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(54) Title: METHODS OF PREDICTING EFFICACY OF AN ANTI-VEGFA TREATMENT FOR SOLID TUMORS

(57) Abstract: Provided are methods of predicting the efficacy of an anti-vascular endothelial growth factor A (VEGFA) treatment on a subject diagnosed with a solid tumor such as carcinoma, e.g., hepatocellular carcinoma, by determining a presence or an absence of a genomic amplification which comprises a VEGFA gene in a sample of the solid tumor, wherein the presence or the absence of the genomic amplification predicts the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor. Also provided are methods of treating a subject diagnosed with a solid tumor by predicting the efficacy of the anti-VEGFA treatment according to the method of the invention and selecting a treatment regimen based on the prediction.



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METHODS OF PREDICTING EFFICACY OF AN ANTI-VEGFA TREATMENT FOR SOLID TUMORS

FIELD AND BACKGROUND OF THE INVENTION

5 The present invention, in some embodiments thereof, relates to personalized anti-cancer therapy by methods of predicting efficacy of anti-cancer therapies on a subject, and, more particularly, but not exclusively, to methods of treating solid tumors by predicting the efficacy of an anti-VEGFA treatment on the subject and selecting a treatment regimen based on the prediction of efficacy.

10 Many of the anti-cancer drugs are efficient in some patients but exhibit no therapeutic effect on other patients having apparently the same diagnosis. In addition, while anti-cancer therapies are associated with varying degrees of side effects, their high costs are often taken into consideration when designing a treatment regimen. In order to develop targeted therapies for cancer, specific subgroups of the cancerous tumors have
15 to be identified in terms of specific molecular aberrations of individual tumors. Thus, identification of defining and recurring genetic abnormalities, which distinguish susceptible tumors, is mandatory for optimization of cancer treatment.

 Predictive biomarkers, which are quantifiable parameters identifying subsets of disease that are more likely to respond to a specific treatment, are usually based on
20 specific pathogenetic mechanisms that are related to a specific drug, and are considered the most important aspect of personalized medicine. Prominent examples for clinically validated cancer biomarkers include ERBB2 amplification in breast and gastric cancer and K-RAS mutations in colorectal cancer. These biomarkers serve as key determinates of treatment with Trastuzumab or Cetuximab, respectively.

25 Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide, and the fifth most common cancer. It is generally accepted that HCC is most commonly the outcome of chronic injury and inflammation, resulting in hepatocyte regeneration and dysregulated growth factor signaling. In recent years it has become clear that inflammatory signaling pathways can support survival, growth and progression
30 of cancer.

 The first line treatment for HCC is the multi-kinase inhibitor Sorafenib, which although not specific, is a strong inhibitor of VEGF receptors signaling. Sorafenib

blocks several receptor tyrosine kinases including: VEGFR1, 2 and 3, PDGFR, c-Kit and RET, as well as inhibiting downstream Raf kinase isoforms (Kamimura, S. & Tsukamoto, H. Cytokine gene expression by Kupffer cells in experimental alcoholic liver disease. *Hepatology* 22, 1304-1309, 1995). Sorafenib was recently shown to extend median survival from 7.9 months to 10.7 months in patients with advanced HCC (Stage C), establishing a new standard of care. Yet, the overall success of this treatment is modest and Sorafenib therapy is associated with a substantial degree of side effects (Llovet, J.M., *et al.* 2008, Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 359, 378-390). A clear picture of the molecular pathogenesis that underlies the development of HCC is lacking; furthermore, no molecular biomarker predictive of treatment outcome has been found yet.

One of the ways to study cancer initiation and progression is to develop mouse models that faithfully recapitulate specific human malignant processes. Mdr2 is an ortholog of a human gene mutated in progressive familial intrahepatic cholestasis (PFIC3). Mdr2 deficiency (Mdr2^{-/-}) results in chronic inflammation of the portal tracts, eventually leading to inflammation-induced liver tumors that share many features with human HCC, and therefore was shown to be an effective tool for studying HCC [Mauad, T.H., *et al.*, 1994, *Am. J. Pathol.* 145, 1237-45; Pikarsky, E. *et al.*, 2004, *Nature* 431, 461-466; Katzenellenbogen, M., *et al.*, 2006, *Cancer Res* 66, 4001-10; Oude Elferink, R.P. and Groen, A.K., 1995, *J. Hepatol.* 23, 617-25].

VEGF-A is a master regulator of angiogenesis whose role in tumor vessel recruitment is very well established. However, other roles for VEGF-A in tumorigenesis are now emerging. It was recently shown that VEGF-A can act synergistically with EGFR to promote proliferation of skin cancer cells which express VEGF receptor 1 (FLT1). Moreover, in the context of the liver, it was shown that VEGF-A elicits hepatocyte proliferation by elevating the expression of several mitogens in the liver sinusoidal endothelial cells [Ding, B.S., *et al.* Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature* 468, 310-315 (Published November 11, 2010); LeCouter, J., *et al.* Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science* 299, 890-893, 2003].

Murukesh N., *et al.* 2010 (Minireview. Biomarkers of angiogenesis and their role in the development of VEGF inhibitors; *British Journal of Cancer*, 102:8-18, which is

hereby incorporated by reference in its entirety), review several clinical studies which aim at identifying biomarkers for predicting efficacy of VEGF inhibitors and conclude that none of the clinical studies published to date have been qualified as having a predictive value on efficacy of treatment. In addition, studies aiming at using the expression levels of VEGF as a biomarker for predicting efficacy to anti-VEGFA treatment resulted in vague results (Siegel AB, et al., 2008; Thomas MB, et al., 2009), thus excluding expression levels of VEGFA from being a predictive marker for treatment efficacy.

Chiang, D.Y., et al., 2008, describe overexpression of VEGFA via gain of 6p21 in hepatocellular carcinoma. In addition, the Chr6p21 amplification was found to be correlated with advanced stage HCC (Chiang, D.Y., et al., 2008, Cancer Res. 68, 6779-88; Chochi, Y., et al., 2009, J. Pathol. 217, 677-84; Patil, M.A., et al., 2005, Carcinogenesis 26, 2050-7).

Siegel AB, et al., 2008; Zhu AX, et al., 2006; and Thomas MB, et al., 2009 describe phase II clinical trials using bevacizumab (AvastinTM) alone or in combination with additional anti-cancer drugs for the treatment of hepatocellular carcinoma.

Komorowski J, et al., 2006 show that in cells the anti-angiogenic action of thalidomide [α -(N-phthalimido)-glutarimide] is due to direct inhibitory action on VEGF secretion and capillary microvessel formation.

Additional background art includes Ouchi, K., et al. *Dig Surg* 17, 42-48, 2000; Moinzadeh, P., et al., *Br J Cancer* 92, 935-941, 2005; Weir, B.A., et al. 2007, *Nature* 450, 893-898; Tsafirir, D., et al. 2006, *Cancer Res.* 66, 2129-2137.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of predicting an efficacy of an anti-vascular endothelial growth factor A (VEGFA) treatment on a subject diagnosed with a solid tumor, comprising: determining a presence or an absence of a genomic amplification which comprises a VEGFA gene in a sample of the solid tumor, wherein the presence or the absence of the genomic amplification predicts the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor, thereby predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor.

According to an aspect of some embodiments of the present invention there is provided a method of treating of a subject diagnosed with a solid tumor, the method comprising: (a) predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor according to the method of some embodiments of the invention, and (b) selecting a treatment regimen based on the prediction; thereby treating of the subject diagnosed with the solid tumor.

According to an aspect of some embodiments of the present invention there is provided a method of selecting a treatment regimen for treating a subject diagnosed with a solid tumor, the method comprising: (a) predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor according to the method of some embodiments of the invention, and (b) selecting a treatment regimen based on the prediction; thereby selecting the treatment regimen for treating the subject diagnosed with a solid tumor.

According to some embodiments of the invention, the solid tumor is carcinoma.

According to some embodiments of the invention, the carcinoma is hepatocellular carcinoma.

According to some embodiments of the invention, determining the presence or the absence of the genomic amplification is effected by comparing a ratio determined in a sample of the solid tumor between a copy number of the VEGFA and a copy number of a centromeric marker of human chromosome 6, or visa versa, to a reference ratio determined in at least one sample devoid of the solid tumor between a copy number of the VEGFA and a copy number of the centromeric marker of human chromosome 6, or visa versa, respectively.

According to some embodiments of the invention, an increase above a predetermined threshold in the ratio determined in the sample of the solid tumor relative to the reference ratio indicates the presence of the genomic amplification.

According to some embodiments of the invention, an identical ratio or a change below a predetermined threshold in the ratio determined in the sample of the solid tumor as compared to the reference ratio indicates the absence of the genomic amplification.

According to some embodiments of the invention, determining a presence or an absence of a genomic amplification is effected using a DNA detection method.

According to some embodiments of the invention, determining a presence or an absence of a genomic amplification is effected using a chromosomal detection method.

According to some embodiments of the invention, the method further comprising comparing an expression level of the VEGFA in the sample of the solid tumor to a reference expression data obtained from at least one sample devoid of cancer.

According to some embodiments of the invention, an increase above a predetermined threshold in the expression level of the VEGFA in the sample of the solid tumor relative to the reference expression data predicts the efficacy of the anti-VEGFA treatment on the solid tumor.

According to some embodiments of the invention, the sample devoid of cancer is a liver sample.

According to some embodiments of the invention, the expression level is determined using an RNA detection method.

According to some embodiments of the invention, the expression level is determined using a protein detection method.

According to some embodiments of the invention, the anti-VEGFA treatment comprises Sorafenib.

According to some embodiments of the invention, the anti-VEGFA treatment comprises bevacizumab.

According to some embodiments of the invention, the anti-VEGFA treatment comprises a soluble form of the VEGF-receptor.

According to some embodiments of the invention, the anti-VEGFA treatment comprises thalidomide.

According to some embodiments of the invention, the anti-VEGFA treatment comprises a combination of at least two anti-VEGFA drugs selected from the group consisting of Sorafenib, bevacizumab, a soluble form of the VEGF-receptor and thalidomide.

According to some embodiments of the invention, the treatment regimen comprises administering of at least one anti-VEGFA drug selected from the group consisting of Sorafenib, bevacizumab, a soluble form of the VEGF-receptor and thalidomide.

According to some embodiments of the invention, the treatment regimen further comprises administering a drug selected from the group consisting of erlotinib, oxaliplatin, cisplatin, platinum, rIFN α -2b, doxorubicin, fluorouracil, DX-8951f, thalidomide, doxorubicin, epirubicin, and taxol.

5 According to some embodiments of the invention, the genomic amplification is of a human chromosome 6p21.

According to some embodiments of the invention, the genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:23.

10 According to some embodiments of the invention, the genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:24.

According to some embodiments of the invention, the genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:25.

According to some embodiments of the invention, the VEGFA comprises the genomic nucleic acid sequence set forth by SEQ ID NO:1.

15 According to some embodiments of the invention, the solid tumor is hepatocellular, and wherein the hepatocellular solid tumor is associated with hepatitis C infection.

20 According to some embodiments of the invention, the efficacy of the anti-VEGFA treatment is determined by tumor regression following at least 8 weeks of the anti-VEGFA treatment.

According to some embodiments of the invention, the efficacy of the anti-VEGFA treatment is determined by progression-free survival (PFS) time of at least one year.

25 According to some embodiments of the invention, the efficacy of the anti-VEGFA treatment is determined by a proliferation assay.

According to some embodiments of the invention, the DNA detection method comprises DNA quantitative PCR (qPCR).

30 Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent

specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how
10 embodiments of the invention may be practiced.

 In the drawings:

 FIG. 1 is an image of raw data obtained from array comparative genome hybridization (aCGH) analysis showing the amplification (right shift of dots) in a specific region of chromosome 17. Samples produced from ten different *Mdr2*^{-/-} derived
15 HCCs were subjected to array CGH. Note the 2 Mbp long genomic region on the qB3 band of murine chromosome 17 which includes the VEGFA gene (human GeneID: 7422), *Mrps18a* (human GeneID: 55168), *Parc* (CCL18, human GeneID: 6362), Exportin 5 (XPO5, human GeneID:57510). CGH reveals amplification in the genomic locus of VEGFa

20 FIGs. 2A-H are images depicting chromogenic *in situ* hybridization (CISH) of murine HCC tumors harboring the Chr17qB3 genomic region amplification. CISH was performed using probes specific for Chr17qB3. Two different nuclei from two different tumors from each subgroup are shown. Figures 2A-B - Tumor #1-; Figures 2C-D - Tumor #2; Figures 2E-F -Tumor #3; Figure 2G-H – Tumor #4. Note that tumors #1 and
25 #2 are negative for the genomic amplification and tumors #3 and #4 are positive for the genomic amplification. Scale bar 3 μ m. Arrows point to positive CISH signals.

 FIG. 2I is a schematic representation of the murine Chr17qB3 genomic region amplification in various HCC tumors which map the critical region of the genomic amplification. DNA qPCR analysis was performed using primers specific for different
30 loci on the qB3 arm of chromosome 17. Each line represents a different Amp^{pos} tumor. Thin line represents non-amplified region, thick line represents amplified (>2 fold

increase) regions. The list includes several of the residing genes (the full list is presented in Table 5 in the Examples section which follows).

FIGs. 2J-O are graphs depicting relative mRNA expression of various genes residing on the amplified region in HCC tumors with or without the genomic amplification. qPCR analysis was performed on wild type liver tissues (WT liver), HCC tumors without the amplification (Amp^{neg} tumor) and HCC tumors harboring the genomic amplification (Amp^{pos} tumor) using primers which specifically detect Cdc5L (Figure 2J), HSP90ab1 (Figure 2K), VEGF-A (Figure 2L), Tjap1 (Figure 2M), Xpo5 (Figure 2N) and Parc (Figure 2O). Cross line signifies geometric mean (*p<0.01, **p<0.0001). Note the significant increase in mRNA expression of Cdc5L, VEGF-A, Tjap1, Xpo5 and Parc in tumors harboring the genomic amplification as compared to tumors devoid of the genomic amplification.

FIG. 3 is a histogram depicting real time quantitative PCR (qPCR) and ELISA analysis of VEGF mRNA and protein levels, respectively, of amplified versus non-amplified murine Mdr2^{-/-} tumors. Note the significant increase in VEGFA expression level (on both RNA and protein levels) in amplified HCC tumors.

FIGs. 4A-F are representative images of murine HCC tumors with (Figures 4B, 4D, 4F) or without (Figures 4A, 4C and 4E) the genomic amplification stained by IHC with antibodies specific to vWF (Figures 4A-B), BrdU (Figures 4C-D), and F4/80 (Figures 4E-F). Scale bar 100 μm. Note that the Amp^{pos} tumors are a distinct subpopulation.

FIGs. 4G-I are histograms depicting quantization of the IHC analyses (for which representative images are shown in Figures 4A-F). IHC stainings were quantified using automated image analysis. Figure 4G – vWF; Figure 4H – BrdU; Figure 4I – F4/80. White bars = WT liver; Light grey bars = AMP^{neg} Tumors; Dark grey bars = AMP^{pos} tumors. WT n = 2, Amp^{neg} n = 9, Amp^{pos} n = 7. *p < 0.01, **p < 0.05).

FIGs. 5A-H are images of IHC for BrdU (Figures 5A-D) and Ki67 (Figures 5E-H) of HCC tumors with or without the genomic amplification of mice which were treated with sFLT or remained untreated. Mdr2^{-/-} mice were treated with adenovectors expressing either GFP alone (Figures 5A, 5C, 5E and 5G) or GFP with sFLT (Figures 5B, 5D, 5F and 5H) for 10 days. Shown are representative photomicrographs of IHC for

BrdU and Ki67. Tumor infiltrating cells remain proliferative. Scale bars: Figures 5A-D – 50 μ m, Figures 5E-H - 100 μ m.

FIGs. 5I-J are histograms depicting quantization of the IHC results (for which representative images are shown in Figures 5A-H). The percent of positive nuclei in IHC staining for BrdU (Figure 5I) and Ki67 (Figure 5J) was quantified using automated image analysis. Mice were treated as described in Figures 5A-H above. Amp^{neg} GFP treated (n = 11, white bars), Amp^{neg} sFLT treated (n = 10, light grey bars), Amp^{pos} GFP treated (n = 7, dark grey bars) and Amp^{pos} sFLT treated (n = 6, black bars). * indicates significance of $p < 0.05$; ** indicates significance of $p < 0.05$. Note that in Amp^{pos} sFLT there is a significant decrease in the number of BrdU or Ki67 positive nuclei, thus demonstrating a significant response to the anti-VEGF-A sFLT treatment. These results demonstrate that the VEGF-A amplicon predicts a response to VEGF blockade by sFLT.

FIGs. 5K-L are images depicting IHC for the mitosis-specific marker phosphohistone 3 (pHH3) in amplified tumors treated with adeno-sFLT (Figure 5L) or adeno-GFP (Figure 5K). Note the decrease in pHH3 positive hepatocytes in adeno-sFLT treated tumors, indicating reduced proliferation.

FIGs. 5M-N are histograms depicting the results of qPCR analysis of VEGF-A (Figure 5M), HGF (Figure 5N) of Amp^{neg} and Amp^{pos} tumors treated with the indicated adenovectors (as described in Figures 5AH above). Cross line signifies geometric mean (* $p < 0.0001$). Note that in tumors harboring the amplification, the anti-VEGF-A treatment (sFLT) results in a significant decrease in HGF mRNA levels as compared to tumors harboring the amplification which were transformed with a control adenovirus GFP vector (Figure 5N).

FIGs. 5O-R are histograms (Figures 5O-P) and images (Figures 5Q-R) depicting high expression level of the HIF1 α target genes Glut1 (Figure 5O) and PGK1 (Figure 5P) in Amp^{pos} tumors treated with the indicated adenovectors (as described in Figures 5AH above) and the associated necrosis (Figures 5Q-R). Cross line signifies geometric mean. Figure 5Q - a histological section stained with H&E, showing necrosis. Scale bar 500 μ m; Figure 5R - a macroscopic picture of a tumor showing areas of hemorrhagic necrosis. Results are representative for three out of the six sFLT treated Amp^{pos} tumors. Note that the tumors that show increased expression of the HIF1 α

target genes Glut1 and PGK1 (markers for hypoxia) also display necrosis on histological evaluation.

FIGs. 6A-C are a histogram and images depicting the results of qPCR analysis of HGF mRNA (Figure 6A) and IHC of HGF in non-amplified (Figure 6B) and amplified (Figure 6C) tumors. qPCR analysis of HGF mRNA was performed on WT livers, non-amplified tumors and amplified tumors. Immunostaining for HGF reveals staining in endothelial and inflammatory cell populations, predominantly in the amplified tumor.

FIG. 7 is a histogram depicting VEGF-A relative gene dose in various murine HCC tumors which is used for screening for Amp^{pos} tumors. qPCR was performed on DNA extracted from Mdr2^{-/-} tumors (Tumors numbers 1-42) and wild-type (WT) liver samples (samples 1-3) using a set of primers targeting the 3'-UTR (untranslated region) (white bars) and the promoter (grey bars) regions of the VEGF-A gene. Primers' sequences are provided in Table 4 in the EXAMPLES section which follows. The results were compared to those obtained by CGH testing of the same tumors (tumors 1-10) and validated that the VEGF-A gene dose (determined by DNA qPCR) can detect the genomic amplification.

FIGs. 8A-D are images of vWF IHC analysis performed on amplified (Figures 8A-B) and non-amplified (Figures 8C-D) HCC tumors treated with adeno-sFLT (Figures 8B and 8D) or adeno-GFP (Figures 8A and 8C) vectors.

FIGs. 9A-H are images depicting representative photomicrographs of BrdU (Figures 9A-D) or vWF (Figures 9E-H) IHC in amplified tumors (Figures 9C, 9D, 9G and 9H) or non-amplified tumors (Figures 9A, 9B, 9E and 9F) which were treated with Sorafenib (Figures 9B, 9D, 9F and 9H) or vehicle (Figures 9A, 9C, 9E and 9G). Note the decrease in stained nuclei (as determined by BrdU staining) in the treated Amp^{pos} group alone (Figure 9D) while no decrease in blood vessel (as determined by vWF staining; Figure 9H) is evident.

FIG. 9I is a histogram depicting quantization of the IHC staining for vWF using automated image analysis. Amp^{neg} vehicle treated (n=13; Amp^{neg} Veh), Amp^{neg} Sorafenib treated (n=13, Amp^{neg} Sor), Amp^{pos} vehicle treated (n=7; Amp^{pos} Veh) and Amp^{pos} sorafenib treated (n=5; Amp^{pos} Sor). P = 0.06.

FIGs. 9J-M are histograms depicting qPCR analysis of Amp^{neg} and Amp^{pos} for VEGF-A (Figure 9J), HGF (Figure 9K), PGK1 (Figure 8L) and Glut1 (Figure 9M). Cross line signifies geometric mean (*p<0.05). Note that Amp^{pos} tumors are sensitive to short term treatment with Sorafenib as indicated by a reduction in HGF mRNA levels.

FIG. 9N is a histogram depicting quantization of the IHC staining for BrdU using automated image analysis, demonstrating the effect of Sorafenib treatment on the proliferation of mice HCC tumors. Mdr2^{-/-} mice were treated with Sorafenib or vehicle alone for 3 days. Shown are the percentages of BrdU positive nuclei in tumors bearing the amplification ("Biomarker +") or being devoid of the amplification ("Biomarker -"), following treatment with Sorafenib or vehicle. Note the decline in the proliferation rate of tumor cells in tumors bearing the amplification which were treated with Sorafenib, as compared to the proliferation rate of the tumor cells in tumors devoid of the genomic amplification.

FIGs. 10A-K are histograms depicting qPCR analysis of the mRNA levels of several of the 63 genes in Amp^{pos} and Amp^{neg}, demonstrating the expression profile of several of the amplicon residing genes. Cross line signifies geometric mean (*p<0.05, **p<0.01). Figure 10A – Runx2; Figure 10B – Supt3h; Figure 10C – Clic5; Figure 10D – Aars2; Figure 10E – Nfkbic; Figure 10F – Tmem63b; Figure 10G – Mrpl14; Figure 10H – Capn11; Figure 10I – Mrps18a; Figure 10J- EGFL9; Figure 10K – Gnm1.

FIGs. 11A-E are histological analyses demonstrating that Amp^{pos} HCC hold distinct histological features. Figures 11A-B - Representative H&E stained sections of Amp^{neg} and Amp^{pos} tumors showing steatosis (lipid droplets) in the Amp^{pos} but not Amp^{neg} group. Scale bar 100 μm. Figures 11C-D - Representative H&E stained sections of Amp^{neg} and Amp^{pos} tumors demonstrating the differences in the size of tumor cell and cytoplasm between the two groups. Scale bar 50 μm. Figure 11E -H&E stained sections of Amp^{neg} and Amp^{pos} tumors were examined by a pathologist and evaluated for the presence of large cells and steatosis. A χ^2 test was used to determine statistical significance.

FIGs. 12A-J are images (Figures 12A-H) and histograms (Figures 12I-J) demonstrating that 10 days inhibition of VEGF-A in Amp^{pos} tumors does not alter the microenvironmental content. Figures 12A-D – IHC of vWF; Figures 12E-H – IHC of F4/80. Amplified tumors (Figures 12C, 12D, 12G, 12H) and non-amplified tumors

(Figures 12A, 12B, 12E, 12F) were treated for 10 days with adenovirus vector expressing GFP (Figures 12A, 12C, 12E and 12G) or sFTL-GFP (Figures 12B, 12D, 12F and 12H). Figures 12I-J – quantization of the IHC for vWF (Figure 12I) and F4/80 (Figure 12J) using automated image analysis. Amp^{neg} GFP treated (n = 12; white bars), Amp^{neg} sFLT treated (n = 12; light grey bars), Amp^{pos} GFP treated (n = 5; dark grey bars) and Amp^{pos} sFLT treated (n = 5; black bars). Differences between the Amp^{pos} with GFP or sFLT were statistically insignificant.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to personalized anti-cancer therapy by methods of predicting efficacy of anti-cancer therapies on a subject, and, more particularly, but not exclusively, to methods of treating solid tumors by predicting the efficacy of an anti-VEGFA treatment on the subject and selecting a treatment regimen based on the prediction of efficacy.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

The present inventors have uncovered that the variability in response to an anti-VEGFA treatment such as Sorafenib (a multi-tyrosine kinase inhibitor, marketed as Nexavar by Bayer) or a soluble VEGF-A receptor, depends on the presence or absence of the genomic amplification on murine chromosome 17qB3 (a syntenic region of human chromosome 6p21) which comprises the VEGFA gene, such that subjects having a solid tumor which comprises the genomic amplification respond well (*i.e.*, in an efficient manner) to the anti-VEGFA treatment, and subjects having the same solid tumor albeit devoid of the genomic amplification respond in a less efficient manner (or do not respond at all) to the anti-VEGFA treatment.

As shown in the Examples section which follows, the present inventors have uncovered that a subset of HCCs tumors in Mdr2-deficient mice harbor a specific amplicon at murine chromosome 17 (Chr17qB3) spanning the VEGF-A gene (Figures 1, 2A-O, 7 and 10A-K, and Table 5, Example 1); that the genomic amplification elevates VEGF-A mRNA and protein levels (Figure 3, Example 2), that tumors

harboring the amplification (Amp^{pos}) are characterized by larger tumor cells, extensive steatosis (Figures 11A-E), increased vessel density (Figure 4A-B and 4G, Example 2) and increased proliferation rate (Figures 4C-D and 4H, Example 2). In addition, the present inventors found that genomic amplification induces a unique tumor environment with higher expression of the macrophage marker F4/80 (Figures 4E-F and 4I, Example 3) and higher levels of hepatocyte growth factor (HGF, Figures 6A-C, Example 3). In addition, as shown in Example 4 of the Examples section which follows, the Amp^{pos}-HCC tumors were significantly more susceptible to treatment with a soluble form of the VEGF-A receptor (sFLT) as compared to Amp^{neg}-HCC tumors, as shown by an efficient inhibition of tumor cell proliferation (Figures 5A-L, Example 4), which was accompanied by a decrease in HGF mRNA levels in sFLT-treated tumors (Figure 5N, Example 4) and with an increase in tissue hypoxia (Figures 5O-P, Example 4) and necrosis (Figures 5Q-R, Example 4). Moreover, as shown in Example 6 of the Examples section which follows, the present inventors found that Amp^{pos}-HCC tumors are uniquely sensitive to Sorafenib, a multi-tyrosine kinase inhibitor (which inhibits VEGF-A activity), which is currently the first line treatment of advanced HCC in human beings, as shown by decrease in tumor cell proliferation (Figures 9A-D, Figure 9N, Example 6) and HGF levels (Figure 9K, Example 6). Altogether, these data demonstrate that the genomic amplification which comprises the VEGF-A gene distinguishes a subgroup of HCC tumors that are sensitive to direct VEGF-A blocking (e.g., sFLT) and Sorafenib treatment, and suggest the use of the genomic amplification as a prognostic marker to predict the efficacy of an anti-VEGF-A treatment in a subject having a solid tumor.

Thus, according to an aspect of some embodiments of the invention, there is provided a method of predicting an efficacy of an anti-vascular endothelial growth factor A (VEGFA) treatment on a subject diagnosed with a solid tumor, comprising determining a presence or an absence of a genomic amplification which comprises a VEGFA gene in a sample of the solid tumor, wherein the presence or the absence of the genomic amplification predicts the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor, thereby predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor.

According to some embodiments of the invention, presence of the genomic amplification which comprises the VEGFA gene in a solid tumor sample, predicts that the anti-VEGFA treatment will be efficient in treating the solid tumor in the subject.

As used herein the phrase "predicting efficacy of an anti-VEGFA treatment" refers to determining the likelihood that an anti-VEGFA treatment will be efficient or non-efficient in treating the solid tumor, e.g., the success or failure of the anti-VEGFA treatment in treating the solid tumor in a subject in need thereof. The term "efficacy" as used herein refers to the extent to which the anti-VEGFA treatment produces a beneficial result, e.g., an improvement in one or more symptoms of the pathology (caused by the solid tumor) and/or clinical parameters related to the pathology as described hereinbelow. For example, the efficacy of an anti-VEGFA treatment may be evaluated using standard therapeutic indices for solid tumors.

According to some embodiments of the invention, the efficacy of treatment is a long-term efficacy.

As used herein the phrase "long-term efficacy" refers to the ability of a treatment to maintain a beneficial result over a period of time, e.g., at least about 16 weeks, at least about 26 weeks, at least about 32 weeks, at least about 36 weeks, at least about 40 weeks, at least about 48 weeks, at least about 52 weeks, at least about 18 months, at least about 24 months, at least about 3 years, at least about 4 years, at least about 5 years, at least about 6 years, at least about 7 years, at least about 8 years, at least about 9 years, at least about 10 years, or longer.

According to some embodiments of the invention, the efficacy of the anti-VEGFA treatment is determined by tumor regression following at least 8 weeks of the anti-VEGFA treatment.

According to some embodiments of the invention, an anti-VEGFA treatment is considered efficient in treating a solid tumor if it exerts an improvement in at least one relevant clinical parameter related to the solid tumor in the treated subject as compared to an untreated subject diagnosed with the same solid tumor (e.g., the same type, stage, degree and/or classification of the solid tumor), or as compared to the clinical parameters related to the solid tumor of the same subject prior to the anti-VEGFA treatment.

Non-limiting examples of the clinical parameters related to the solid tumor which can be monitored in order to determine the efficacy of the anti-VEGFA treatment

include the number of tumor lesions, dimensions (e.g., size) of each of the tumor lesion, tumor stage, differentiation state of tumor, presence and/or degree of tumor metastases, effect of the tumor on physiological function of the subject affected by the solid tumor, and the like.

5 Evaluation of the efficacy of an anti-VEGFA treatment can be also performed using acceptable clinical criteria, such as the criteria proposed by the "Response Evaluation Criteria in Solid Tumors (RECIST) Committee" described in Therasse P., Arbuck SG., Eisenhauer EA et al. (New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National
10 Cancer Institute of the United States, National Cancer Institute of Canada. J. Natl. Cancer Inst. 92:205-216, 2000), which is incorporated by reference in its entirety. The (RECIST) is a set of published rules that define when cancer patients improve ("respond"), stay the same ("stabilize"), or worsen ("progression") during treatments.

According to some embodiments of the invention, the efficacy of the anti-
15 VEGFA treatment can be determined using at least one, two or all three of the response criteria included in the RECIST which include evaluation of target lesions [target lesions are selected on the basis of their size (lesions with the longest diameter)], evaluation of non-target lesions [all other lesions (or sites of disease) identified as non-target lesions], and evaluation of best overall response.

20 Evaluation of target lesions classifies the response as follows: (i) Complete Response (CR) - Disappearance of all target lesions; (ii) Partial Response (PR) - At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD; (iii) Stable Disease (SD) - Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for Progressive Disease (PD), taking as
25 reference the smallest sum LD since the treatment started; (iv) Progressive Disease (PD) - At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Evaluation of non-target lesions classifies the response as follows: (i) Complete
30 Response (CR) - Disappearance of all non-target lesions and normalization of tumor marker level; (ii) Incomplete Response/ Stable Disease (SD) - Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal

limits; (iii) Progressive Disease (PD) - Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Evaluation of the best overall response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

According to some embodiments of the invention, an anti-VEGFA treatment is considered efficient in treating the solid tumor if it results in at least a partial response (PR) according to the RECIST criteria.

According to some embodiments of the invention, an anti-VEGFA treatment is considered efficient in treating the solid tumor if it results in a complete response (CR) according to the RECIST criteria.

According to some embodiments of the invention, the efficacy of the anti-VEGFA treatment is determined by progression-free survival (PFS) time of at least one year.

According to some embodiments of the invention, determining the efficacy of an anti-VEGF-A treatment is performed by monitoring the proliferation state of the tumor cells.

The proliferation state of tumor cells can be determined using various methods known in the art, such as using the synthetic nucleoside analogue of thymidine, bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU). BrdU is commonly used in the detection of proliferating cells in living tissues. BrdU is incorporated into the newly synthesized DNA of replicating cells, substituting for thymidine during DNA replication. Antibodies specific for BrdU can then be used to detect the incorporated chemical, thus indicating cells that were actively replicating their DNA.

Non-limiting examples of solid tumors (cancers) which can be treated by the anti-VEGFA treatment according to some embodiments of the invention include tumors of the gastrointestinal tract (colon solid tumor, rectal carcinoma, colorectal carcinoma, colorectal cancer, colorectal adenoma, hereditary nonpolyposis type 1, hereditary nonpolyposis type 2, hereditary nonpolyposis type 3, hereditary nonpolyposis type 6; colorectal cancer, hereditary nonpolyposis type 7, small and/or large bowel carcinoma, esophageal carcinoma, tylosis with esophageal cancer, stomach carcinoma, pancreatic carcinoma, pancreatic endocrine tumors), endometrial carcinoma, dermatofibrosarcoma protuberans, gallbladder carcinoma, Biliary tract tumors, prostate cancer, prostate adenocarcinoma, renal cancer (e.g., Wilms' tumor type 2 or type 1), liver cancer (e.g., hepatoblastoma, hepatocellular carcinoma, hepatocellular cancer), bladder cancer, embryonal rhabdomyosarcoma, germ cell tumor, trophoblastic tumor, testicular germ cells tumor, immature teratoma of ovary, uterine, epithelial ovarian, sacrococcygeal tumor, choriocarcinoma, placental site trophoblastic tumor, epithelial adult tumor, ovarian carcinoma, serous ovarian cancer, ovarian sex cord tumors, cervical carcinoma, uterine cervix carcinoma, small-cell and non-small cell lung carcinoma, nasopharyngeal, breast carcinoma (e.g., ductal breast cancer, invasive intraductal breast cancer, sporadic; breast cancer, susceptibility to breast cancer, type 4 breast cancer, breast cancer-1, breast cancer-3; breast-ovarian cancer), squamous cell carcinoma (e.g., in head and neck), neurogenic tumor, astrocytoma, ganglioblastoma, neuroblastoma, lymphomas (e.g., Hodgkin's disease, non-Hodgkin's lymphoma, B cell, Burkitt, cutaneous T cell, histiocytic, lymphoblastic, T cell, thymic), gliomas, adenocarcinoma, adrenal tumor, hereditary adrenocortical carcinoma, brain malignancy (tumor), various other carcinomas (e.g., bronchogenic large cell, ductal, Ehrlich-Lette ascites, epidermoid, large cell, Lewis lung, medullary, mucoepidermoid, oat cell, small cell, spindle cell, spinocellular, transitional cell, undifferentiated, carcinosarcoma, choriocarcinoma, cystadenocarcinoma), ependimoblastoma, epithelioma, fibrosarcoma, giant cell tumor, glial tumor, glioblastoma (e.g., multiforme, astrocytoma), glioma hepatoma, heterohybridoma, heteromyeloma, histiocytoma, hybridoma (e.g., B cell), hypernephroma, insulinoma, islet tumor, keratoma, leiomyoblastoma, leiomyosarcoma, leukemia (e.g., acute lymphatic, acute lymphoblastic, acute lymphoblastic pre-B cell, acute lymphoblastic T cell leukemia, acute - megakaryoblastic, monocytic, acute

myelogenous, acute myeloid, acute myeloid with eosinophilia, B cell, basophilic, chronic myeloid, chronic, B cell, eosinophilic, Friend, granulocytic or myelocytic, hairy cell, lymphocytic, megakaryoblastic, monocytic, monocytic-macrophage, myeloblastic, myeloid, myelomonocytic, plasma cell, pre-B cell, promyelocytic, subacute, T cell, lymphoid neoplasm, predisposition to myeloid malignancy, acute nonlymphocytic leukemia), lymphosarcoma, melanoma, mammary tumor, mastocytoma, medulloblastoma, mesothelioma, metastatic tumor, monocyte tumor, multiple myeloma, myelodysplastic syndrome, myeloma, nephroblastoma, nervous tissue glial tumor, nervous tissue neuronal tumor, neurinoma, neuroblastoma, oligodendroglioma, osteochondroma, osteomyeloma, osteosarcoma (e.g., Ewing's), papilloma, transitional cell, pheochromocytoma, pituitary tumor (invasive), plasmacytoma, retinoblastoma, rhabdomyosarcoma, sarcoma (e.g., Ewing's, histiocytic cell, Jensen, osteogenic, reticulum cell), schwannoma, subcutaneous tumor, teratocarcinoma (e.g., pluripotent), teratoma, testicular tumor, thymoma and trichoepithelioma, gastric cancer, fibrosarcoma, glioblastoma multiforme; multiple glomus tumors, Li-Fraumeni syndrome, liposarcoma, lynch cancer family syndrome II, male germ cell tumor, mast cell leukemia, medullary thyroid, multiple meningioma, endocrine neoplasia myxosarcoma, paraganglioma, familial nonchromaffin, pilomatricoma, papillary, familial and sporadic, rhabdoid predisposition syndrome, familial, rhabdoid tumors, soft tissue sarcoma, and Turcot syndrome with glioblastoma.

According to some embodiments of the invention, the solid tumor which is treated by the anti-VEGFA treatment is a carcinoma.

The term "carcinoma" as used herein refers to any malignant tumor derived from epithelial cells or tissue.

According to some embodiments of the invention, the carcinoma is selected from the group consisting of hepatocellular carcinoma, cervical cancer, Nasopharyngeal carcinoma (NPC), bladder cancer, lung cancer (e.g., non-small cell lung cancer), esophageal squamous cell carcinoma, multiple myeloma, kidney cancer (renal cell carcinoma), metastatic renal cell carcinoma, colon cancer, colorectal cancer, pancreatic cancer and ovarian cancer.

According to some embodiments of the invention the carcinoma is lung cancer.

According to some embodiments of the invention the carcinoma is colorectal cancer.

According to some embodiments of the invention the carcinoma is hepatocellular carcinoma.

5 According to some embodiments of the invention the hepatocellular solid tumor is associated with hepatitis C infection.

According to some embodiments of the invention the carcinoma is cancer metastases.

10 Table 1 provides a non-limiting list of solid tumors which include the 6p21 genomic amplification.

Table 1

<i>Tumor Type</i>	<i>Reference</i>
Lung cancer	Nature 450, 893-898 (2007)
Hepatocellular Carcinoma	Cancer Res 68, 6779-6788 (2008)
Colon cancer	PNAS 106, 7131-7136 (2009)

Table 1.

15 Table 2 provides a non-limiting list of solid tumors which include the 6p21-p23 gain or amplification.

Table 2

20

Type of tumor	Number of tumors	Tumors with gain in 6p21-p23 (%)	Tumors with amplification in 6p21-p23 (%)
Carcinomas*			
Hepatocellular carcinoma	409	22.61	0.20
Merkel cell carcinoma	48	27.10	2.10
Basal cell carcinoma	16	40.30	0.00
Ovarian serous carcinoma	56	28.60	0.00
Transitional cell carcinoma	133	9.68	0.51
Lymphoid tumors			
Large B cell lymphoma	360	8.36	0.07

Type of tumor	Number of tumors	Tumors with gain in 6p21-p23 (%)	Tumors with amplification in 6p21-p23 (%)
Plasmacytoma	21	22.76	0.00
Sarcomas			
Osteosarcoma	137	33.19	3.39
Malignant peripheral nerve sheath	70	23.93	0.00
Leiomyosarcoma	136	9.98	1.64
Melanomas	91	31.78	0.00
Retinoblastoma	133	39.36	10.50
Glioblastoma	108	4.60	0.90
Neuroblastoma	303	22.10	0.17
Carcinosarcoma	23	21.70	6.31

Table 2. Tumors with 6p21-p23 gain or amplification. Derived from J Clin Pathol 2007, 60: 1-7 (Santos GC).

As used herein the term “vascular endothelial growth factor A (VEGFA)” (also known as VPF, VEGF, MVCD1, MGC70609) refers to synthetic, recombinant and/or naturally occurring polynucleotide and polypeptide sequences assigned to the gene symbol VEGFA. These include but are not limited to the genomic sequence encoding VEGFA [nucleotides 43737953-43754224 of GenBank Accession No. NC_000006.11 as set forth by SEQ ID NO:1], the mRNA transcripts encoded thereby [e.g., GenBank Accession Nos. NM_001025366.1 (SEQ ID NO:2); NM_003376.4 (SEQ ID NO:3); NM_001025367.1 (SEQ ID NO:4); NM_001025368.1 (SEQ ID NO:5); NM_001033756.1 (SEQ ID NO:6); NM_001025369 (SEQ ID NO:7); NM_001025370 (SEQ ID NO:8)] and/or the polypeptide variants encoded thereby [e.g., GenBank Accession Nos. NP_001020537.2 (SEQ ID NO:9); NP_003367.4 (SEQ ID NO:10); NP_001020538.2 (SEQ ID NO:11); NP_001020539.2 (SEQ ID NO:12); NP_001028928.1 (SEQ ID NO:13); NP_001020540.2 (SEQ ID NO:14); NP_001020541.2 (SEQ ID NO:15)].

As used herein the phrase “genomic amplification which comprises a VEGFA gene” refers to the presence of more than one copy per chromosome homolog of at least the genomic sequence encoding VEGFA.

As used herein the phrase “chromosome homolog” refers to a single chromosome of the pair of chromosomes that pair (synapse) during meiosis.

According to some embodiments of the invention the genomic amplification which comprises the VEGFA gene is of the human chromosome 6p21 [nucleotide
5 coordinates chr6:30,400,001-46,200,000 (SEQ ID NO:23) according to UCSC on Human GRCh37 Assembly (human genome 19 (hg19)]. The 6p21 region includes the chromosomal bands 6p21.33, 6p21.32, 6p21.31, 6p21.2, 6p21.1. The specific band in 6p21 region which comprises the VEGFA genomic sequence is 6p21.1, which is encompassed by nucleotide coordinates chr6:40,500,001-46,200,000 (SEQ ID NO:24)
10 according to UCSC on Human GRCh37 Assembly [human genome 19 (hg19)].

According to some embodiments of the invention the genomic amplification which comprises the VEGFA gene is set forth in SEQ ID NO:24.

According to some embodiments of the invention the genomic amplification which comprises the VEGFA gene is set forth in SEQ ID NO: 25 (Chr6: 43684022-
15 44002022 in the hg19 assembly). This sequence comprises the VEGFA (SEQ ID NO:1, LOC100132354 (SEQ ID NO:405) and C6orf223 (SEQ ID NO:406) genomic sequences.

Non-limiting examples of BACs (bacterial artificial chromosomes) which are derived from the 6p21.1 region and which can be used to detect the genomic
20 amplification which comprises VEGFA gene include RP11-710L16 [chromosome 6:43,633,251-43,817,196; according to UCSC (University California Santa Cruz) on Human GRCh37 Assembly (human genome 19 (hg19); SEQ ID NO:18] which fully covers the VEGFA genomic sequence; and RP11-21M9 [chr6:43,743,280-43,929,157 according to UCSC on Human GRCh37 Assembly (human genome 19 (hg19); SEQ ID
25 NO:19] which partially covers the VEGFA genomic sequence (data not shown).

According to some embodiments of the invention the genomic amplification which comprises the VEGFA gene is set forth in SEQ ID NO:18 and/or 19.

According to some embodiments of the invention, the genomic amplification comprises at least 2, e.g., at least 3, e.g., at least 4, e.g., at least 5, e.g., at least 6, e.g.,
30 at least 7, e.g., at least 8, e.g., at least 9, e.g., at least 10, e.g., at least 15, e.g., at least 20, e.g., at least 30, e.g., at least 40, e.g., at least 50, e.g., at least 60, e.g., at least 70, e.g., at least 80, e.g., at least 90, e.g., at least 100, e.g., at least 200, e.g., at least 300, e.g., at

least 400, e.g., at least 600, e.g., at least 1000 copies per chromosome homolog, or more of the genomic sequence encompasses the coding region of VEGFA (e.g., SEQ ID NO:1).

According to some embodiments of the invention, the genomic amplification
5 comprises at least 2, e.g., at least 3, e.g., at least 4, e.g., at least 5, e.g., at least 6, e.g.,
at least 7, e.g., at least 8, e.g., at least 9, e.g., at least 10, e.g., at least 15, e.g., at least 20,
e.g., at least 30, e.g., at least 40, e.g., at least 50, e.g., at least 60, e.g., at least 70, e.g., at
least 80, e.g., at least 90, e.g., at least 100, e.g., at least 200, e.g., at least 300, e.g., at
least 400, e.g., at least 600, e.g., at least 1000 copies per chromosome homolog, or more
10 of the genomic sequence selected from the group consisting of SEQ ID NOs:1, 18, 19,
23, 24, and 25.

According to some embodiments of the invention, determining the presence or
the absence of the genomic amplification is effected by comparing a ratio determined in
a sample of the solid tumor between a copy number of the VEGFA and a copy number
15 of a centromeric marker of human chromosome 6, or visa versa, namely, comparing a
ratio determined in a sample of the cancer between the copy number of a centromeric
marker of human chromosome 6 and a copy number of the VEGFA to a reference ratio
determined in at least one sample devoid of the solid tumor between a copy number of
the VEGFA and a copy number of the centromeric marker of human chromosome 6, or
20 visa versa, namely, a reference ratio determined in at least one sample devoid of the
solid tumor between a copy number of the centromeric marker of human chromosome 6
and a copy number of the VEGFA, respectively.

According to some embodiments of the invention, the centromeric marker of
human chromosome 6 includes nucleotide coordinates 6:605000000-633000000 according
25 to UCSC on Human GRCh37 Assembly (human genome 19 (hg19)). Non-limiting
examples of BAC clones which are encompassed by the human pericentromeric
chromosome 6 and which can be used for detection of the copy number of human
chromosome 6 include: RP1-91N13 (SEQ ID NO:20), RP5-1194O12 (SEQ ID NO:21),
and RP1-271N20 (SEQ ID NO:22). Non-limiting examples of centromeric markers
30 which can be used to detect the copy number of human chromosome 6 include the CEP
6 SPECTRUM GREEN (ABBOTT Molecular); ZYTODOT CEN 6 probe (PD2;
ZytoVision, C-3002-400); alphoid clone 308 (D6Z1; a 3-kb DNA fragment that is

repeated in centromer 6; Jabs Wang E et al., Characterization of human centromeric regions of specific chromosomes by means of alphoid DNA sequences. Am. J. Hum. Genet. 41:374-390, 1987); and SKU CEN006 (Empire Genomics, Buffalo, NY, USA).

As used herein a “sample” refers to any biological sample which contains a cell
5 of a subject or a cellular component.

Non-limiting examples of biological samples include body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, lymph fluids, and various external secretions of the respiratory, intestinal and genitourinary tracts, tears, saliva, milk as well as white blood cells, tissue biopsies (including those obtained by fine needle aspiration,
10 a surgical tool) e.g., of malignant tissues.

According to some embodiments of the invention, the sample comprises tumor cells and/or tissue.

According to some embodiments of the invention, the sample is a tissue section (e.g., a paraffin-embedded section, e.g., an archive paraffin-embedded tissue section, or
15 a frozen tissue section).

According to some embodiments of the invention, the sample is a fine-needle aspiration sample of a solid tumor.

According to some embodiments of the invention, an increase above a predetermined threshold in the ratio determined in the sample of the carcinoma relative
20 to the reference ratio indicates the presence of the genomic amplification.

As used herein the phrase “an increase above a predetermined threshold” refers to an increase in the ratio determined in the sample of the carcinoma relative to the reference ratio which is higher than a predetermined threshold such as a about 10 %, e.g., higher than about 20 %, e.g., higher than about 30 %, e.g., higher than about 40 %, e.g., higher than about 50 %, e.g., higher than about 60 %, higher than about 70 %, higher than about 80 %, higher than about 90 %, higher than about 2 times, higher than about three times, higher than about four time, higher than about five times, higher than about six times, higher than about seven times, higher than about eight times, higher than about nine times, higher than about 20 times, higher than about 50 times, higher
25 than about 100 times, higher than about 200 times, higher than about 350, higher than
30 than about 500 times, higher than about 1000 times, or more relative to the reference ratio.

According to some embodiments of the invention, an identical ratio or a change below a predetermined threshold in the ratio determined in the sample of the carcinoma as compared to the reference ratio indicates the absence of the genomic amplification.

As used herein the phrase "changed below a predetermined threshold" refers to
5 an increase or a decrease in the level of expression in the cell of the subject relative to the reference ratio which is lower than a predetermined threshold, such as lower than about 2 times, e.g., lower than about 90%, e.g., lower than about 80%, e.g., lower than about 70%, e.g., lower than about 60%, e.g., lower than about 50%, e.g., lower than about 40%, e.g., lower than about 30%, e.g., lower than about 20%, e.g., lower than
10 about 10%, e.g., lower than about 9%, e.g., lower than about 8%, e.g., lower than about 7%, e.g., lower than about 6%, e.g., lower than about 5%, e.g., lower than about 4%, e.g., lower than about 3%, e.g., lower than about 2%, e.g., lower than about 1% relative to the reference ratio.

According to some embodiments of the invention, determining a presence or an
15 absence of a genomic amplification is effected using a chromosomal detection method.

Non-limiting examples of chromosomal detection methods include fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH), primed *in situ* labeling (PRINS), quantitative FISH (Q-FISH) and/or multicolor-banding (MCB).

Methods of employing FISH analysis on interphase chromosomes are known in
20 the art. Briefly, directly-labeled probes [e.g., a probe derived from the amplified region which comprises the VEGF-A gene such as SEQ ID NO: 18 (BAC RP11-710L16), 19 (BAC RP11-21M9), 23, 24 and/or 25 labeled in one color (e.g., green) and a probe derived from a chromosome 6 centromer such as SEQ ID NO:20 (BAC RP1-91N13), 21 (BAC RP5-1194012) and/or 22 (BAC RP1-271N20) labeled in another color (e.g., red)]
25 are mixed with hybridization buffer (e.g., LSI/WCP, Abbott) and a carrier DNA (e.g., human Cot 1 DNA, available from Abbott). The probe solution is applied on microscopic slides containing the biological sample (e.g., tissue sections from a tumor biopsy) and the slides are covered using a coverslip. The probe-containing slides are denatured for 4-5 minutes at 71 °C (or 3 minutes at 70 °C) and are further incubated for
30 24-60 hours at 37 °C using an hybridization apparatus (e.g., HYBrite, Abbott Cat. No. 2J11-04). To increase the specificity, following hybridization, the slides are washed for 2 minutes at 70-72 °C in a solution of 0.3 % NP-40 (Abbott) in 60 mM NaCl and 6 mM

NaCitrate (0.4XSSC). Slides are then immersed for 1 minute in a solution of 0.1 % NP-40 in 2XSSC at room temperature, following which the slides are allowed to dry in the dark. Counterstaining is performed using, for example, DAPI II counterstain (Abbott).

For CISH a labeled complementary DNA or RNA strand is used to localize a specific DNA or RNA sequence in a tissue specimen. CISH can be used to detect various chromosomal abnormalities such as gene amplification, gene deletion, chromosome translocation, and chromosome number. Usually, CISH utilizes conventional peroxidase or alkaline phosphatase reactions, and is applicable to formalin-fixed, paraffin-embedded tissues, blood or bone marrow smears, metaphase chromosome spreads, and fixed cells.

PRINS analysis has been employed in the detection of gene deletion (Tharapel SA and Kadandale JS, 2002. *Am. J. Med. Genet.* 107: 123-126), determination of fetal sex (Orsetti, B., et al., 1998. *Prenat. Diagn.* 18: 1014-1022), and identification of chromosomal aneuploidy (Mennicke, K. et al., 2003. *Fetal Diagn. Ther.* 18: 114-121).

Methods of performing PRINS analysis are known in the art and include for example, those described in Coullin, P. et al. (*Am. J. Med. Genet.* 2002, 107: 127-135); Findlay, I., et al. (*J. Assist. Reprod. Genet.* 1998, 15: 258-265); Musio, A., et al. (*Genome* 1998, 41: 739-741); Mennicke, K., et al. (*Fetal Diagn. Ther.* 2003, 18: 114-121); Orsetti, B., et al. (*Prenat. Diagn.* 1998, 18: 1014-1022). Briefly, slides containing interphase chromosomes are denatured for 2 minutes at 71 °C in a solution of 70 % formamide in 2XSSC (pH 7.2), dehydrated in an ethanol series (70, 80, 90 and 100 %) and are placed on a flat plate block of a programmable temperature cycler (such as the PTC-200 thermal cycler adapted for glass slides which is available from MJ Research, Waltham, Massachusetts, USA). The PRINS reaction is usually performed in the presence of unlabeled primers and a mixture of dNTPs with a labeled dUTP (e.g., fluorescein-12-dUTP or digoxigenin-11-dUTP for a direct or indirect detection, respectively). Alternatively, or additionally, the sequence-specific primers can be labeled at the 5' end using e.g., 1-3 fluorescein or cyanine 3 (Cy3) molecules. Thus, a typical PRINS reaction mixture includes sequence-specific primers (50-200 pmol in a 50 µl reaction volume), unlabeled dNTPs (0.1 mM of dATP, dCTP, dGTP and 0.002 mM of dTTP), labeled dUTP (0.025 mM) and Taq DNA polymerase (2 units) with the appropriate reaction buffer. Once the slide reaches the desired annealing temperature

the reaction mixture is applied on the slide and the slide is covered using a cover slip. Annealing of the sequence-specific primers is allowed to occur for 15 minutes, following which the primed chains are elongated at 72 °C for another 15 minutes. Following elongation, the slides are washed three times at room temperature in a solution of 4XSSC/0.5 % Tween-20 (4 minutes each), followed by a 4-minute wash at PBS. Slides are then subjected to nuclei counterstain using DAPI or propidium iodide. The fluorescently stained slides can be viewed using a fluorescent microscope and the appropriate combination of filters (e.g., DAPI, FITC, TRITC, FITC-rhodamin).

It will be appreciated that several primers which are specific for several targets can be used on the same PRINS run using different 5' conjugates. Thus, the PRINS analysis can be used as a multicolor assay for the determination of the presence, and/or location of several genes or chromosomal loci.

In addition, as described in Coullin et al., (2002, Supra) the PRINS analysis can be performed on the same slide as the FISH analysis, preferably, prior to FISH analysis.

High-resolution multicolor banding (MCB) on interphase chromosomes - This method, which is described in detail by Lemke et al. (Am. J. Hum. Genet. 71: 1051-1059, 2002), uses YAC/BAC and region-specific microdissection DNA libraries as DNA probes for interphase chromosomes. Briefly, for each region-specific DNA library 8-10 chromosome fragments are excised using microdissection and the DNA is amplified using a degenerated oligonucleotide PCR reaction. For example, for MCB staining of chromosome 5, seven overlapping microdissection DNA libraries were constructed, two within the p arm and five within the q arm (Chudoba I., et al., 1999; Cytogenet. Cell Genet. 84: 156-160). Each of the DNA libraries is labeled with a unique combination of fluorochromes and hybridization and post-hybridization washes are carried out using standard protocols (see for example, Senger et al., 1993; Cytogenet. Cell Genet. 64: 49-53). Analysis of the multicolor-banding can be performed using the isis/mFISH imaging system (MetaSystems GmbH, Altlussheim, Germany). It will be appreciated that although MCB staining on interphase chromosomes was documented for a single chromosome at a time, it is conceivable that additional probes and unique combinations of fluorochromes can be used for MCB staining of two or more chromosomes at a single MCB analysis.

Quantitative FISH (Q-FISH) – In this method chromosomal abnormalities are detected by measuring variations in fluorescence intensity of specific probes. Q-FISH can be performed using Peptide Nucleic Acid (PNA) oligonucleotide probes. PNA probes are synthetic DNA mimics in which the sugar phosphate backbone is replaced by repeating N-(2-aminoethyl) glycine units linked by an amine bond and to which the nucleobases are fixed (Pellestor F and Paulasova P, 2004; Chromosoma 112: 375-380). Thus, the hydrophobic and neutral backbone enables high affinity and specific hybridization of the PNA probes to their nucleic acid counterparts (e.g., chromosomal DNA). Such probes have been applied on interphase nuclei to monitor telomere stability (Slijepcevic, P. 1998; Mutat. Res. 404:215-220; Henderson S., et al., 1996; J. Cell Biol. 134: 1-12), the presence of Fanconi anemia (Hanson H, et al., 2001, Cytogenet. Cell Genet. 93: 203-6) and numerical chromosome abnormalities such as trisomy 18 (Chen C, et al., 2000, Mamm. Genome 10: 13-18), as well as monosomy, duplication, and deletion (Taneja KL, et al., 2001, Genes Chromosomes Cancer. 30: 57-63).

Alternatively, Q-FISH can be performed by co-hybridizing whole chromosome painting probes (e.g., for chromosomes 21 and 22) on interphase nuclei as described in Truong K et al, 2003, Prenat. Diagn. 23: 146-51.

According to some embodiments of the invention, determining a presence or an absence of a genomic amplification is effected using a DNA detection method.

Comparative Genome Hybridization (CGH) - is based on a quantitative two-color fluorescence *in situ* hybridization (FISH) on metaphase chromosomes. In this method a test DNA (e.g., DNA extracted from the biological sample which includes tumor cells, e.g., obtained from a tumor tissue biopsy) is labeled in one color (e.g., green) and mixed in a 1:1 ratio with a reference DNA (e.g., DNA extracted from a control cell) which is labeled in a different color (e.g., red). Methods of amplifying and labeling whole-genome DNA are well known in the art (see for example, Wells D, et al., 1999; Nucleic Acids Res. 27: 1214-8). Briefly, genomic DNA is amplified using a degenerate oligonucleotide primer [e.g., 5'-CCGACTCGAGNNNNNNATGTGG, SEQ ID NO: 404 (Telenius, H., et al., 1992; Genomics 13:718-25)] and the amplified DNA is labeled using e.g., the Spectrum Green-dUTP (for the test DNA) or the Spectrum Red-dUTP (for the reference DNA). The mixture of labeled DNA samples is precipitated with Cot1 DNA (Gibco-BRL) and resuspended in an hybridization mixture containing

e.g., 50 % formamide, 2XSSC, pH 7 and 10 % dextrane sulfate. Prior to hybridization, the labeled DNA samples (*i.e.*, the probes) are denatured for 10 minutes at 75 °C and allowed to cool at room temperature for 2 minutes. Likewise, the metaphase chromosome spreads are denatured using standard protocols (e.g., dehydration in a series of ethanol, denaturation for 5 minutes at 75 °C in 70 % formamide and 2XSSC). Hybridization conditions include incubation at 37 °C for 25-30 hours in a humidified chamber, following by washes in 2XSSC and dehydration using an ethanol series, essentially as described elsewhere (Wells, D., et al., 2002; Fertility and Sterility, 78: 543-549). Hybridization signal is detected using a fluorescence microscope and the ratio of the green-to-red fluorescence can be determined using e.g., the Applied Imaging (Santa Clara, CA) computer software. If both genomes are equally represented in the metaphase chromosomes (*i.e.*, no deletions, duplication or insertions in the DNA derived from the tumor cells) the labeling on the metaphase chromosomes is orange. However, regions which are either deleted or duplicated in the tumor cell(s) are stained with red or green, respectively.

DNA array-based comparative genomic hybridization (CGH-array) - This method, which is fully described in Hu, D.G., et al., 2004, Mol. Hum. Reprod. 10: 283-289, is a modified version of CGH and is based on the hybridization of a 1:1 mixture of the test and reference DNA probes on an array containing chromosome-specific DNA libraries. Methods of preparing chromosome-specific DNA libraries are known in the art (see for example, Bolzer A., et al., 1999; Cytogenet. Cell. Genet. 84: 233-240). Briefly, single chromosomes are obtained using either microdissection or flow-sorting and the genomic DNA of each of the isolated chromosomes is PCR-amplified using a degenerated oligonucleotide primer. To remove repetitive DNA sequences, the amplified DNA is subjected to affinity chromatography in combination with negative subtraction hybridization (using e.g., human Cot-1 DNA or centromer-specific repetitive sequence as subtractors), essentially as described in Craig JM., et al., 1997; Hum. Genet. 100: 472-476. Amplified chromosome-specific DNA libraries are then attached to a solid support [(e.g., SuperAmine slides (TeleChem, USA)], dried, baked and washed according to manufacturer's recommendation. Labeled genomic DNA probes (a 1:1 mixture of the test and reference DNAs) are mixed with non-specific carrier DNA (e.g., human Cot-1 and/or salmon sperm DNA, Gibco-BRL), ethanol-precipitated and re-

suspended in an hybridization buffer such as 50 % deionized formamide, 2XSSC, 0.1 % SDS, 10 % Dextran sulphate and 5 X Denhardt's solution. The DNA probes are then denatured (80 °C for 10 minutes), pre-annealed (37 °C for 80 minutes) and applied on the array for hybridization of 15-20 hours in a humid incubator. Following hybridization the arrays are washed twice for 10 minutes in 50 % formamide/2XSSC at 45 °C and once for 10 minutes in 1XSSC at room temperature, following which the arrays are rinsed three times in 18.2 MΩ deionized water. The arrays are then scanned using any suitable fluorescence scanner such as the GenePix 4000B microarray reader (Axon Instruments, USA) and analyzed using the GenePix Pro. 4.0.1.12 software (Axon).

The genomic amplification which comprises the VEGFA gene can be detected using a quantitative DNA-based techniques such as quantitative DNA PCR (qDNA PCR) or quantitative Southern blot analysis. The quantitative DNA assays (qPCR or qSouthern blot) can determine the absolute number of gene copies or relative amount of gene copies (of a DNA sequence derived from the genomic amplification) when normalized to normalizing genes, and those of ordinary skills in the art are capable of assessing the results of such assays in order to determine presence or absence of the genomic amplification (see e.g., Figure 7).

Briefly, genomic DNA is extracted using known methods from a "test" biological sample (e.g., a tumor biopsy, or a fine needle aspiration sample for which the presence or absence of the genomic amplification is unknown) and from a reference sample with a known status with regard to presence or absence of the genomic amplification. The reference sample can be a positive control, *i.e.*, a sample which is known to have the genomic amplification as determined by other methods such as FISH (e.g., a tumor having the genomic amplification), or it can be a negative control, *i.e.*, a sample which is known to be devoid of the genomic amplification (having only a single copy per chromosome homologue). The negative control sample can be derived from a non-tumor tissue (derived from the same species, e.g., from human) or from a tumor tissue devoid of the genomic amplification as determined based on other methods, such as FISH.

Methods of performing qDNA PCR analysis are known to the ordinary skilled in the art and further exemplified in the Examples section which follows and in Figure 7.

For example, qPCR analyses can be carried out with SYBR green (Invitrogen) in 7900HT Fast Real-Time PCR System (Applied BioSystems), and the results can be analyzed using the qBase v1.3.5 software. For example, for detecting the genomic amplification in chromosome 6p21, which comprises the VEGF-A gene, qPCR can be performed using primers pairs specific to any of the genes encompassed in the genomic amplification (e.g., SEQ ID NOs: 1, 26-403 and 405-433). Non-limiting examples of such primers pairs are provided in Table 3 below and in SEQ ID NOs: 456-1269.

Table 3
Genes encompassed within the human 6p21 genomic amplification region and primers and probes for quantifying the copy number of the genomic amplification

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
LOC646520	26	30436659	30438025	456	457	1270
RANP1	27	30453662	30454724	458	459	1271
HLA-E	28	30457183	30461982	460	461	1272
GNL1	29	30509154	30525371	462	463	1273
PRR3	30	30524486	30532473	464	465	1274
ABCF1	31	30539170	30559309	466	467	1275
MIR877	32	30552109	30552194	468	469	1276
PPP1R10	33	30568182	30585020	470	471	1277
MRPS18B	34	30585486	30594174	472	473	1278
C6orf134	35	30594613	30614598	474	475	1279
PTMAP1	36	30601227	30603024	476	477	1280
C6orf136	37	30614816	30620987	478	479	1281
DHX16	38	30620896	30640830	480	481	1282
KIAA1949	39	30644166	30655672	482	483	1283
NRM	40	30655826	30658769	484	485	1284
RPL7P4	41	30664523	30665313	486	487	1285
MDC1	42	30667584	30685458	488	489	1286
TUBB	43	30688157	30693199	490	491	1287
LOC100287146	44	30691146	30694154	492	493	1288
FLOT1	45	30695511	30710453	494	495	1289
IER3	46	30710976	30712327	496	497	1290
DDR1	47	30851861	30867933	498	499	1291

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
GTF2H 4	48	30875977	30881880	500	501	1292
VARS2	49	30881982	30894236	502	503	1293
SFTA2	50	30899127	30899952	504	505	1294
DPCR1	51	30908777	30921998	506	507	1295
LOC100 422429	52	30929178	30929755	508	509	1296
LOC100 420530	53	30931992	30933937	510	511	1297
MUC21	54	30951485	30957675	512	513	1298
HCG22	55	31021984	31027653	514	515	1299
C6orf15	56	31079000	31080332	516	517	1300
PSORS1 C1	57	31082608	31107869	518	519	1301
CDSN	58	31082865	31088252	520	521	1302
PSORS1 C2	59	31105311	31107127	522	523	1303
CCHCR 1	60	31110216	31126015	524	525	1304
TCF19	61	31126303	31131992	526	527	1305
POU5F1	62	31132114	31138451	528	529	1306
PSORS1 C3	63	31141512	31145676	530	531	1307
HCG27	64	31165537	31171745	532	533	1308
HLA-C	65	31236529	31239855	534	535	1309
LOC100 287272	66	31243352	31246531	536	537	1310
RPL3P2	67	31248108	31249348	538	539	1311
WASF5 P	68	31255162	31256941	540	541	1312
HLA-B	69	31321649	31324989	542	543	1313
DHFRP 2	70	31331244	31334742	544	545	1314
LOC100 462812	71	31345494	31345805	546	547	1315
HLA-S	72	31349346	31350264	548	549	1316
LOC100 507436	73	31367561	31433586	550	551	1317
HLA-X	74	31429623	31430267	552	553	1318
HCP5	75	31430957	31433586	554	555	1319
HCG26	76	31439006	31440185	556	557	1320
MICB	77	31465855	31478901	558	559	1321
PPIAP9	78	31486654	31488179	560	561	1322
RPL15P 4	79	31495853	31496498	562	563	1323
MCCD1	80	31496739	31498008	564	565	1324
BAT1	81	31497996	31510225	566	567	1325

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
SNORD 117	82	31504151	31504226	568	569	1326
SNORD 84	83	31508878	31508955	570	571	1327
ATP6V 1G2	84	31512239	31514627	572	573	1328
NFKBI L1	85	31514628	31526606	574	575	1329
LTA	86	31539876	31542098	576	577	1330
TNF	87	31543350	31546112	578	579	1331
LTB	88	31548335	31550202	580	581	1332
LST1	89	31553956	31556686	582	583	1333
NCR3	90	31556660	31560762	584	585	1334
LOC100 130756	91	31578860	31579133	586	587	1335
AIF1	92	31582994	31584798	588	589	1336
BAT2	93	31588450	31605554	590	591	1337
SNORA 38	94	31590856	31590987	592	593	1338
BAT3	95	31606805	31620477	594	595	1339
APOM	96	31623671	31625987	596	597	1340
C6orf47	97	31626075	31628549	598	599	1341
BAT4	98	31629862	31633163	600	601	1342
CSNK2 B	99	31633657	31637843	602	603	1343
LY6G5 B	100	31638728	31640227	604	605	1344
LY6G5 C	101	31644461	31648150	606	607	1345
BAT5	102	31654726	31671137	608	609	1346
LY6G6 F	103	31674684	31678372	610	611	1347
LY6G6 E	104	31679753	31681842	612	613	1348
LY6G6 D	105	31683133	31685581	614	615	1349
LY6G6 C	106	31686425	31689510	616	617	1350
C6orf25	107	31691161	31692851	618	619	1351
DDAH2	108	31694817	31698039	620	621	1352
CLIC1	109	31698358	31704341	622	623	1353
MSH5	110	31707725	31730455	624	625	1354
C6orf26	111	31730773	31732627	626	627	1355
C6orf27	112	31733371	31745108	628	629	1356
VARS	113	31745295	31763712	630	631	1357
LSM2	114	31765173	31774743	632	633	1358
HSPA1 L	115	31777396	31782835	634	635	1359

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
HSPA1A	116	31783291	31785719	636	637	1360
HSPA1B	117	31795512	31798031	638	639	1361
C6orf48	118	31802693	31807541	640	641	1362
SNORD48	119	31803040	31803103	#N/A	#N/A	1363
SNORD52	120	31804853	31804916	#N/A	#N/A	1364
NEU1	121	31826829	31830709	642	643	1365
SLC44A4	122	31830969	31846823	644	645	1366
EHMT2	123	31847536	31865464	646	647	1367
ZBTB12	124	31867394	31869769	648	649	1368
C2	125	31895266	31913449	650	651	1369
CFB	126	31913721	31919861	652	653	1370
RDBP	127	31919864	31926864	654	655	1371
MIR1236	128	31924616	31924717	656	657	1372
SKIV2L	129	31926581	31937532	658	659	1373
DOM3Z	130	31937588	31940032	660	661	1374
STK19	131	31938952	31949223	662	663	1375
C4A	132	31949834	31970457	664	665	1376
CYP21A1P	133	31972719	31976761	666	667	1377
TNXA	134	31976197	31980800	668	669	1378
STK19P	135	31981047	31981961	670	671	1379
C4B	136	31982572	32003195	672	673	1380
CYP21A2	137	32006082	32009419	674	675	1381
TNXB	138	32008932	32077153	676	677	1382
ATF6B	139	32083045	32096030	678	679	1383
FKBP1	140	32096484	32098067	680	681	1384
PRRT1	141	32116140	32119720	682	683	1385
LOC100507547	142	32120579	32122119	684	685	1386
PPT2	143	32121301	32131452	686	687	1387
EGFL8	144	32132405	32136062	688	689	1388
AGPAT1	145	32135983	32145888	690	691	1389
RNF5	146	32146162	32148570	692	693	1390
AGER	147	32148746	32152023	694	695	1391
PBX2	148	32152510	32157963	696	697	1392
GPSM3	149	32158543	32163300	698	699	1393
NOTCH4	150	32162620	32191844	700	701	1394
C6orf10	151	32260475	32339656	702	703	1395

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
HNRNP A1P2	152	32293175	32294298	704	705	1396
BTNL2	153	32362513	32374900	706	707	1397
HLA-DRA	154	32407619	32412823	708	709	1398
HLA-DRB9	155	32427597	32427866	710	711	1399
HLA-DRB5	156	32485151	32498006	712	713	1400
HLA-DRB6	157	32520490	32527779	714	715	1401
HLA-DRB1	158	32546546	32557562	716	717	1402
HLA-DQA1	159	32605183	32611429	718	719	1403
HLA-DQB1	160	32627657	32634466	720	721	1404
HLA-DQA2	161	32709163	32714664	722	723	1405
HLA-DQB2	162	32723875	32731330	724	725	1406
HLA-DOB	163	32780540	32784825	726	727	1407
TAP2	164	32789610	32806547	728	729	1408
PSMB8	165	32808494	32812712	730	731	1409
LOC100507463	166	32811908	32814277	732	733	1410
TAP1	167	32812986	32821748	734	735	1411
PSMB9	168	32821938	32827628	736	737	1412
PPP1R2 P1	169	32844255	32847851	738	739	1413
LOC100507444	170	32846948	32847619	740	741	1414
LOC100294145	171	32861953	32871535	742	743	1415
HLA-Z	172	32864179	32864266	744	745	1416
HLA-DMB	173	32902406	32908847	746	747	1417
HLA-DMA	174	32916391	32920899	748	749	1418
BRD2	175	32936437	32949282	750	751	1419
HLA-DOA	176	32971959	32977389	752	753	1420
HLA-DPA1	177	33032794	33041378	754	755	1421
HLA-DPB1	178	33043760	33054976	756	757	1422

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
RPL32P 1	179	33047076	33047788	758	759	1423
HLA- DPA2	180	33059259	33061091	760	761	1424
COL11 A2P	181	33071570	33074821	762	763	1425
HLA- DPB2	182	33080293	33096890	764	765	1426
HLA- DPA3	183	33098974	33099120	766	767	1427
COL11 A2	184	33130469	33160245	768	769	1428
RXRB	185	33161365	33168432	770	771	1429
RNY4P 10	186	33167378	33167469	772	773	#N/A
SLC39A 7	187	33168603	33172214	774	775	1430
HSD17 B8	188	33172419	33174607	776	777	1431
MIR219 -1	189	33175612	33175721	778	779	1432
RING1	190	33176286	33180499	780	781	1433
LOC100 419609	191	33183351	33183960	782	783	1434
VPS52	192	33218049	33239662	784	785	1435
RPS18	193	33239852	33244281	786	787	1436
B3GAL T4	194	33244917	33246602	788	789	1437
WDR46	195	33246880	33257304	790	791	1438
PFDN6	196	33257378	33258711	792	793	1439
RGL2	197	33259431	33267176	794	795	1440
TAPBP	198	33267471	33282164	796	797	1441
ZBTB22	199	33282182	33285719	798	799	1442
DAXX	200	33286335	33290793	800	801	1443
MYL8P	201	33306755	33307272	802	803	1444
LYPLA 2P1	202	33332501	33334139	804	805	1445
RPL35A P4	203	33357133	33357256	806	807	1446
KIFC1	204	33359313	33377701	808	809	1447
RPL12P 1	205	33367792	33368421	810	811	1448
PHF1	206	33378773	33384230	812	813	1449
CUTA	207	33384319	33386065	814	815	1450
SYNGA P1	208	33387847	33421466	816	817	1451
ZBTB9	209	33422356	33425321	818	819	1452
BAK1	210	33540323	33548070	820	821	1453

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
GGNBP1	211	33551476	33556803	822	823	1454
C6orf227	212	33553883	33561115	824	825	1455
ITPR3	213	33589161	33664351	826	827	1456
LOC100507563	214	33599021	33601413	828	829	1457
C6orf125	215	33665346	33679504	830	831	1458
IP6K3	216	33689443	33714762	832	833	1459
LEMD2	217	33738990	33756906	834	835	1460
MLN	218	33762449	33771793	836	837	1461
LOC100507584	219	33857288	33864684	838	839	1462
MIR1275	220	33967749	33967828	840	841	1463
GRM4	221	33989628	34101443	842	843	1464
KRT18P9	222	34157509	34158918	844	845	1465
CYCSL1	223	34187149	34188714	846	847	1466
HMGA1	224	34204577	34214008	848	849	1467
C6orf1	225	34214157	34216904	850	851	1468
RPL35P2	226	34231047	34231500	852	853	1469
LOC100507620	227	34247456	34250571	854	855	1470
NUDT3	228	34255997	34360441	856	857	1471
RPS10	229	34385231	34393876	858	859	1472
PACSIN1	230	34433905	34503000	860	861	1473
SPDEF	231	34505580	34524091	864	865	1474
LOC100101247	232	34543878	34544534	866	867	1475
C6orf106	233	34555065	34664625	868	869	1476
RPL7P25	234	34584299	34585101	870	871	1477
LOC100131607	235	34664094	34665247	872	873	1478
RPS10P13	236	34711917	34712495	874	875	1479
SNRPC	237	34724871	34741634	876	877	1480
UHRF1BP1	238	34759794	34845291	878	879	1481
TAF11	239	34845555	34855819	880	881	1482
ANKS1A	240	34857038	35059190	882	883	1483

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
TCP11	241	35085849	35109187	884	885	1484
SCUBE3	242	35182190	35218609	886	887	1485
ZNF76	243	35227510	35263760	888	889	1486
DEF6	244	35265595	35289548	890	891	1487
PPARD	245	35310335	35395968	892	893	1488
LOC100507672	246	35316761	35324633	894	895	1489
MKRNP2	247	35413987	35417642	896	897	1490
FANCE	248	35420138	35434881	898	899	1491
RPL10A	249	35436178	35438558	900	901	1492
TEAD3	250	35441374	35464861	902	903	1493
TULP1	251	35465651	35480647	904	905	1494
RPS15A P19	252	35523623	35524081	906	907	1495
FKBP5	253	35541362	35696360	908	909	1496
RPL36P9	254	35575370	35575757	910	911	1497
LOC285847	255	35694539	35704724	912	913	1498
C6orf81	256	35704859	35716685	914	915	1499
C6orf126	257	35744392	35747329	916	917	1500
C6orf127	258	35748831	35755841	918	919	1501
CLPS	259	35762760	35765102	920	921	1502
LHFPL5	260	35773071	35791852	922	923	1503
SRPK1	261	35800811	35888957	924	925	1504
SLC26A8	262	35911291	35992413	926	927	1505
DPRXP2	263	35957267	35958269	928	929	1506
MAPK14	264	35995454	36079013	930	931	1507
LOC100505482	265	36059260	36060417	932	933	1508
MAPK13	266	36098262	36107842	934	935	1509
BRPF3	267	36164550	36200567	936	937	1510
PNPLA1	268	36210945	36276372	938	939	1511
C6orf222	269	36283534	36304662	940	941	1512
ETV7	270	36333971	36355467	942	943	1513
PXT1	271	36358328	36410666	944	945	#N/A
KCTD20	272	36410544	36458319	946	947	1514

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
STK38	273	36461669	36515247	948	949	1515
SFRS3	274	36562090	36572244	950	951	1516
LOC389 386	275	36641527	36643222	952	953	1517
CDKN1 A	276	36646459	36655109	954	955	1518
GPR166 P	277	36704782	36705483	956	957	1519
LOC100 420942	278	36704887	36705660	958	959	1520
CPNE5	279	36708555	36807220	960	961	1521
LOC100 127961	280	36808556	36812209	962	963	1522
PPIL1	281	36822605	36842800	964	965	1523
C6orf89	282	36853640	36896740	966	967	1524
LOC100 505509	283	36891451	36901406	968	969	1525
PI16	284	36922209	36932613	970	971	1526
MTCH1	285	36935917	36953949	972	973	1527
FGD2	286	36973423	36996845	974	975	1528
COX6A 1P2	287	37012610	37013157	976	977	1529
RPL12P 2	288	37059003	37059621	978	979	1530
PIM1	289	37137922	37143204	980	981	1531
TMEM2 17	290	37179954	37225931	982	983	1532
TBC1D 22B	291	37225548	37300746	984	985	1533
RNF8	292	37321748	37362514	986	987	1534
FTSJD2	293	37400907	37449284	988	989	1535
C6orf12 9	294	37450696	37467700	990	991	1536
LOC100 505530	295	37506128	37509166	992	993	1537
LOC100 505550	296	37511329	37514538	994	995	1538
MDGA1	297	37600284	37665766	996	997	1539
ZFAND 3	298	37787307	38122400	998	999	1540
BTBD9	299	38136227	38607924	1000	1001	1541
LOC100 505567	300	38449372	38450303	1002	1003	1542
LOC100 128379	301	38555099	38556305	1004	1005	1543
GLO1	302	38643701	38670952	1006	1007	1544
DNAH8	303	38690552	38998567	1008	1009	1545
ZRF1PS	304	38730632	38734659	1010	1011	1546

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
LOC100 128655	305	39007444	39010968	1012	1013	1547
GLP1R	306	39016557	39055520	1014	1015	1548
C6orf64	307	39071840	39082865	1016	1017	1549
KCNK5	308	39156747	39197251	1018	1019	1550
KCNK1 7	309	39266777	39282237	1020	1021	1551
KCNK1 6	310	39282474	39290330	1022	1023	#N/A
KIF6	311	39302876	39693181	1024	1025	1552
LOC100 124373	312	39321621	39322274	1026	1027	1553
LOC100 131899	313	39521593	39522716	1028	1029	1554
DAAM2	314	39760793	39872641	1030	1031	1555
LOC100 505635	315	39849580	39865028	1032	1033	1556
MOCS1	316	39872034	39902290	1034	1035	1557
RPL23P 6	317	39926090	39926598	1036	1037	1558
LOC442 210	318	39960554	39967943	1038	1039	1559
FLJ4164 9	319	40312084	40323745	1040	1041	1560
TDRG1	320	40346163	40347631	1042	1043	1561
LRFN2	321	40359373	40555126	1044	1045	1562
LOC100 505697	322	40484082	40491672	1046	1047	1563
UNC5C L	323	40994640	41006938	1048	1049	1564
TSPO2	324	41010237	41012076	1050	1051	1565
APOBE C2	325	41020940	41032630	1052	1053	1566
C6orf13 0	326	41034531	41040188	1054	1055	1567
NFYA	327	41040707	41070146	1056	1057	1568
LOC221 442	328	41068773	41108573	1058	1059	1569
TREML 1	329	41117342	41122070	1060	1061	1570
TREM2	330	41126246	41130922	1062	1063	1571
TREML 2	331	41157552	41168925	1064	1065	1572
TREML 3	332	41176292	41185685	1066	1067	1573
TREML 4	333	41196062	41206120	1068	1069	1574

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
TREML 2P	334	41217115	41217327	1070	1071	1575
TREM1	335	41243712	41254457	1072	1073	1576
RPL32P 15	336	41275696	41278043	1074	1075	1577
NCR2	337	41303528	41318625	1076	1077	1578
LOC100 505711	338	41349130	41350823	1078	1079	#N/A
LOC100 505730	339	41491633	41516359	1080	1081	1579
FOXP4	340	41514164	41570122	1082	1083	1580
MDFI	341	41606195	41621982	1084	1085	1581
LOC100 130606	342	41634644	41635393	1086	1087	1582
TFEB	343	41651716	41703997	1088	1089	1583
PGC	344	41704449	41715139	1090	1091	#N/A
FRS3	345	41737914	41747630	1092	1093	1584
PRICKL E4	346	41748500	41755110	1094	1095	1585
TOMM 6	347	41755181	41757634	1096	1097	1586
USP49	348	41765383	41863099	1098	1099	1587
MED20	349	41873092	41888877	1100	1101	1588
BYSL	350	41888965	41900784	1102	1103	1589
CCND3	351	41902671	42016610	1104	1105	1590
TAF8	352	42018251	42048644	1106	1107	1591
C6orf13 2	353	42068857	42110715	1108	1109	1592
GUCA1 A	354	42123144	42147794	1110	1111	1593
GUCA1 B	355	42151022	42162694	1112	1113	1594
MRPS1 0	356	42174539	42185633	1114	1115	1595
TRERF 1	357	42192669	42419783	1116	1117	1596
RPL36A P5	358	42467416	42467817	1118	1119	1597
UBR2	359	42531760	42661243	1120	1121	1598
PRPH2	360	42664333	42690358	1122	1123	1599
LOC442 211	361	42694972	42695932	1124	1125	1600
TBCC	362	42712234	42713884	1126	1127	1601
FLJ3871 7	363	42750665	42753299	1128	1129	1602
KIAA02 40	364	42788794	42836296	1130	1131	1603
RPL7L1	365	42847671	42854731	1132	1133	1604

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
C6orf22 6	366	42858003	42858554	1134	1135	1605
PTCRA	367	42883727	42893576	1136	1137	1606
CNPY3	368	42896860	42907008	1138	1139	1607
RPL24P 4	369	42924074	42924504	1140	1141	1608
GNMT	370	42928500	42931618	1142	1143	1609
PEX6	371	42931611	42946981	1144	1145	1610
PPP2R5 D	372	42952330	42980080	1146	1147	1611
MEA1	373	42979965	42981618	1148	1149	1612
KLHDC 3	374	42981977	42989032	1150	1151	1613
C6orf15 3	375	42989385	42997337	1152	1153	1614
LOC100 505808	376	43000252	43002143	1154	1155	1615
CUL7	377	43005355	43021683	1156	1157	1616
MRPL2	378	43021767	43027242	1158	1159	1617
KLC4	379	43027372	43042833	1160	1161	1618
PTK7	380	43044029	43129457	1162	1163	1619
SRF	381	43138920	43149244	1164	1165	1620
CUL9	382	43149922	43192325	1166	1167	1621
C6orf10 8	383	43193367	43197211	1168	1169	1622
TTBK1	384	43211222	43255997	1170	1171	1623
SLC22A 7	385	43265998	43273276	1172	1173	1624
CRIP3	386	43273211	43276530	1174	1175	1625
RPL34P 14	387	43295590	43296214	1176	1177	1626
ZNF318	388	43303808	43337181	1178	1179	1627
RPS2P2 8	389	43331153	43331957	1180	1181	1628
ABCC1 0	390	43399489	43418163	1182	1183	1629
DLK2	391	43418090	43423786	1184	1185	1630
TJAP1	392	43445261	43474294	1186	1187	1631
C6orf15 4	393	43474707	43478424	1188	1189	1632
YIPF3	394	43479565	43484728	1190	1191	1633
POLR1 C	395	43484791	43497114	1192	1193	1634
XPO5	396	43490068	43543812	1194	1195	1635
RPS2P2 9	397	43506522	43507463	1196	1197	1636
POLH	398	43543878	43588260	1198	1199	1637

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
GTPBP 2	399	43588218	43596936	1200	1201	1638
MAD2L 1BP	400	43597279	43608689	1202	1203	1639
RSPH9	401	43612767	43638748	1204	1205	1640
MRPS1 8A	402	43638934	43655549	1206	1207	1641
LOC100 132242	403	43673593	43674494	1208	1209	1642
VEGFA	1	43737946	43754224	1210	1211	1643
LOC100 132354	405	43858765	43905944	1212	1213	1644
C6orf22 3	406	43968337	43973695	1214	1215	1645
RPL29P 16	407	44056862	44057500	1216	1217	1646
MRPL1 4	408	44081372	44095191	1218	1219	1647
TMEM6 3B	409	44095376	44123256	1220	1221	1648
CAPN1 1	410	44126548	44152139	1222	1223	1649
SLC29A 1	411	44187242	44201888	1224	1225	1650
LOC100 505819	412	44209727	44221620	1226	1227	1651
HSP90A B1	413	44214849	44221614	1228	1229	1652
SLC35B 2	414	44221838	44225283	1230	1231	1653
NFKBI E	415	44225903	44233525	1232	1233	1654
TMEM1 51B	416	44238480	44247182	1234	1235	1655
TCTE1	417	44247897	44265458	1236	1237	1656
AARS2	418	44266463	44281063	1238	1239	1657
SPATS1	419	44310397	44344904	1240	1241	1658
CDC5L	420	44355302	44414780	1242	1243	1659
LOC100 128935	421	44440641	44445639	1244	1245	1660
LOC100 505862	422	44513903	44519618	1246	1247	1661
SUPT3 H	423	44796469	45345670	1248	1249	1662
LOC100 422452	424	44866370	44867032	1250	1251	1663
LOC100 271870	425	45065696	45067086	1252	1253	1664

<i>Gene Symbol</i>	<i>Gene SEQ ID NO:</i>	<i>Start</i>	<i>End</i>	<i>For. SEQ ID NO:</i>	<i>Rev. SEQ ID NO:</i>	<i>Probe SEQ ID NO:</i>
LOC100420052	426	45126583	45127268	1254	1255	1665
MIR586	427	45165411	45165507	1256	1257	1666
RUNX2	428	45296054	45518819	1258	1259	1667
CLIC5	429	45866188	46048085	1260	1261	1668
ENPP4	430	46097701	46114436	1262	1263	1669
ENPP5	431	46127762	46138717	1264	1265	1670
ACTG1 P9	432	46172649	46174298	1266	1267	1671
RCAN2	433	46188469	46293531	1268	1269	1672

Table 3. Provided are the genes encompassed within the human chromosome 6p21 genomic amplification region [shown by gene symbol, sequence identifier and the start and end nucleotide positions on human chromosome 6 according to UCSC on Human GRCh37 Assembly (human genome 19 (hg19)], and means to quantifying the copy number of the genomic amplification in 6p21 using PCR primers for qDNA PCR (provided by sequence identifiers) and DNA probes for quantitative Southern blot (provided by sequence identifiers). “Gene SEQ ID NO:” - Genomic sequence SEQ ID NO.; “Start” - The first nucleotide corresponding to the gene as indicated by position on human Chr.6; “End” - The last nucleotide corresponding to the gene as indicated by position on human Chr. 6; “For. SEQ ID NO:” - Forward primer (5'→3') for qDNA PCR (SEQ ID NO.); “Rev. SEQ ID NO:” - Reverse primer for qDNA PCR (SEQ ID NO.); “probe SEQ ID NO:” - Probe for qSouthern blot. #N/A - not available.

Methods of performing quantitative Southern blot are known to the ordinary skilled in the art. Once the DNA is extracted from a test and a reference sample, the concentration of the DNA can be determined with a spectrophotometer, following which the DNA is digested with restriction enzyme endonucleases such as *HindIII* and/or with combination of a few restriction enzymes (e.g., *HindIII* and *BglII*). Once digested, equal amounts of DNA (e.g., 5-10 µg per lane) from both the “test” sample(s) and the reference sample(s) are loaded onto an agarose gel (e.g., 0.6-1.3% agarose which contains ethidium bromide) and are subject to gel electrophoresis. Prior to blotting, the amount in each lane is equalized based on the intensity of DNA smear stained with ethidium bromide. After blotting to a membrane (e.g., Hybond N+; Amersham) the blotted DNA is hybridized with a labeled DNA probe. The probe can be labeled using methods well known in the art with a radioactive isotope such as ³²P, or with a non-radioactive labeling such as using Digoxigenin labeling.

Suitable DNA probes for quantitative Southern blot can be prepared from the genomic region of-interest, and/or from a complementary DNA (cDNA, complementary DNA probes, which are in the antisense direction with respect to the mRNA sense

sequence) of any of the coding sequences comprised in the sequence of the genomic amplification which comprises the VEGF-A gene. The sequence of the cDNA can be obtained from the NCBI web site [Hypertext Transfer Protocol://World Wide Web (dot) ncbi (dot) nlm (dot) nih (dot) gov/] by searching “GENE” with the “Gene Symbol” listed in Table 3 above, or by performing a sequence alignment (e.g., BLASTN) using as a “Query Sequence” any of the genomic sequences provided in SEQ ID NOs:1, 26-403 and 405-433 (Table 3 above). The length of the probe for Southern blot analysis can vary from few tens of nucleotides to several kilobases of nucleotides.

Suitable polynucleotide probes are those referred to as “unique probes” which specifically hybridize to the target DNA sequence but not to other DNA sequences in the sample under the same hybridization conditions. By way of example, hybridization of short polynucleotide probes (below 200 nucleotides in length) can be effected by the following hybridization protocols depending on the desired stringency; By way of example, hybridization of short polynucleotide probes (below 200 nucleotides in length) can be effected by the following hybridization protocols depending on the desired stringency; (i) an hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 1 - 1.5 °C below the T_m , final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m ; (ii) hybridization solution of 6 x SSC and 0.1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 2 - 2.5 °C below the T_m , final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the T_m , final wash solution of 6 x SSC, and final wash at 22 °C; (iii) hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 37 °C, final wash solution of 6 x SSC and final wash at 22 °C. Variations in the hybridization conditions (e.g., hybridization solution, wash solutions and temperatures) can be made by one skilled in the art according to the probe length and its G/C content. For example, for polynucleotide probes larger than 200 nucleotides, the

hybridization/washes temperatures can be about 55-80 °C (e.g., 65 °C) and the hybridization/wash solutions can include formamide (e.g., 50 %).

By way of example, hybridization with longer DNA probes (e.g., about 1-2 kb) can be performed using the following hybridization solution (i) 5X Denhardt's Solution [50X Denhardt's Solution consists of 1% BSA, 1% Polyvinylpyrrolidone, 1% Ficoll], 100 mg/ml Salmon or Herring Sperm DNA, 0.1% SDS, 5XSSPE [SSPE (20X) consists of: 3M NaCl, 0.2M Sodium Phosphate, pH 7.4, 25 mM EDTA], 50% formamide; hybridization temperature of 20°C below the calculated T_m (melting temperature); and washes 1 x 20 minutes in 1X SSC, 0.1% SDS at 45° C, followed by 3 x 20 minutes in 0.2X SSC, 0.1 % SDS at 65° C.

According to some embodiments of the invention, the probe has a nucleotide sequence having as a 5' nucleotide the nucleotide at position "X" in SEQ ID NO:23 and as a 3' nucleotide the nucleotide at position "Y" in SEQ ID NO:23; wherein X is a numerator selected from nucleotide position 1-15,799,979 in SEQ ID NO:23, wherein Y is a numerator selected from nucleotide position 21-15,799,999 in SEQ ID NO:23, and wherein numerator Y is larger than the numerator X by at least about 20 nucleotides, e.g., by at least about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1600, about 1700 nucleotides or more.

According to some embodiments of the invention, the probe has a nucleotide sequence having as a 5' nucleotide the nucleotide at position "X" in SEQ ID NO:24 and as a 3' nucleotide the nucleotide at position "Y" in SEQ ID NO:24; wherein X is a numerator selected from nucleotide position 1-5,699,979 in SEQ ID NO:24, wherein Y is a numerator selected from nucleotide position 21-5,699,999 in SEQ ID NO:24, and wherein numerator Y is larger than the numerator X by at least about 20 nucleotides, e.g., by at least about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1600, about 1700 nucleotides or more.

According to some embodiments of the invention, the probe has a nucleotide sequence having as a 5' nucleotide the nucleotide at position "X" in SEQ ID NO:25 and

as a 3' nucleotide the nucleotide at position "Y" in SEQ ID NO:25; wherein X is a numerator selected from nucleotide position 1-317,980 in SEQ ID NO:25, wherein Y is a numerator selected from nucleotide position 21-318,000 in SEQ ID NO:25, and wherein numerator Y is larger than the numerator X by at least about 20 nucleotides, e.g., by at least about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 70, about 80, about 90, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1600, about 1700 nucleotides or more.

According to some embodiments of the invention, the length of the probe varies from about 20 bases to about 10 kilobase (kb), e.g., from about 20 bases to about 7 kb, e.g., 100 bases to about 5 kb, e.g., from about 200 bases to about 2 kb, e.g., from about 300 bases to about 1.5 kb, e.g., from about 500-1300 bases. Various software are available to design appropriate DNA probes for genomic hybridization using Southern blot analysis. Examples include, but are not limited to G2C::Software [Hypertext Transfer Protocol://World Wide Web (dot) genes2cognition (dot) org/software/southern_blot/probe_tiling_method (dot) html].

It should be noted that a probe for Southern blot analysis should be preferably devoid of repetitive DNA sequences, such that when hybridized to genomic DNA will result in a single or a few distinct bands (see e.g., determination of gene copy number by quantitative Southern blot analysis in the Dystrophin gene, Hiraishi Y., et al., 1992, J. Med. Genet. 29: 897-901, which is hereby incorporated by reference in its entirety).

Bioinformatic tools for removing repetitive sequences from nucleotide sequences are known in the art. For example, the RepeatMasker tool (Institute for System Biology, available via the Hypertext Transfer Protocol://World Wide Web (dot) repeatmasker (dot) org/ web site can be used. Another convenient tool for excluding repetitive sequences from a DNA sequence is via the UCSC Genome Browser [Hypertext Transfer Protocol://genome (dot) ucsc (dot) edu/cgi-bin/hgGateway] in which one can insert the genomic region of interest (e.g., human chromosome 6p21) and in the "DNA" tool select the option of "Mask Repeats". The outcome of the repeat masking tools can be such that all repetitive sequences are converted to "NNN..." and can be further excluded from the sequence.

Non-limiting examples of DNA probes (devoid of repetitive sequences) from the genomic region encompassed by the amplification (6p21) are provided in SEQ ID NOs:1270-1672 as described in the “Probe” column in Table 3 above.

Qualifying of suitable probes for quantitative Southern blot can be done by Southern blot analysis using the identified probe (according to the teachings provided herein) using known reference DNA samples derived from a positive control biological sample (which is known to include the genomic amplification based on other methods such as FISH) and/or a negative control biological sample (which is known to be devoid of the genomic amplification based on other methods such as FISH).

According to some embodiments of the invention the method further comprising comparing an expression level of the VEGFA in the sample of the carcinoma to a reference expression data obtained from at least one sample devoid of cancer.

According to some embodiments of the invention an increase above a predetermined threshold in the expression level of the VEGFA in the sample of the carcinoma relative to the reference expression data predicts the efficacy of the anti-VEGFA treatment on the carcinoma (i.e., that the anti-VEGFA treatment is efficient in treating the solid tumor).

According to some embodiments of the invention the sample devoid of cancer is a liver sample.

The expression level of VEGFA can be detected using various RNA detection methods such as Northern Blot analysis, RT-PCR analysis, RNA in situ hybridization stain, In situ RT-PCR stain, DNA microarrays/DNA chips, Oligonucleotide microarray.

The expression and/or activity level of the VEGFA protein can be determined using methods known in the arts such as Enzyme linked immunosorbent assay (ELISA), Western blot, radio-immunoassay (RIA), fluorescence activated cell sorting (FACS), immunohistochemical analysis, in situ activity assay, in vitro activity assays.

As used herein the phrase “anti-VEGFA” refers to an agent (e.g., drug) which reduces, inhibits, or suppresses VEGFA levels (expression level and/or activity) in cells or tissue.

According to some embodiments of the invention, the anti-VEGF agent inhibits VEGF-A activity by blocking the VEGF-A tyrosine kinase receptor.

According to some embodiments of the invention, the anti-VEGF agent is a small molecule, which blocks the VEGF-A tyrosine kinase receptor.

According to some embodiments of the invention, the anti-VEGF agent reduces, inhibits or suppresses VEGFA levels in the tumor cells of a subject in need thereof.

5 As used herein the phrase “anti-VEGFA treatment” refers to administration of an anti-VEGF drug into a subject in need thereof. It should be noted that administration of an anti-VEGF drug may comprise a single or multiple dosages, as well as a continuous administration, depending on the pathology to be treated and the subject receiving the treatment.

10 Non-limiting examples of anti-VEGF agents which can be used according to the method of the invention include an anti-VEGF antibody (e.g., the monoclonal antibody bevacizumab such as AVASTINTM; ranibizumab), an anti-VEGFA RNA silencing agents (e.g., antisense, siRNA, shRNA), an anti-VEGFA Ribozyme, an anti-VEGFA DNAzyme, a soluble form of the VEGF-receptor (e.g., GenBank Accession No. AAC50060; SEQ ID NO:16), a small molecule, thalidomide or an analogue thereof (e.g., as described in Miguel Fernández Braña et al., European Journal of Medicinal Chemistry, Volume 44, Issue 9, 2009, Pages 3533-3542; Magdy A.-H. Zahran et al., Bioorganic & Medicinal Chemistry, Volume 16, Issue 22, 15 November 2008, Pages 9708-971; all of which are incorporated by reference in their entirety), anti-VEGFA
15 aptamers (e.g., Pegaptanib, a pegylated anti-VEGF aptamer which specifically binds to VEGF 165; pegaptanib sodium), small molecules which are anti-VEGF-A tyrosine kinase inhibitors such as Sorafenib (Nexavar®, Bayer HealthCare Pharmaceuticals), ABT-869 (Linifanib; N-[4-(3-Amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea), Axitinib (also known as AG013736, a small molecule tyrosine
20 kinase inhibitor under development by Pfizer), BIBF1120 (oral potent triple angiokine inhibitor targeting VEGFR, PDGFR, FGFR kinases), Brivanib (BMS-582664), Brivanib alaninate, Cediranib (AZD2171), CHIR-258, E7080, Motesanib Diphosphate (AMG-706), Pazopanib Hydrochloride, Sunitinib Malate (Sutent), Vandetanib, Vatalanib, Dihydrochloride Salt, XL184, and/or XL880 (GSK1363089, EXEL-2880). Additional
25 anti-VEGFA agents, which can be used according to the method of the invention, include those described in Murukesh N., et al., 2010, which is fully incorporated herein in its entirety.

According to some embodiments of the invention, the anti-VEGFA comprises Sorafenib.

According to some embodiments of the invention, the anti-VEGFA comprises bevacizumab.

5 According to some embodiments of the invention, the anti-VEGFA comprises a soluble form of the VEGF-receptor.

According to some embodiments of the invention, the anti-VEGFA comprises thalidomide.

10 According to some embodiments of the invention, the anti-VEGFA comprises a combination of at least two-anti VEGFA drugs selected from the group consisting of Sorafenib, bevacizumab, a soluble form of the VEGF-receptor and thalidomide.

Non-limiting examples of such combination therapy with anti-VEGFA drugs comprises Sorafenib and bevacizumab; Sorafenib and a soluble form of the VEGF-receptor; Sorafenib and thalidomide; bevacizumab and a soluble form of the VEGF-receptor; and bevacizumab and thalidomide; a soluble form of the VEGF-receptor and thalidomide.

20 According to some embodiments of the invention, the anti-VEGFA antibody comprises an antigen binding region capable of specifically binding VEGFA. Preferably, the antigen binding region specifically binds at least one epitope of VEGFA. As used herein, the term "epitope" refers to any antigenic determinant on an antigen to which the paratope of an antibody binds.

Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or carbohydrate side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

25 The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof, such as Fab, F(ab')₂, and Fv that are capable of binding to macrophages. These functional antibody fragments are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab'

fragments are obtained per antibody molecule; (3) (Fab')₂, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds; (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988, incorporated herein by reference).

Antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647, and references contained therein, which patents are hereby incorporated by reference in their entirety. See also Porter, R. R. [Biochem. J. 73: 119-126 (1959)]. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Fv fragments comprise an association of VH and VL chains. This association may be noncovalent, as described in Inbar et al. [Proc. Nat'l Acad. Sci. USA 69:2659-62

(19720]. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise VH and VL chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the VH and VL domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by [Whitlow and Filpula, *Methods* 2: 97-105 (1991); Bird et al., *Science* 242:423-426 (1988); Pack et al., *Bio/Technology* 11:1271-77 (1993); and U.S. Pat. No. 4,946,778, which is hereby incorporated by reference in its entirety.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick and Fry [*Methods*, 2: 106-10 (1991)].

Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are

those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.*, 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introduction of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10,: 779-783 (1992); Lonberg et

al., Nature 368: 856-859 (1994); Morrison, Nature 368 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13, 65-93 (1995).

According to some embodiments of the invention the anti-VEGFA agent is an anti-VEGFA RNA silencing agent. As used herein, the phrase "RNA silencing" refers to a group of regulatory mechanisms [e.g. RNA interference (RNAi), transcriptional gene silencing (TGS), post-transcriptional gene silencing (PTGS), quelling, co-suppression, and translational repression] mediated by RNA molecules which result in the inhibition or "silencing" of the expression of a corresponding protein-coding gene. RNA silencing has been observed in many types of organisms, including plants, animals, and fungi.

As used herein, the term "RNA silencing agent" refers to an RNA which is capable of inhibiting or "silencing" the expression of a target gene (e.g., VEGFA). In certain embodiments, the RNA silencing agent is capable of preventing complete processing (e.g. the full translation and/or expression) of an mRNA molecule through a post-transcriptional silencing mechanism. RNA silencing agents include noncoding RNA molecules, for example RNA duplexes comprising paired strands, as well as precursor RNAs from which such small non-coding RNAs can be generated. Exemplary RNA silencing agents include dsRNAs such as siRNAs, miRNAs and shRNAs. In one embodiment, the RNA silencing agent is capable of inducing RNA interference. In another embodiment, the RNA silencing agent is capable of mediating translational repression.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla. Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and
5 comprise about 19 base pair duplexes. The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex.

10 Accordingly, the present invention contemplates use of dsRNA to downregulate protein expression from mRNA.

According to one embodiment, the dsRNA is greater than 30 bp. The use of long dsRNAs (i.e. dsRNA greater than 30 bp) has been very limited owing to the belief that these longer regions of double stranded RNA will result in the induction of the interferon
15 and PKR response. However, the use of long dsRNAs can provide numerous advantages in that the cell can select the optimal silencing sequence alleviating the need to test numerous siRNAs; long dsRNAs will allow for silencing libraries to have less complexity than would be necessary for siRNAs; and, perhaps most importantly, long dsRNA could prevent viral escape mutations when used as therapeutics.

20 Various studies demonstrate that long dsRNAs can be used to silence gene expression without inducing the stress response or causing significant off-target effects - see for example [Strat et al., Nucleic Acids Research, 2006, Vol. 34, No. 13 3803–3810; Bhargava A et al. Brain Res. Protoc. 2004;13:115–125; Diallo M., et al., Oligonucleotides. 2003;13:381–392; Paddison P.J., et al., Proc. Natl Acad. Sci. USA.
25 2002;99:1443–1448; Tran N., et al., FEBS Lett. 2004;573:127–134].

In particular, the present invention also contemplates introduction of long dsRNA (over 30 base transcripts) for gene silencing in cells where the interferon pathway is not activated (e.g. embryonic cells and oocytes) see for example Billy et al., PNAS 2001, Vol 98, pages 14428-14433. and Diallo et al, Oligonucleotides, October 1,
30 2003, 13(5): 381-392. doi:10.1089/154545703322617069.

The present invention also contemplates introduction of long dsRNA specifically designed not to induce the interferon and PKR pathways for down-regulating gene

expression. For example, Shinagwa and Ishii [*Genes & Dev.* 17 (11): 1340-1345, 2003] have developed a vector, named pDECAP, to express long double-strand RNA from an RNA polymerase II (Pol II) promoter. Because the transcripts from pDECAP lack both the 5'-cap structure and the 3'-poly(A) tail that facilitate ds-RNA export to the cytoplasm, long ds-RNA from pDECAP does not induce the interferon response.

Another method of evading the interferon and PKR pathways in mammalian systems is by introduction of small inhibitory RNAs (siRNAs) either via transfection or endogenous expression.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 18-30 basepairs) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned the RNA silencing agent of the present invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the

nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-UUCAAGAGA-3' (Brummelkamp, T. R. et al. (2002) Science 296: 550) and 5'-UUUGUGUAG-3' (Castanotto, D. et al. (2002) RNA 8:1454). It will be recognized
5 by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

According to another embodiment the RNA silencing agent may be a miRNA. miRNAs are small RNAs made from genes encoding primary transcripts of various
10 sizes. They have been identified in both animals and plants. The primary transcript (termed the "pri-miRNA") is processed through various nucleolytic steps to a shorter precursor miRNA, or "pre-miRNA." The pre-miRNA is present in a folded form so that the final (mature) miRNA is present in a duplex, the two strands being referred to as the miRNA (the strand that will eventually basepair with the target) The pre-miRNA is a
15 substrate for a form of dicer that removes the miRNA duplex from the precursor, after which, similarly to siRNAs, the duplex can be taken into the RISC complex. It has been demonstrated that miRNAs can be transgenically expressed and be effective through expression of a precursor form, rather than the entire primary form (Parizotto et al. (2004) Genes & Development 18:2237-2242 and Guo et al. (2005) Plant Cell 17:1376-
20 1386).

Unlike, siRNAs, miRNAs bind to transcript sequences with only partial complementarity (Zeng et al., 2002, Molec. Cell 9:1327-1333) and repress translation without affecting steady-state RNA levels (Lee et al., 1993, Cell 75:843-854; Wightman et al., 1993, Cell 75:855-862). Both miRNAs and siRNAs are processed by Dicer and
25 associate with components of the RNA-induced silencing complex (Hutvagner et al., 2001, Science 293:834-838; Grishok et al., 2001, Cell 106: 23-34; Ketting et al., 2001, Genes Dev. 15:2654-2659; Williams et al., 2002, Proc. Natl. Acad. Sci. USA 99:6889-6894; Hammond et al., 2001, Science 293:1146-1150; Moulatos et al., 2002, Genes Dev. 16:720-728). A recent report (Hutvagner et al., 2002, Sciencexpress 297:2056-
30 2060) hypothesizes that gene regulation through the miRNA pathway versus the siRNA pathway is determined solely by the degree of complementarity to the target transcript. It

is speculated that siRNAs with only partial identity to the mRNA target will function in translational repression, similar to an miRNA, rather than triggering RNA degradation.

Synthesis of RNA silencing agents suitable for use with the present invention can be effected as follows. First, the VEGFA mRNA sequence is scanned downstream of the
5 AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex
10 [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level (www.ambion.com/techlib/tn/91/912.html).

Second, potential target sites are compared to an appropriate genomic database
15 (e.g., human, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server (world wide web (dot) ncbi (dot) nlm (dot) nih (dot) gov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for siRNA synthesis.
20 Preferred sequences are those including low G/C content as these have proven to be more effective in mediating gene silencing as compared to those with G/C content higher than 55 %. Several target sites are preferably selected along the length of the target gene for evaluation. For better evaluation of the selected siRNAs, a negative control is preferably used in conjunction. Negative control siRNA preferably include
25 the same nucleotide composition as the siRNAs but lack significant homology to the genome. Thus, a scrambled nucleotide sequence of the siRNA is preferably used, provided it does not display any significant homology to any other gene.

It will be appreciated that the RNA silencing agent of the present invention need not be limited to those molecules containing only RNA, but further encompasses
30 chemically-modified nucleotides and non-nucleotides.

In some embodiments, the RNA silencing agent provided herein can be functionally associated with a cell-penetrating peptide." As used herein, a "cell-

penetrating peptide" is a peptide that comprises a short (about 12-30 residues) amino acid sequence or functional motif that confers the energy-independent (i.e., non-endocytotic) translocation properties associated with transport of the membrane-permeable complex across the plasma and/or nuclear membranes of a cell. The cell-
5 penetrating peptide used in the membrane-permeable complex of the present invention preferably comprises at least one non-functional cysteine residue, which is either free or derivatized to form a disulfide link with a double-stranded ribonucleic acid that has been modified for such linkage. Representative amino acid motifs conferring such properties are listed in U.S. Pat. No. 6,348,185, the contents of which are expressly incorporated
10 herein by reference. The cell-penetrating peptides of the present invention preferably include, but are not limited to, penetratin, transportan, pIsl, TAT(48-60), pVEC, MTS, and MAP.

mRNAs to be targeted using RNA silencing agents include, but are not limited to, those whose expression is correlated with an undesired phenotypic trait. Exemplary
15 mRNAs that may be targeted are those that encode truncated proteins i.e. comprise deletions. Accordingly the RNA silencing agent of the present invention may be targeted to a bridging region on either side of the deletion. Introduction of such RNA silencing agents into a cell would cause a down-regulation of the mutated protein while leaving the non-mutated protein unaffected.

20 Another anti-VEGFA agent is a DNAzyme molecule capable of specifically cleaving an mRNA transcript or DNA sequence of the VEGFA. DNAzymes are single-stranded polynucleotides which are capable of cleaving both single and double stranded target sequences (Breaker, R.R. and Joyce, G. Chemistry and Biology 1995;2:655; Santoro, S.W. & Joyce, G.F. Proc. Natl. Acad. Sci. USA 1997;94:4262) A general
25 model (the "10-23" model) for the DNAzyme has been proposed. "10-23" DNAzymes have a catalytic domain of 15 deoxyribonucleotides, flanked by two substrate-recognition domains of seven to nine deoxyribonucleotides each. This type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions (Santoro, S.W. & Joyce, G.F. Proc. Natl. Acad. Sci. USA 199; for rev of DNAzymes see
30 Khachigian, LM [Curr Opin Mol Ther 4:119-21 (2002)].

Examples of construction and amplification of synthetic, engineered DNAzymes recognizing single and double-stranded target cleavage sites have been disclosed in U.S.

Pat. No. 6,326,174 to Joyce et al. DNazymes of similar design directed against the human Urokinase receptor were recently observed to inhibit Urokinase receptor expression, and successfully inhibit colon cancer cell metastasis *in vivo* (Itoh et al, 2002, Abstract 409, Ann Meeting Am Soc Gen Ther world wide web(dot) asgt (dot) org). In
5 another application, DNazymes complementary to bcr-abl oncogenes were successful in inhibiting the oncogenes expression in leukemia cells, and lessening relapse rates in autologous bone marrow transplant in cases of CML and ALL.

Another anti-VEGFA agent is an antisense polynucleotide capable of specifically hybridizing with an mRNA transcript encoding the VEGFA.

10 Design of antisense molecules which can be used to efficiently downregulate a VEGFA must be effected while considering two aspects important to the antisense approach. The first aspect is delivery of the oligonucleotide into the cytoplasm of the appropriate cells, while the second aspect is design of an oligonucleotide which specifically binds the designated mRNA within cells in a way which inhibits translation
15 thereof.

The prior art teaches of a number of delivery strategies which can be used to efficiently deliver oligonucleotides into a wide variety of cell types [see, for example, Luft J Mol Med 76: 75-6 (1998); Kronenwett et al. Blood 91: 852-62 (1998); Rajur et al. Bioconjug Chem 8: 935-40 (1997); Lavigne et al. Biochem Biophys Res Commun 237:
20 566-71 (1997) and Aoki et al. (1997) Biochem Biophys Res Commun 231: 540-5 (1997)].

In addition, algorithms for identifying those sequences with the highest predicted binding affinity for their target mRNA based on a thermodynamic cycle that accounts for the energetics of structural alterations in both the target mRNA and the
25 oligonucleotide are also available [see, for example, Walton et al. Biotechnol Bioeng 65: 1-9 (1999)].

Such algorithms have been successfully used to implement an antisense approach in cells. For example, the algorithm developed by Walton et al. enabled scientists to successfully design antisense oligonucleotides for rabbit beta-globin (RBG) and mouse
30 tumor necrosis factor-alpha (TNF alpha) transcripts. The same research group has more recently reported that the antisense activity of rationally selected oligonucleotides against three model target mRNAs (human lactate dehydrogenase A and B and rat

gp130) in cell culture as evaluated by a kinetic PCR technique proved effective in almost all cases, including tests against three different targets in two cell types with phosphodiester and phosphorothioate oligonucleotide chemistries.

In addition, several approaches for designing and predicting efficiency of specific oligonucleotides using an in vitro system were also published (Matveeva et al., Nature Biotechnology 16: 1374 - 1375 (1998)).

Several clinical trials have demonstrated safety, feasibility and activity of antisense oligonucleotides. For example, antisense oligonucleotides suitable for the treatment of cancer have been successfully used [Holmund et al., Curr Opin Mol Ther 1:372-85 (1999)], while treatment of hematological malignancies via antisense oligonucleotides targeting c-myc gene, p53 and Bcl-2 had entered clinical trials and had been shown to be tolerated by patients [Gerwitz Curr Opin Mol Ther 1:297-306 (1999)].

More recently, antisense-mediated suppression of human heparanase gene expression has been reported to inhibit pleural dissemination of human cancer cells in a mouse model [Uno et al., Cancer Res 61:7855-60 (2001)].

Thus, the current consensus is that recent developments in the field of antisense technology which, as described above, have led to the generation of highly accurate antisense design algorithms and a wide variety of oligonucleotide delivery systems, enable an ordinarily skilled artisan to design and implement antisense approaches suitable for downregulating expression of known sequences without having to resort to undue trial and error experimentation.

Another anti-VEGFA agent is a ribozyme molecule capable of specifically cleaving an mRNA transcript encoding a VEGFA. Ribozymes are being increasingly used for the sequence-specific inhibition of gene expression by the cleavage of mRNAs encoding proteins of interest [Welch et al., Curr Opin Biotechnol. 9:486-96 (1998)]. The possibility of designing ribozymes to cleave any specific target RNA has rendered them valuable tools in both basic research and therapeutic applications. In the therapeutics area, ribozymes have been exploited to target viral RNAs in infectious diseases, dominant oncogenes in cancers and specific somatic mutations in genetic disorders [Welch et al., Clin Diagn Virol. 10:163-71 (1998)]. Most notably, several ribozyme gene therapy protocols for HIV patients are already in Phase 1 trials. More recently, ribozymes have been used for transgenic animal research, gene target

validation and pathway elucidation. Several ribozymes are in various stages of clinical trials. ANGIOZYME was the first chemically synthesized ribozyme to be studied in human clinical trials. ANGIOZYME specifically inhibits formation of the VEGF-r (Vascular Endothelial Growth Factor receptor), a key component in the angiogenesis pathway. Ribozyme Pharmaceuticals, Inc., as well as other firms have demonstrated the importance of anti-angiogenesis therapeutics in animal models. HEPTAZYME, a ribozyme designed to selectively destroy Hepatitis C Virus (HCV) RNA, was found effective in decreasing Hepatitis C viral RNA in cell culture assays (Ribozyme Pharmaceuticals, Incorporated - WEB home page).

Qualifying agents which can reduce, inhibit or suppress the expression level and/or activity of VEGF can be performed using various *in vitro* (e.g., using a biological sample of the subject), *ex vivo* (e.g., in cells of a subject which are treated by the agent and are then injected into an animal model) or *in vivo* (e.g., by testing the expression level and/or activity of VEGFA in cells of a subject following treatment of the subject with the anti-VEGFA agent) methods.

Each of the anti-VEGFA agents described hereinabove or the expression vector encoding same can be administered to the individual per se or as part of a pharmaceutical composition which also includes a physiologically acceptable carrier. The purpose of a pharmaceutical composition is to facilitate administration of the active ingredient to an organism.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the anti-VEGFA agent accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of the present invention may be manufactured by
5 processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically
10 acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such
15 as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers
20 well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain
25 tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired,
30 disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of

the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (anti-VEGFA agent) effective to prevent, alleviate or ameliorate symptoms of the cancer (e.g., carcinoma) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can

be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide tissue levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

According to some embodiments of the invention, the method further comprising informing the subject on the results of the prediction of anti-VEGFA treatment efficacy test. For example, informing the subject that based on the presence of the genomic

amplification (which comprises a VEGFA gene) in the solid tumor the anti-VEGFA treatment is predicted to be efficient in treating the cancer. On the other hand, if the solid tumor is devoid of the genomic amplification (which comprises a VEGFA gene), then informing the subject that, based on the absence of the genomic amplification, the anti-VEGFA treatment is likely not to be efficient for treating the cancer, and that alternative therapeutic approaches should be explored.

According to an aspect of some embodiments of the invention there is provided a method of treating a subject diagnosed with a solid tumor, the method comprising: (a) predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor according to the method of some embodiments of the invention, and (b) selecting a treatment regimen based on the prediction; thereby treating of the subject diagnosed with the solid tumor.

The term “treating” refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or condition, e.g., the cancer, e.g., the carcinoma) and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

As used herein, the term “subject” includes mammals, preferably human beings at any age which suffer from the pathology.

According to an aspect of some embodiments of the present invention there is provided a method of selecting or designing a treatment regimen for treating a subject diagnosed with a solid tumor, the method comprising: (a) predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor according to the method of some embodiments of the invention, and (b) selecting a treatment regimen based on the prediction; thereby selecting or designing the treatment regimen for treating the subject diagnosed with a solid tumor.

As used herein the phrase “treatment regimen” refers to a treatment plan that specifies the type of treatment, dosage, schedule and/or duration of a treatment provided to a subject in need thereof (e.g., a subject diagnosed with a pathology). The selected treatment regimen can be an aggressive one which is expected to result in the best clinical outcome (e.g., complete cure of the pathology) or a more moderate one which

may relief symptoms of the pathology yet results in incomplete cure of the pathology. It will be appreciated that in certain cases the more aggressive treatment regimen may be associated with some discomfort to the subject or adverse side effects (e.g., a damage to healthy cells or tissue). The type of treatment can include the anti-VEGFA agent of the invention alone or in combination with other chemotherapeutic drugs, a surgical intervention (e.g., removal of lesion, diseased cells, tissue, or organ), a cell replacement therapy, an administration of a therapeutic drug (e.g., receptor agonists, antagonists, hormones, chemotherapy agents) in a local or a systemic mode, an exposure to radiation therapy using an external source (e.g., external beam) and/or an internal source (e.g., brachytherapy) and/or any combination thereof. The dosage, schedule and duration of treatment can vary, depending on the severity of pathology and the selected type of treatment, and those of skills in the art are capable of adjusting the type of treatment with the dosage, schedule and duration of treatment.

Non-limiting examples of chemotherapy agents which can be administered in combination with the anti-VEGFA agent of some embodiments of the invention include Mechlorethamine, (HN 2), Cyclophosphamide, Ifosfamide, Melphalan, Chlorambucil, Estramustine, Hexamethyl-melamine, Thiotepa, Busulfan, Carmustine, Lomustine, Semustine, Streptozocin, Dacarbazine, Procarbazine, Aziridine, Methotrexate, Trimetrexate, Fluorouracil, Floxuridine, Cytarabine, Azacitidine, Mercaptopurine, Thioguanine, Pentostatin, Fludarabine, Vinblastine (VLB), Vincristine, Vindesine, Etoposide, Teniposide, Dactinomycin, Daunorubicin, Doxorubicin, 4'-Deoxydoxorubicin, Bleomycin, Plicamycin, Mitomycin, L-Asparaginase, Docetaxel, Paclitaxel, Interferon Alfa, Tumor Necrosis Factor, Cisplatin, Carboplatin, Mitoxantrone, Hydroxyurea, Procarbazine, Mitotane, Aminoglutethimide, Hydroxyprogesterone, caproate, Medroxy-progesterone, acetate, Megestrol acetate, Diethylstilbestrol, Ethinyl estradiol, Tamoxifen, Testosterone, propionate, Fluoxymesterone, Flutamide, Leuprolide, Goserelin.

Non-limiting examples of approved oncology drugs which can be administered in combination with the anti-VEGFA agent of some embodiments of the invention include Aldesleukin, Alemtuzumab, alitretinoin, allopurinol, altretamine, amifostine, anastrozole, arsenic trioxide, Asparaginase, BCG Live, bexarotene capsules, bexarotene gel, bleomycin, busulfan intravenous, busulfan oral, calusterone, capecitabine,

carboplatin, carmustine, carmustine with Polifeprosan 20 Implant, celecoxib, chlorambucil, cisplatin, cladribine, cyclophosphamide, cyclophosphamide, cytarabine, cytarabine liposomal, dacarbazine, dactinomycin, actinomycin D, dactinomycin, actinomycin D, Darbepoetin alfa, Darbepoetin alfa, daunorubicin liposomal, daunorubicin, daunomycin, Denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, doxorubicin liposomal, DROMOSTANOLONE PROPIONATE, Elliott's B Solution, epirubicin, Epoetin alfa, estramustine, etoposide phosphate, etoposide VP-16, exemestane, Filgrastim, floxuridine (intraarterial), fludarabine, fluorouracil, 5-FU, fulvestrant, gemcitabine, gemtuzumab ozogamicin, goserelin acetate, hydroxyurea, Ibritumomab Tiuxetan, idarubicin, ifosfamide, imatinib mesylate, imatinib mesylate, Interferon alfa-2a, Interferon alfa-2b, irinotecan, letrozole, leucovorin, levamisole, lomustine, CCNU, mecllorethamine, nitrogen mustard, megestrol acetate, melphalan, L-PAM, mercaptopurine, 6-MP, mesna, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone phenpropionate, Nofetumomab, Oprelvekin, oxaliplatin, paclitaxel, pamidronate, pegademase, Pegaspargase, Pegfilgrastim, pentostatin, pipobroman, plicamycin, mithramycin, porfimer sodium, porfimer sodium, procarbazine, quinacrine, Rasburicase, Rituximab, Sargramostim, streptozocin, talc, tamoxifen, temozolomide, teniposide, VM-26, testolactone, thioguanine, 6-TG, thiotepa, topotecan, toremifene, Tositumomab, Trastuzumab, tretinoin, ATRA, Uracil Mustard, valrubicin, vinblastine, vincristine, vinorelbine, and zoledronate.

According to some embodiments of the invention the treatment regimen further comprises administering an anti-VEGFA agent in combination with a drug selected from the group consisting of erlotinib, oxaliplatin, cisplatin, platinum, rIFNa-2b, doxorubicin, fluorouracil, DX-8951f, thalidomide, doxorubicin, epirubicin, and taxol.

According to some embodiments of the invention the treatment regimen further comprises administering an anti-VEGFA agent in combination with a treatment selected from the group consisting of percutaneous ethanol injection, radiofrequency ablation, transcatheter arterial chemoembolization.

As used herein the term "about" refers to $\pm 10\%$.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination

in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various
5 embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

10

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized
15 in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons,
20 Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659
25 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are
30 extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219;

5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

GENERAL MATERIALS AND EXPERIMENTAL METHODS

Mice - Mdr2^{-/-} mice on FVB background were held in specific pathogen free conditions. Wild-type (WT) control mice were aged matched FVB mice. Two hours prior to sacrifice, mice were injected with 100 µl BrdU (Cell Proliferation labeling reagent, Amersham; Catalogue number: RPN201) per 10 gram body weight. Mice were anesthetized using Ketamine and Xylazine and were perfused via the heart with PBS-Heparin solution followed by perfusion with 4% formaldehyde. All animal experiments were performed in accordance with the guidelines of the institutional committee for the use of animals for research.

Adenoviral vectors and Sorafenib - Adenoviral vectors encoding green fluorescence protein (GFP) or GFP and sFLT (Soluble fms-like tyrosine kinase-1) were prepared in GH354 cells using standard procedures. A titer of 10⁹ transducing units was injected to mice tail veins. Sorafenib (Xingcheng Chempharm Co., Ltd Taizhou, China) was administered daily (50 mg/kg) by oral gavage. Cremophor EL/ethanol/water; (12.5:12.5:75) was used as vehicle solution.

Immunohistochemistry (IHC) and ELISA - Antibodies used for IHC were vWF (DAKO Corp, Carpinteria, CA, USA, Catalogue number A0082), Cleaved Caspase 3 [Cell Signaling, USA, Catalogue number: 9661 (Rabbit polyclonal)], F4/80 (Serotec Raleigh, NC, USA; Catalogue number MCA497GA), HGF (R&D Systems Inc,

Minneapolis, MN, USA; Catalogue number: AF2207), BrdU (NeoMarkers, Thermo Scientific, Fremont, USA, BrdU Ab-3, Catalogue number: MS-1058-P), Ki67 (NeoMarkers, Thermo Scientific, Fremont, USA, Catalogue number: RM-9106), pHH3 (phospho-Histone H2A.X, MILLIPORE, Catalogue number: 05-636). IHC was performed on 5 µm paraffin sections. Antigen retrieval was performed in a Decloaking ChamberTM (Biocare Medical, Concord, CA, USA) in Citrate buffer for all antibodies except vWF and F4/80 for which retrieval was performed with Pronase (Sigma, St Louis, MO, USA, Catalogue number: P8038). VEGF-A ELISA was performed using Quantikine® mouse ELISA kit (R&D Systems Inc, Minneapolis, MN, USA). Secondary antibodies for all antibodies used were Histofine® (Nichirei Biosciences, Chuo-ku, Tokyo 104-8402 Japan), except for mouse derived antibodies that were detected with EnvisionTM (Corp, Carpinteria, CA, USA).

In-Situ hybridization - Probes for CISH analysis were prepared from the BAC clones RP24-215A3 for Chromosome 17 and RP23-174D11 for the pericentromeric region (BACPAC resources center). BAC clones were labeled with Digoxigenin (DIG) using Nick-Translation mix (Roche, Indianapolis, IN, USA, Catalogue No. 11745808910). Mouse Cot-1 DNA (Gibco-Invitrogen Corporation products, Grand Island, NY, USA) and sonicated murine genomic DNA were added to the probe for background block. Tissues were prepared by boiling in pretreatment buffer and digestion with Pepsin (Zymed® Catalog Number - 00-3009). Hybridization was performed overnight in 37°C after 5 minutes of denaturation in 95° C. The Spot-Light® detection kit (Invitrogen) was used for anti DIG antibody and secondary antibody.

qPCR and array-based Comparative Genomic Hybridization (aCGH):

aCGH - Genomic DNA was isolated using the QIAGEN DNAeasy Tissue kit. Samples were hybridized to mouse CGH 60-mer oligonucleotides microarrays (Agilent Technologies, Santa Clara, CA, United States), washed and scanned according to Agilent Technologies instructions.

RNA qPCR - RNA was extracted from tissues by mechanical grinding in TriReagent® (Sigma, St Louis, MO, USA) with a Polytron tissue homogenizer (Kinematica, Bohemia, NY, USA). cDNA was prepared with MMLV reverse transcriptase (Invitrogen by Life Technologies). RNA qPCR analyses were carried out with SYBR® Green qPCR Detection (Invitrogen by Life Technologies) in 7900HT Fast

Real-Time PCR System (Applied BioSystems). Results were analyzed using the qBase v1.3.5 software. Primer sequences are shown in Table 4 below. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) and PPIA [peptidylprolyl isomerase A (cyclophilin A)] together were used as reference genes in all analyses.

- 5 **DNA Real-Time PCR (qPCR) Method** - Genomic DNA was isolated using the QIAGEN DNAeasy Tissue kit. Primers sets were designed using the Oligo Design and Analysis Tools provided by Integrated DNA Technologies (IDT). qPCR analyses were carried out with SYBR green (Invitrogen) in 7900HT Fast Real-Time PCR System (Applied BioSystems), and the results were analyzed using the qBase v1.3.5 software.
- 10 Primer sequences are shown in Table 4 below.

Table 4: Primers for quantitative PCR

<i>Gene</i>	<i>Forward primer</i>	<i>SEQ ID NO:</i>	<i>Reverse primer</i>	<i>SEQ ID NO:</i>
Exportin 5	AGATCCTGGAGCATGT TGTC AAG	434	CCAATCGGGACATGCTGTT C	435
Glut1	TGCTGTGCTCATGACCA TC	436	GACCAGGGCCTACTTCAA AG	437
HGF	CCTCACAAGCATGATA TCACTC	438	ACTCGGATGTTTGGGTCAG	439
HPRT	GTTAAGCAGTACAGCC CCAAA	440	AGGGCATATCCAACAACA AACTT	441
Parc	GCAACAAGAACAACACTGA GACCTC	442	GACTCATGGTACGGCCTGC	443
PGK1	TTGATGAAGAAGGAGC TGA ACTAC	444	GCTGGATCTTGTCTGCAAC TTTAG	445
PPIA	CGCGTCTCCTTCGAGCT GTTTG	446	TGTAAAGTCACCACCCTGG CACAT	447
UBC	CAGCCGTATATCTTCCC AGACT	448	CTCAGAGGGATGCCAGTA ATCTA	449
VEGF-A	GGACGTCTACCAGCGA AGCT	450	CACAGGACGGCTTGAAGA TGT	451
VEGF-A 3' UTR (for qDNA PCR)	TGAAAGGCTTCAGTGT GGTCTG	452	GGGTTTGTCGTGTTTCTGG AAG	453
VEGF-A promoter (for qDNA PCR)	CCCTATCCTCATACGTC CCTG	454	GAGTGAGGTTATATGCGG ACAG	455
Supt3h	CCGTGCCTCCACGTGTC TTA	1673	GTCTTCCCTGCATTTCTTCC AC	1674

<i>Gene</i>	<i>Forward primer</i>	<i>SEQ ID NO:</i>	<i>Reverse primer</i>	<i>SEQ ID NO:</i>
Clic5	CCAAGCTGCATGTGGT CAAG	1675	CTGCACAGGTGTTGGTGAA CTC	1676
Aars2	CGACCTCTTCTCTCCAC TGCTC	1677	TGTCTATCCGTCCCTCATC TGC	1678
Nfkbie	ACTCGAGGCGCTCACA TACATC	1679	CAGGAAAGCCAAGCAACA GAAT	1680
Tmem63b	ACCGGTATGAGCGTCT CACCTC	1681	GCTGTCAGCCAGGAACAG AAG	1682
Mrpl14	CGGGAGATTCCCAGGC TGTTA	1683	CTGGCTGAATGCTCTGCTG AC	1684
Capn11	TGCTAAGGCTCCTCAA GAAAGC	1685	CTCGCACCAGCATGTTATG TGT	1686
Mrps18a	ATTGAAGGCCGAATCA CAGAAA	1687	ATAAGGCCGGATGAACTG ACTG	1688
Mrps18a	ATTGAAGGCCGAATCA CAGAAA	1689	ATAAGGCCGGATGAACTG ACTG	1690
Gnmt	CCCACATGGTAACCCT GGACTA	1691	AGACGCCAAACAGTGTGG GTAG	1692
EGF19	CCT CAC AAT CAC CCT GCC	1693	CTG CTC ACA AGG TCC AGC	1694
Runx2	CCAACCCACGAATGCA CTATC	1695	TAGTGAGTGGTGGCGGAC ATAC	1696

Table 4. Provided are the primer sequences 5'→3' (with sequence identifiers) which were used in the qPCR analysis. Unless indicated otherwise, the provided primers were used for qRNA-PCR. The primers from VEGFA-3'-UTR and VEGFA-promoter regions were used for DNA qPCR.

5

IHC quantification and statistics - Immunohistochemical stainings were quantitated when indicated using an Ariol SL-50 system (Applied Imaging, Grand Rapids, MI, USA). Data was analyzed using a paired two tails Student's T-Test at $p < 0.05$. Histological differences were analyzed using Pearson's χ^2 test at $p < 0.05$. Data was processed using Microsoft Excel 2007. Graphs were generated using either GraphPad Prism 5.0 or excel softwares.

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EXAMPLE 1

CHARACTERIZATION OF A GENOMIC AMPLIFICATION WHICH INCLUDES THE VEGF-A GENE

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Experimental Results

Identification of a chromosomal gain in the genomic region encompassing the VEGF-A gene - In search of new candidate targets for tailored therapy, the present

inventors have applied both array Comparative Genomic Hybridization (aCGH) and cDNA expression array to samples produced from ten HCCs obtained from 16 months old *Mdr2*^{-/-} mice. The aCGH revealed several recurring genomic amplifications in the qB3 band of murine chromosome 17 (Chr17qB3, data not shown) encoding among others the VEGF-A (GeneID: 7422), Mrps18a (human GeneID: 55168), Parc (CCL18, human GeneID: 6362) and Exportin 5 (XPO5, human GeneID:57510) (Figure 1).

The incidence of the genomic amplification on murine chromosome Chr17qB3 is about 14% in Mdr2^{-/-} tumors - Out of the 93 successfully tested HCC tumors, 13 were found to bear this amplification (13.97%). To determine the rate of this amplification, the present inventors tested a larger cohort of *Mdr2*^{-/-} tumors by quantitative PCR (qPCR) of tumor DNA (Figure 7) and chromogenic *in situ* hybridization (CISH, Figures 2A-H).

Mapping of the minimal genomic region on murine chromosome 17qB3 which is amplified in the HCC tumors - To map the minimally amplified region of this amplicon, DNA qPCR of several loci along the murine chromosome 17 was performed in a cohort of tumors bearing this amplification (Figure 2I). The present inventors found that the common proximal border lies between 43.3 and 43.8 mega base pairs (mbp) from the chromosome start and the common distal border lies between 46.9 and 48.5 mbp. This region encodes 63 genes as listed in Table 5, hereinbelow.

Table 5
Genes found in the genomic amplification on murine chromosome 17 (17qB3)

	<i>Gene</i>	<i>Start</i>	<i>End</i>	<i>Description</i>
1	Clic5	44325521	44417117	chloride intracellular channel 5
2	Runx2	44740950	44951746	runt related transcription factor 2
3	4930564C03Rik	44325521	45505103	RIKEN cDNA 4930564C03 gene
4	Supt3h	44914120	45256233	suppressor of Ty 3 homolog (S. cerevisiae)
5	EG210562	44740950	45570656	predicted gene, EG210562
6	EG668314	44914120	45611887	predicted gene, EG668314
7	Cdc5l	45528836	45570656	cell division cycle 5-like (S. pombe)
8	Spats1	45585886	45611887	spermatogenesis associated, serine-rich 1
9	EG668319	45459310	45686626	predicted gene, EG668319
10	Aars2	45643790	45657792	alanyl-tRNA synthetase 2, mitochondrial (putative)

	<i>Gene</i>	<i>Start</i>	<i>End</i>	<i>Description</i>
11	Tcte1	45660383	45679628	t-complex-associated testis expressed 1
12	Gm323	45585886	45704618	gene model 323, (NCBI)
13	Nfkbie	45692665	45700118	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
14	Slc35b2	45701101	45704618	solute carrier family 35, member B2
15	Hsp90ab1	45704727	45710210	heat shock protein 90kDa alpha (cytosolic), class B member 1
16	Slc29a1	45722171	45729342	solute carrier family 29 (nucleoside transporters), member 1
17	EG653016	45704727	45823167	predicted gene, EG653016
18	Capn11	45767153	45796258	calpain 11
19	Tmem63b	45797126	45823167	transmembrane protein 63b
20	Mrpl14	45823321	45835444	mitochondrial ribosomal protein L14
21	LOC100043363	45868077	46231621	hypothetical protein LOC100043363
22	Vegfa	46153942	46169326	vascular endothelial growth factor A
23	Mrps18a	46247953	46265857	mitochondrial ribosomal protein S18A
24	1700027N10Rik	46231448	46306319	RIKEN cDNA 1700027N10 gene
25	Mad2l1bp	46284334	46290500	MAD2L1 binding protein
26	Gtpbp2	46297981	46306319	GTP binding protein 2
27	Polh	46308942	46339574	polymerase (DNA directed), eta (RAD 30 related)
28	Xpo5	46339804	46379248	exportin 5
29	Rpo1-1	46380869	46384994	RNA polymerase 1-1
30	Yipf3	46385029	46389486	Yip1 domain family, member 3
31	Gm88	46391114	46394265	gene model 88, (NCBI)
32	Tjap1	46394825	46419962	tight junction associated protein 1
33	Egfl9	46434370	46440220	EGF-like-domain, multiple 9
34	Abcc10	46440179	46464972	ATP-binding cassette, sub-family C (CFTR/MRP), member 10
35	BC040756	46481389	46494098	cDNA sequence BC040756
36	Zfp318	46520715	46557869	zinc finger protein 318
37	Crip3	46565891	46568720	cysteine-rich protein 3
38	EG328825	46565891	46636567	predicted gene, EG328825
39	Ttbk1	46579397	46624624	tau tubulin kinase 1
40	BC048355	46633738	46636567	cDNA sequence BC048355
41	Parc	46637554	46683337	p53-associated parkin-like cytoplasmic protein
42	Srf	46683788	46693111	serum response factor
43	Ptk7	46701400	46766453	PTK7 protein tyrosine kinase 7
44	Klc4	46767580	46782093	kinesin light chain 4

	<i>Gene</i>	<i>Start</i>	<i>End</i>	<i>Description</i>
45	Mrpl2	46783197	46787081	mitochondrial ribosomal protein L2
46	Cul7	46787287	46801313	cullin 7
47	BC011248	46804391	46811204	cDNA sequence BC011248
48	Klhd3	46811499	46817879	kelch domain containing 3
49	Meal	46818086	46820054	male enhanced antigen 1
50	Ppp2r5d	46819940	46841951	protein phosphatase 2, regulatory subunit B (B56), delta isoform
51	Pex6	46848412	46862490	peroxisomal biogenesis factor 6
52	Gnmt	46862613	46866114	glycine N-methyltransferase
53	Tnrc5	46872662	46889161	trinucleotide repeat containing 5
54	Ptra	46892612	46900661	pre T-cell antigen receptor alpha
55	2310039H08Rik	46872662	46968362	RIKEN cDNA 2310039H08 gene
56	Rpl7l1	46910856	46919605	ribosomal protein L7-like 1
57	BC032203	46909584	47029412	cDNA sequence BC032203
58	A330017A19Rik	46910856	47061875	RIKEN cDNA A330017A19 gene
59	Tbcc	47027570	47029412	tubulin-specific chaperone c
60	Prph2	47047434	47061875	peripherin 2
61	Ubr2	47065240	47147481	ubiquitin protein ligase E3 component n-recognin 2
62	Gm16494	47147397	47153905	similar to high mobility group nucleosomal binding domain 2
63	EG240110	47047434	47515628	predicted gene, EG240110

Table 5. Provided is a list of 63 genes found in the genomic amplification on murine Chr17qB3 (which is the syntenic region of chromosome 6p21 of the human) along with the full gene names and the location of their genomic sequences on murine chromosome 17. The start and end numbers relate to nucleotide position on mouse chromosome 17 according to accepted chromosomal nucleotide positions.

The genomic amplification matched increases in the mRNA levels of VEGF-A, Exportin 5 and Parc - These results were cross compared with the mRNA analysis looking for concomitant amplification and mRNA overexpression. The mRNA levels of several of these genes was analyzed in a set of Chr17qB3 amplicon positive (herein denoted Amp^{pos}) and negative (Amp^{neg}) by qPCR. This analysis revealed amplification matched increases in the mRNA levels of several genes such as VEGF-A, Exportin 5, Parc, Aars2, Mrpl14, Tmem63b, Mrps18a, and Tjap1 [Figures 2J-O and Figures 10A-K]. Some genes were not differentially elevated (e.g. Calpain 11 and IkBe) while others were elevated only in some of the Amp^{pos} tumors regardless of the copy number (e.g. HSP90ab1 and EGFL9). Thus, the amplification of the murine genomic region Chr17qB3 is a recurrent event in HCC related with a unique expression signature of several of its residing genes.

EXAMPLE 2**AMPLIFICATION OF VEGF-A CORRELATES WITH A SPECIFIC VEGF-A
RELATED TUMOR CHARACTERISTICS*****Experimental Results***

5 The present inventors checked whether the genomic amplification which includes the VEGF-A gene is necessary in order to elevate VEGF-A protein levels. A tight correlation was found between the changes in VEGF-A mRNA and protein levels in Mdr2^{-/-} derived tumors (Figure 3), suggesting that in these tumors, VEGF-A protein levels are dictated by mRNA change. Concordantly, the present inventors also found a
10 6-fold higher vessel density in Amp^{pos} compared to Amp^{neg} HCCs as measured by immunostaining for the endothelial marker vWF (Figure 4A-B and 4G).

The Amp^{pos} tumor group as a subgroup of HCCs characterized by specific histological changes and an increased rate of proliferation - Analysis of hematoxylin and eosin (H&E) stained sections of Amp^{pos} and Amp^{neg} HCCs revealed that the murine
15 Amp^{pos} tumors are characterized by larger tumor cells, and extensive steatosis (Figures 11A-E). BrdU immunostaining revealed that Amp^{pos} HCCs displayed a 2-fold increase in tumor cell proliferation (Figures 4C-D and 4H). Of note, proliferation is one of the strongest and most consistent markers of poor prognosis in human HCC (Ouchi, K., *et al.* Mitotic index is the best predictive factor for survival of patients with resected
20 hepatocellular carcinoma. *Dig Surg* 17, 42-48, 2000). Cleaved Caspase 3 staining revealed no difference in the rate of apoptosis (data not shown). Altogether, these data signify the Amp^{pos} tumor group as a subgroup of HCCs characterized by specific histological changes and an increased rate of proliferation.

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EXAMPLE 3**AMPLIFICATION OF VEGF-A INDUCES A UNIQUE TUMOR
MICROENVIRONMENT VIA A HEPATOCYTE-MACROPHAGE CROSSTALK*****Experimental Results***

The Amp^{pos} HCC tumors have a distinct microenvironment content with enhanced numbers of macrophages – VEGF-A was shown to exert its proangiogenic
30 effects by attracting naïve myeloid cells that in turn modulate the microenvironment (Grunewald, M., *et al.* VEGF-induced adult neovascularization: recruitment, retention,

and role of accessory cells. *Cell* 124, 175-189, 2006). Immunostaining for the macrophage marker F4/80 showed a 4-fold higher macrophage content in Amp^{pos} compared with Amp^{neg} HCCs (Figures 4E-F and 4I) while no differences in the neutrophil content between tumor groups were detected (data not shown). Furthermore, levels of several known markers of tumor associated macrophages including Arginase 1, TGFβ And Ym1 were elevated in the Amp^{pos} group while markers of classically activated macrophages, including TNFα, iNOS and CXCL10 were not changed (data not shown). Other markers of these macrophage subsets such as TNFα, INOS, CXCL10, Arginase 1, TGFβ and Ym1 did not show any significant differences (data not shown). Thus, among the Mdr2^{-/-} HCCs tumors, those harboring the genomic amplification (Amp^{pos}) have a distinct microenvironment content with enhanced numbers of both macrophages and endothelial cells.

The Amp^{pos} HCC tumors express higher levels of hepatocyte growth factor (HGF) as compared to Amp^{neg} HCC tumors - The present inventors have detected a nearly 3-fold elevation of HGF mRNA levels in Amp^{pos} versus Amp^{neg} HCCs (Figures 5N and 6A). Furthermore, immunostaining for HGF showed HGF expression in the Amp^{pos} tumors alone (Figures 6B-C). Interestingly, HGF staining in these tumors was restricted to non-neoplastic intratumoral cells (Figure 6C).

EXAMPLE 4

INHIBITION OF VEGF-A ATTENUATES PROLIFERATION SELECTIVELY IN AMP^{POS} TUMORS

Experimental Results

The VEGF-A inhibitor, soluble FLT receptor (sFLT), inhibits proliferation of hepatocytes in Amp^{pos} HCC tumors - To test the functional role of VEGF in Amp^{pos} and Amp^{neg} tumors, the present inventors injected adenoviral vectors encoding GFP alone or GFP and a soluble VEGF-A receptor 1 (sFLT), a well known potent inhibitor of VEGF-A (Mahasreshti, P.J., *et al.* 2001. Adenovirus-mediated soluble FLT-1 gene therapy for ovarian carcinoma. *Clin Cancer Res* 7, 2057-2066). Mice were sacrificed at ten days post injection and tumors were collected and examined. VEGF-A amplification status was determined after sacrifice by both DNA qPCR (data not shown) and VEGF-A mRNA expression (Figure 5M). The efficiency of adenovirus transduction was

assessed by immunostaining for GFP (data not shown). Immunostaining for markers of proliferation such as Ki67, BrdU and phospho-histone 3 (pHH3) revealed that blocking VEGF markedly inhibited tumor cell proliferation in Amp^{pos} tumors, yet did not affect proliferation in Amp^{neg} tumors (Figures 5A-L). The decrease in proliferation was
5 accompanied with a nearly 3 fold reduction in HGF mRNA levels in sFLT treated tumors (Figure 5N). Notably, HGF levels in Amp^{neg} tumors were unaffected by VEGF blockade (Figures 5N), suggesting that the increase in HGF levels in the Amp^{pos} tumors can be attributed to VEGF-A, expression of which is driven by the amplicon. This effect was irrelevant of tumor size as the distribution of tumor diameter was similar between
10 all four groups (data not shown). Treatment with adenovirus encoding GFP alone did not induce any change in either of the groups. Other Amp^{pos} associated traits like macrophage infiltration and steatosis were not affected significantly by VEGF-A inhibition (FIGs 12A-J and data not shown).

The anti-VEGF-A treatment resulted in a specific increase in the HIF1 α target genes Glut1 and PGK1 - Macroscopic inspection and histological analysis of
15 H&E stained sections revealed multiple foci of coagulative necrosis in three out of the six sFLT treated Amp^{pos} tumors (Figures 5Q-R). In these three specific tumors, the present inventors also found an elevation of the HIF1 α target genes Glut1 and PGK1 (Figures 5O-P), indicative of tissue hypoxia. No evidence of necrosis was detected in
20 any of the other treatment groups (data not shown). Immunostaining for vWF revealed a trend (yet, statistically insignificant) towards decrease in vasculature only in the VEGF-A blocked Amp^{pos} tumors, mainly in the hypoxic tumors (Figures 8A-D, Figure 12A-D, 12I), possibly explaining the induction of hypoxia in these tumors. Treatment with GFP alone did not induce any change in either of the groups. In addition, macrophage
25 infiltration was not affected after 10 days of VEGF-A inhibition (Figure 12 E-H, 12J). Thus, 10 days inhibition of VEGF-A does not alter significantly the microenvironmental content.

EXAMPLE 5**PROFILING OF PROANGIOGENIC FACTORS EXPRESSION IN MDR2^{-/-} HCC****Experimental Results**

In addition to VEGF-A, Angiopoietin 1 & 2, FGF 1 & 2, PDGF A, B & C, PLGF and VEGF-B are known to regulate angiogenesis. Analysis of their mRNA levels revealed that several of these factors are overexpressed in HCCs compared with normal livers, irrespective of amplicon status (data not shown). Notably, PDGF-C levels are significantly higher (2.4 fold) in Amp^{neg} compared with Amp^{pos} tumors (data not shown). This stands in line with previous reports showing that PDGF-C can promote angiogenesis in a VEGF-A independent manner (Crawford, Y., *et al.* PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. *Cancer Cell* 15, 21-34, 2009) and could explain the lack of decrease in vessel density in the Amp^{neg} tumors in response to VEGF-A inhibition. Furthermore, the levels of Angiopoietin 2, a known inhibitor of vessel maturation along with VEGF-A, were higher in the Amp^{pos} tumors accounting for the decrease in Amp^{pos} tumors vascularity upon treatment (data not shown).

EXAMPLE 6**AMP^{POS} TUMORS ARE UNIQUELY SENSITIVE TO SORAFENIB****Experimental Results**

The present inventors have identified a sub-group of liver tumors that bears a specific genomic amplification and is distinguished by several traits from other tumors. This tumor sub population is extremely sensitive to treatment with an anti VEGF-A agent (sFLT). As this amplification was previously found in aCGH analyses performed on human HCCs, the present inventors suggest that detection of this amplification by either *in situ* hybridization or qPCR in biopsies taken from HCC patients could serve as a prognostic marker that would predict the tumor responsiveness to anti-VEGF-A treatments such as Bevacizumab, soluble VEGF-receptor or other antibody driven or pharmacological inhibitors of this pathway.

The multi-tyrosine kinase receptor inhibitor Sorafenib is the current preferred treatment for patients with unresectable HCC (Llovet, J.M., *et al.* Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 359, 378-390, 2008). Sorafenib inhibits the

VEGF receptors and B-Raf, a downstream effector of both the VEGF receptors and c-Met (Wilhelm, S.M., et al. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 7, 3129-3140, 2008). The present inventors tested whether Sorafenib could have a selective advantage towards Amp^{pos} tumors, as follows.

Mdr2^{-/-} mice (age 1+ year) were treated with Sorafenib or vehicle alone for three days, after which they were sacrificed and tumor tissue was analyzed. Amplification status was assessed after sacrifice by DNA qPCR and VEGF-A mRNA expression (Figure 9J and data not shown). Immunostaining for BrdU demonstrated decreased proliferation in the Amp^{pos} tumor group only (Figures 9A-D, Figure 9N). Similarly to VEGF-A inhibition, HGF levels were decreased only in the treated Amp^{pos} tumors (Figure 9K). At the same time, the non-amplified group was not different from the vehicle treated control in either proliferation (Figure 9N) or HGF expression (Figure 9K). No decrease in blood vessel density (Figures 9E-H, Figure 9I) or signs of hypoxia (Figures 9L-M) were observed, possibly due to the short duration of treatment (only 3 days), implying that the effect of VEGF-A amplification on proliferation could be independent of angiogenesis.

These data suggest that the VEGF-A amplification distinguishes a subgroup of Mdr2^{-/-} HCCs that are sensitive to direct VEGF-A blocking, as well as to Sorafenib treatment.

Analysis and Discussion

Using a mouse model of inflammation-induced HCC, the present inventors identified and characterized a specific subtype of HCCs. These tumors are defined by an amplification of a genomic region encompassing the VEGF-A gene. The present inventors show that tumors bearing this amplification (Amp^{pos}) differ from Amp^{neg} tumors in several aspects: histological appearance, rate of proliferation and microenvironmental interactions. A cytokine-based crosstalk between malignant Amp^{pos} hepatocytes and tumor associated macrophages was delineated. Most importantly, the present inventors show that Amp^{pos} tumors are uniquely sensitive to VEGF-A inhibition, suggesting that VEGF-A copy number could serve as a predictive biomarker for response to VEGF blocking agents in human HCC.

The amplicon spanning the VEGF-A locus was previously reported in human HCC, lung and colorectal cancers. Cumulative analysis of human HCC [Chiang, D.Y., et al. *Cancer Res* 68, 6779-6788 (2008); Chochi, Y., et al. A copy number gain of the 6p arm is linked with advanced hepatocellular carcinoma: an array-based comparative genomic hybridization study. *J Pathol* 217, 677-684 (2009); Moinzadeh, P., et al., Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade--results of an explorative CGH meta-analysis. *Br J Cancer* 92, 935-941 (2005); Patil, M.A., et al. Array-based comparative genomic hybridization reveals recurrent chromosomal aberrations and Jab1 as a potential target for 8q gain in hepatocellular carcinoma. *Carcinogenesis* 26, 2050-2057 (2005); Weir, B.A., et al. Characterizing the cancer genome in lung adenocarcinoma. *Nature* 450, 893-898 (2007); Tsafrir, D., et al. Relationship of gene expression and chromosomal abnormalities in colorectal cancer. *Cancer Res* 66, 2129-2137 (2006)] shows that the VEGF-A specific region (VEGF-A amplicon) is amplified in between 7-24% of human HCCs and the whole chromosome arm is amplified in nearly 30% of human HCCs. In the Mdr2^{-/-} mouse model, the present inventors found the VEGF-A amplicon in 14% of HCCs, irrespective of tumor size, suggesting that this could be an early event.

Inhibition of VEGF-A was already tested in human HCC. Treatment with Bevacizumab alone or in combination with Erlotinib was tested and yielded positive results in clinical experiments (Siegel, A.B., et al. 2008, *J. Clin. Oncol.* 26, 2992-2998; Thomas, M.B., et al. 2009, *J. Clin. Oncol.* 27, 843-850).

Importantly however, to date, there are no clinically reliable biomarkers for personalizing HCC treatment, identifying the most suitable patients and reducing the overall treatment morbidity. The findings described herein suggest that response to anti VEGF-A treatment or Sorafenib can be predicted by quantifying VEGF-A copy number in the tumor tissue from HCC patients. If this is the case, it could lead to substantial improvement in treatment selection for this highly aggressive tumor. Notably, the same amplification was found also in lung and colorectal cancers (Weir, B.A., et al. 2007, *Nature* 450, 893-898; Tsafrir, D., et al. 2006, *Cancer Res.* 66, 2129-2137), suggesting that similar treatment guidelines could be applied to all VEGF-A Amp^{pos} tumors.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

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WHAT IS CLAIMED IS:

1. A method of predicting an efficacy of an anti-vascular endothelial growth factor A (VEGFA) treatment on a subject diagnosed with a solid tumor, comprising
determining a presence or an absence of a genomic amplification which comprises a VEGFA gene in a sample of the solid tumor,
wherein said presence or said absence of said genomic amplification predicts the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor,
thereby predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor.
2. A method of treating of a subject diagnosed with a solid tumor, the method comprising:
 - (a) predicting the efficacy of the anti-VEGFA treatment on the subject diagnosed with the solid tumor according to the method of claim 1, and
 - (b) selecting a treatment regimen based on said prediction;
thereby treating of the subject diagnosed with the solid tumor.
3. The method of claim 1 or 2, wherein the solid tumor is carcinoma.
4. The method of claim 3, wherein said carcinoma is hepatocellular carcinoma.
5. The method of claim 1, 2, 3 or 4, wherein said determining said presence or said absence of said genomic amplification is effected by comparing a ratio determined in a sample of the solid tumor between a copy number of said VEGFA and a copy number of a centromeric marker of human chromosome 6, or visa versa, to a reference ratio determined in at least one sample devoid of the solid tumor between a copy number of said VEGFA and a copy number of said centromeric marker of human chromosome 6, or visa versa, respectively.

6. The method of claim 5, wherein an increase above a predetermined threshold in said ratio determined in said sample of the solid tumor relative to said reference ratio indicates said presence of said genomic amplification.

7. The method of claim 5, wherein an identical ratio or a change below a predetermined threshold in said ratio determined in said sample of the solid tumor as compared to said reference ratio indicates said absence of said genomic amplification.

8. The method of claim 1, 2, 3, 4, 5, 6 or 7, wherein said determining a presence or an absence of a genomic amplification is effected using a DNA detection method.

9. The method of claim 1, 2, 3, 4, 5, 6 or 7, wherein said determining a presence or an absence of a genomic amplification is effected using a chromosomal detection method.

10. The method of any of claims 1-9, further comprising comparing an expression level of said VEGFA in said sample of the solid tumor to a reference expression data obtained from at least one sample devoid of cancer.

11. The method of claim 10, wherein an increase above a predetermined threshold in said expression level of said VEGFA in said sample of the solid tumor relative to said reference expression data predicts the efficacy of the anti-VEGFA treatment on the solid tumor.

12. The method of claim 10 or 11, wherein said sample devoid of cancer is a liver sample.

13. The method of claim 10, 11 or 12, wherein said expression level is determined using an RNA detection method.

14. The method of claim 10, 11 or 12, wherein said expression level is determined using a protein detection method.

15. The method of any of claims 1-14, wherein the anti-VEGFA treatment comprises Sorafenib.

16. The method of any of claims 1-14, wherein the anti-VEGFA treatment comprises bevacizumab.

17. The method of any of claims 1-14, wherein the anti-VEGFA treatment comprises a soluble form of the VEGF-receptor.

18. The method of any of claims 1-14, wherein the anti-VEGFA treatment comprises thalidomide.

19. The method of any of claims 1-14, wherein the anti-VEGFA treatment comprises a combination of at least two anti-VEGFA drugs selected from the group consisting of Sorafenib, bevacizumab, a soluble form of the VEGF-receptor and thalidomide.

20. The method of any of claims 2-18, wherein said treatment regimen comprises administering of at least one anti-VEGFA drug selected from the group consisting of Sorafenib, bevacizumab, a soluble form of the VEGF-receptor and thalidomide.

21. The method of claim 20, wherein said treatment regimen further comprises administering a drug selected from the group consisting of erlotinib, oxaliplatin, cisplatin, platinum, rIFNa-2b, doxorubicin, fluorouracil, DX-8951f, thalidomide, doxorubicin, epirubicin, and taxol.

22. The method of any of claims 1-18, wherein said genomic amplification is of a human chromosome 6p21.

23. The method of any of claims 1-18, wherein said genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:23.

24. The method of any of claims 1-18, wherein said genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:24.

25. The method of any of claims 1-18, wherein said genomic amplification comprises the nucleotide sequence set forth in SEQ ID NO:25.

26. The method of any of claims 5-22, wherein said VEGFA comprises the genomic nucleic acid sequence set forth by SEQ ID NO:1.

27. The method of any of claims 1-26, wherein the solid tumor is hepatocellular, and wherein the hepatocellular solid tumor is associated with hepatitis C infection.

28. The method of any of claims 1-27, wherein the efficacy of the anti-VEGFA treatment is determined by tumor regression following at least 8 weeks of said anti-VEGFA treatment.

29. The method of any of claims 1-28, wherein the efficacy of the anti-VEGFA treatment is determined by progression-free survival (PFS) time of at least one year.

30. The method of any of claims 1-29, wherein the efficacy of the anti-VEGFA treatment is determined by a proliferation assay.

31. The method of claim 8, wherein said DNA detection method comprises DNA quantitative PCR (qPCR).

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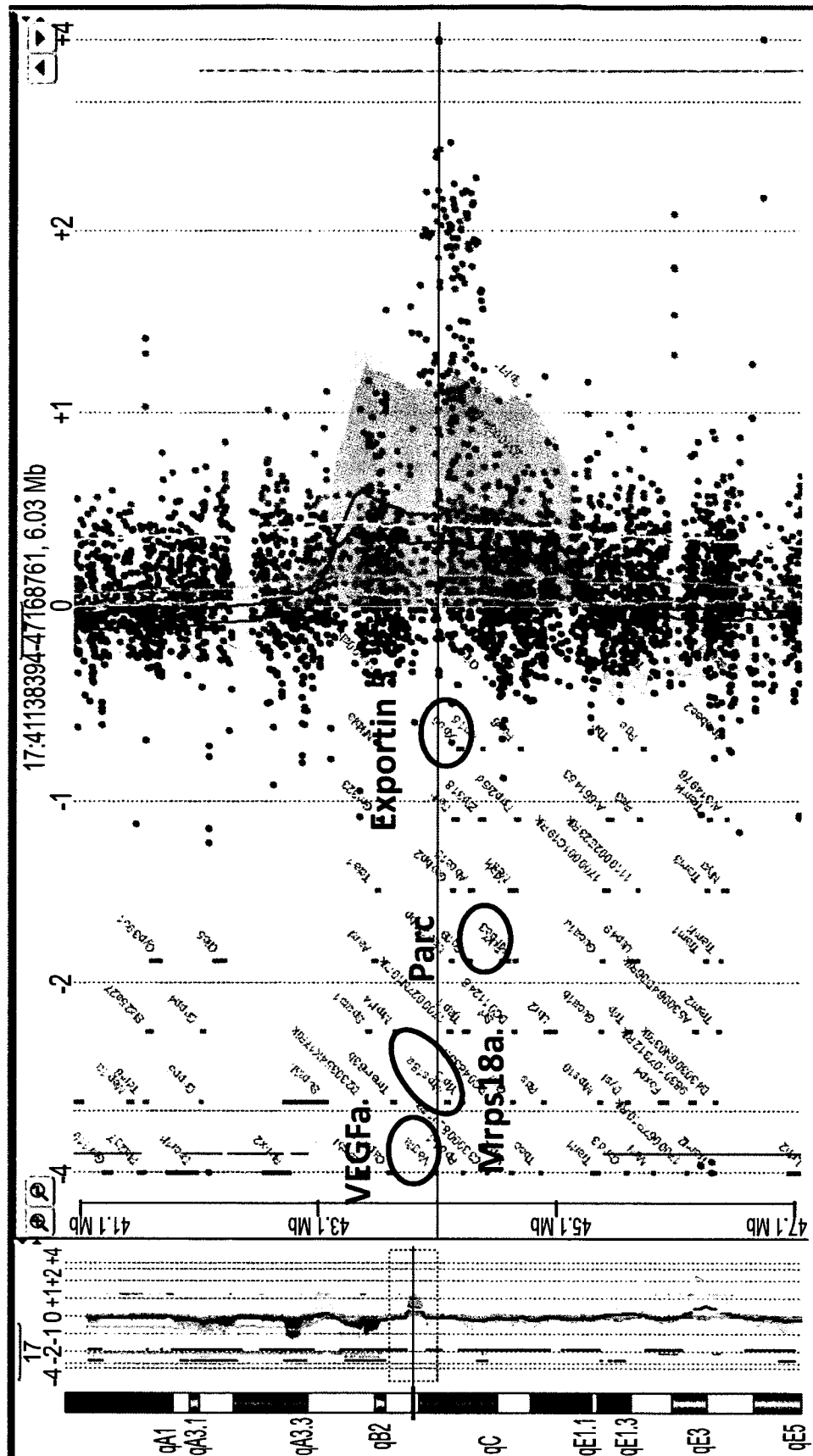


FIG. 1

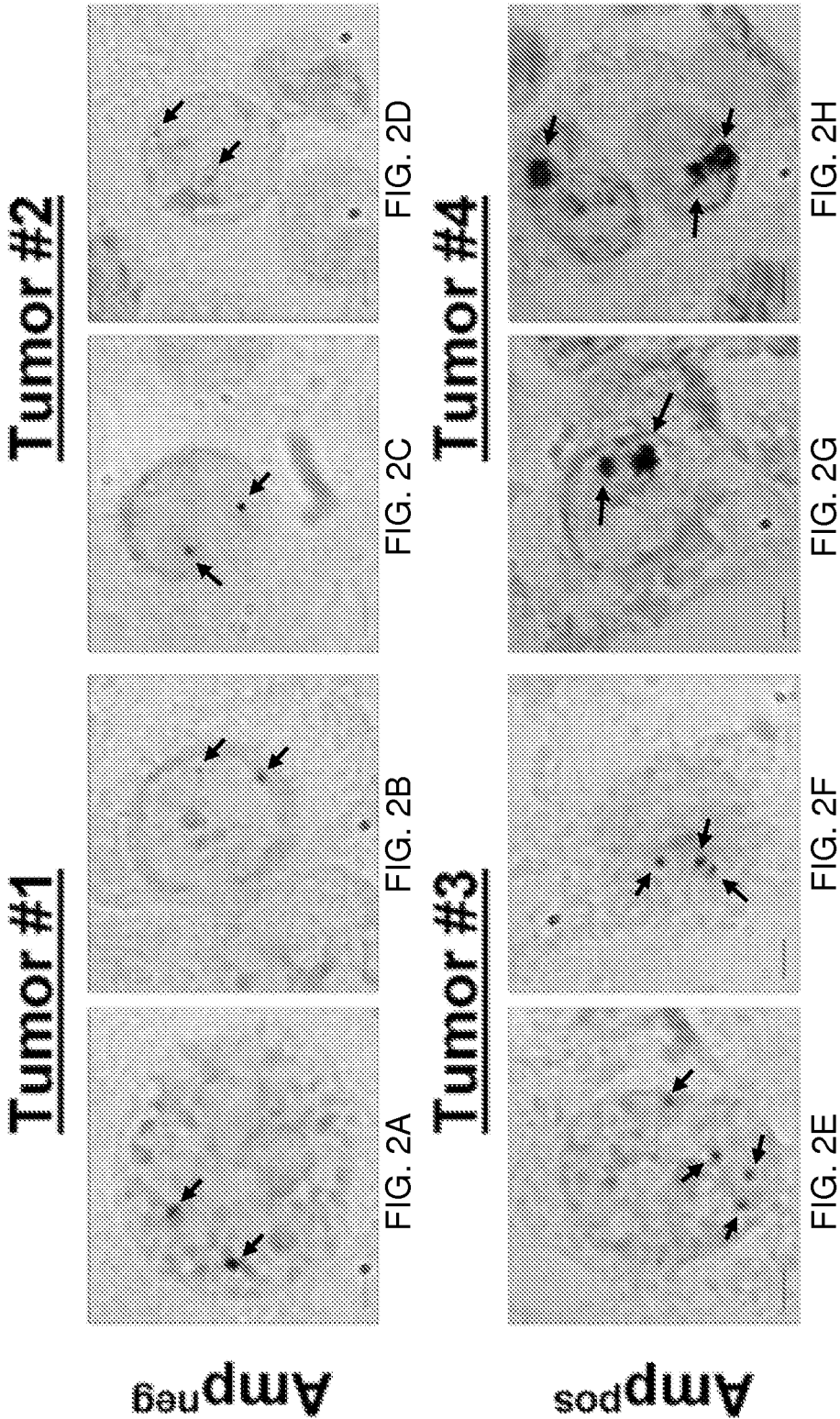
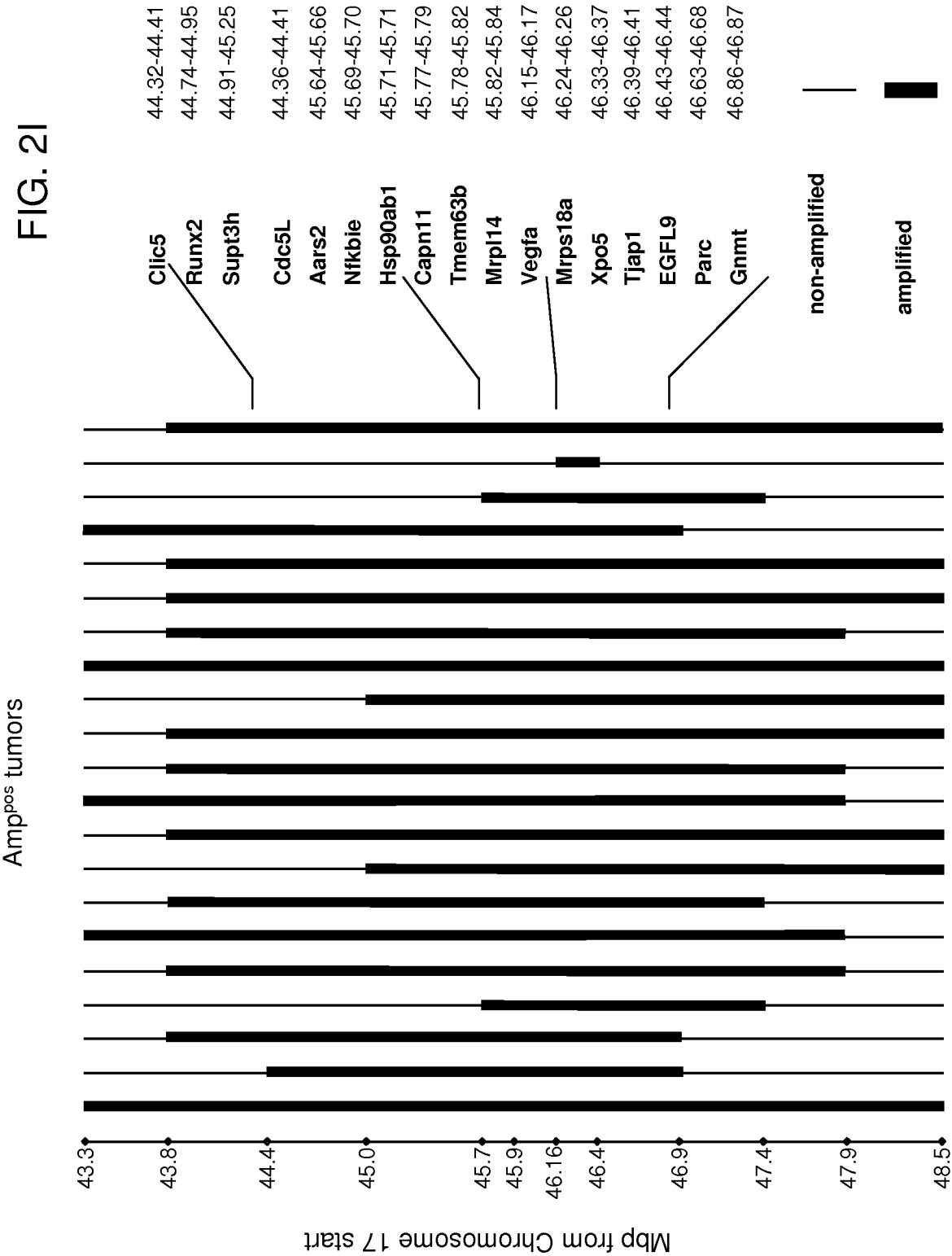
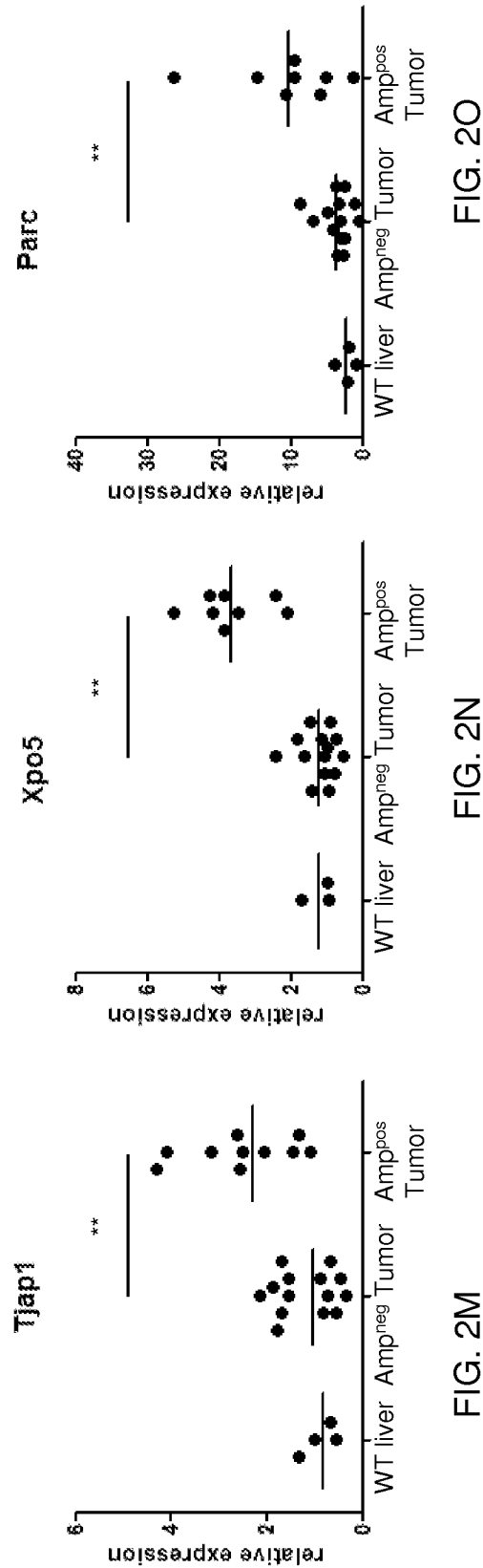
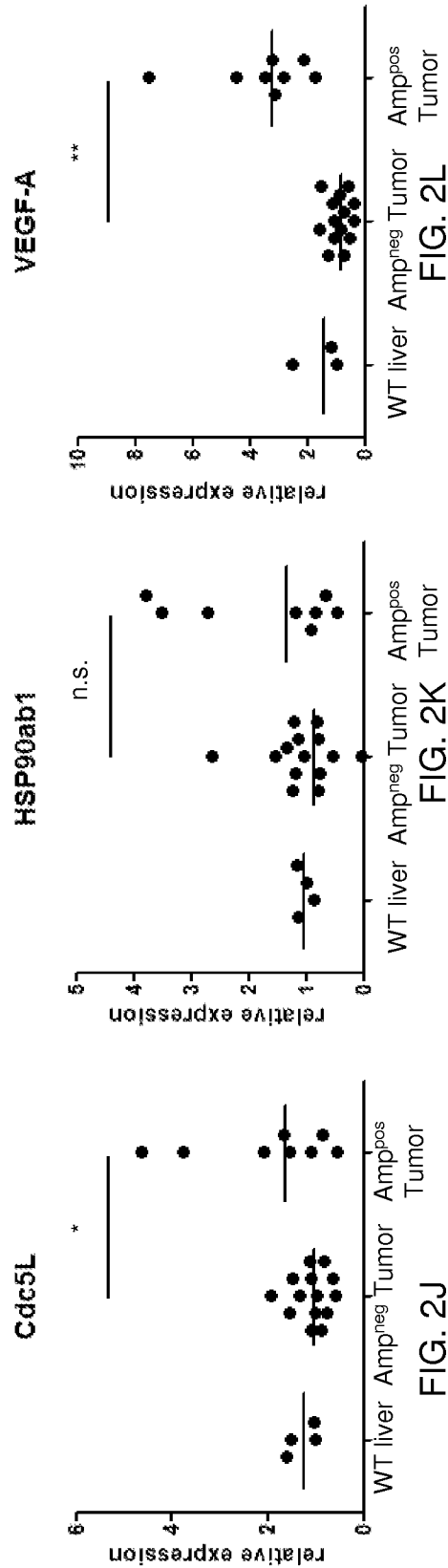
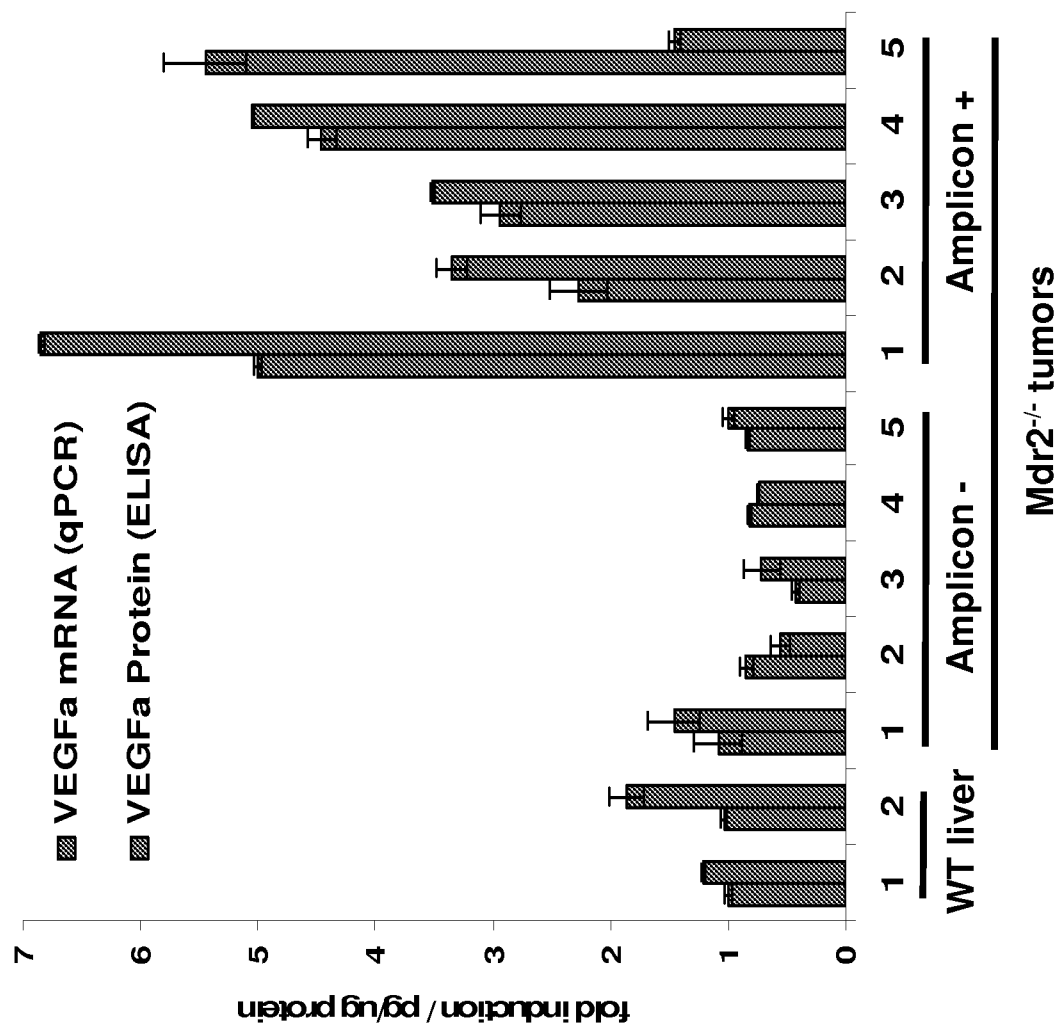
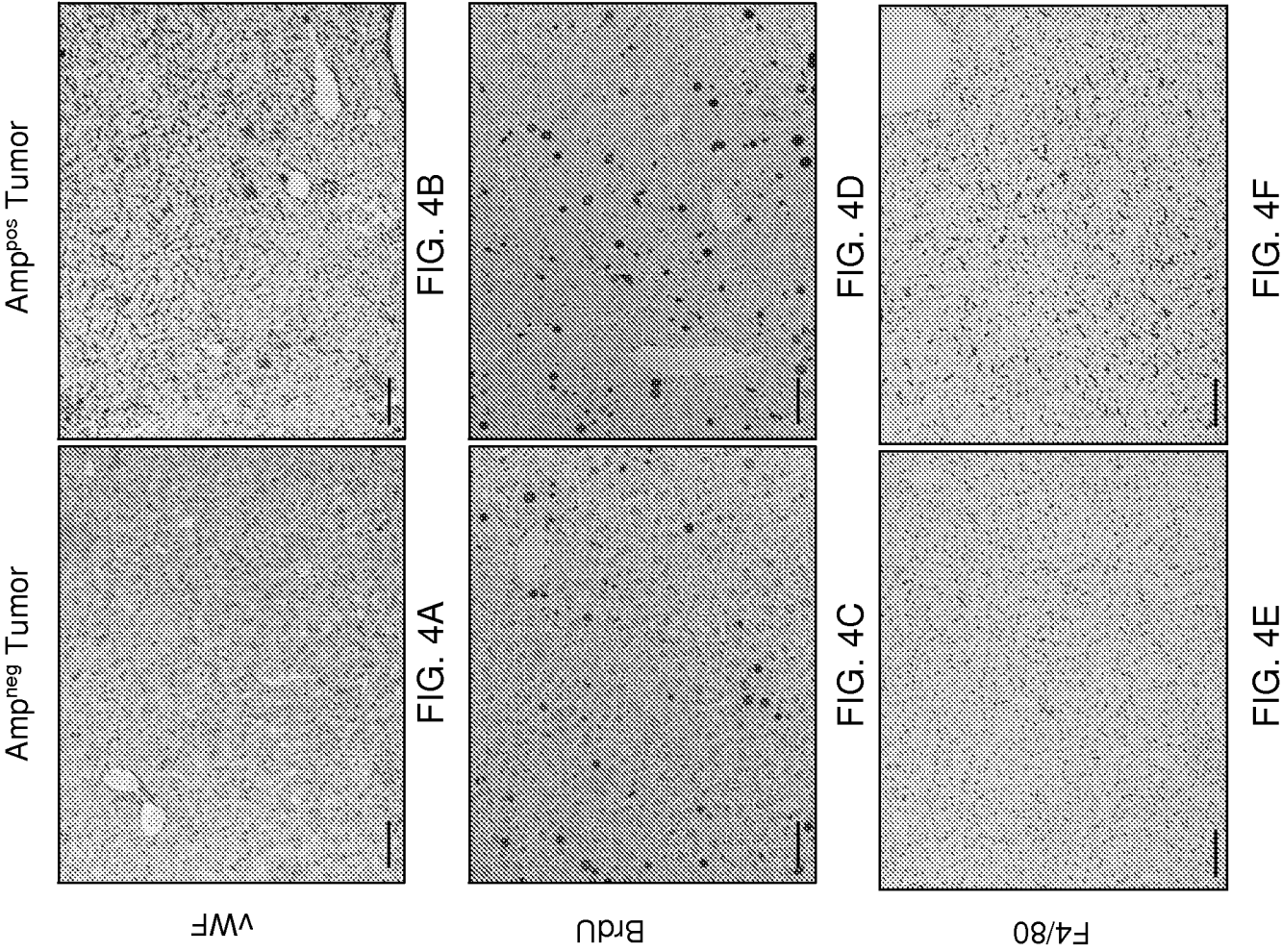


FIG. 2I









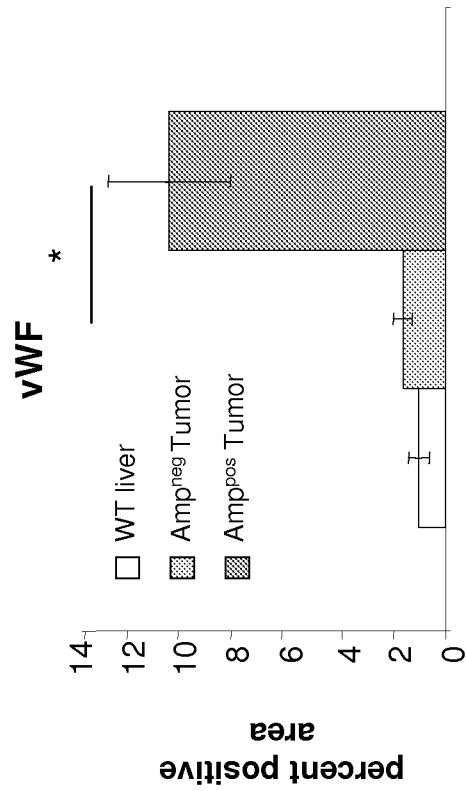


FIG. 4G

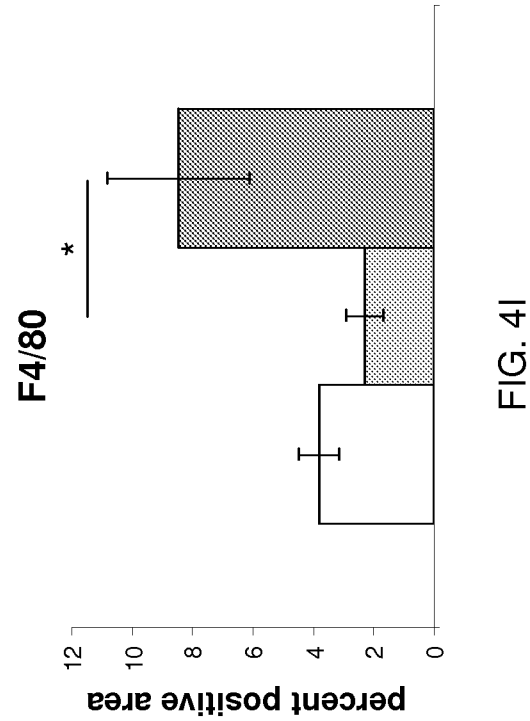


FIG. 4I

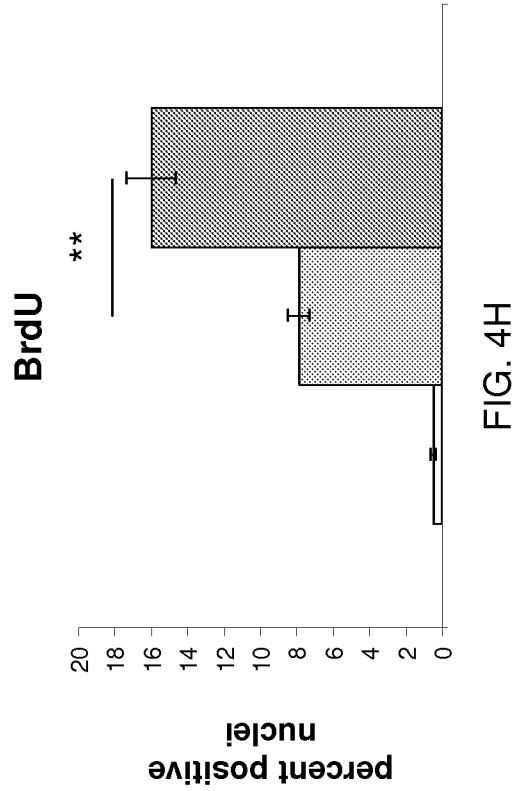
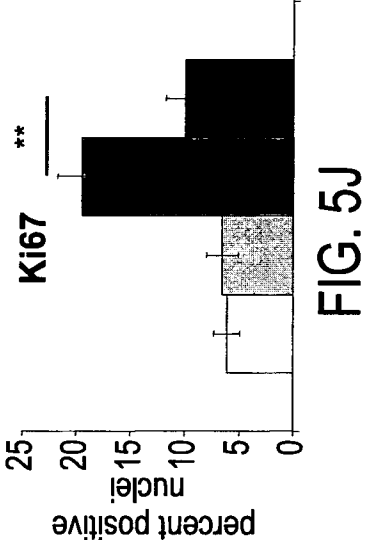
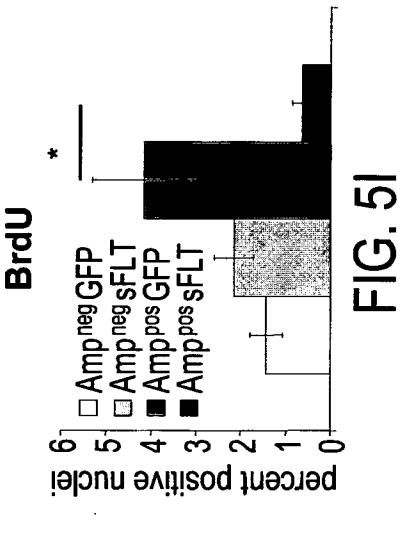
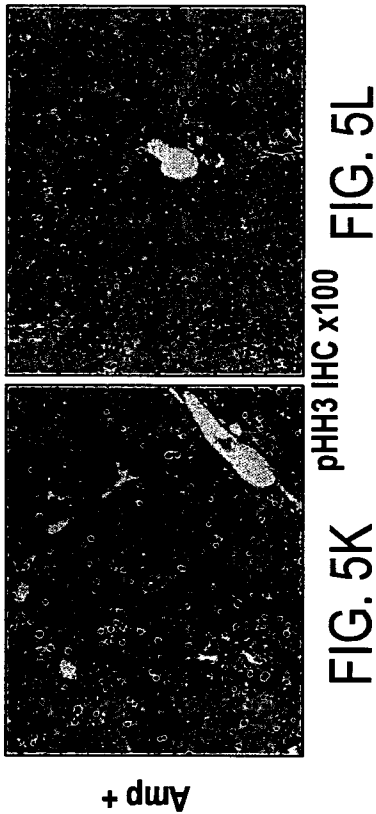
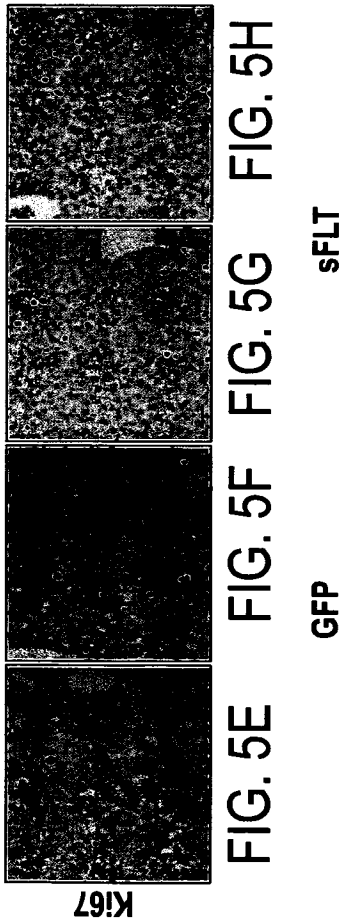
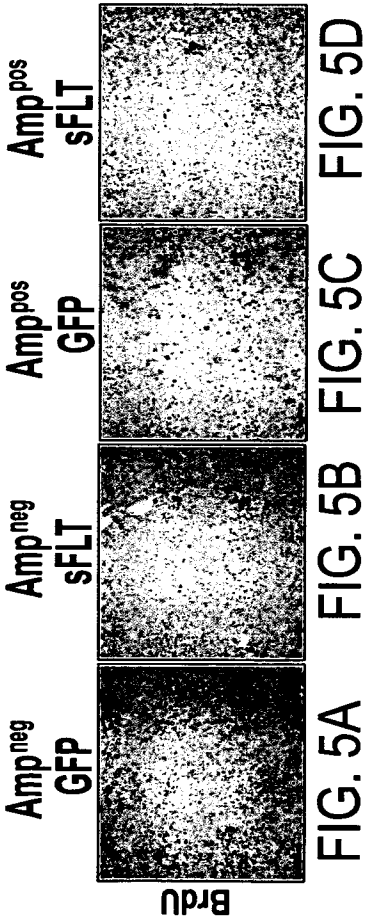


FIG. 4H



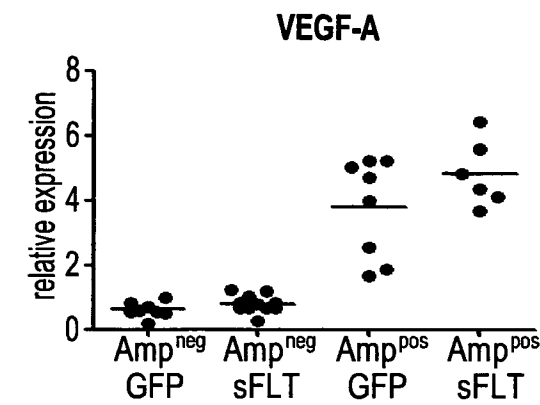


FIG. 5M

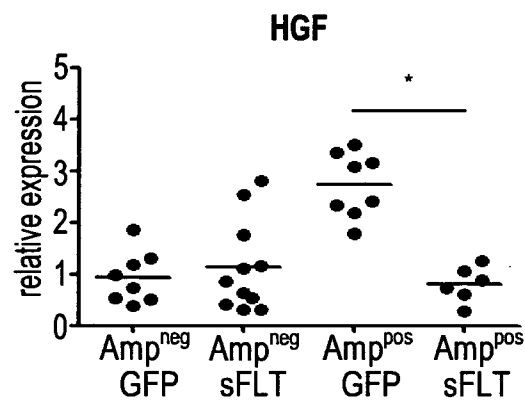


FIG. 5N

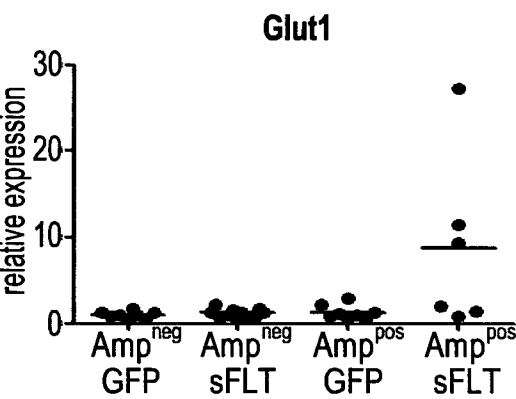


FIG. 5O

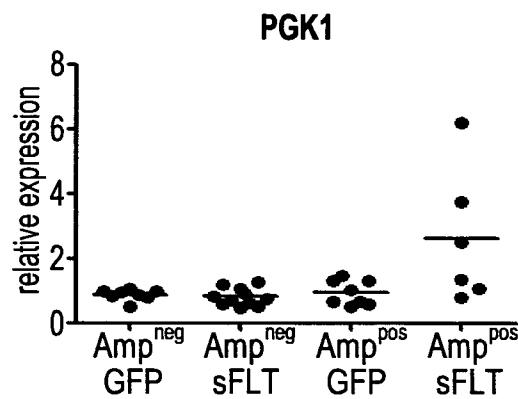


FIG. 5P

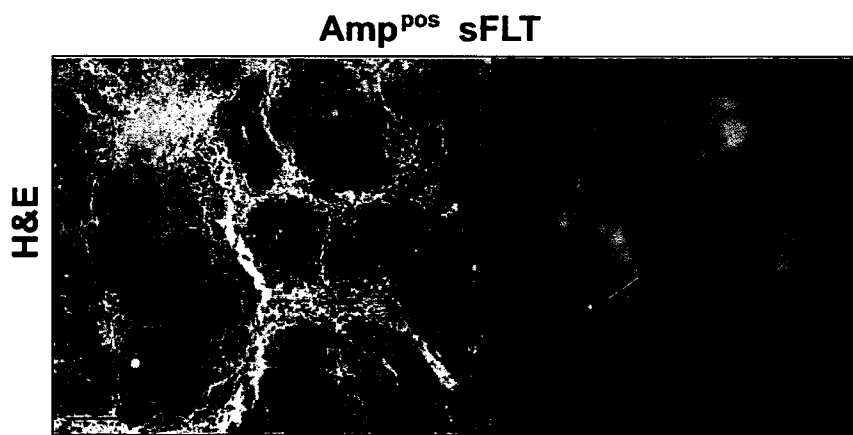


FIG. 5Q

FIG. 5R

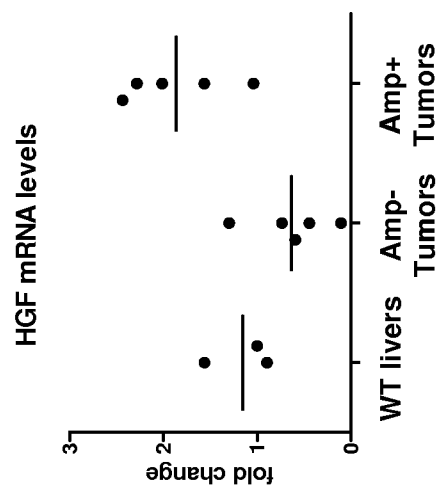


FIG. 6A

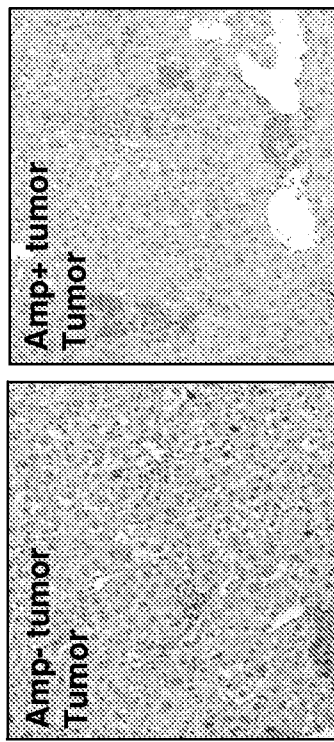


FIG. 6B HGF IHC x200 FIG. 6C

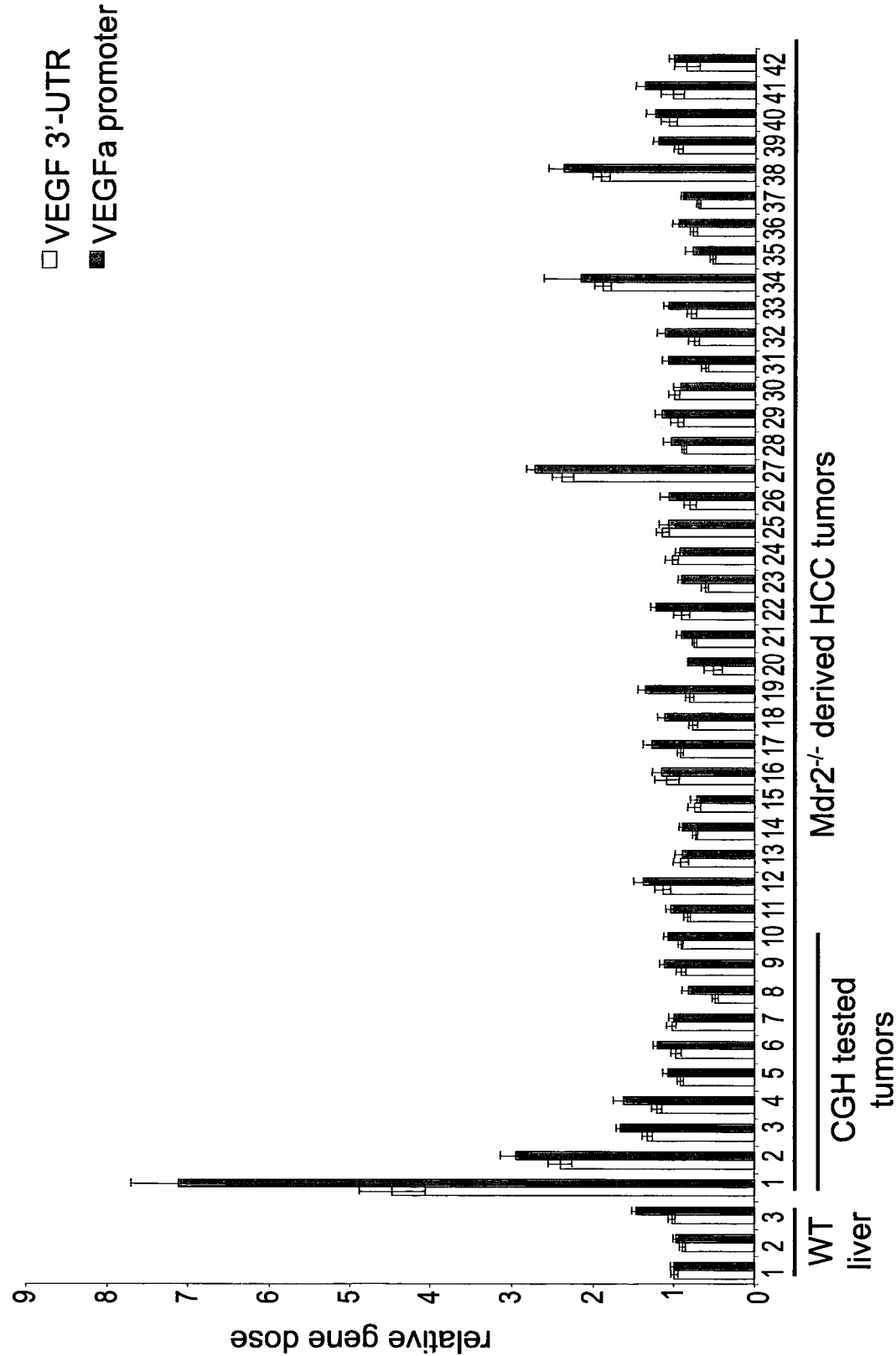
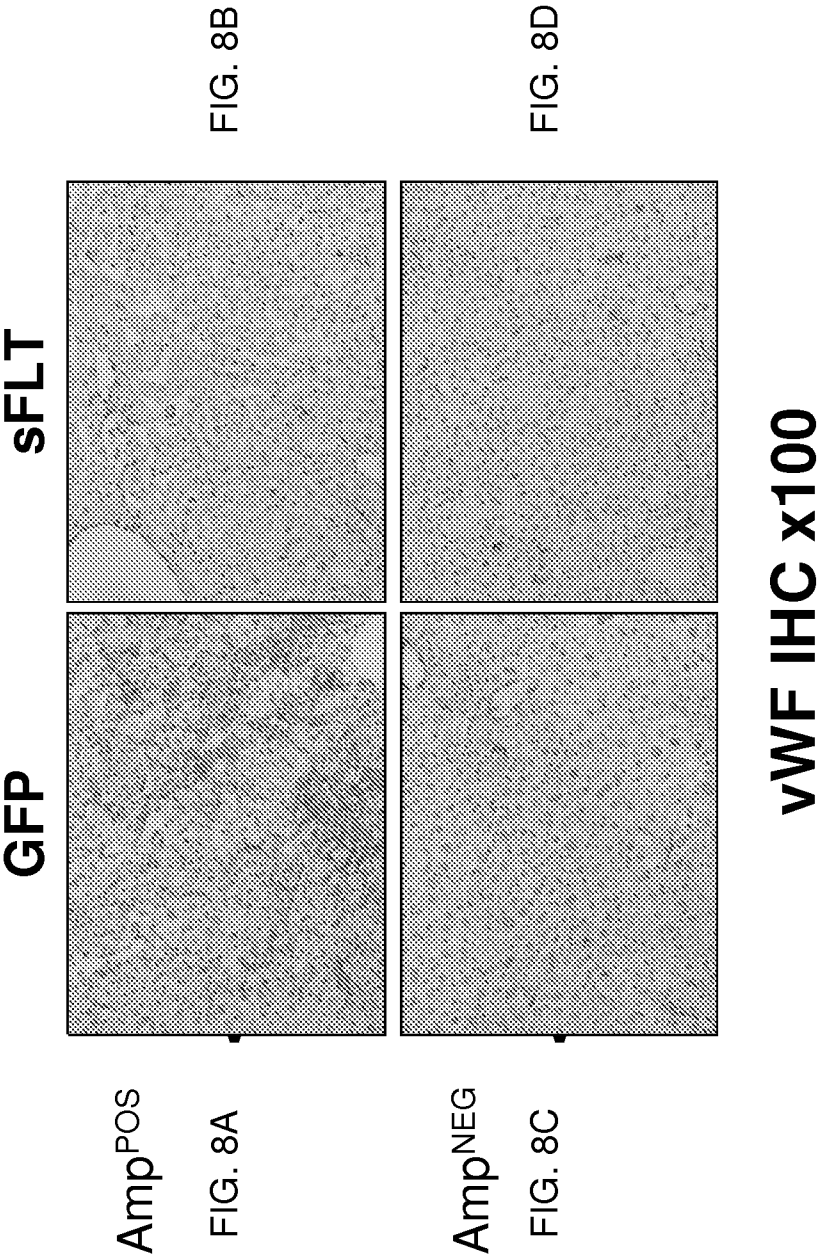


FIG. 7



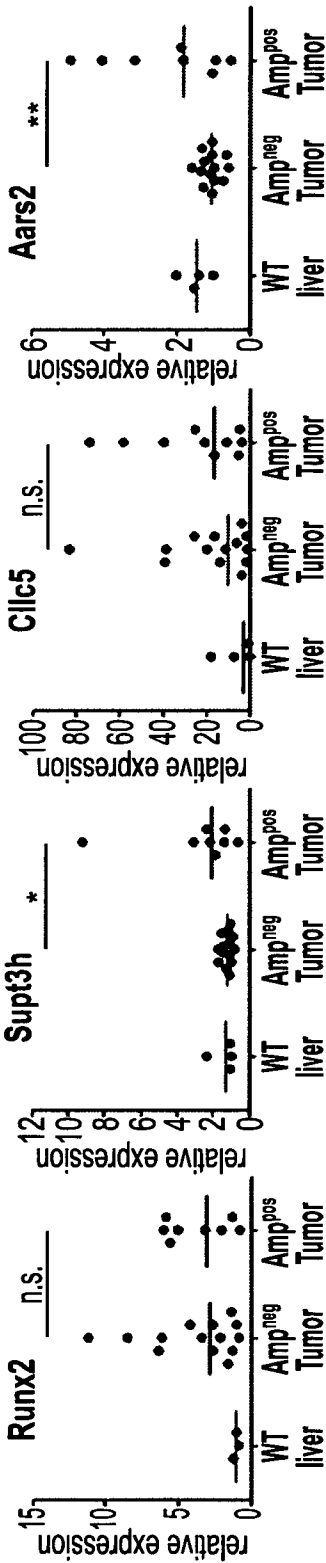


FIG. 10A

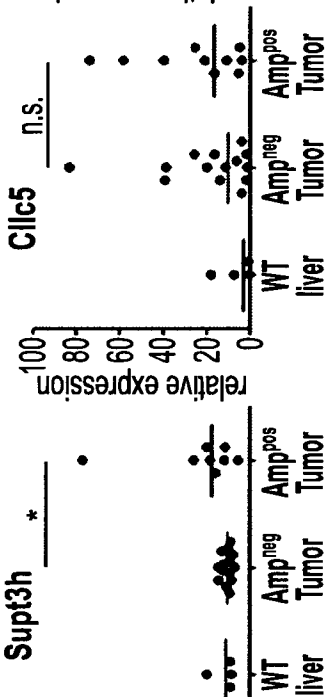


FIG. 10B

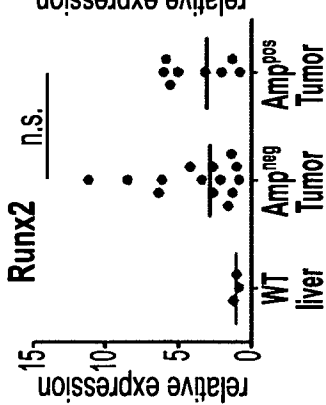


FIG. 10C

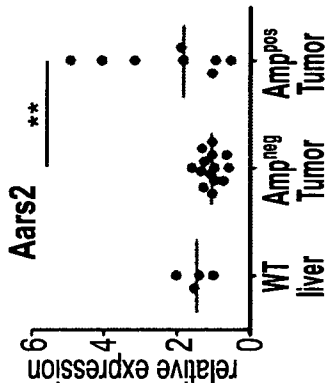


FIG. 10D

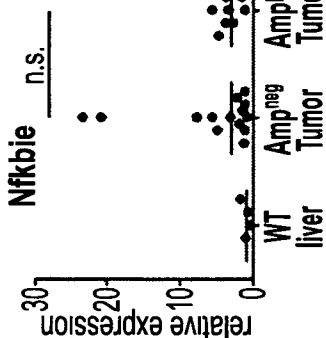


FIG. 10E

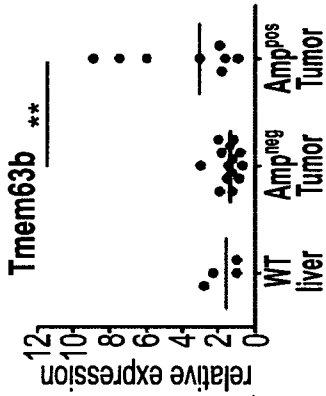


FIG. 10F

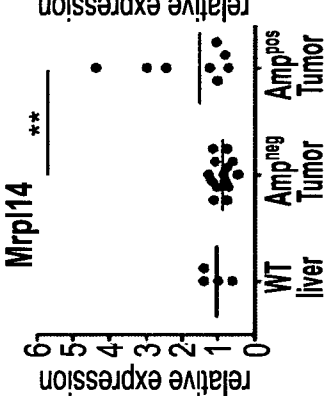


FIG. 10G

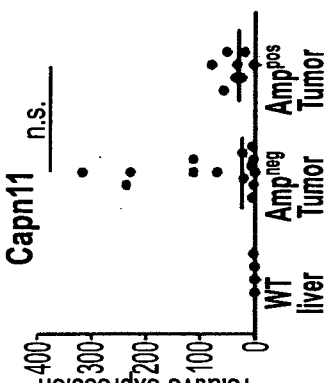


FIG. 10H

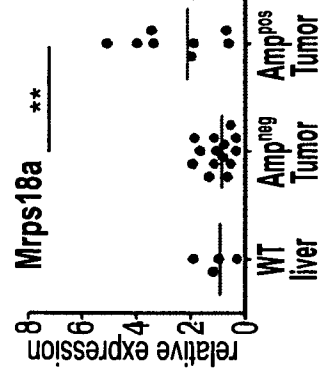


FIG. 10I

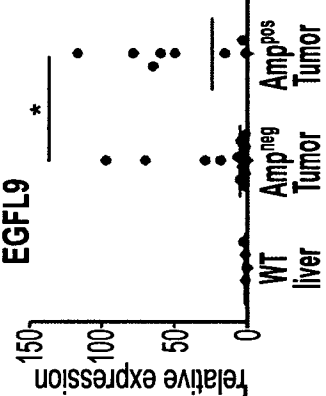


FIG. 10J

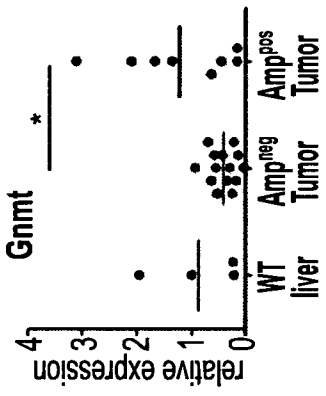


FIG. 10K

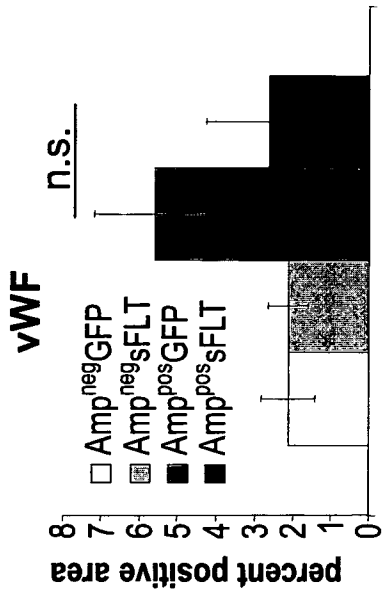


FIG. 12I

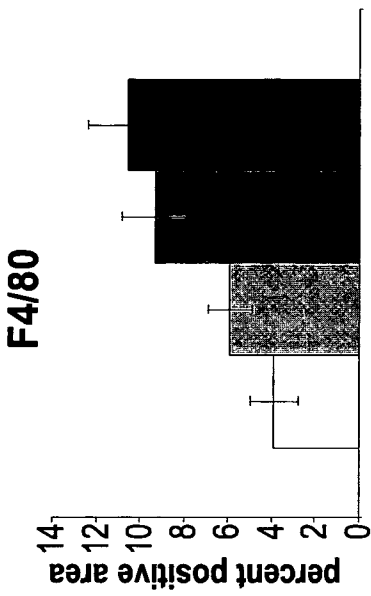


FIG. 12J

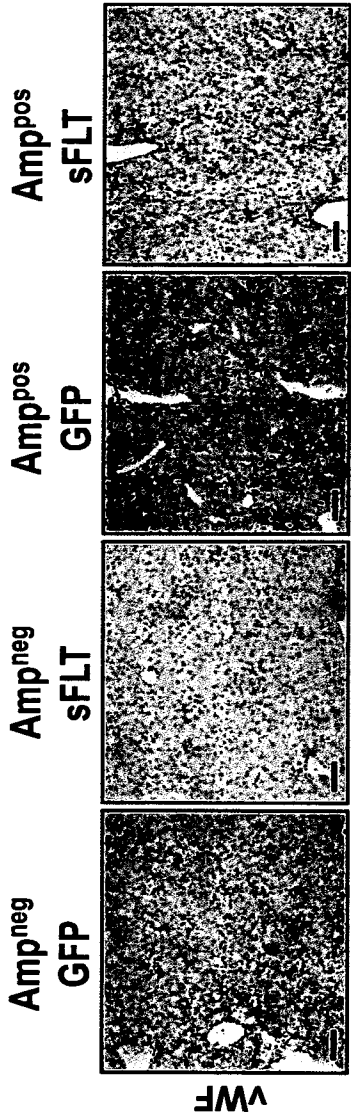


FIG. 12A FIG. 12B FIG. 12C FIG. 12D

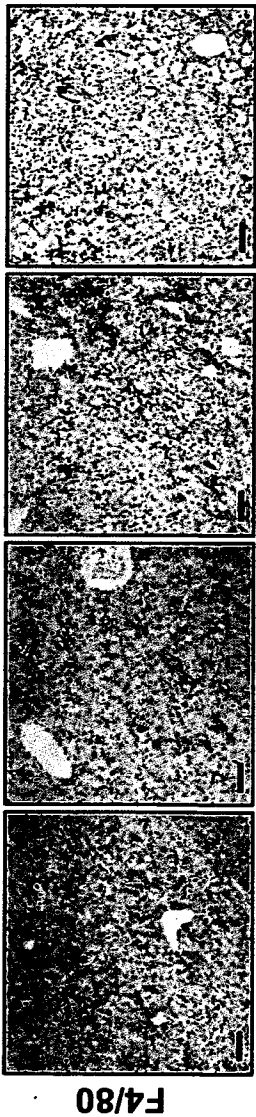


FIG. 12E FIG. 12F FIG. 12G FIG. 12H

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2011/050868

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHIANG DEREK Y ET AL: "Focal gains of VEGFA and molecular classification of hepatocellular carcinoma", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 68, no. 16, 15 August 2008 (2008-08-15), pages 6779-6788, XP002598557, ISSN: 0008-5472, DOI: DOI:10.1158/0008-5472.CAN-08-0742 cited in the application page 6787	1-31
Y	----- WO 2009/140556 A2 (UNIV SOUTHERN CALIFORNIA [US]; LENZ HEINZ-JOSEF [US]; ZHANG WU [US]) 19 November 2009 (2009-11-19) page 121; example 9 ----- -/-	1-31



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"&" document member of the same patent family

Date of the actual completion of the international search

28 June 2011

Date of mailing of the international search report

11/07/2011

Name and mailing address of the ISA/

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NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Gabriels, Jan

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/050868

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	M. CABALLERO ET AL: "Serum Vascular Endothelial Growth Factor as a Predictive Factor in Metronomic (Weekly) Paclitaxel Treatment for Advanced Head and Neck Cancer", ARCHIVES OF OTOLARYNGOLOGY - HEAD AND NECK SURGERY, vol. 133, no. 11, 1 November 2007 (2007-11-01), pages 1143-1148, XP55001472, ISSN: 0886-4470, DOI: 10.1001/archotol.133.11.1143 the whole document	1-31
Y	----- F V NEGRI ET AL: "Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy", BRITISH JOURNAL OF CANCER, vol. 98, no. 1, 15 January 2008 (2008-01-15), pages 143-147, XP55001473, ISSN: 0007-0920, DOI: 10.1038/sj.bjc.6604131 the whole document	1-31
Y	----- DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; June 2006 (2006-06), MILANO M ET AL: "Recent advances in targeted therapies for colorectal cancer.", XP002645493, Database accession no. NLM16984744 the whole document & JOURNAL OF ONCOLOGY PHARMACY PRACTICE : OFFICIAL PUBLICATION OF THE INTERNATIONAL SOCIETY OF ONCOLOGY PHARMACY PRACTITIONERS JUN 2006 LNKD- PUBMED:16984744, vol. 12, no. 2, June 2006 (2006-06), pages 69-73, ISSN: 1078-1552 ----- -/--	1-31

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2011/050868

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LISA RYDÉN ET AL: "Tumor-specific VEGF-A and VEGFR2 in postmenopausal breast cancer patients with long-term follow-up. Implication of a link between VEGF pathway and tamoxifen response", BREAST CANCER RESEARCH AND TREATMENT, KLUWER ACADEMIC PUBLISHERS, BO, vol. 89, no. 2, 1 January 2005 (2005-01-01), pages 135-143, XP019274717, ISSN: 1573-7217 the whole document	1-31
Y	WO 2008/118846 A1 (PREC THERAPEUTICS INC [US]; HEINZMAN JAMIE [US]; BROWER STACEY [US]; B) 2 October 2008 (2008-10-02) claim 10	1-31
Y	WO 2008/128008 A2 (SQUIBB BRISTOL MYERS CO [US]; PARADA JESUS PLATERO [US]; GALBRAITH SUS) 23 October 2008 (2008-10-23) the whole document	1-31
Y	US 2007/105142 A1 (WILHELM SCOTT [US]) 10 May 2007 (2007-05-10) claim 1	1-31
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Y	WO 2008/027013 A2 (NAT CANCER CT [SG]; HUYNH THE HUNG [SG]; CHOW PIERCE KAH-HOE [SG]; SOO) 6 March 2008 (2008-03-06) page 141	1-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2011/050868

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WO 2008118846 A1	02-10-2008	CA 2680528 A1 EP 2136630 A1 US 2010216168 A1	02-10-2008 30-12-2009 26-08-2010
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