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(54) Title: METHOD FOR PREDICTING THE OUTCOME OF A TREATMENT WITH AFLIBERCEPT OF A PATIENT SUSPECTED TO SUFFER FROM A CANCER BY MEASURING THE LEVEL OF A PLASMA BIOMARKER

(57) Abstract: The present invention concerns the use of VCAM-1, ICAM-1 and/or PIGF as biomarkers for predicting the outcome of the treatment with aflibercept, or ziv-aflibercept of a patient suspected to suffer from a cancer.

METHOD FOR PREDICTING THE OUTCOME OF A TREATMENT WITH AFLIBERCEPT OF A PATIENT SUSPECTED TO SUFFER FROM A CANCER BY MEASURING THE LEVEL OF A PLASMA BIOMARKER

The present invention concerns the use of VCAM-1, ICAM-1 and PlGF as a biomarker for predicting the outcome of the treatment with aflibercept, or ziv-aflibercept, of a patient suspected to suffer from cancer.

Aflibercept, or ziv-aflibercept, also referred to as VEGFR1R2-Fc.DELTA.C1 Flt1D2.Flk1D3.Fc.DELTA.C1 or AVE0005, is a homo dimer protein, with each dimer comprising two identical monomers, each of which is a fusion protein comprising the signal sequence of VEGFR1 fused to the D2 Ig domain of the VEGFR1 receptor, itself fused to the D3 Ig domain of the VEGFR2 receptor, in turn fused to the Fc domain of IgG1.

The protein chain is glycosylated, with N-acetyl-glucosamine, fucose, galactose, mannose and sialic acids contributing to the carbohydrate structures. The N-linked oligosaccharides consist of mainly bi-antennary structures with zero, one or two terminal sialic acids. The monomer has the amino acid sequence SEQ ID N°1.

The U.S. Food and Drug Administration (FDA) already approved aflibercept under the trade name EYLEA® for the treatment of patients with neovascular (wet) age-related macular degeneration (AMD). In particular, EYLEA® is the trade name for aflibercept as generated, processed and formulated for intravitreal injection.

At the time of registration of aflibercept (ZALTRAP®) for cancer indication, and In light of aflibercept's approved use in treating AMD, the FDA requested that a different name (ziv-aflibercept) be given for the compound's use in the treatment of cancer. Thus, ziv-aflibercept is the United States Adopted Name (USAN) accepted by FDA to designate a pharmaceutical composition comprising aflibercept as generated, processed and formulated for injection via intravenous infusion. Ziv-aflibercept has been approved by the FDA for sale under the tradename ZALTRAP® for the treatment of metastatic colorectal cancer (mCRC).

The European Medicines Agency (EMA) approved ZALTRAP® as well however did not request separate names for the compound. Thus, in the European Union the name "aflibercept" is used regardless of the indication.

ZALTRAP® and EYLEA® are obtained by slightly different processes. They both contain aflibercept or ziv-aflibercept, but the ratio of aggregates of aflibercept or ziv-aflibercept is slightly different in ZALTRAP® and EYLEA®.

ZALTRAP® approval was based on data obtained from the VELOUR trial – a multicenter, randomized, placebo-controlled phase III trial, which compared the efficacy of aflibercept *versus* placebo in combination with the FOLFIRI regimen for patients with mCRC previously treated with an oxaliplatin containing regimen.

Despites the efficacy and the safety of the treatment of cancer by aflibercept it remains a goal to better identify patients who should benefit more from the treatment. Indeed the ability to identify Metastatic Colorectal Cancer (mCRC) patients who will benefit from aflibercept would further improve clinical utility of this drug.

To date, no validated predictive serum or plasma biomarkers have been identified that correlate with treatment outcomes to aflibercept.

The profiling of tumor and plasma samples derived from patients involved in clinical trials and subsequent analysis of their genomic / proteomic and clinical data could allow the discovery and potential validation of predictive biomarkers.

It has now been discovered that high levels of VCAM-1, ICAM-1 and/or PlGF at baseline correlated with shorter survival times.

Vascular cell adhesion molecule-1 (VCAM-1) also known as CD106 has the sequence SEQ ID N°1 (NCBI Reference Sequence: NP_001069.1). The term "VCAM-1" encompasses its homologues and isoforms and variants thereof, as well as fragments of the sequences, provided that the variant proteins (including isoforms), homologous proteins and/or fragments are recognized by one or more VCAM-1 specific antibodies.

Intercellular Adhesion Molecule 1 (ICAM-1) also known as CD54 has the sequence SEQ ID N°2 (NCBI Reference Sequence: NP_000192.2). The term "ICAM-1" encompasses its homologues and isoforms and variants thereof, as well as fragments of the sequences, provided that the variant proteins (including isoforms), homologous proteins and/or fragments are recognized by one or more ICAM-1 specific antibodies.

"Placental Growth Factor" or "PlGF" comprises the 2 isoforms PlGF1 et PlGF2 which have respectively the sequences SEQ ID N°3 (NCBI Reference Sequence: NP_002623.2) and SEQ ID N°4 (NCBI Reference Sequence: NP_001193941.1). The term " PlGF " encompasses PlGF1 et PlGF2 their homologues and isoforms and variants thereof, as well as fragments of the sequences, provided that the variant proteins (including isoforms), homologous proteins and/or fragments are recognized by one or more PlGF specific antibodies.

The correlation of ICAM-1 with patient outcome was tested in clinical trials where patients were treated with bevacizumab.

The correlation of ICAM-1 with patient outcome was found in a trial wherein lung cancer patients were treated with bevacizumab, cisplatin and etoposide (Horn et al, J Clin Oncol 2009; 27:6006-6011). The authors found that "patients who had high ICAM levels had a non significant trend towards improved OS compared with patients who had low levels", whereas in another study (Dowlati et al, 2008, Clin Cancer Res 14(5), 1407) "Patients with low baseline ICAM had a higher response than those with high ICAM".

But the correlation of ICAM-1 with patient outcome was not found in colorectal cancer patients treated with bevacizumab, capecitabine and oxaliplatin (Liu et al; Cancer Medicine 2013; 2(2): 234–242).

These articles show the level of unpredictability between various biomarkers studies with the same biologics i.e. bevacizumab.

Furthermore bevacizumab is an anti-VEGF-A antibody. Aflibercept is not an antibody but a chimeric protein. It consists of portions of extracellular domains of human VEGF receptors 1 and 2 fused to human IgG1 Fc portion. Aflibercept binds not only to VEGF-A but also to VEGF-B and placental growth factor (PlGF).

Thus bevacizumab and aflibercept have structure and functions that are very different and the man skilled in the art would not transpose directly the results obtained with bevacizumab to aflibercept.

The correlation of VCAM-1 with patient outcome was found in the two clinical trials mentioned above (Liu et al; Cancer Medicine 2013; 2(2): 234–242 and Horn et al, J Clin Oncol 2009; 27:6006-6011). However in these two studies there is not a placebo arm to let us know if the effect is predictive or prognostic.

The invention relates to the use of VCAM-1, ICAM-1 and/or PlGF as biomarkers for predicting the outcome of the treatment with aflibercept, or ziv-aflibercept of a patient suspected to suffer from a cancer.

In one aspect, the present invention provides a method of determining whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy for the said cancer comprising the step of subjecting a patient's biological sample to at least one assay to measure at baseline the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF, wherein when the biological sample of the biomarker level is low relative to a reference level of expression of the biomarker, the patient is identified as a candidate for therapy for cancer.

In another aspect, the present invention provides a method of determining whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy for the said cancer comprising the step of subjecting a patient's biological sample to at least one assay to measure at baseline the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF, wherein when the biological sample level of the biomarker is high relative to a reference level of expression of the biomarker or threshold, the patient is identified as not being a candidate for therapy for cancer. The threshold or reference level of expression of the biomarker allows to define sensitive and non-sensitive populations.

In an embodiment the reference level of expression of VCAM-1 is comprised between around 406 ng/mL and around 577 ng/mL. Yet in another the reference level of expression of VCAM-1 is around 553 ng/mL .

In another embodiment the reference level of expression of ICAM-1 is comprised between around 92 ng/mL and around 145 ng/mL. Yet in another the reference level of expression of ICAM-1 is around 144 ng/mL.

In another embodiment the reference level of expression of PlGF is comprised between around 12 ng/mL and around 19 ng/mL. Yet in another the reference level of expression of PlGF is around 17 ng/mL.

The invention relates also to a method for treating a patient with a cancer with aflibercept, or ziv-aflibercept, comprising administering a therapeutically effective amount of aflibercept, or ziv-aflibercept to the patient, wherein the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF in the patient's biological sample is low relative to a reference level of expression of the biomarker.

Yet in another aspect the invention relates to a method for improving the progression- free survival (PFS) and/or the overall survival (OS) of a patient with a cancer, comprising administering a therapeutically effective amount of aflibercept, or ziv-aflibercept to the patient, wherein the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF in the patient's biological sample is low relative to a reference level of expression of the biomarker.

In an embodiment of one of the methods described above the biological sample is chosen from the group consisting of blood, serum and plasma.

In an embodiment of one of the methods described above the cancer is a colon cancer, a colorectal cancer or a rectal cancer.

In a further embodiment of one of the the colorectal cancer is a metastatic colorectal cancer.

In another embodiment of the invention, the subject is treated with aflibercept and further undergoes a chemotherapeutic treatment with oxaliplatin, 5-fluorouracil (5-FU) and folinic acid (i.e. the FOLFOX treatment), folinic acid, 5-fluorouracil and irinocetan (i.e. the FOLFIRI treatment), or 5-fluorouracil and folinic acid (i.e. the FUFOL or LV5FU2 treatment).

The chemotherapeutic treatment may combine at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or at most 10, 9, 8, 7, 6, 5, 4, 3, 2 agents, such as e.g. a combination of oxaliplatin, 5-fluorouracil (5-FU) and folinic acid (i.e. the FOLFOX treatment or the modified FOLFOX6 treatment as described in the example below), a combination of folinic acid, 5-fluorouracil and irinocetan (i.e. the FOLFIRI treatment), or a combination of 5-fluorouracil and folinic acid (i.e. the FUFOL or LV5FU2 treatment).

In this regard the application WO2012146610 relates to a method of treatment of the mCRC by aflibercept, or ziv-aflibercept in combination with FOLFIRI. The content of this application is incorporated by reference.

In an embodiment of one of the methods described above therapeutically effective amounts of aflibercept, or ziv-aflibercept, oxaliplatin, 5-fluorouracil (5-FU) and folinic acid are administered to said patient.

In an embodiment of one of the methods described above therapeutically effective amounts of aflibercept, or ziv-aflibercept, folinic acid, 5- fluorouracil (5-FU) and irinocetan are administered to said patient.

In a further embodiment of one of the methods described above folinic acid at a dosage comprised between about 200 mg/m^2 and about 600 mg/m^2, 5-fluorouracil (5-FU)

at a dosage comprised between about 2000 mg/m^2 and about 4000 mg/m^2, irinocetan at a dosage comprised between about 100 mg/m^2 and about 300 mg/m^2 and aflibercept at a dosage comprised between about 1 mg/kg and about 10 mg/kg are administered to patient.

In a further embodiment of one of the methods described above folinic acid at a dosage of about 400 mg/m^2, 5-fluorouracil (5-FU) at a dosage of about 2800 mg/m^2, irinocetan at a dosage of about 180 mg/m^2 and aflibercept at a dosage of about 4 mg/kg are administered to patient.

In a further embodiment of one of the methods described above folinic acid is administered intravenously at a dosage of about 400 mg/m^2, 5-fluorouracil (5-FU) is administered intravenously at a dosage of about 2800 mg/m^2, irinocetan is administered intravenously at a dosage of about 180 mg/m^2 and aflibercept is administered intravenously at a dosage of about 4 mg/kg and wherein the combination is administered every two weeks.

In a further embodiment of one of the methods described above folinic acid, 5-fluorouracil (5-FU), irinocetan and aflibercept are administered intravenously every two weeks for a period comprised between 9 and 18 weeks.

In a further embodiment of one of the methods described above folinic acid is administered intravenously immediately after aflibercept administration. It can be also administered intravenously immediately after aflibercept administration over a period of about 2 hours.

In a further embodiment of one of the methods described above irinocetan is administered intravenously immediately after aflibercept administration. It can be also administered intravenously immediately after aflibercept administration over a period of about 90 minutes.

In a further embodiment of one of the methods described above 5-fluorouracil (5-FU) is administered immediately after aflibercept administration.

In a further embodiment of one of the methods described above a first quantity of 5- fluorouracil (5-FU) is administered intravenously immediately after aflibercept administration and a second quantity of 5-FU is administered intravenously after the first quantity in continous infusion.

In a further embodiment of one of the methods described above about 400 mg/m^2 of 5- fluorouracil (5-FU) is administered intravenously over a period of 2 to 4 minutes after aflibercept administration and wherein 2400 mg/m^2 of 5-FU is administered intravenously over around 46 hours after the administration of the 400 mg/m^2 in continous infusion.

In an embodiment said patient has previously been treated with therapy based on oxaliplatin or on bevacizumab.

In another embodiment said patient has failed with chemotherapy, radiotherapy or surgery.

The invention relates also to aflibercept, or ziv-aflibercept for use in treating a patient suspected to suffer from cancer, wherein the patient has been identified as having lower level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF in biological sample as compared to the reference level of expression of the biomarker.

The invention further relates to a kit for predicting whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy, which kit comprises:

a) means for measuring the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF; and
b) optionally, a label giving instructions for the use of said kit in predicting whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy.

Another aspect of the invention further relates to an article of manufacture comprising:

a) a packaging material;
b) means for measuring the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF; and
c) a label giving instructions for the use of said kit in predicting whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy.

The above methods and use of the invention may be, for instance, in vitro or ex vivo methods and use.

Means for measuring the expression level of VCAM-1, ICAM-1 and PlGF protein are well-known in the art and include immunoassay such as ELISA assay. For instance the means for measuring VCAM-1 include antibodies specifically binding to VCAM-1.

The level of VCAM-1 protein may be, for instance, determined using immunological detection methods such as an ELISA assay. The methods involve an antibody which binds to VCAM-1 protein, for example a monoclonal or polyclonal antibody, an antibody variant or fragments such as a single chain antibody, a diabody, a minibody, a single chain Fv fragment (sc(Fv)), a Sc(Fv)2 antibody, a Fab fragment or a F(ab')2 fragment, or a single domain antibody. Such antibodies are well known in the art and are commercially

available. They may also notably be obtained by immunization of animals (for example rabbits, rats or mice) with VCAM-1 protein. Antibodies may be used to determine protein expression in a range of immunological assays including competitive and non-competitive assay systems using techniques such as western blotting, immunohistochemistry/ immunofluorescence (i.e protein detection on fixed cells or tissues), radioimmunoassay such as RIA (radio-linked immunoassay), ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, e.g. FIA (fluorescence-linked immunoassay), chemiluminescence immunoassays, ECLIA (electrochemiluminescence immunoassay) and protein A immunoassays. Such assays are routine and well known to the person skilled in the art (Ausubel *et al* (1994) *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York).

Protein expression of VCAM-1 may be determined by proteomic method such as mass spectrometry assays (LC-MS or LC-MS/MS). Qualitative and quantitative mass spectrometric techniques are known and used in the art. To this aim, target peptides specific for marker proteins are selected and quantified based on calibration curves established with synthetic peptides labeled with stable isotopes. Enzymatic digests, spiked with a defined amount of isotope labeled target peptides, are analyzed by liquid chromatography coupled with mass spectrometry. The ratio between labeled and non-labeled target peptides is measured to assess target peptide concentrations and therefore protein marker concentration.

The means for measuring the expression level of VCAM-1 may also include reagents such as e.g. reaction and/or washing buffers. The means may be present, e.g., in vials or microtiter plates, or be attached to a solid support such as a microarray as can be the case for primers and probes.

Similar means are at the disposal of the man skilled in the art for detecting ICAM-1 and PlGF.

In an embodiment VCAM-1, ICAM-1 and PlGF proteins can be measured with a bead-based multiplex assay, the Luminex™ technology.

Aflibercept, or ziv-aflibercept is provided in a formulation which is not prejudicial to the patient to be treated.

In an embodiment aflibercept, or ziv-aflibercept is *provided in a formulation* with sucrose and polysorbate 20 (stabilisers), sodium chloride, citrate buffer, and sodium phosphate buffer, adjusted to final pH.

In another embodiment aflibercept, or ziv-aflibercept, is supplied in two drug product presentations:

- a presentation at 100 mg aflibercept, or ziv-aflibercept / 4.0 mL (nominal concentration).
- a second presentation at 200 mg aflibercept, or ziv-aflibercept / 8.0 mL (nominal concentration).

Both presentations are manufactured from the same bulk sterile solution at 25 mg/mL of aflibercept, or ziv-aflibercept.

Prior to infusion to the patient, the concentrate solution is diluted with 0.9% sodium chloride solution or 5% dextrose.

The anti-cancer agents used in the above recited method or use are provided in a pharmaceutically acceptable carrier, excipient or diluent which is not prejudicial to the patient to be treated.

Pharmaceutically acceptable carriers and excipient that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminium stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-a-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

As appreciated by skilled artisans, compositions are suitably formulated to be compatible with the intended route of administration. Examples of suitable routes of administration include parenteral route, including for instance intramuscular, subcutaneous, intravenous, intraperitoneal or local intratumoral injections. The oral route can also be used, provided that the composition is in a form suitable for oral administration, able to protect the active principle from the gastric and intestinal enzymes.

The term "effective amount" refers to an amount of a drug alone or in combination with other drug or treatment regimen effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some

extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in vivo can, for example, be measured by assessing the duration of survival, duration of progression free survival (PFS), the response rates (RR), duration of response, and/or quality of life.

The terms “Therapy”, “therapeutic”, “treatment” and “treating” are used herein to characterize a therapeutic method or process that is aimed at (1) slowing down or stopping the progression, aggravation, or deterioration of the symptoms of the disease state or condition to which such term applies; (2) alleviating or bringing about ameliorations of the symptoms of the disease state or condition to which such term applies; and/or (3) reversing or curing the disease state or condition to which such term applies.

The term "overall survival (OS)" refers to the length of time during and after treatment the patient survives. As the skilled person will appreciate, a patient's overall survival is improved or enhanced, if the patient belongs to a subgroup of patients that has a statistically significant longer mean survival time as compared to another subgroup of patients.

The term "progression- free survival (PFS)" refers to the length of time during and after treatment during which, according to the assessment of the treating physician or investigator, the patient's disease does not become worse, i.e., does not progress. As the skilled person will appreciate, a patient's progression- free survival is improved or enhanced if the patient belongs to a subgroup of patients that has a longer length of time during which the disease does not progress as compared to the average or mean progression free survival time of a control group of similarly situated patients.

A “subject” or a “patient” may be a human or a non-human mammal, such as monkeys, dogs, cats, guinea pigs, hamsters, rabbits, cows, horses, goats and sheep.

The term "reference level" herein refers to a predetermined value. As the skilled artisan will appreciate the reference level is predetermined and set to meet the requirements in terms of e.g. specificity and/or sensitivity. These requirements can vary, e.g. from regulatory body to regulatory body. It may for example be that assay sensitivity or specificity, respectively, has to be set to certain limits, e.g. 80%, 90% or 95%. These

requirements may also be defined in terms of positive or negative predictive values. Nonetheless, based on the teaching given in the present invention it will always be possible to arrive at the reference level meeting those requirements. In one embodiment the reference level is determined in healthy individuals. The reference value in one embodiment has been predetermined in the disease entity to which the patient belongs. In certain embodiments the reference level can e.g. be set to any percentage between 25% and 75% of the overall distribution of the values in a disease entity investigated. In other embodiments the reference level can e.g. be set to the median, tertiles or quartiles as determined from the overall distribution of the values in a disease entity investigated. In one embodiment the reference level is set to the median value as determined from the overall distribution of the values in a disease entity investigated.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents Kaplan-Meier curves for PFS endpoint for sensitive and non-sensitive populations defined with VCAM-1.

Figure 2 represents Kaplan-Meier curves for OS endpoint for sensitive and non-sensitive populations defined with VCAM-1.

Figure 3 represents Kaplan-Meier curves for PFS endpoins for sensitive and non-sensitive populations defined with ICAM-1.

Figure 4 represents Kaplan-Meier curves for OS endpoint for sensitive and non-sensitive populations defined with ICAM-1.

Figure 5 represents Kaplan-Meier curves for PFS endpoint for sensitive and non-sensitive populations defined with VCAM-1 and ICAM-1.

Figure 6 represents Kaplan-Meier curves for OS endpoint for sensitive and non-sensitive populations defined with VCAM-1 and ICAM-1.

Figure 7 represents Kaplan-Meier curves for PFS endpoint for sensitive and non-sensitive populations defined with PlGF.

EXAMPLE: Predictive effect of VCAM-1, ICAM-1 and/or PlGF on PFS in the AFLAME study

STUDY EFC11338 (AFLAME)

EFC11338 was designed as a Multinational, Randomized, Double-Blind Study of Aflibercept Versus Placebo with Irinotecan/5-FU Combination (FOLFIRI) in Patients with Metastatic Colorectal Cancer (MCRC) After Failure of an Oxaliplatin Based Regimen.

Objectives:

To identify potential predictive biomarkers for response to treatment on efficacy endpoints (Progression Free Survival (PFS), Overall Survival (OS) and Overall Response Rate (ORR))

To identify potential prognostic biomarkers for efficacy endpoints (PFS, OS and ORR)

To identify potential correlation between biomarkers and other baseline characteristics

To identify potential correlation of longitudinal plasma measurements with clinical endpoints

To identify potential groups of homogeneous individuals based on their molecular profiles in an unsupervised way and to estimate correlation with clinical outcomes (PFS, OS and ORR)

To assess the safety profile of the population identified by prognostic/predictive biomarkers

Dosage and schedule of administration:

Patients were administered either aflibercept or placebo, depending on arm assigned. Immediately after, patients received irinotecan, 5-FU and leucovorin (FOLFIRI regimen). This treatment was repeated every 2 weeks.

Aflibercept/placebo

Arm A, aflibercept: 4 mg/kg was administered IV over 1 hour on Day 1, every 2 weeks,

OR

Arm B, placebo: 4 mg/kg was administered IV over 1 hour on Day 1, every 2 weeks.

FOLFIRI regimen

Immediately after aflibercept/placebo administration, all the patients received:

- Irinotecan 180 mg/m2 IV infusion in 500 mL D5W over 90 minutes and dl leucovorin* 400 mg/m2 IV infusion over 2 hours, at the same time, in bags using a Y-line, followed by:
- 5-FU 400 mg/m2 IV bolus given over 2-4 minutes, followed by:
- 5-FU 2400 mg/m2 continuous IV infusion in 500 mL D5W (recommended) over 46-hours.

Duration of treatment:

Patient was treated until disease progression, unacceptable toxicity or patient's refusal

Duration of observation:

Patients were followed when on study treatment and during follow up period until death or the study cut-off date for OS, whichever comes first. The cut-off date for OS is one year after the last patient enrolled.

Number of subjects:

Intent-to-Treat (ITT) population: 332 (109 in the placebo group + 223 in the aflibercept group)

Evaluable population for response rate: 295 (96 in the placebo group + 199 in the aflibercept group)

Evaluable for Luminex biomarkers – Group 1 biomarkers: 295 (99 in the placebo group + 196 in the aflibercept group)

Evaluable for Luminex biomarkers – Group 2 biomarkers: 108 (37 in the placebo group + 71 in the aflibercept group)

Evaluable for ELISA biomarkers: 329 (107 in the placebo group + 222 in the aflibercept group)

Due to an operational error, some patients randomized to the aflibercept arm have received placebo for one or several treatment cycles; or vice versa. A total of 198 patients received at least one misallocated treatment kit.

Criteria for evaluation:

Biomarkers:

Different types of biomarker data were investigated in the current translational research proposal in the AFLAME study:

- 107 plasma angiogenic factors and inflammatory cytokines measured at baseline, during and after treatment (end of cycle 1 infusion, 48h post aflibercept/placebo on cycle 2 or 3, 30 days after last aflibercept/placebo administration) with Luminex® technology
- Free VEGF-A and PlGF measured in plasma samples at baseline with ELISA (Enzyme-Linked Immunosorbent Assay) technology

PREPARATION OF SAMPLES AND ANALYSIS

Plasma biomarkers were measured either with Luminex™ technology (bead-based multiplex assay) or with ELISA.

Plasma biomarkers measured with Luminex™ technology

Plasma angiogenic factors and inflammatory cytokines have been measured at baseline pre cycle 1.

Thirty proteins measured on all the samples were defined as Group 1 biomarkers and 77 proteins measured only for some samples were defined as Group 2 biomarkers. The biomarkers from Group 1 were angiogenic/inflammatory molecules and have been selected based on aflibercept mechanism of action (inhibiting 3 angiogenic factors and their receptors), key candidate biomarkers identified on independent aflibercept studies or literature/experts.

Plasma biomarkers measured with ELISA

In addition to plasma biomarkers measured with Luminex™ technology, free VEGF-A and free PlGF concentrations of baseline plasma samples were measured with ELISA technology.

RESULTS

Univariate analysis

Biomarkers have been tested for predictive and prognostic effects for PFS.

VCAM-1 and ICAM-1 have been identified as potentially predictive with corrected Benjamini-Hochberg (BH) p-value ≤ 0.2 (295 subjects).

Then sensitive and non-sensitive populations have been defined using VCAM-1 and ICAM-1 (BH p-value ≤ 0.2).

PlGF has been identified as potentially predictive by ELISA (unadjusted p-value = 0.075).

Identification of sensitive and non-sensitive populations with VCAM-1

The threshold of 6.32 corresponding to 553 ng/mL has been determined to define sensitive (197 individuals) and non-sensitive populations (98 individuals) respectively corresponding to individuals with low values for VCAM-1 and high values for VCAM-1.

Figure 1 and Figure 2 represent Kaplan-Meier curves for PFS and OS endpoints for sensitive and non-sensitive populations illustrating the better treatment effect for low VCAM-1 group compared to high VCAM-1 group. For OS, in addition to a better treatment effect compared to placebo, sensitive population showed globally a better prognostic (increased OS for low VCAM-1 group in placebo and aflibercept arms).

Table 1 shows the response rate for sensitive and non-sensitive populations by treatment group. There was an increased response rate (26%) for the aflibercept/folfiri treatment in sensitive population compared to the non-sensitive population (10%).

Table 1 - Response Rate for non-sensitive/sensitive populations defined with VCAM-1 for PFS - Preprocessed data - VCAM-1 and RR evaluable population

Population	Placebo/Folfiri	Aflibercept/Folfiri
Sensitive VCAM-1 <= 6.32 (N = 189)	3/68 (4%)	31/121 (26%)
Non-sensitive VCAM-1 > 6.32 (N = 94)	1/26 (4%)	7/68 (10%)
Total	4/94 (4%)	38/189 (20%)

Identification of sensitive and non-sensitive populations with ICAM-1

The threshold of 5.04 corresponding to 144 ng/mL has been determined to define sensitive (205 individuals) and non-sensitive populations (90 individuals) respectively corresponding to individuals with low values for ICAM-1 and high values for ICAM-1.

Figure 3 and Figure 4 represent Kaplan-Meier curves for PFS and OS endpoints for sensitive and non-sensitive populations illustrating the better treatment effect for low ICAM-1 group compared to high ICAM-1 group. For OS, in addition to a better treatment effect compared to placebo, sensitive population showed globally a better prognostic (increased OS for low ICAM-1 group in placebo and aflibercept arms).

Table 2 shows the response rate for sensitive and non-sensitive populations by treatment group. There has been an increased response rate (25%) for the aflibercept/folfiri treatment in sensitive population compared to the non-sensitive population (9%).

Table 2 - Response Rate for non-sensitive/sensitive populations defined with ICAM-1 for PFS - Preprocessed data - ICAM-1 and RR evaluable population

Population	Placebo/Folfiri	Aflibercept/Folfiri
Sensitive ICAM-1 <= 5.04 (N = 197)	3/63 (5%)	33/134 (25%)
Non-sensitive ICAM-1 > 5.04 (N = 86)	1/31 (3%)	5/55 (9%)
Total	4/94 (4%)	38/189 (20%)

Multivariate analysis

The multivariate predictive score $0.089 \times \mathrm{ICAM} - 1 + 0.17 \times \mathrm{VCAM} - 1$ has been dichotomized using quantile 10% to 90% as threshold.

Sensitive population showed a significant difference in PFS and in OS in favor of aflibercept over placebo (HR= 0.47 for PFS and HR = 0.66 for OS), which was increased compared to the non-sensitive population (HR= 0.98 for PFS and HR = 1.04 for OS).

Median PFS difference between aflibercept and placebo was equal to 2.59 months in sensitive population, showing a greater but moderate difference compared to non-sensitive population (0.5 month).

Median OS difference between aflibercept and placebo was equal to 3.75 months in sensitive population, showing a greater difference compared to non-sensitive population (-0.39 month). For non-sensitive population there was a decrease in median OS for placebo arm and treated arm (8.90 months and 8.51 months) compared to the other populations illustrating a potential prognostic effect of multivariate score in addition to the predictive effect.

In conclusion sensitive population showed a decreased HR compared to the non-sensitive population for PFS and OS with moderate gain in term of median.

Figure 5 and Figure 6 represent Kaplan-Meier curves for PFS and OS endpoints for sensitive and non-sensitive populations illustrating the better treatment effect for low score group compared to high score group. For OS, in addition to a better treatment effect compared to placebo, sensitive population showed globally a better prognostic (increased OS for low score group in placebo and aflibercept arms).

Identification of sensitive and non-sensitive populations with ***PIGF***

The threshold of 2.82 corresponding to 17 pg/ml has been determined to define sensitive (230 individuals) and non-sensitive populations (99 individuals) respectively corresponding to individuals with low values for PlGF and high values for PlGF.

Figure 7 which represents the Kaplan-Meier curves for PFS endpoint for sensitive and non-sensitive populations defined with PlGF illustrates the determination of the PlGF cut off for sensitive and non-sensitive populations.

CONCLUSIONS

- VCAM-1 and ICAM-1 have been identified as potentially predictive biomarkers for PFS in a univariate framework (unadjusted p-value equal to 0.00017 for VCAM-1 and 0.0043 for ICAM-1).
- The third biomarker that showed up to be potentially predictive was PlGF measured by ELISA (unadjusted p-value = 0.075).
- Linear combination of VCAM-1 and ICAM-1 has been identified as potentially predictive for PFS.

CLAIMS

1. Use of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF for predicting the outcome of the treatment with aflibercept, or ziv-aflibercept of a patient suspected to suffer from a cancer.

2. A method of determining whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy for the said cancer comprising the step of subjecting a patient's biological sample to at least one assay to measure at baseline the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF, wherein when the biomarker level in the biological sample is low relative to a reference level of expression of the biomarker, the patient is identified as a candidate for therapy for cancer.

3. A method of determining whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy for the said cancer comprising the step of subjecting a patient's biological sample to at least one assay to measure at baseline the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF, wherein when the biomarker level in the biological sample is high relative to a reference level of expression of the biomarker, the patient is identified as not being a candidate for therapy for cancer.

4. A method for treating a patient with a cancer with aflibercept, or ziv-aflibercept, comprising administering a therapeutically effective amount of aflibercept, or ziv-aflibercept to the patient, wherein the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGFin the patient's biological sample is lower than the reference level of expression of the biomarker.

5. A method for treating a patient with a cancer with aflibercept, or ziv-aflibercept, comprising administering a therapeutically effective amount of aflibercept, or ziv-aflibercept to the patient, wherein the VCAM-1 and ICAM-1 levels in the patient's biological sample is lower than the respective reference levels of expression VCAM-1 and ICAM-1.

6. A method of determining whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy for the said cancer comprising the step of subjecting a patient's biological sample to at least one assay to measure at

baseline the respective VCAM-1 and ICAM-1 levels, wherein when the biological sample VCAM-1 and ICAM-1 levels are low relative to the respective levels of expression of VCAM-1 and ICAM-1, the patient is identified as a candidate for therapy for cancer.

7. A method according to any of claims 2 to 6, wherein the relative to a reference level of expression of VCAM-1 is between around 406 and around 577 ng/mL.

8. A method according to any of claims 2 to 6, wherein the relative to a reference level of expression of VCAM-1 is around 553 ng/mL.

9. A method according to any of claims 2 to 6, wherein the relative to a reference level of expression of ICAM-1 is between around 92 and around 145 ng/mL .

10. A method according to any of claims 2 to 6, wherein the threshold on ICAM-1 to define sensitive and non-sensitive populations is around 144 ng/mL.

11. A method according to any of claims 2 to 6, wherein the relative to a reference level of expression of PlGF is between around 12 and around 19 pg/mL.

12. A method according to any of claims 2 to 6, wherein the relative to a reference level of expression of PlGF is around 17 pg/mL.

13. A method according to any of claims 4 to 14, wherein the biological sample is chosen from the group consisting of blood, serum and plasma.

14. A method according to any of claims 4 to 15, wherein the cancer is colon cancer, a colorectal cancer or a rectal cancer.

15. A method according to claim 16 wherein the colorectal cancer is a metastatic colorectal cancer.

16. A method according to any of claims 4 to 17, wherein the VCAM-1, ICAM-1 and/or PlGF level which is determined is the circulating level.

17. A method according to any of claims 4 to 18, said method comprising administering to said patient therapeutically effective amounts of aflibercept, or ziv-aflibercept, folinic acid, 5- fluorouracil (5-FU) and irinocetan.

18. Aflibercept, or ziv-aflibercept for use in treating a patient suspected to suffer from cancer, wherein the patient has been identified as having lower level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF in biological sample as compared to the reference level of expression of the biomarker.

19. A kit for predicting whether a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy, which kit comprises:

a) means for measuring the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF; and

b) optionally, a label giving instructions for the use of said kit in predicting whether a a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy.

20. An article of manufacture comprising:

a) a packaging material;

b) means for measuring the level of a biomarker selected from the group consisting of VCAM-1, ICAM-1 and PlGF; and

c) a label giving instructions for the use of said kit in predicting whether a a patient suspected to suffer from cancer is a candidate for aflibercept, or ziv-aflibercept therapy.

FIG.1

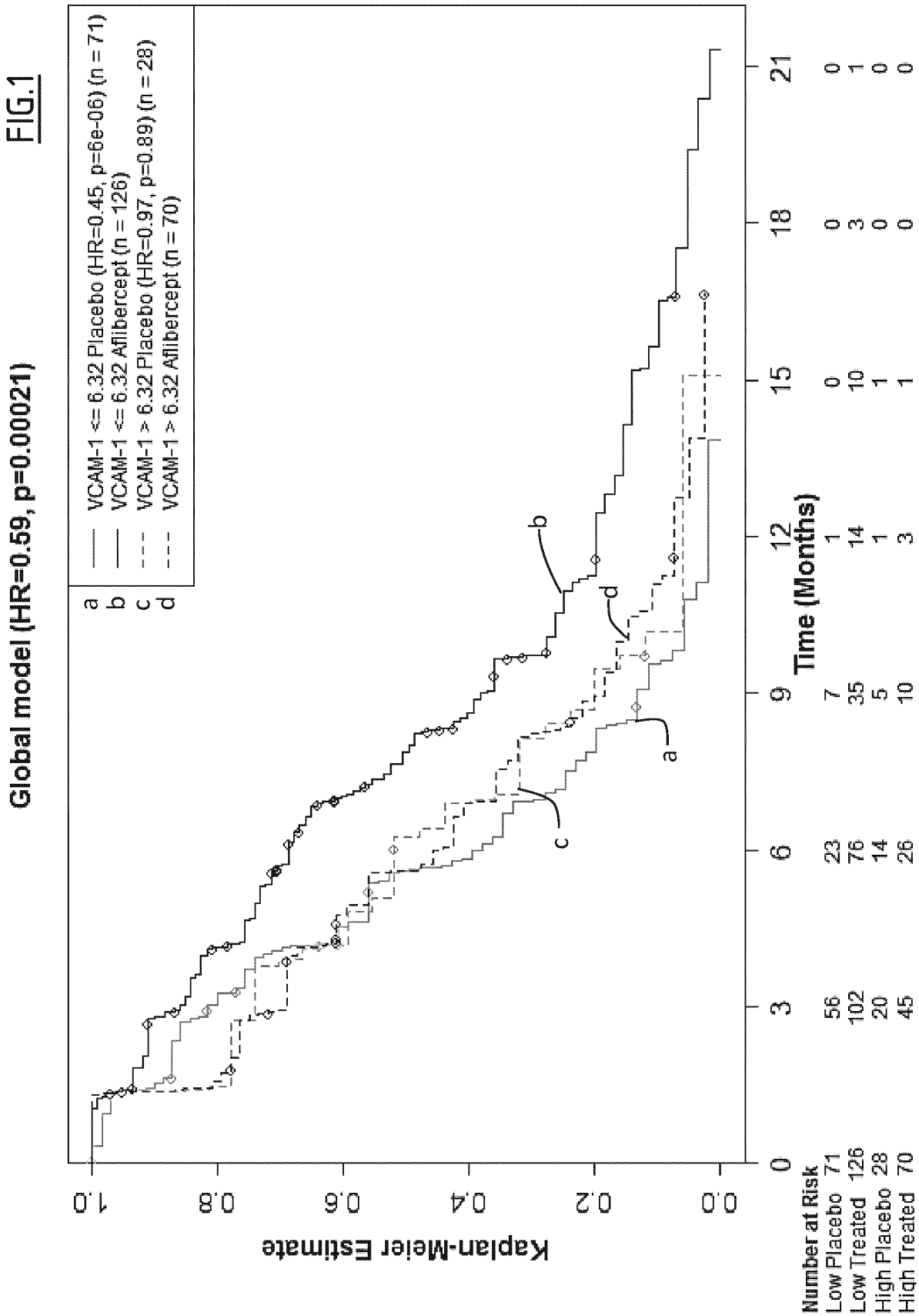

Global model (HR=0.59, p=0.00021)
a VCAM-1 <= 6.32 Placebo (HR=0.45, p=6e-06) (n = 71)
b VCAM-1 <= 6.32 Aflibercept (n = 126)
c VCAM-1 > 6.32 Placebo (HR=0.97, p=0.89) (n = 28)
d VCAM-1 > 6.32 Aflibercept (n = 70)
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21
Number at Risk
Low Placebo 71 56 23 7 1 0 0 0
Low Treated 126 102 76 35 14 10 3 1
High Placebo 28 20 14 5 1 1 0 0
High Treated 70 45 26 10 3 1 0 0

FIG.2

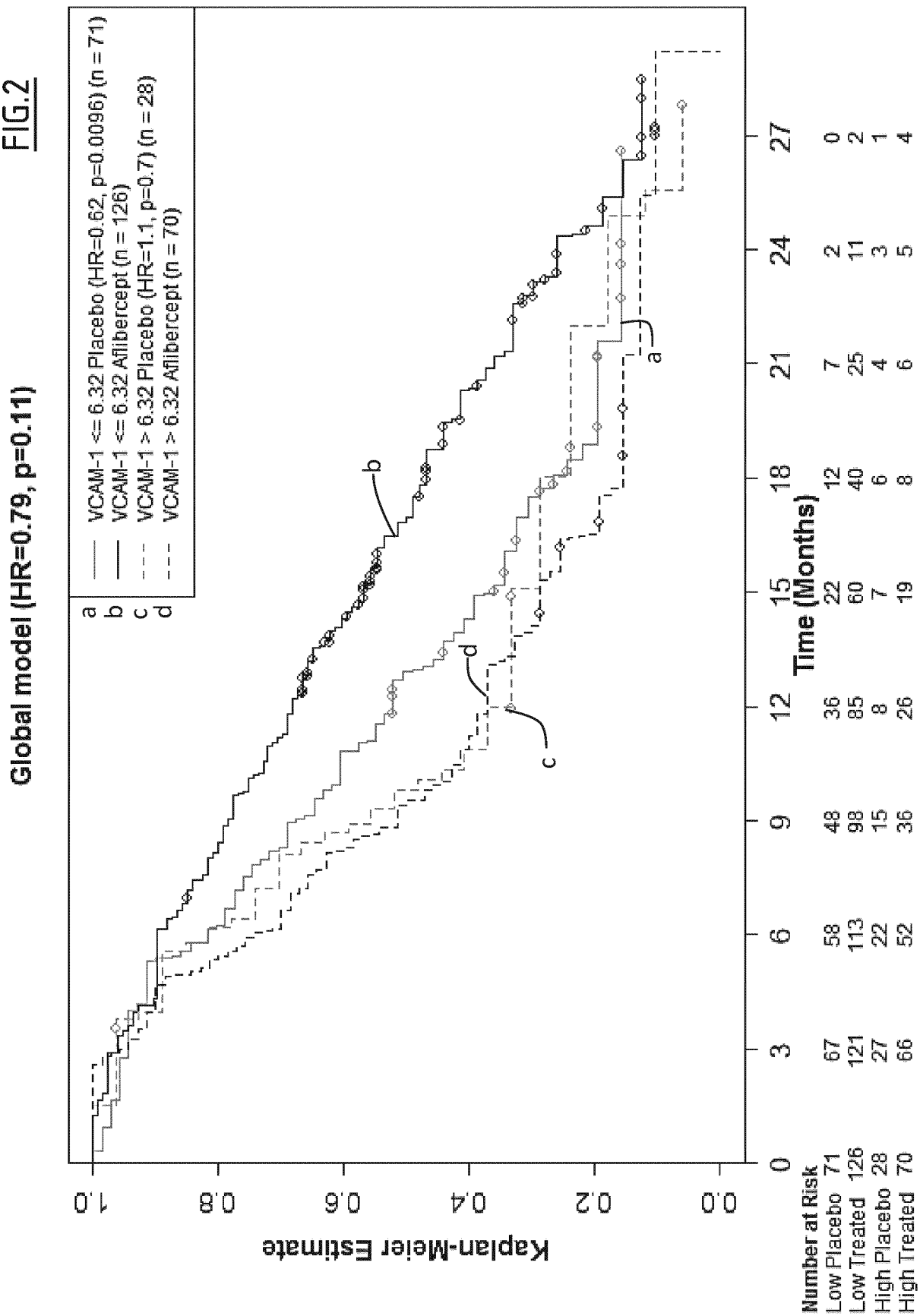

Global model (HR=0.79, p=0.11)
a VCAM-1 <= 6.32 Placebo (HR=0.62, p=0.0096) (n = 71)
b VCAM-1 <= 6.32 Aflibercept (n = 126)
c VCAM-1 > 6.32 Placebo (HR=1.1, p=0.7) (n = 28)
d VCAM-1 > 6.32 Aflibercept (n = 70)
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21 24 27
Number at Risk
Low Placebo 71 67 58 48 36 22 12 7 2 0
Low Treated 126 121 113 98 85 60 40 25 11 2
High Placebo 28 27 22 15 8 7 6 4 3 1
High Treated 70 66 52 36 26 19 8 6 5 4

FIG.3

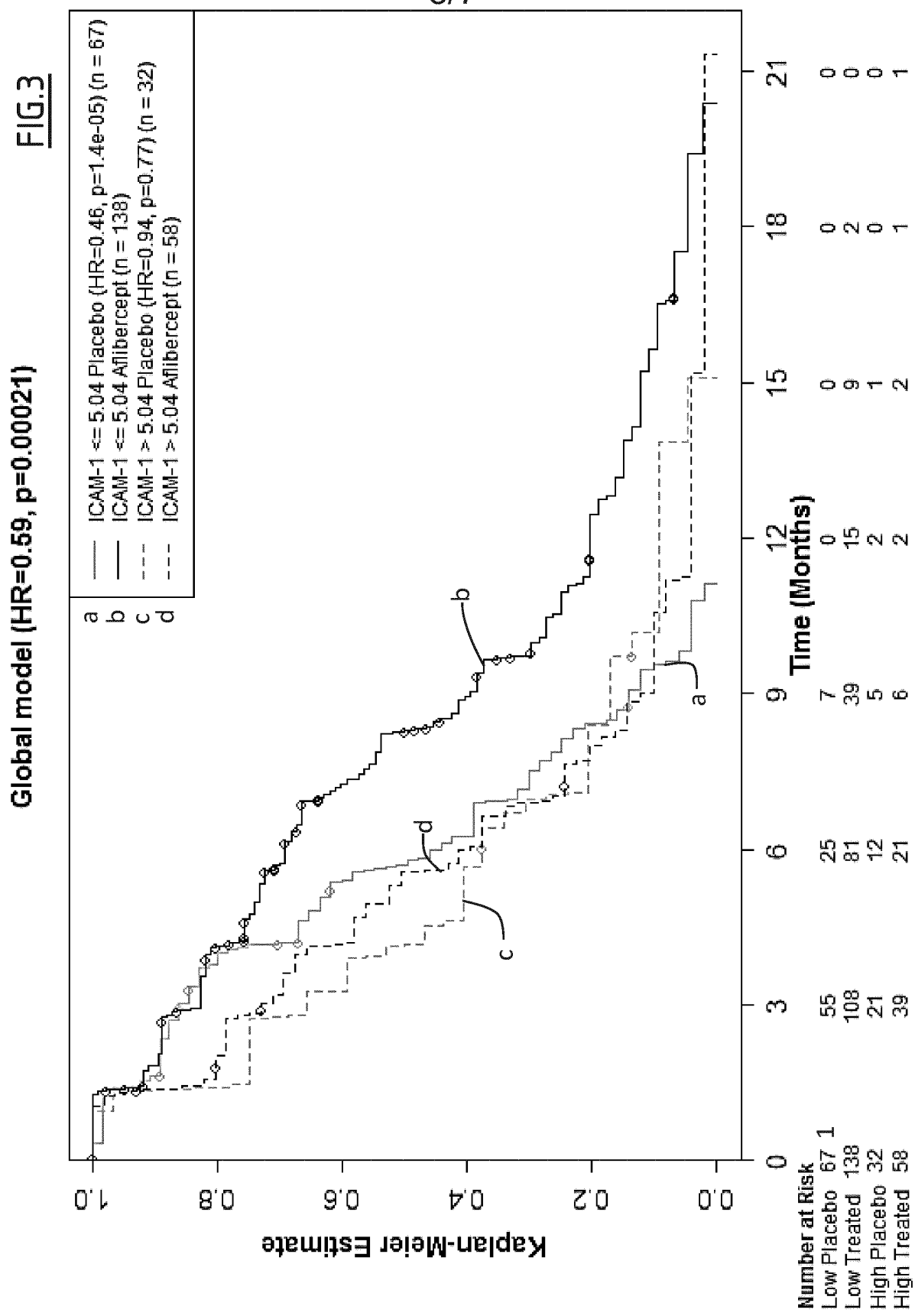

Global model (HR=0.59, p=0.00021)
a ICAM-1 <= 5.04 Placebo (HR=0.46, p=1.4e-05) (n = 67)
b ICAM-1 <= 5.04 Aflibercept (n = 138)
c ICAM-1 > 5.04 Placebo (HR=0.94, p=0.77) (n = 32)
d ICAM-1 > 5.04 Aflibercept (n = 58)
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21
Number at Risk
Low Placebo 67 55 25 7 0 0 0 0
Low Treated 138 108 81 39 15 9 2 0
High Placebo 32 21 12 5 2 1 0 0
High Treated 58 39 21 6 2 2 1 1

FIG.4

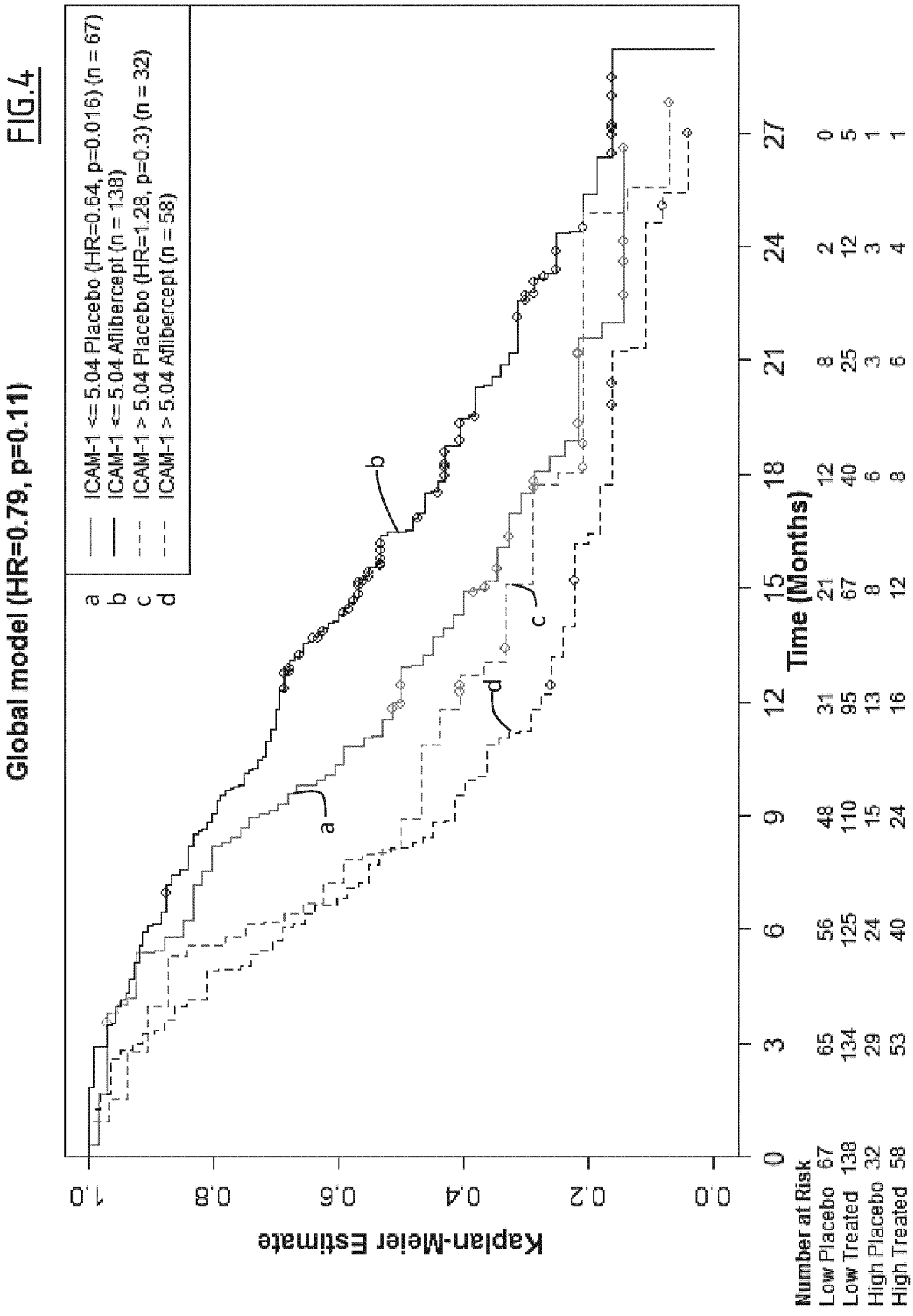

Global model (HR=0.79, p=0.11)
a ICAM-1 <= 5.04 Placebo (HR=0.64, p=0.016) (n = 67)
b ICAM-1 <= 5.04 Aflibercept (n = 138)
c ICAM-1 > 5.04 Placebo (HR=1.28, p=0.3) (n = 32)
d ICAM-1 > 5.04 Aflibercept (n = 58)
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21 24 27
Number at Risk
Low Placebo 67 65 56 48 31 21 12 8 2 0
Low Treated 138 134 125 110 95 67 40 25 12 5
High Placebo 32 29 24 15 13 8 6 3 3 1
High Treated 58 53 40 24 16 12 8 6 4 1

FIG.5

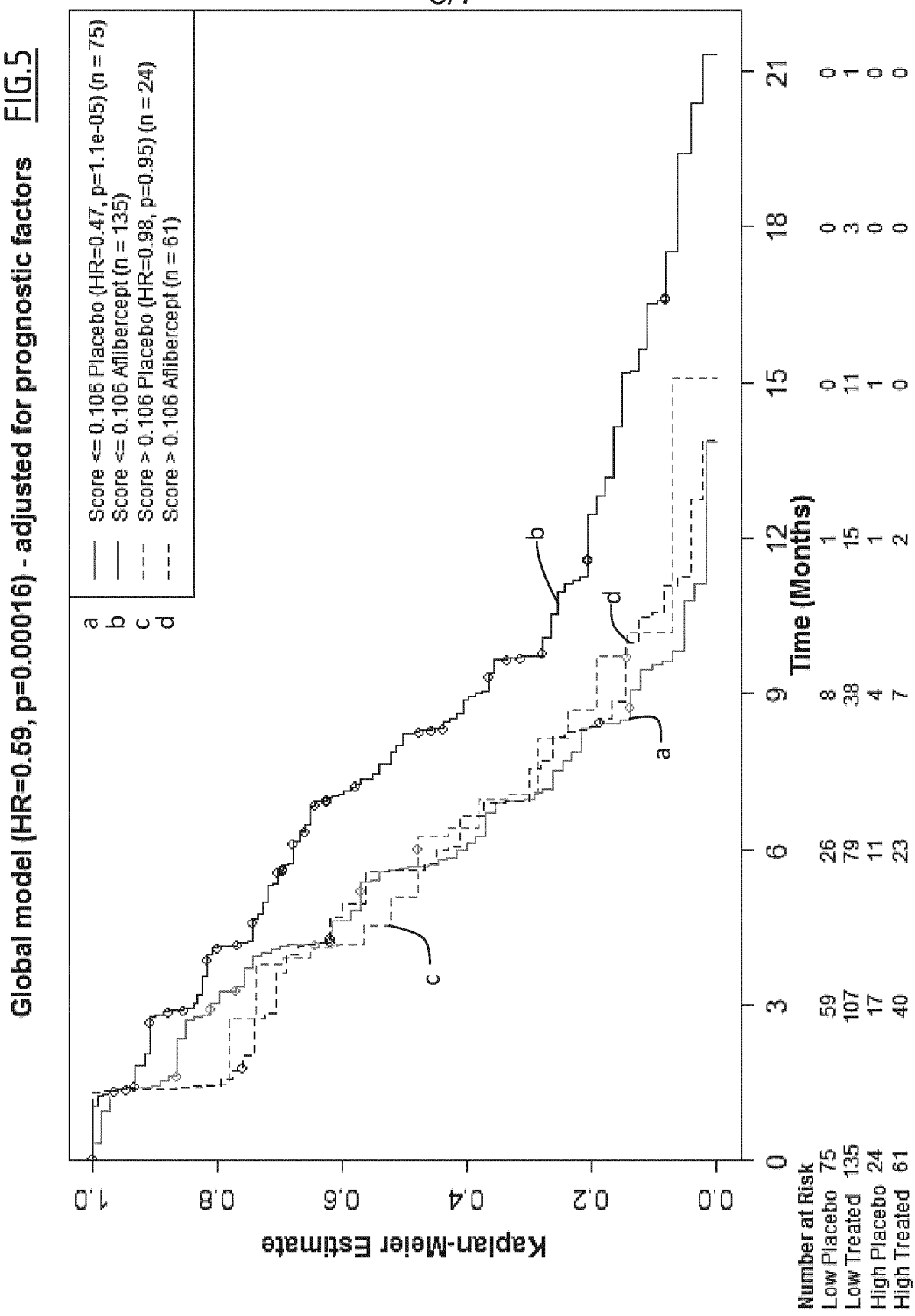

Global model (HR=0.59, p=0.00016) - adjusted for prognostic factors
Score <= 0.106 Placebo (HR=0.47, p=1.1e-05) (n = 75)
Score <= 0.106 Aflibercept (n = 135)
Score > 0.106 Placebo (HR=0.98, p=0.95) (n = 24)
Score > 0.106 Aflibercept (n = 61)
a
b
c
d
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21
Number at Risk
Low Placebo 75 59 26 8 1 0 0 0
Low Treated 135 107 79 38 15 11 3 1
High Placebo 24 17 11 4 1 1 0 0
High Treated 61 40 23 7 2 0 0 0

FIG.6

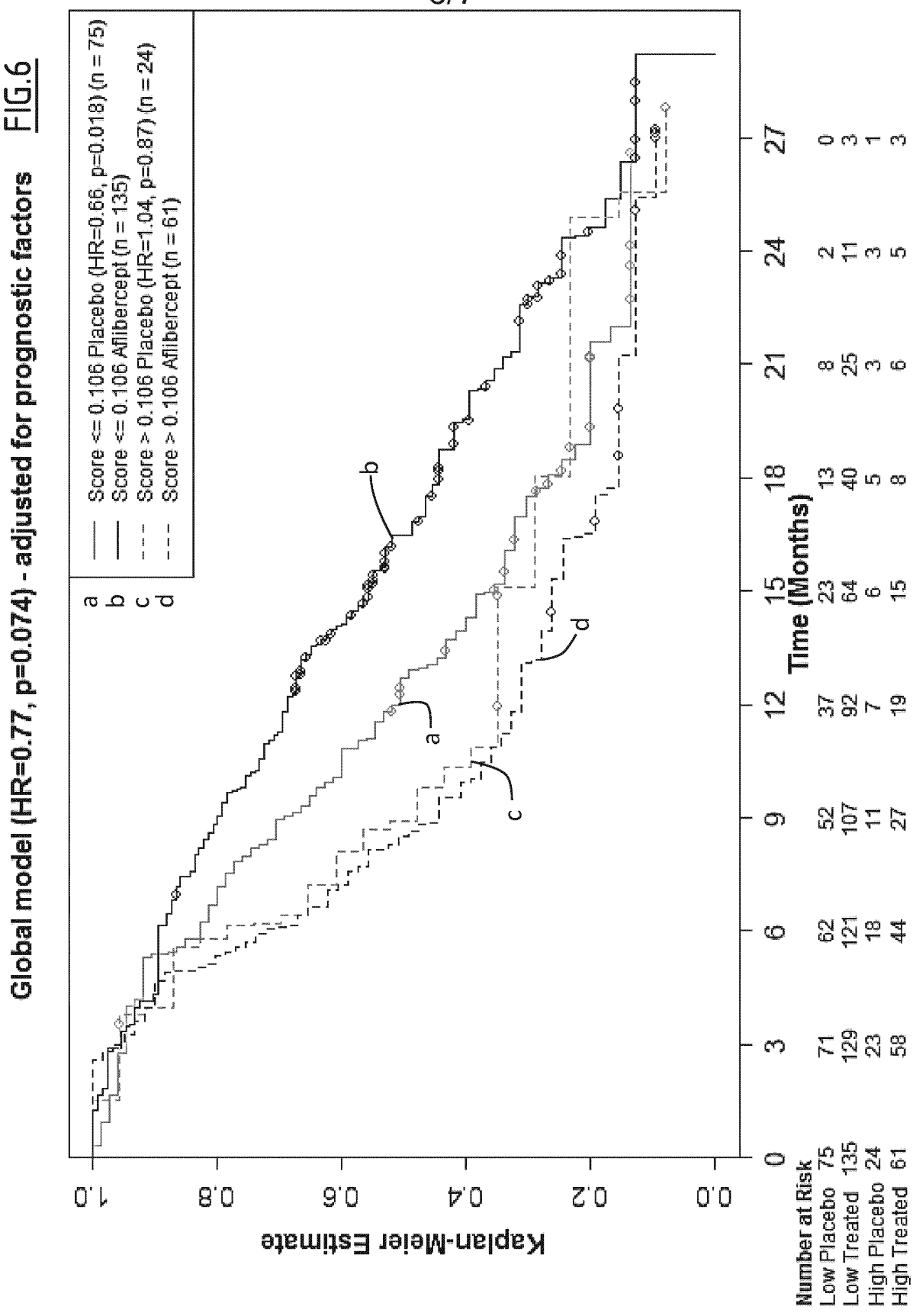
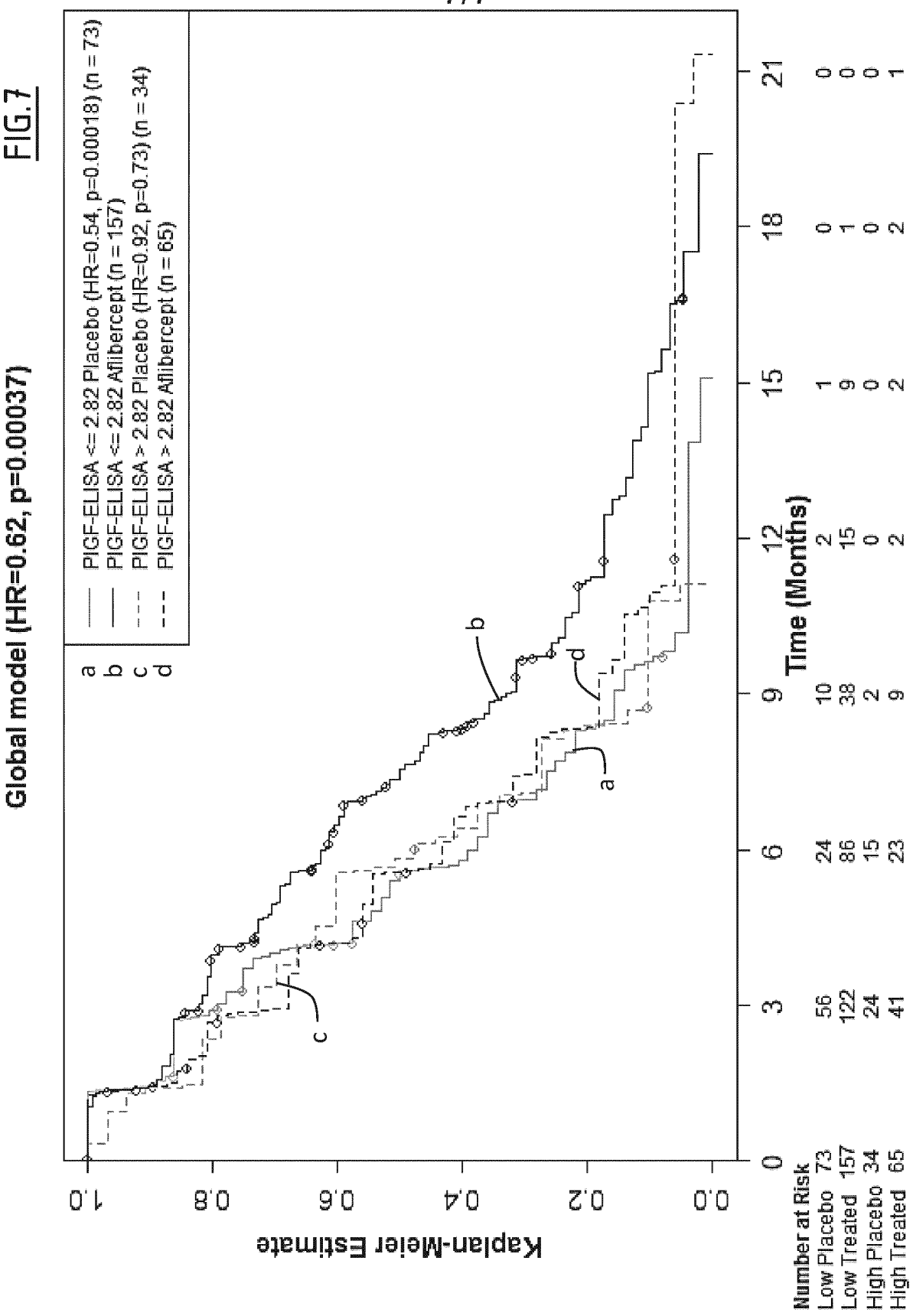

Global model (HR=0.77, p=0.074) - adjusted for prognostic factors
Score <= 0.106 Placebo (HR=0.66, p=0.018) (n = 75)
Score <= 0.106 Aflibercept (n = 135)
Score > 0.106 Placebo (HR=1.04, p=0.87) (n = 24)
Score > 0.106 Aflibercept (n = 61)
a
b
c
d
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21 24 27
Number at Risk
Low Placebo 75 71 62 52 37 23 13 8 2 0
Low Treated 135 129 121 107 92 64 40 25 11 3
High Placebo 24 23 18 11 7 6 5 3 3 1
High Treated 61 58 44 27 19 15 8 6 5 3

FIG.7


Global model (HR=0.62, p=0.00037)
a PIGF-ELISA <= 2.82 Placebo (HR=0.54, p=0.00018) (n = 73)
b PIGF-ELISA <= 2.82 Aflibercept (n = 157)
c PIGF-ELISA > 2.82 Placebo (HR=0.92, p=0.73) (n = 34)
d PIGF-ELISA > 2.82 Aflibercept (n = 65)
Kaplan-Meier Estimate
0.0
0.2
0.4
0.6
0.8
1.0
Time (Months)
0 3 6 9 12 15 18 21
Number at Risk
Low Placebo 73 56 24 10 2 1 0 0
Low Treated 157 122 86 38 15 9 1 0
High Placebo 34 24 15 2 0 0 0 0
High Treated 65 41 23 9 2 2 2 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/051363

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

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, BIOSIS, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DIETHER LAMBRECHTS ET AL: "Evaluation of efficacy and safety markers in a phase II study of metastatic colorectal cancer treated with aflibercept in the first-line setting", BRITISH JOURNAL OF CANCER, vol. 113, no. 7, 10 September 2015 (2015-09-10), pages 1027-1034, XP055283970, GB ISSN: 0007-0920, DOI: 10.1038/bjc.2015.329 the whole document In particular: Title; Abstract; Materials and methods section; Figure 1. ----- -/--	1-20

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

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2017	19/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	C.F. Angioni

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/008975 A1 (SANOFI SA [FR]) 21 January 2016 (2016-01-21) the whole document In particular the claims.	1-20
X	US 6 749 853 B1 (THORPE PHILIP E [US] ET AL) 15 June 2004 (2004-06-15)	19,20
A	the whole document In particular: Claims 6, 14, 15 and 27.	1-18
Y	J. BAAR ET AL: "A Vasculature-Targeting Regimen of Preoperative Docetaxel with or without Bevacizumab for Locally Advanced Breast Cancer: Impact on Angiogenic Biomarkers", CLINICAL CANCER RESEARCH, vol. 15, no. 10, 5 May 2009 (2009-05-05), pages 3583-3590, XP055284319, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-08-2917 the whole document In particular: Title; Abstract; Patients and methods section.	1-20
Y	MARKUS WEHLAND ET AL: "Biomarkers for Anti-Angiogenic Therapy in Cancer", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 2012 MDPI AG CHE, vol. 14, no. 5, 1 January 2013 (2013-01-01), pages 9338-9364, XP055283946, ISSN: 1661-6596, DOI: 10.3390/ijms14059338 the whole document In particular: Title; Abstract; pp. 9340-9342, sections 2 and 3.1; pp. 9344-9345, bridging paragraph.	1-20
X	J. F. DE GROOT ET AL: "Myeloid Biomarkers Associated with Glioblastoma Response to Anti-VEGF Therapy with Aflibercept", CLINICAL CANCER RESEARCH, vol. 17, no. 14, 1 June 2011 (2011-06-01), pages 4872-4881, XP55367887, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-11-0271 the whole document In particular: Title; Abstract; Materials and methods section; Page 4875, first paragraph; Figure 1c; Discussion, 3rd to 5th paragraphs.	1-20

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. CHIRON ET AL: "Differential Antitumor Activity of Aflibercept and Bevacizumab in Patient-Derived Xenograft Models of Colorectal Cancer", MOLECULAR CANCER THERAPEUTICS, vol. 13, no. 6, 31 March 2014 (2014-03-31) , pages 1636-1644, XP55367889, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.MCT-13-0753 the whole document -----	1-20
A	CLAUS ZEHETNER ET AL: "Systemic Counterregulatory Response of Placental Growth Factor Levels to Intravitreal Aflibercept Therapy", INVESTIGATIVE OPTHALMOLOGY & VISUAL SCIENCE, vol. 56, no. 5, 21 May 2015 (2015-05-21), pages 3279-3286, XP55367860, US ISSN: 1552-5783, DOI: 10.1167/iovs.15-16686 the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/051363

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20(partially)

   Use, methods and kits for predicting the outcome of aflibercept/ziv-aflibercept treatment, wherein the biomarker is a cellular adhesion molecule such as VCAM-1 and/or ICAM-1.

   ---

2. claims: 1-20(partially)

   Use, methods and kits for predicting the outcome of aflibercept/ziv-aflibercept treatment, wherein the biomarker is PIGF.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/051363

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016008975 A1	21-01-2016	AU 2015289124 A1	09-02-2017
		EP 3170005 A1	24-05-2017
		KR 20170030631 A	17-03-2017
		SG 11201700284U A	27-02-2017
		TW 201619608 A	01-06-2016
		WO 2016008975 A1	21-01-2016
US 6749853 B1	15-06-2004	US 6749853 B1	15-06-2004
		US 2003219441 A1	27-11-2003