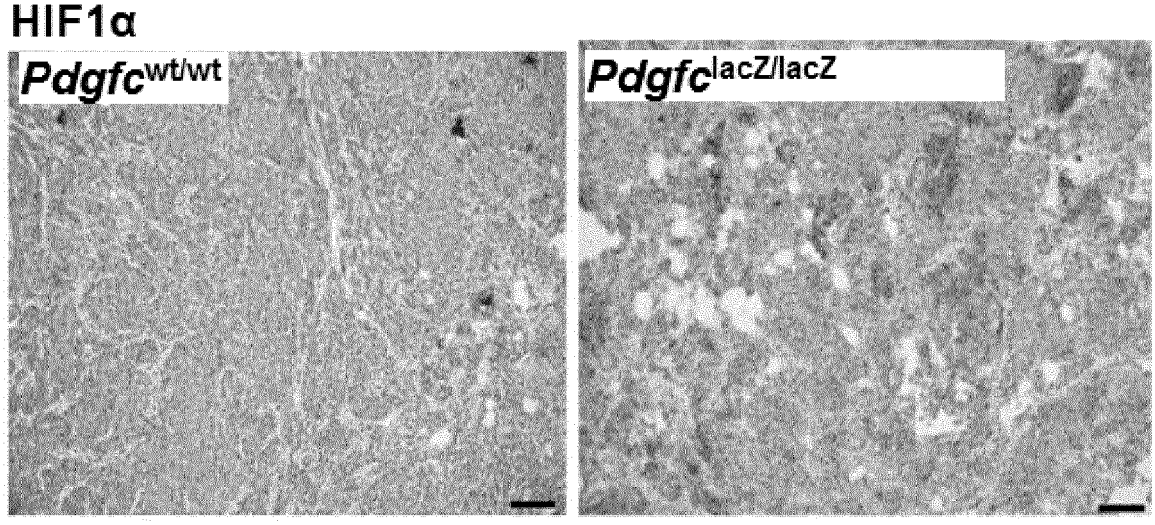

Pdgfc wt/wt
Pdgfc lacZ/lacZ

Fig. 3

C


HIF1α
Pdgfc wt/wt
Pdgfc lacZ/lacZ

D

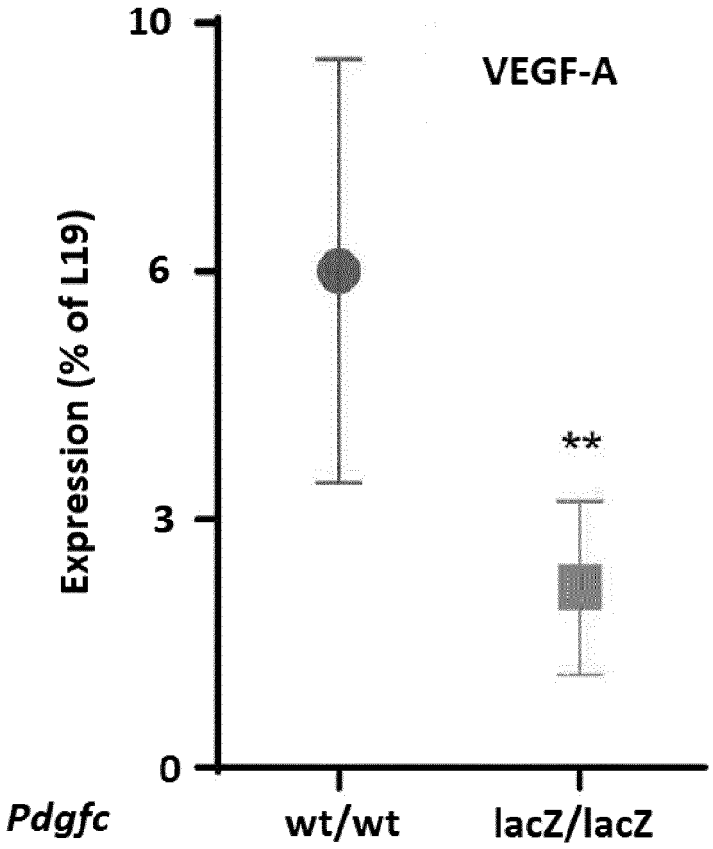

VEGF-A
Expression (% of L19)
10
6
3
0
**
Pdgfc
wt/wt
lacZ/lacZ

Fig. 3 cont'

E

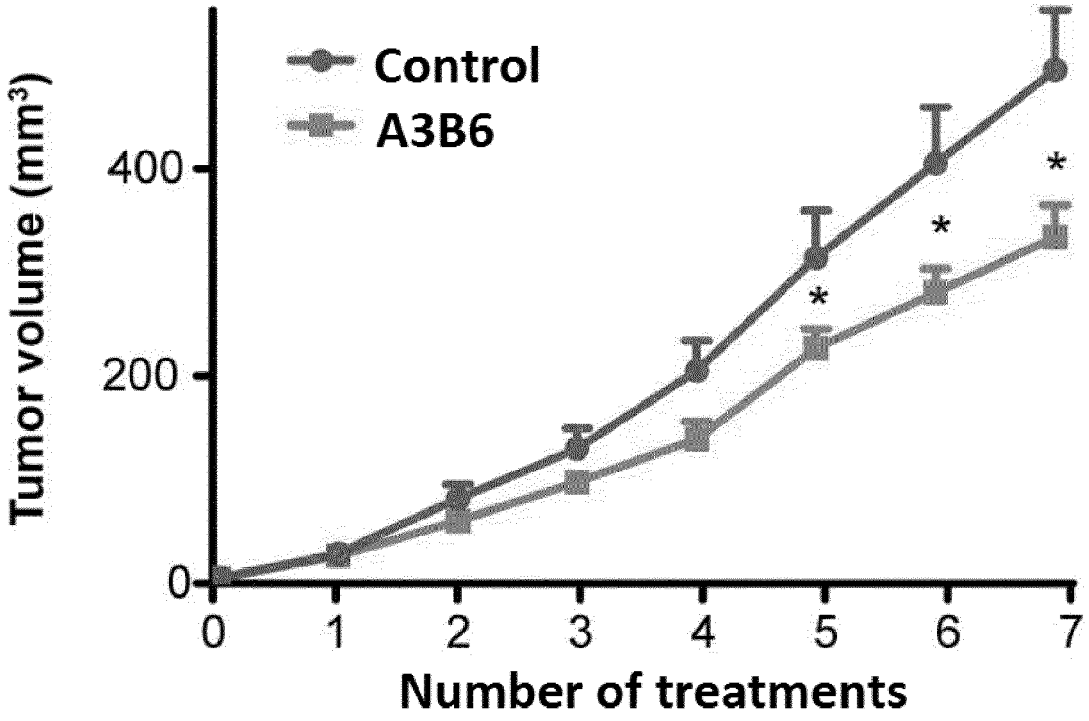

Control
A3B6
Tumor volume (mm3)
400
200
0
0
1
2
3
4
5
6
7
Number of treatments
*
*
*

Fig. 3 cont'

F

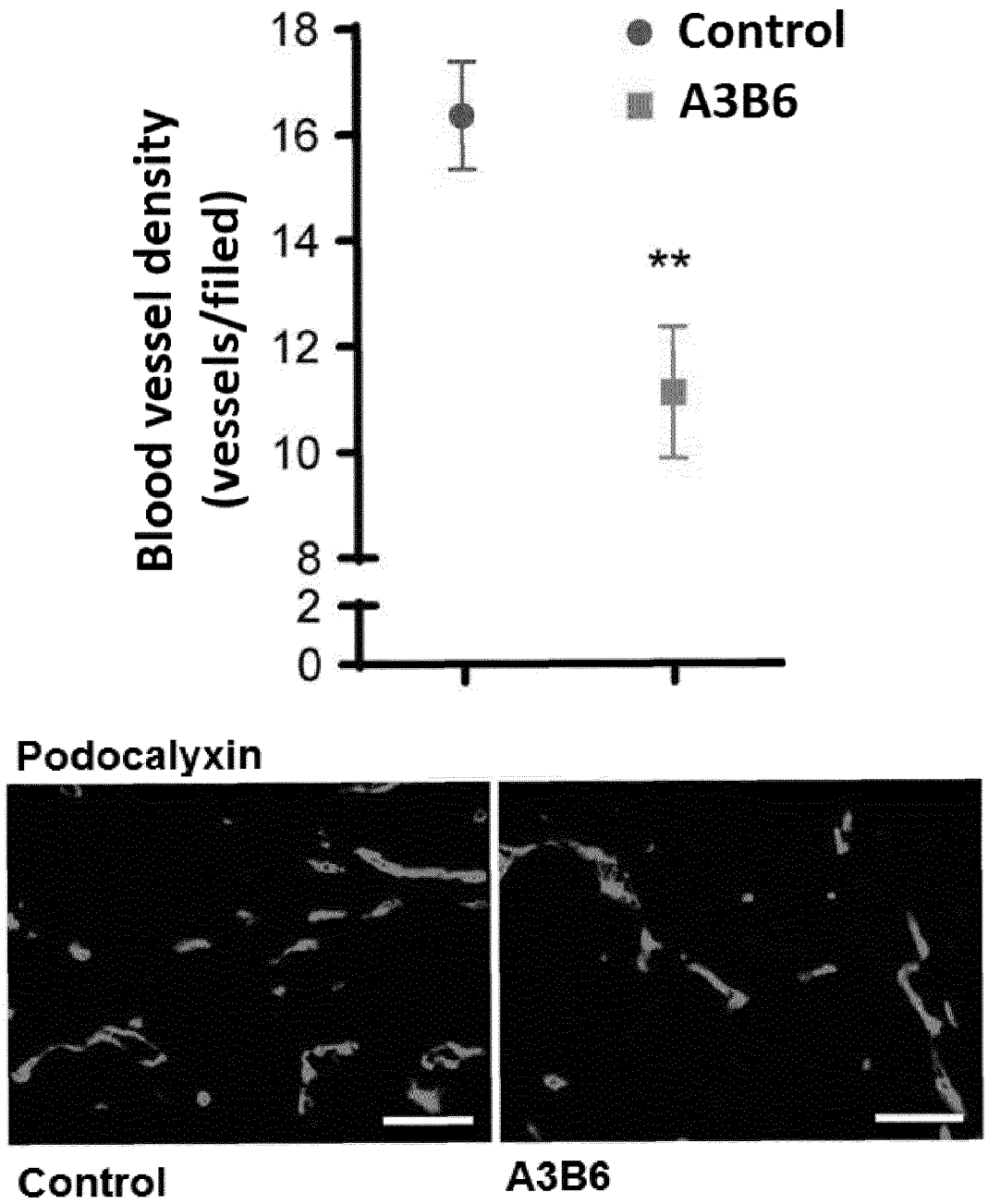
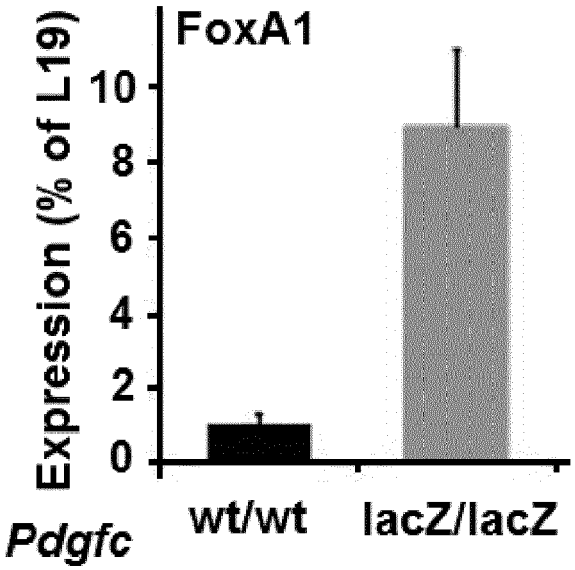

Blood vessel density
(vessels/filed)
18
16
14
12
10
8
2
0
Control
A3B6
**
Podocalyxin
Control
A3B6

Fig. 3 cont'

A


FoxA1
Expression (% of L19)
10
8
6
4
2
0
Pdgfc
wt/wt
lacZ/lacZ

B

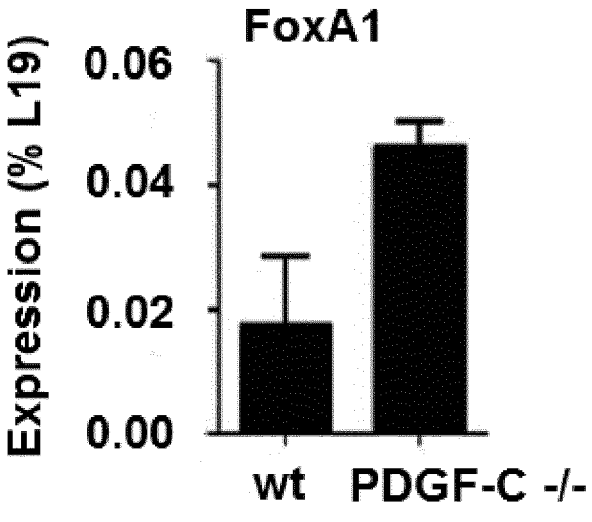

FoxA1
Expression (% L19)
0.06
0.04
0.02
0.00
wt
PDGF-C -/-

Fig. 4

C

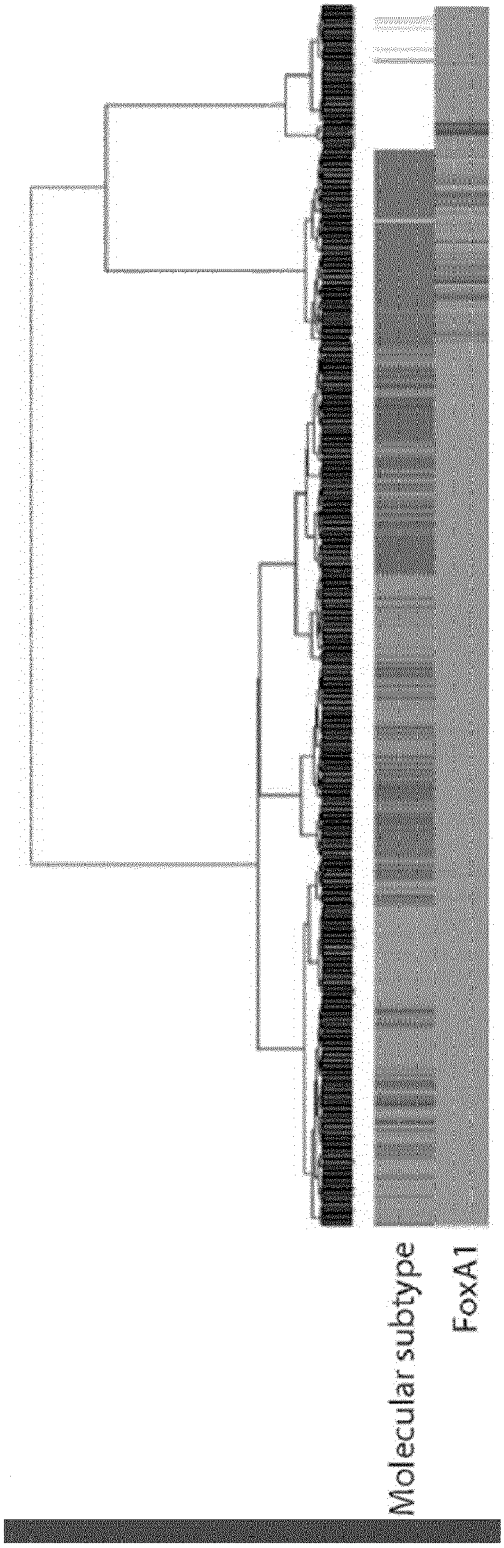
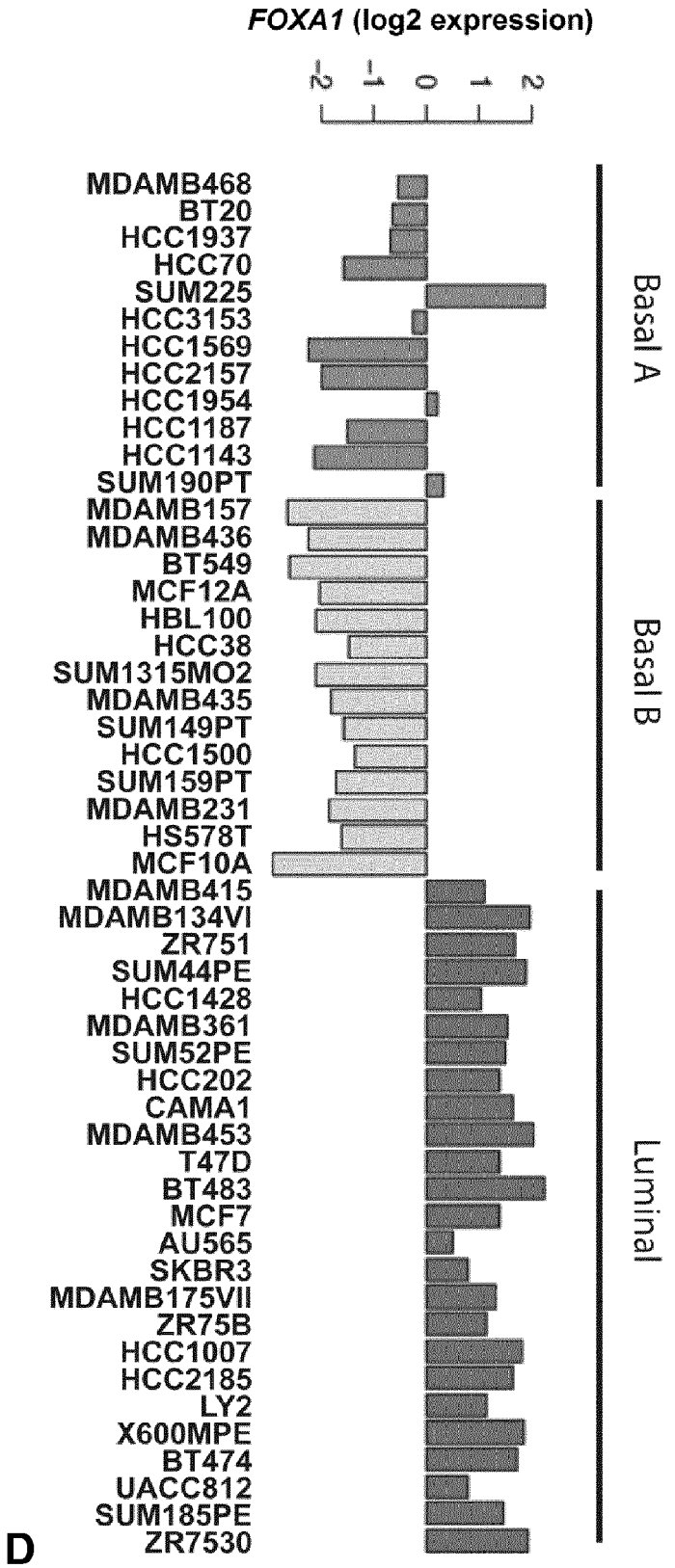


Fig. 4 cont'


FOXA1 (log2 expression)
-2
-1
0
1
2
MDAMB468
BT20
HCC1937
HCC70
SUM225
HCC3153
HCC1569
HCC2157
HCC1954
HCC1187
HCC1143
SUM190PT
MDAMB157
MDAMB436
BT549
MCF12A
HBL100
HCC38
SUM1315MO2
MDAMB435
SUM149PT
HCC1500
SUM159PT
MDAMB231
HS578T
MCF10A
MDAMB415
MDAMB134VI
ZR751
SUM44PE
HCC1428
MDAMB361
SUM52PE
HCC202
CAMA1
MDAMB453
T47D
BT483
MCF7
AU565
SKBR3
MDAMB175VII
ZR75B
HCC1007
HCC2185
LY2
X600MPE
BT474
UACC812
SUM185PE
ZR7530
Basal A
Basal B
Luminal

D

Fig. 4 cont'

E

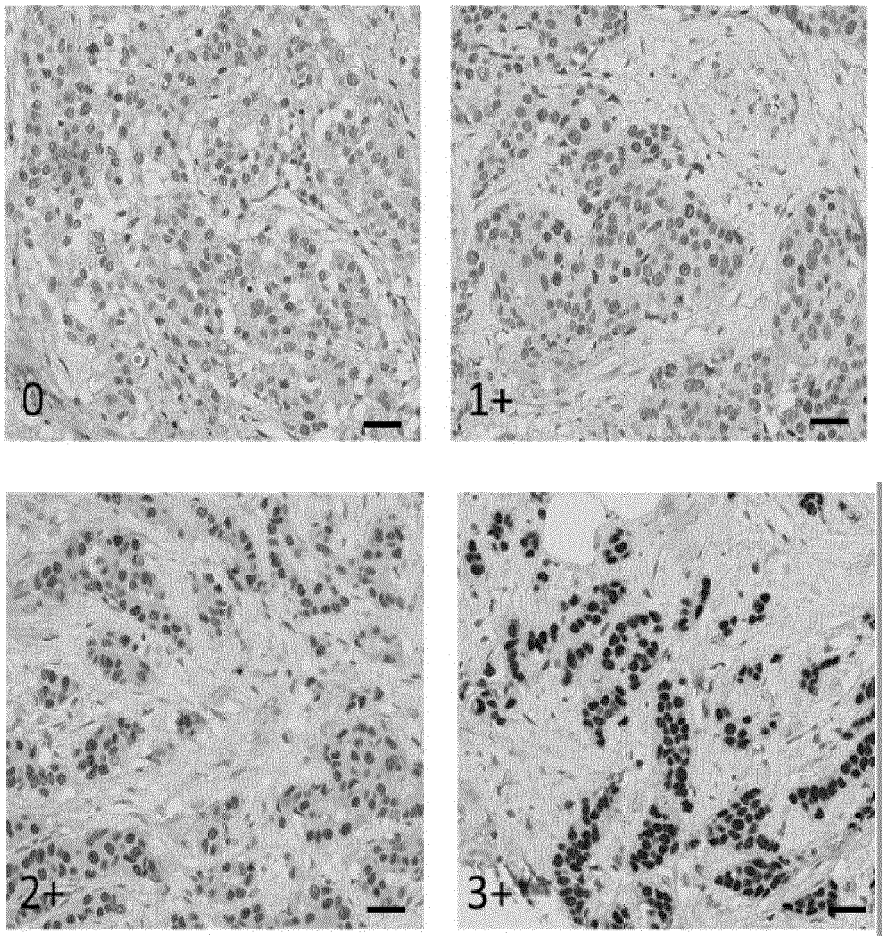
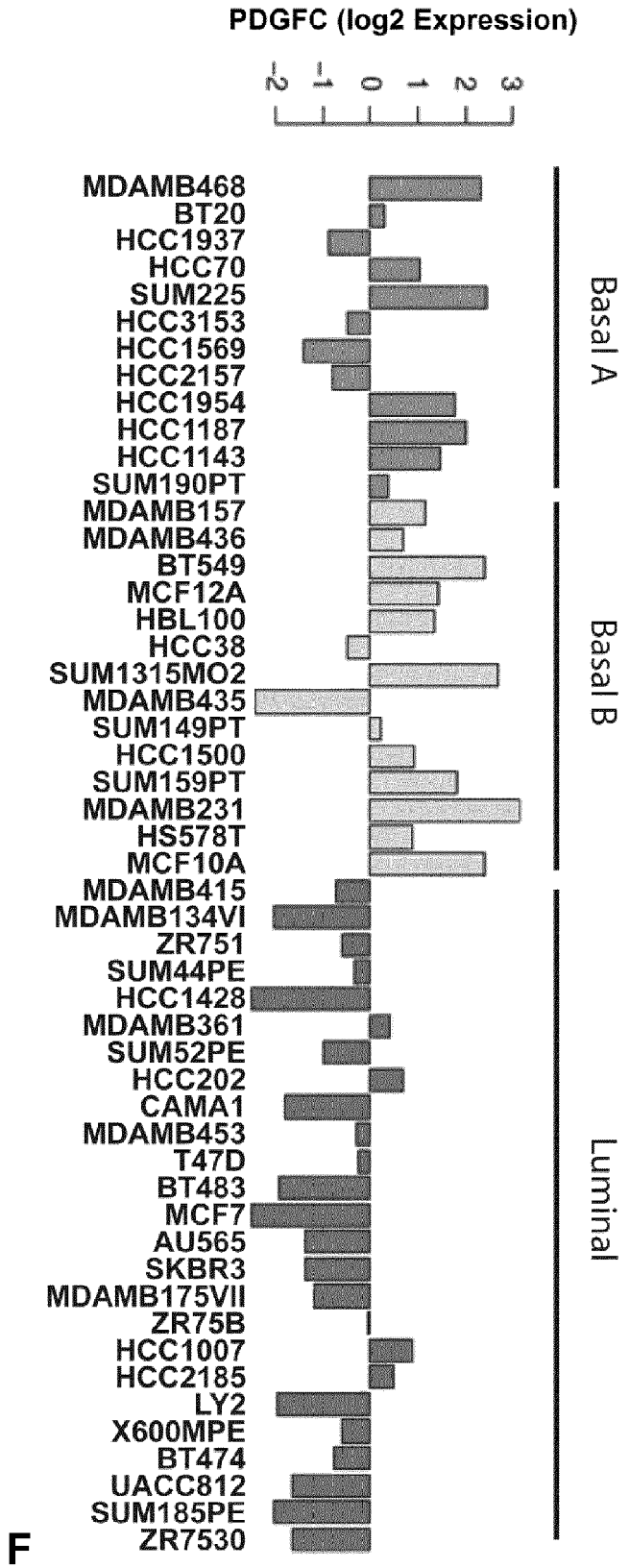


Fig. 4 cont'


PDGFC (log2 Expression)
-2
-1
0
1
2
3
MDAMB468
BT20
HCC1937
HCC70
SUM225
HCC3153
HCC1569
HCC2157
HCC1954
HCC1187
HCC1143
SUM190PT
MDAMB157
MDAMB436
BT549
MCF12A
HBL100
HCC38
SUM1315MO2
MDAMB435
SUM149PT
HCC1500
SUM159PT
MDAMB231
HS578T
MCF10A
MDAMB415
MDAMB134VI
ZR751
SUM44PE
HCC1428
MDAMB361
SUM52PE
HCC202
CAMA1
MDAMB453
T47D
BT483
MCF7
AU565
SKBR3
MDAMB175VII
ZR75B
HCC1007
HCC2185
LY2
X600MPE
BT474
UACC812
SUM185PE
ZR7530
Basal A
Basal B
Luminal

F

Fig. 4 cont'

G

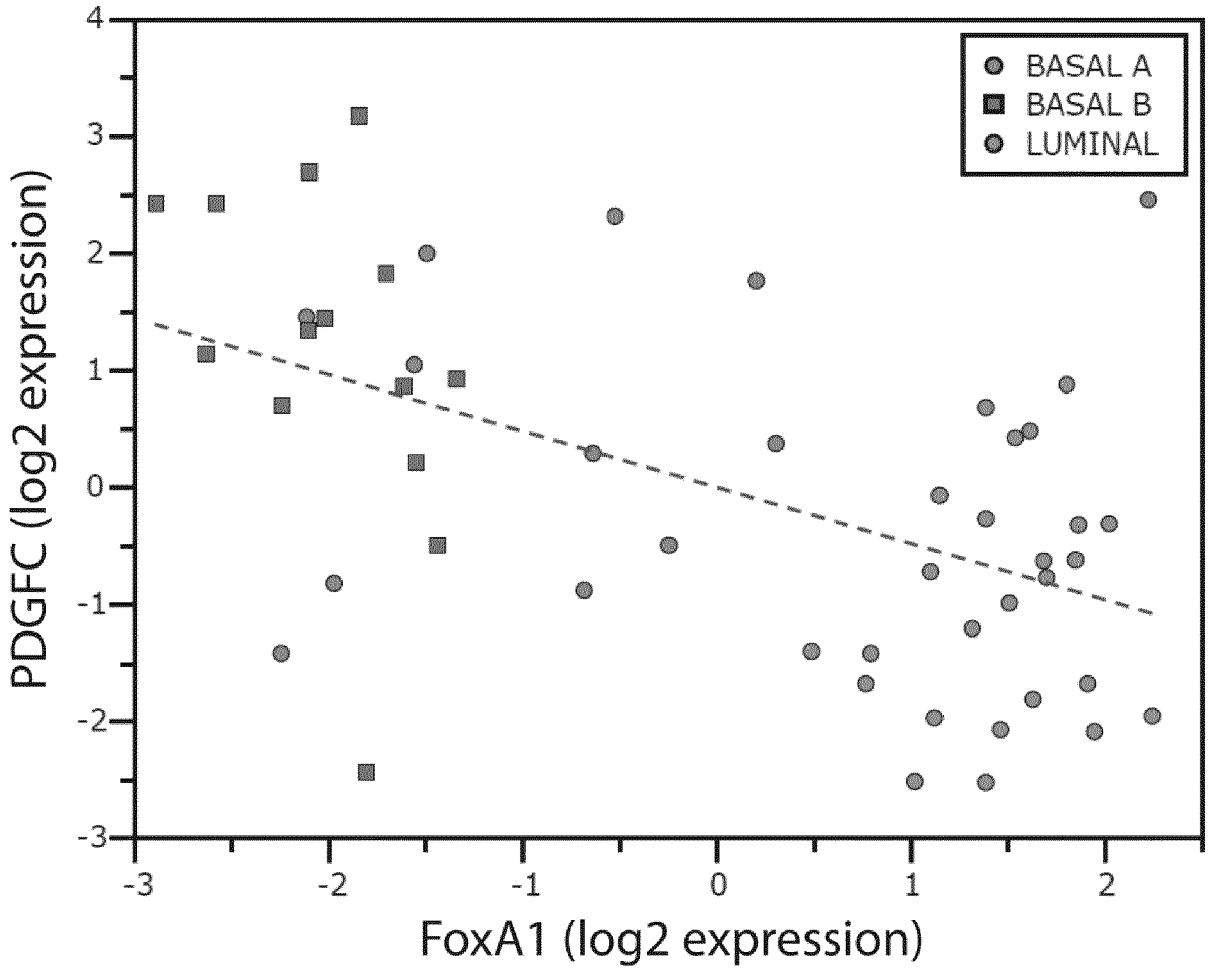
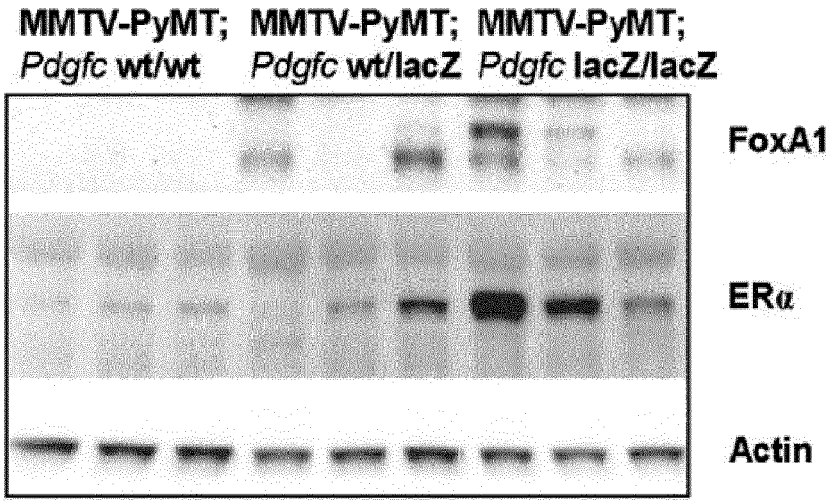

BASAL A
BASAL B
LUMINAL
PDGFC (log2 expression)
FoxA1 (log2 expression)
4
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2

Fig. 4 cont'

A


MMTV-PyMT; Pdgfc wt/wt
MMTV-PyMT; Pdgfc wt/lacZ
MMTV-PyMT; Pdgfc lacZ/lacZ
FoxA1
ERα
Actin

B

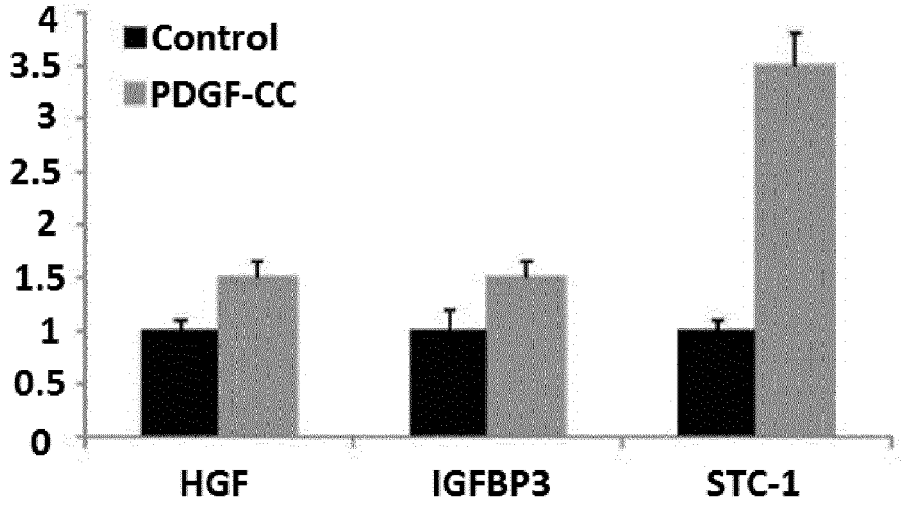

Control
PDGF-CC
4
3.5
3
2.5
2
1.5
1
0.5
0
HGF
IGFBP3
STC-1

Fig. 5

C

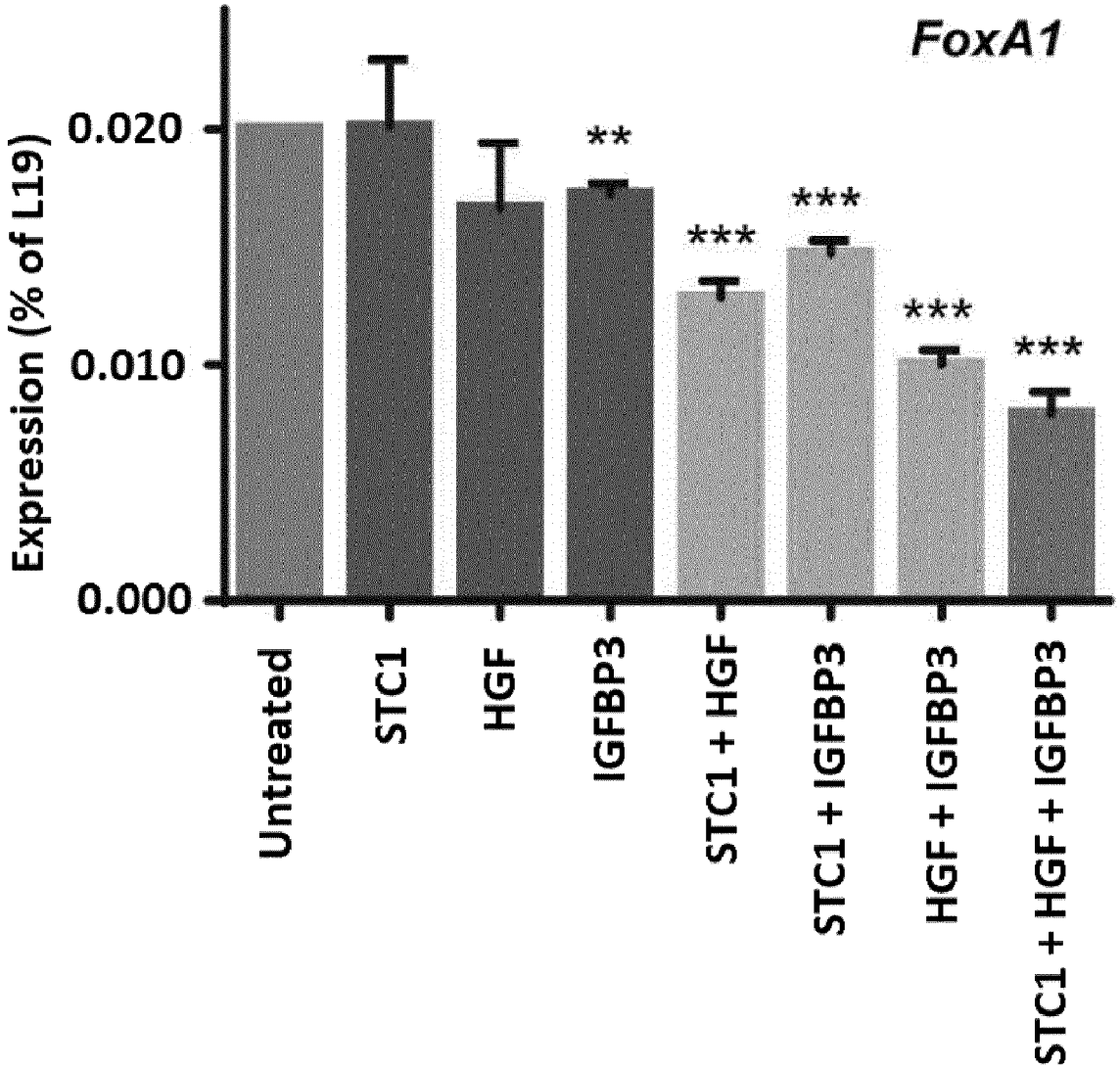

FoxA1
Expression (% of L19)
0.020
0.010
0.000
**
***
***
***
***
Untreated
STC1
HGF
IGFBP3
STC1 + HGF
STC1 + IGFBP3
HGF + IGFBP3
STC1 + HGF + IGFBP3

Fig. 5 cont'

D

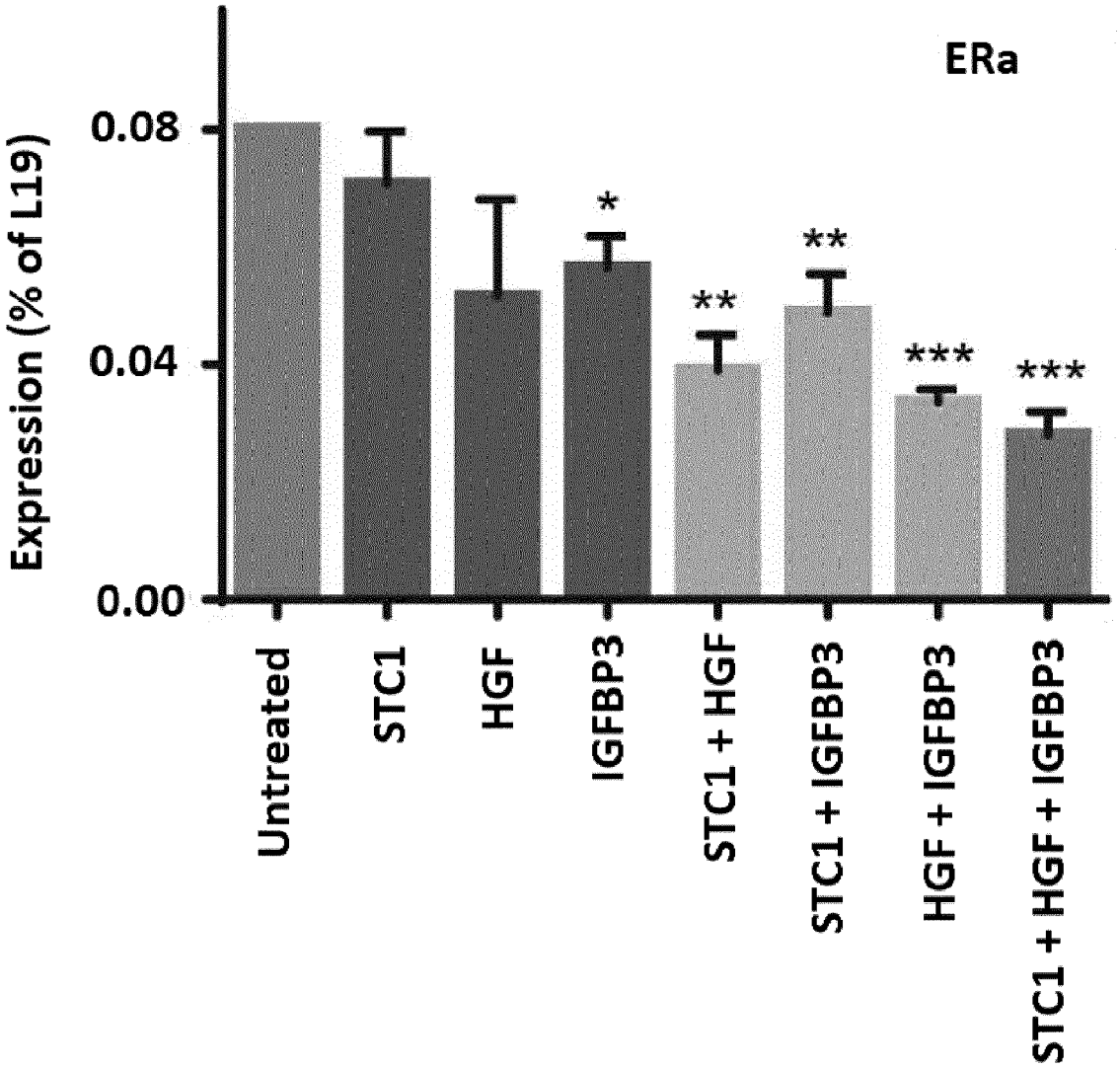

ERa
Expression (% of L19)
0.08
0.04
0.00
*
**
**
***
***
Untreated
STC1
HGF
IGFBP3
STC1 + HGF
STC1 + IGFBP3
HGF + IGFBP3
STC1 + HGF + IGFBP3

Fig. 5 cont'

E

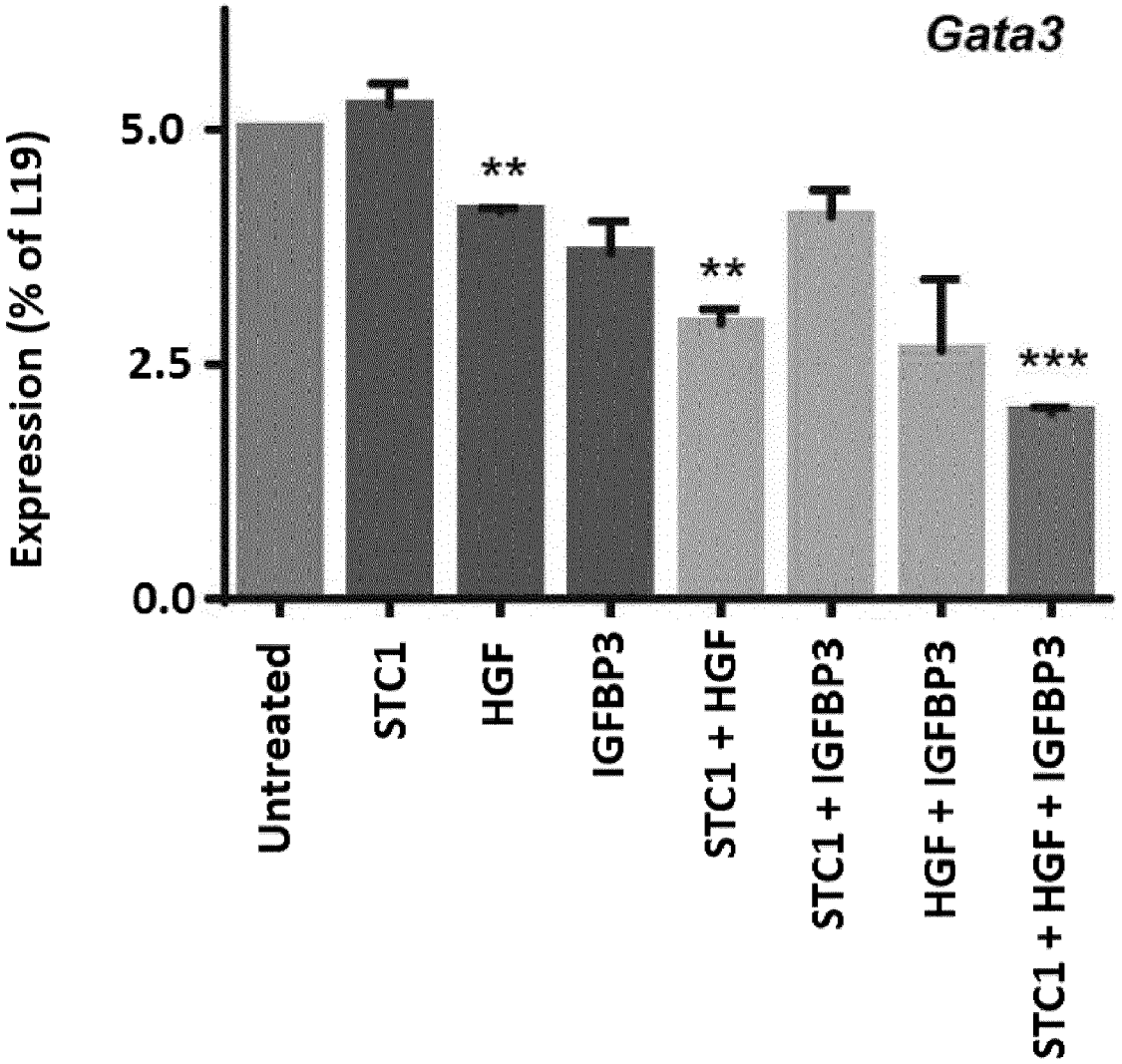
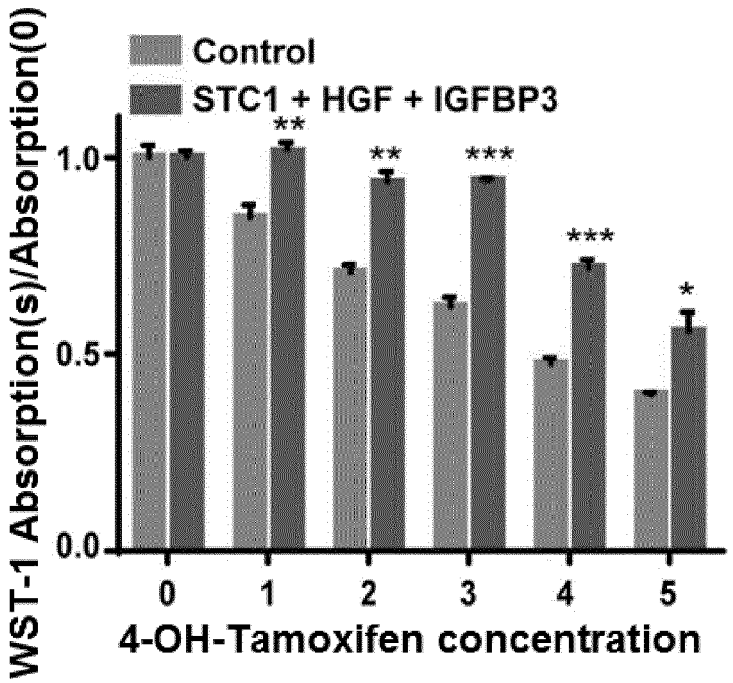

Gata3
Expression (% of L19)
5.0
2.5
0.0
**
**
***
Untreated
STC1
HGF
IGFBP3
STC1 + HGF
STC1 + IGFBP3
HGF + IGFBP3
STC1 + HGF + IGFBP3

Fig. 5 cont'

F


Control
STC1 + HGF + IGFBP3
WST-1 Absorption(s)/Absorption(0)
1.0
0.5
0.0
**
**
***
***
*
0
1
2
3
4
5
4-OH-Tamoxifen concentration

G

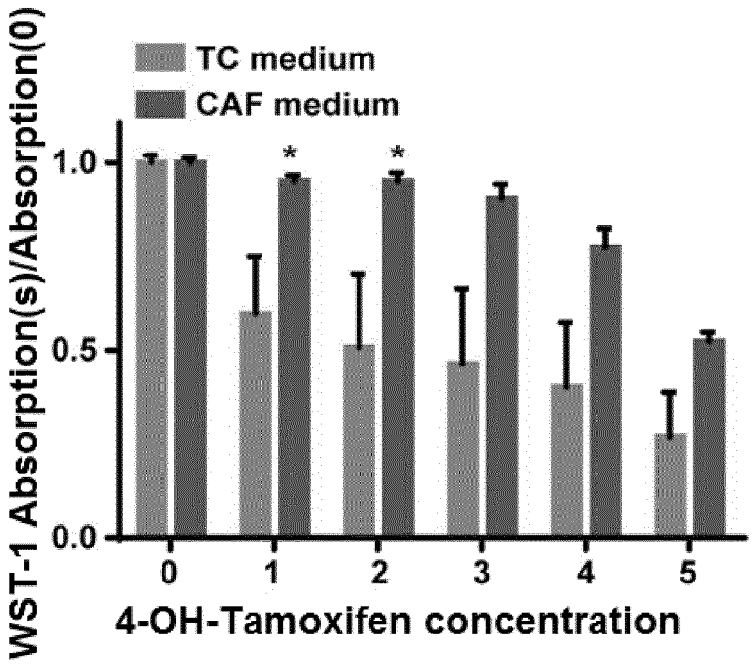

TC medium
CAF medium
WST-1 Absorption(s)/Absorption(0)
1.0
0.5
0.0
*
*
0
1
2
3
4
5
4-OH-Tamoxifen concentration

Fig. 5 cont'

H

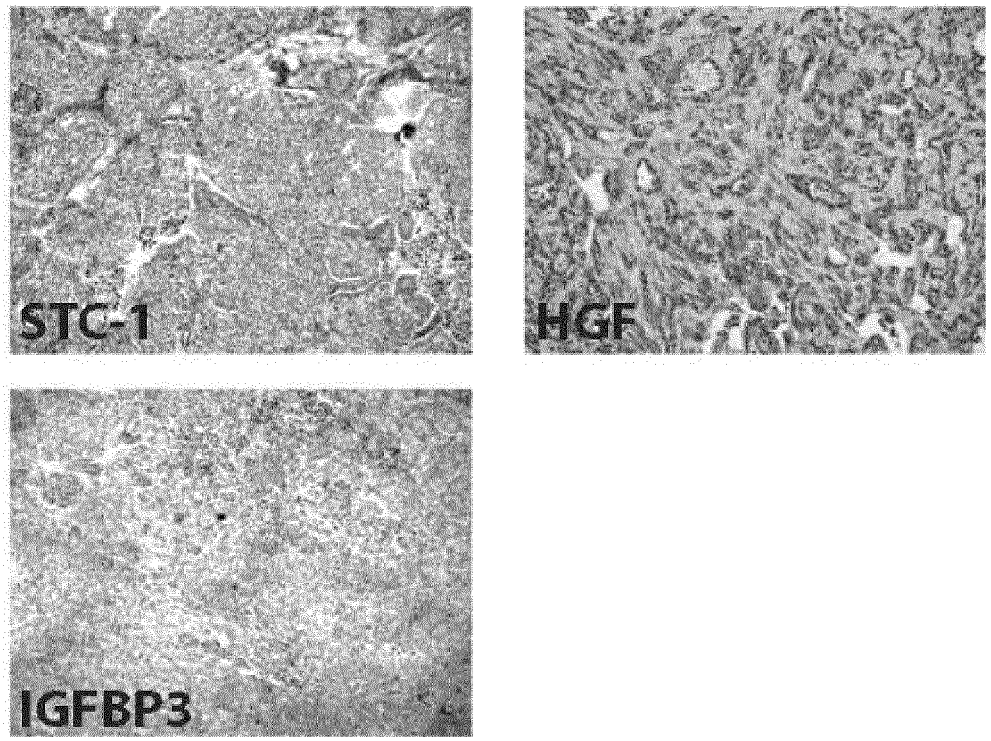
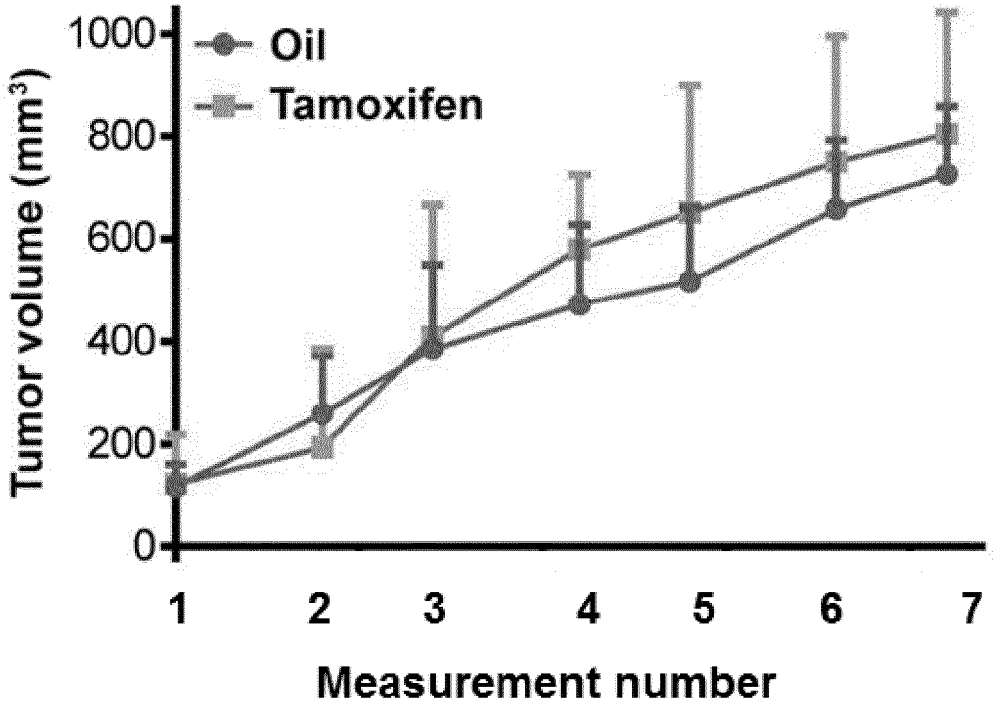


Fig. 5 cont'

A


Oil
Tamoxifen
Tumor volume (mm³)
1000
800
600
400
200
0
1
2
3
4
5
6
7
Measurement number

B

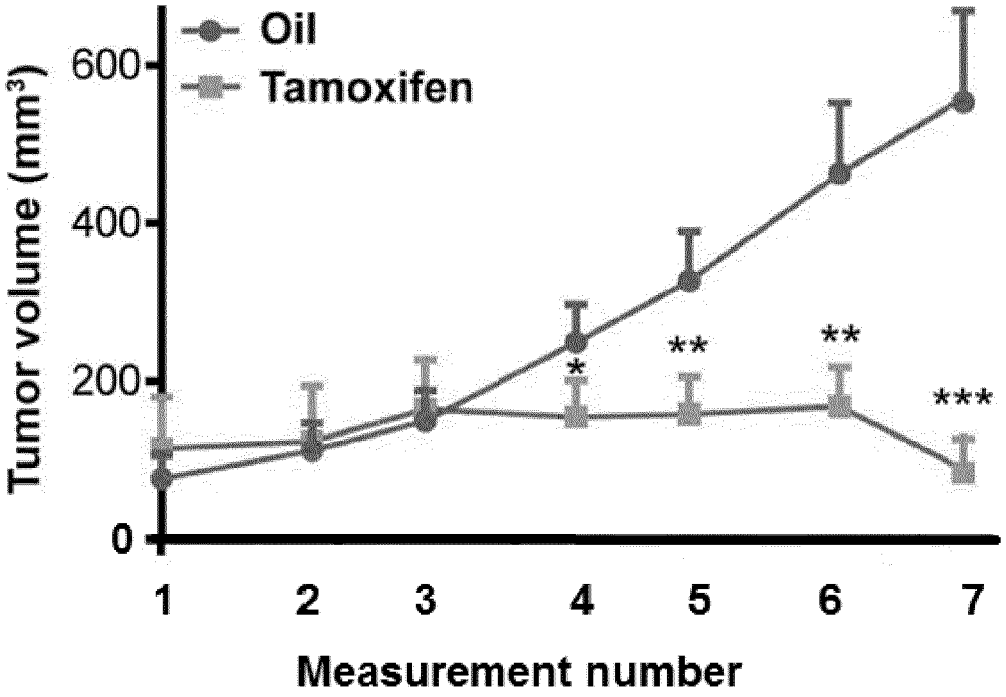
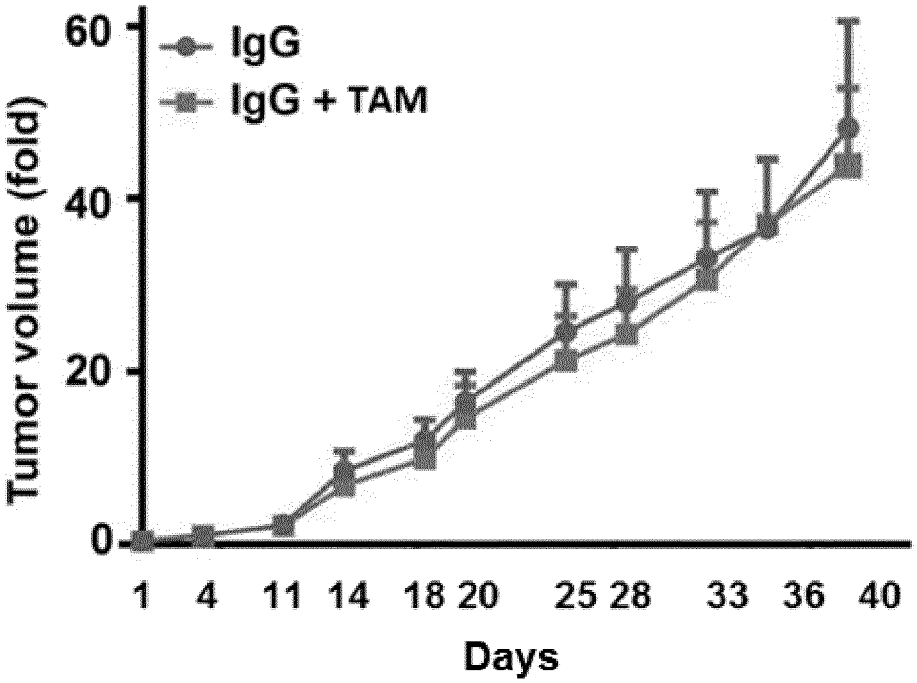

Oil
Tamoxifen
Tumor volume (mm³)
600
400
200
0
*
**
**
***
1
2
3
4
5
6
7
Measurement number

Fig. 6

C


60
40
20
0
Tumor volume (fold)
IgG
IgG + TAM
1 4 11 14 18 20 25 28 33 36 40
Days

D

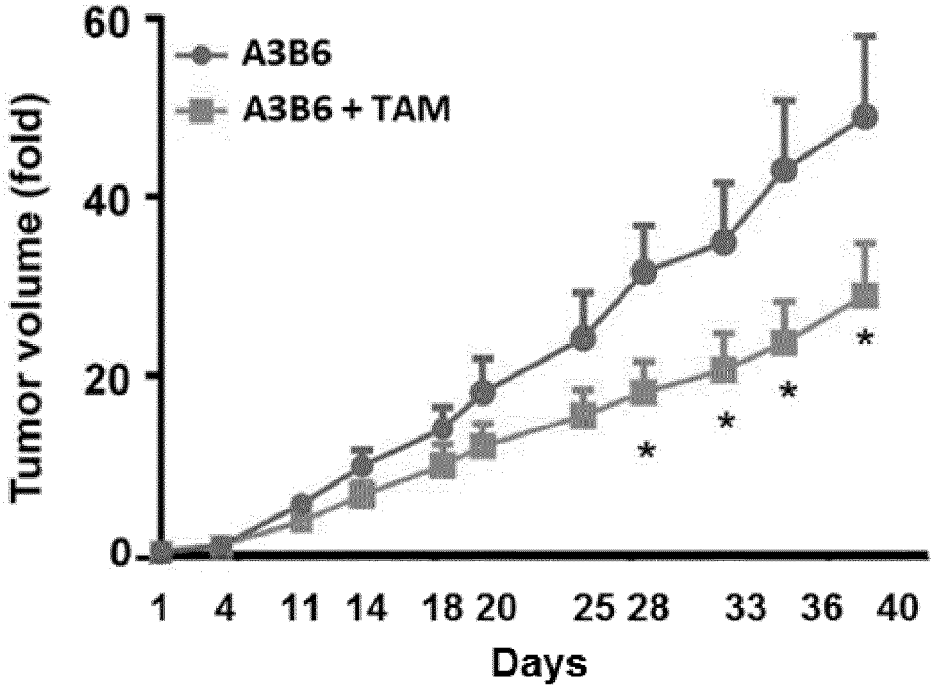
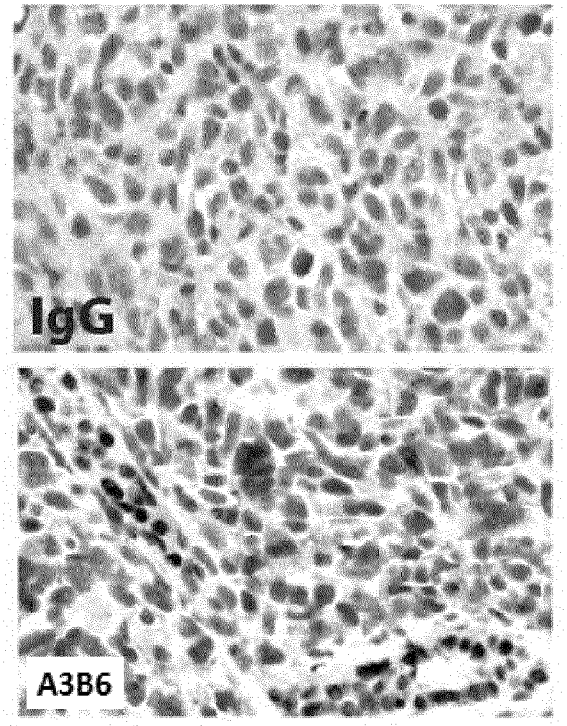

60
40
20
0
Tumor volume (fold)
A3B6
A3B6 + TAM
*
*
*
*
1 4 11 14 18 20 25 28 33 36 40
Days

Fig. 6 cont'

E


IgG
A3B6

F

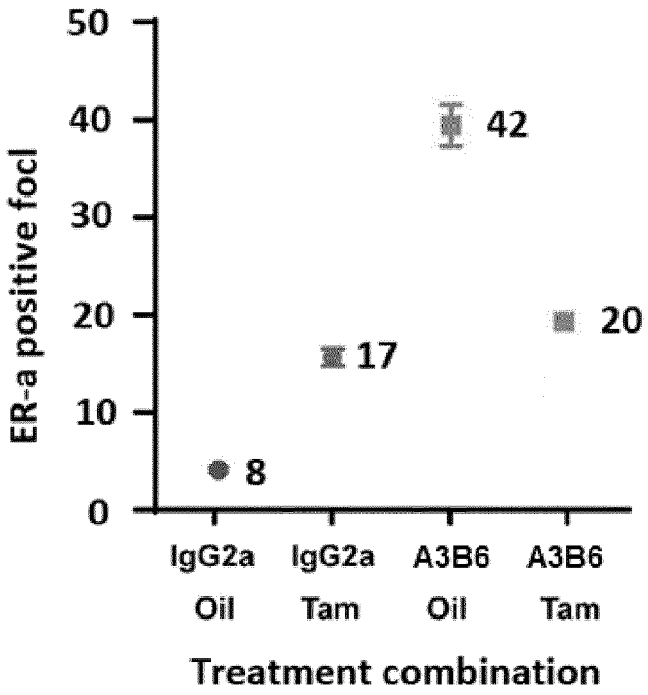

50
40
30
20
10
0
ER-a positive focl
42
20
17
8
IgG2a Oil
IgG2a Tam
A3B6 Oil
A3B6 Tam
Treatment combination

Fig. 6 cont'

G

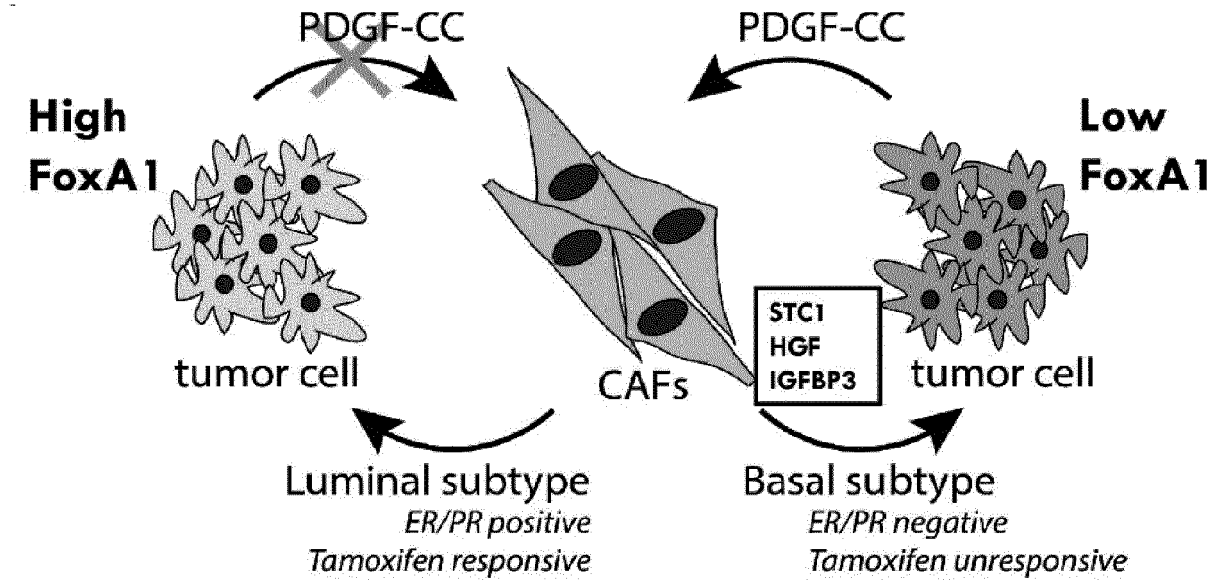

PDGF-CC
PDGF-CC
High
FoxA1
Low
FoxA1
tumor cell
CAFs
STC1
HGF
IGFBP3
tumor cell
Luminal subtype
ER/PR positive
Tamoxifen responsive
Basal subtype
ER/PR negative
Tamoxifen unresponsive

Fig. 6 cont'

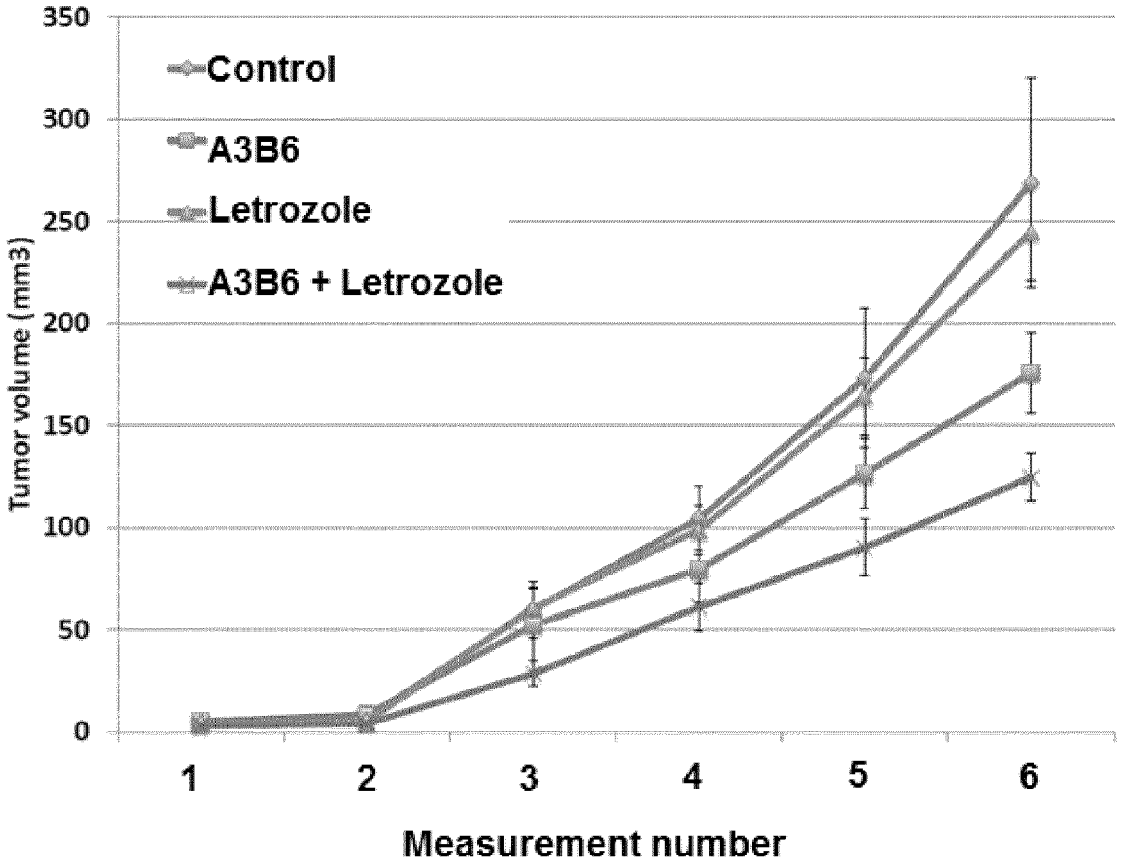

350
300
250
200
150
100
50
0
Tumor volume (mm3)
Control
A3B6
Letrozole
A3B6 + Letrozole
1
2
3
4
5
6
Measurement number

Fig. 7

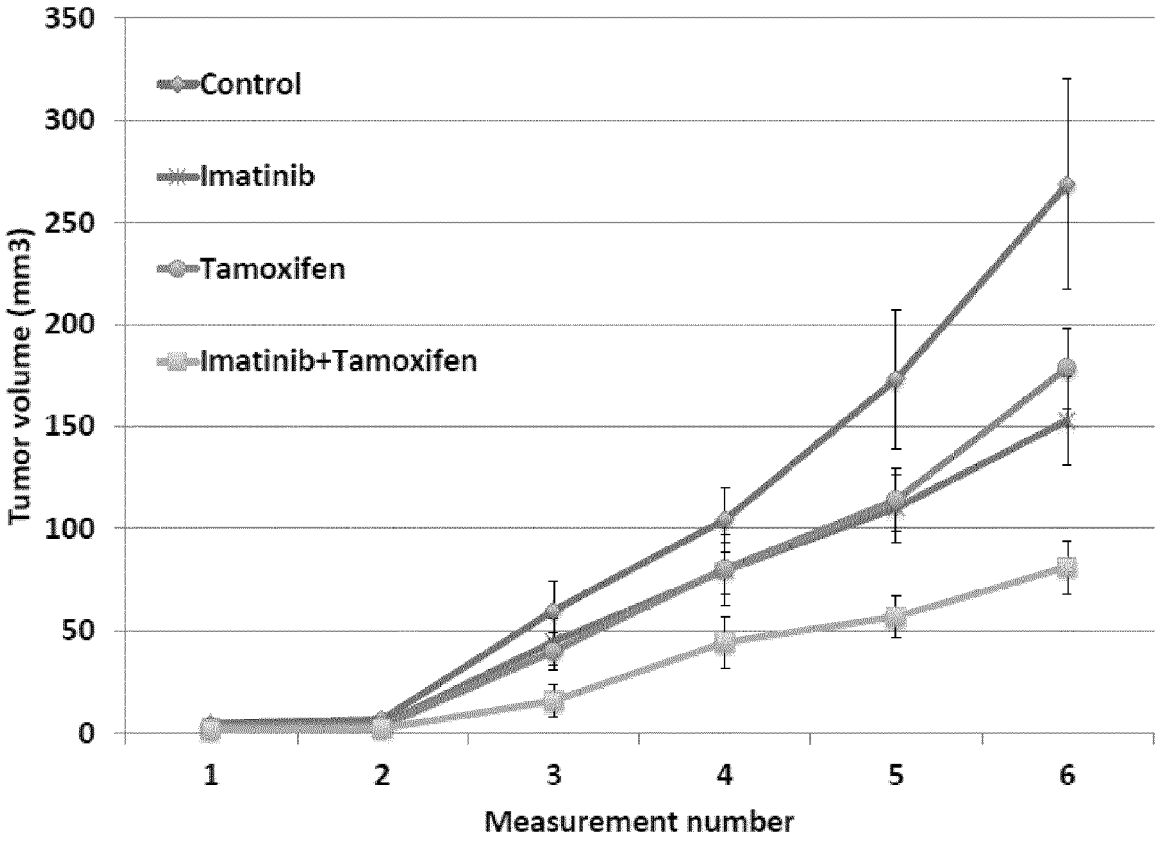

350
300
250
200
150
100
50
0
Tumor volume (mm3)
Control
Imatinib
Tamoxifen
Imatinib+Tamoxifen
1
2
3
4
5
6
Measurement number

Fig. 8

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