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(54) Title: USE OF VEGFR1 AS A BIOMARKER FOR PKN3 INHIBITOR ADMINISTRATION

(57) Abstract: The present invention is related to the use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in a
method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

USE OF VEGFR1 AS A BIOMARKER FOR PKN3 INHIBITOR ADMINISTRATION

The present invention is related to the use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker, a method for deciding whether a subject is a responder to a method of treatment, a method for deciding whether a subject having undergone a first method of treatment, wherein the first method of treatment comprises administering to the subject a PKN3 inhibitor, shall be subject to a second method of treatment, wherein the second method of treatment comprises administering to the subject a PKN3 inhibitor, a method for deciding whether a subject shall undergo a method of treatment, whereby the method of treatment comprises the administration of a PKN3 inhibitor, and a PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease.

Oncogenesis was described by Foulds (Foulds, L. (1958), *J Chronic Dis*, 8, 2-37) as a multistep biological process, which is presently known to occur by the accumulation of genetic damage. On a molecular level, the multistep process of tumorigenesis involves the disruption of both positive and negative regulatory effectors (Weinberg, R.A. (1989); Cancer Res, 49, 3713-3721). The molecular basis for human colon carcinomas has been postulated, by Vogelstein and coworkers (Fearon, E.R. and Vogelstein, B. (1990), Cell, 61, 759-767), to involve a number of oncogenes, tumor suppressor genes and repair genes. Similarly, defects leading to the development of retinoblastoma have been linked to another tumor suppressor gene (Lee, W.H., et al. (1987) Science, 235, 1394-1399). Still other oncogenes and tumor suppressors have been identified in a variety of other malignancies. Unfortunately, there remains an inadequate number of treatable cancers, and the effects of cancer are catastrophic - over half a million deaths per year in the United States alone.

Cancer is fundamentally a genetic disease in which damage to cellular DNA leads to disruption of the normal mechanisms that control cellular proliferation. Two of the mechanisms of action by which tumor suppressors maintain genomic integrity is by cell arrest, thereby allowing for repair of damaged DNA, or removal of the damaged DNA by apoptosis (Ellisen, L.W. and Haber, D.A. (1998), Annu Rev Med, 49, 425-436.). Apoptosis, otherwise called "programmed cell death," is a carefully regulated network of biochemical

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events which act as a cellular suicide program aimed at removing irreversibly damaged cells. Apoptosis can be triggered in a number of ways including binding of tumor necrosis factor, DNA damage, withdrawal of growth factors, and antibody cross-linking of Fas receptors. Although several genes have been identified that play a role in the apoptotic process, the pathways leading to apoptosis have not been fully elucidated. Many investigators have attempted to identify novel apoptosis-promoting genes with the objective that such genes would afford a means to induce apoptosis selectively in neoplastic cells to treat lancer in a patient.

An alternative approach to treating cancer involves the suppression of angiogenesis with an agent such as Endostatin^{TM} or anti-VEGF antibodies. In this approach, the objective is to prevent further vascularization of the primary tumor and potentially to constrain the size of metastatic lesions to that which can support neoplastic cell survival without substantial vascular growth.

A particular group of cancer diseases are those cancer diseases which are aggressive in terms of growth rate of the tumor, invasion into normal tissue, resistance to chemotherapy or other conventional treatments and the formation of metastasis throughout the body. In the case of more aggressive cancer, the cancer tissue is more different from the normal tissue and the tumor is more likely to spread. Therefore one objective in current cancer research is to develop agents which are inhibiting tumor growth and/or reducing the spreading of cancer cells throughout the body.

Definitions for what is an aggressive cancer disease may be taken from the homepage of the National Cancer Institute which is http://www.cancer.gov/Templates/db_alpha.aspx?CdrID=46053. Also, for the description of the aggressivity of a cancer disease, typically grading is used which is a system for classifying cancer cells in terms of how abnormal they appear when examined under a microscope. The objective of a grading system is to provide information about the probable growth rate of the tumor and its tendency to spread. The systems used to grade tumors vary with each type of cancer. Grading plays a role in treatment decisions.

Such grading systems are known to the ones skilled in the art. One of them is the Gleason score which is a system of grading prostate cancer tissue based on how it looks under a microscope. Gleason scores range from 2 to 10 and indicate how likely it is that a tumor will spread. A low Gleason score means the cancer tissue is similar to normal prostate tissue and the tumor is less likely to spread; a high Gleason score means the cancer tissue is very different from normal and the tumor is more likely to spread.

PKN3 which is also referred to as protein kinase N beta or PKN beta has been, among others, described by Oishi K, et al. (Oishi K. et al. *Biochem. Biophys. Res. Commun.* **261** (3): 808–14) or Shibata, H et al. (Shibata, H. et al.; *J. Biochem.* (Japan) **130** (1): 23–31). PKN3 has also been identified as a target molecule in tumors, in particular solid tumors, cancers, in particualr metastatic cancers, and in pre-eclampsia. Also, as described in international patent application WO 2004/019973 protein kinase N beta is a downstream target of the PI-3 kinase/PTEN pathway which is linked to tumorigenesis and metastasis. Particularly the latter effect seems to be strongly related to the loss of suppressor function, more particularly PTEN tumour suppressor function.

The treatment of any disease is intended to provide relief or even cure to a patient while avoiding any undesired side effects or any adverse effects. In order to comply with this task there is a need for biomarkers which allow, in the broadest sense, to distinguish responders to a therapy from non-responders to such therapy. Furthermore, biomarkers are, for example, needed and useful as inclusion criterion for clinical trials, establishment or identification of optimum drug, dose and schedule of therapy and for identifcying patients at high risk for adverse effects.

In the light of the above, there is a need for identifying biomarkers which can be used in the context of diseases and more specifically of diseases which can be treated by administering to a patient suffering from or being at risk of suffering from a disease which can be treated by a PKN3 inhibitor. Insofar, one problem underlying the present invention is the identification of such biomarkers.

A further problem underlying the present invention is the provision of a medicament for a particular group of patients suffering from or being at risk of suffering from a diseases which

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can be treated by administering to such patients a PKN3 inhibitor, whereby preferably such particular group of patients comprises a very high percentage of responders to such therapy comprising administering to the patients a PKN3 inhibitor.

These and further problems are solved by the subject matter of the attached independent claims. Particularly preferred embodiments may be taken from the attached dependent claims.

The problem underlying the present invention is also solved in a first aspect which is also the first embodiment of the first aspect by the use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

In a second embodiment of the first aspect which is also an embodiment of the first embodiment of the first aspect, the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 is a or the biomarker.

The problem underlying the present invention is also solved in a second aspect which is also the first embodiment of the second aspect by the use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in designing a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

In a second embodiment of the second aspect which is also an embodiment of the first embodiment of the second aspect, the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 is a or the biomarker.

In a third embodiment of the first aspect which is also an embodiment of the first and the second embodiment of the first aspect, and in a third embodiment of the second aspect which is also an embodiment of the first and the second embodiment of the second aspect the VEGFR1 is selected from the group comprising VEGFR1 variant 2, VEGFR1 variant 1, VEGFR1 variant 3 and VEGFR1 variant 4.

In a fourth embodiment of the first aspect which is also an embodiment of the first, second and third embodiment of the first aspect, and in a fourth embodiment of the second aspect

which is also an embodiment of the first, second and third embodiment of the second aspect the nucleic acid coding for VEGFR1 is selected from the group comprising VEGFR1 transcript variant 2, VEGFR1 transcript variant 1, VEGFR1 transcript variant 3 and VEGFR1 transcript variant 4.

In a fifth embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a fifth embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the VEGFR1 is soluble VEGFR1 or VEGFR1 variant 2.

In a sixth embodiment of the first aspect which is also an embodiment of the fifth embodiment of the first aspect, and in a sixth embodiment of the second aspect which is also an embodiment of the fifth embodiment of the second aspect the soluble VEGFR1 or VEGFR1 variant 2 comprises an amino acid sequence according to SEQ ID NO:1 or is encoded by a nucleotide sequence according to SEQ ID NO: 2.

In a seventh embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a seventh embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the nucleic acid coding for VEGFR1 is a nucleic acid coding for soluble VEGFR1 or VEGFR1 variant 2.

In an eight embodiment of the first aspect which is also an embodiment of the seventh embodiment of the first aspect, and in an eighth embodiment of the second aspect which is also an embodiment of the seventh embodiment of the second aspect the nucleic acid coding for soluble VEGFR1 or VEGFR1 variant 2 comprises a nucleotide sequence according to SEQ ID NO:2 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 1.

In a ninth embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a ninth embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the VEGFR1 is VEGFR1 variant 1.

In a tenth embodiment of the first aspect which is also an embodiment of the ninth embodiment of the first aspect, and in a tenth embodiment of the second aspect which is also an embodiment of the ninth embodiment of the second aspect the VEGFR1 variant 1 comprises an amino acid sequence according to SEQ ID NO: 3 or is encoded by a nucleotide sequence according to SEQ ID NO: 4.

In an eleventh embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in an eleventh embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 1.

In a twelfth embodiment of the first aspect which is also an embodiment of the eleventh embodiment of the first aspect, and in a twelfth embodiment of the second aspect which is also an embodiment of the eleventh embodiment of the second aspect the nucleic acid coding for VEGFR1 variant 1 comprises a nucleotide sequence according to SEQ ID NO: 4 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 3.

In a 13th embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a 13th embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the VEGFR1 is VEGFR1 variant 3.

In a 14th embodiment of the first aspect which is also an embodiment of the 13th embodiment of the first aspect, and in a 14th embodiment of the second aspect which is also an embodiment of the 13th embodiment of the second aspect the VEGFR1 variant 3 comprises an amino acid sequence according to SEQ ID NO: 5 or is encoded by a nucleotide sequence according to SEQ ID NO: 6.

In a 15th embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a 15th embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 3.

In a 16th embodiment of the first aspect which is also an embodiment of the 15th embodiment of the first aspect, and in a 16th embodiment of the second aspect which is also an embodiment of the 15th embodiment of the second aspect the nucleic acid coding for VEGFR1 variant 3 comprises a nucleotide sequence according to SEQ ID NO: 6 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 5.

In a 17th embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a 17th embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the VEGFR1 is VEGFR1 variant 4.

In an 18th embodiment of the first aspect which is also an embodiment of the 17th embodiment of the first aspect, and in an 18th embodiment of the second aspect which is also an embodiment of the 17th embodiment of the second aspect the VEGFR1 variant 4 comprises an amino acid sequence according to SEQ ID NO: 7 or is encoded by a nucleotide sequence according to SEQ ID NO: 8.

In a 19th embodiment of the first aspect which is also an embodiment of the first, second, third and fourth embodiment of the first aspect, and in a 19th embodiment of the second aspect which is also an embodiment of the first, second, third and fourth embodiment of the second aspect the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 4.

In a 20th embodiment of the first aspect which is also an embodiment of the 19th embodiment of the first aspect, and in a 20th embodiment of the second aspect which is also an embodiment of the 19th embodiment of the second aspect the nucleic acid coding for VEGFR1 variant 4 comprises a nucleotide sequence according to SEQ ID NO: 8 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 7.

In a 21st embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the first aspect, and in a 21st embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh,

eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the second aspect the biomarker is a pharmacodynamic biomarker.

In a 22nd embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the first aspect, and in a 22nd embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the second aspect the biomarker is a predictive biomarker.

In a 23rd embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the first aspect, and in a 23rd embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the second aspect the biomarker is a prognostic biomarker.

In a 24th embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the first aspect, and in a 24th embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th and 20th embodiment of the second aspect the biomarker is a surrogate biomarker.

In a 25th embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th , 20th , 21st, 22nd, 23rd and 24th embodiment of the first aspect, and in a 25th embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th , 20th , 21st, 22nd, 23rd and 24th embodiment of the second aspect the subject is suffering from or at risk of suffering from a disease, wherein the disease is a disease which can be treated, ameliorated and/or cured by a PKN3 inhibitor.

In a 26th embodiment of the first aspect which is also an embodiment of the 25th embodiment of the first aspect, and in a 26th embodiment of the second aspect which is also an embodiment of the 25th embodiment of the second aspect the disease is selected from the group comprising tumor diseases, cancer diseases and pre-eclampsia.

In a 27th embodiment of the first aspect which is also an embodiment of the 25th and 26th embodiment of the first aspect, and in a 27th embodiment of the second aspect which is also an embodiment of the 25th and 26th embodiment of the second aspect the disease is selected from the group comprising lung cancer, pancreas cancer, liver cancer, endometrial cancer, colorectal carcinomas, gliomas, adenocarcinomas, endometrial hyperplasias, hereditary non-polyposis colorectal carcinoma, breast-ovarian cancer, prostate cancer, gastrointestinal harmatomas, lipomas, thryroid adenomas, fibrocystic disease of the breast, cerebellar dysplastic gangliocytoma, breast and thyroid malignancies, large cell carcinoma, small cell carcinoma and squamous cell carcinoma.

In a 28th embodiment of the first aspect which is also an embodiment of the 25th and 26th embodiment of the first aspect, and in a 28th embodiment of the second aspect which is also an embodiment of the 25th and 26th embodiment of the second aspect the disease is a disease is selected from the group comprising cancers, metastatic cancers and any pathological conditions involving the PI 3-kinase pathway.

In a 29th embodiment of the first aspect which is also an embodiment of the 25th and 26th embodiment of the first aspect, and in a 29th embodiment of the second aspect which is also an embodiment of the 25th and 26th embodiment of the second aspect the disease is characterized in that the cells being involved in said disease lack PTEN activity, show an increased aggressive behavior, or are cells of a late stage tumor.

In a 30th embodiment of the first aspect which is also an embodiment of the 25th and 26th embodiment of the first aspect, and in a 30th embodiment of the second aspect which is also an embodiment of the 25th and 26th embodiment of the second aspect the disease is selected from the group comprising metastatic cancers and any pathological conditions involving the PI 3-kinase pathway, whereby such pathological condition consists of endometrial cancer, colorectal carcinomas, gliomas, adenocarcinomas, endometrial hyperplasias, Cowden's

syndrome, hereditary non-polyposis colorectal carcinoma, Li-Fraumene's syndrome, breast-ovarian cancer, prostate cancer, Bannayan-Zonana syndrome, LDD (Lhermitte-Duklos' syndrome) hamartoma-macrocephaly diseases including Cow disease (CD) and Bannayan-Ruvalcaba-Rily syndrome (BRR), mucocutaneous lesions such as trichilemmonmas, macrocephaly, mental retardation, gastrointestinal harmatomas, lipomas, thryroid adenomas, fibrocystic disease of the breast, cerebellar dysplastic gangliocytoma, and breast and thyroid malignancies and large cell carcinoma, small cell carcinoma and squamous cell carcinoma.

In a 31st embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th and 30th embodiment of the first aspect, and in a 31st embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th and 30th embodiment of the second aspect the PKN3 inhibitor is selected from the group comprising an siRNA directed against an mRNA coding for PKN3, an antisense oligonucleotide directed against an mRNA coding for PKN3, a ribozyme directed against an mRNA coding for PKN3, an shRNA directed against an mRNA coding for PKN3, an miRNA or antagomir directed against an mRNA coding for PKN3, an aptamer directed against PKN3, a spiegelmer directed against PKN3, an antibody directed against PKN3, an anticalin directed against PKN3, and a small molecule.

In a 32nd embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th and 31st embodiment of the first aspect, and in a 32nd embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th and 31st embodiment of the second aspect the PKN3 inhibitor is an siRNA wherein the siRNA is as follows:

5' 5u8u7c6g8a6g5c7u7a6g5c5 3'
3' a6c6g8u7c5u7a8g6g5u7a8a 5'

with unmodified ribonuclotides being as indicated and modified ribonucleotides being represented as follows:

     5: 2'-O-Methyl-u,

     6: 2'-O-Methyl-a,

     7: 2'-O-Methyl-c,

     8:2'-O-Methyl-g.

In a 33rd embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th, 31st and 32nd embodiment of the first aspect, and in a 33rd embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th, 31st and 32nd embodiment of the second aspect PKN3 comprises an amino acid sequence according to SEQ ID NO: 9.

In a 34th embodiment of the first aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th, 31st and 32nd embodiment of the first aspect, and in a 34th embodiment of the second aspect which is also an embodiment of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth tenth, eleventh, twelfth, 13th, 14th, 15th, 16th, 17th, 18th, 19th, 20th, 21st, 22nd, 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th, 31st and 32nd embodiment of the second aspect PKN3 is encoded by a nucleotide sequence according to SEQ ID NO: 10 or a nucleotide sequence coding for an amino acid sequence according to SEQ ID NO: 9.

The problem underlying the present invention is also solved in a third aspect which is also the first embodiment of the third aspect by a method for deciding whether a subject is a responder to a method of treatment, wherein the method of treatment comprises administering to the subject a PKN3 inhibitor, comprising:

     - determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to the subject being subjected to the method of treatment,

- determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after the subject having been subjected to the method of treatment;

wherein if the level of VEGFR1 or a nucleic acid coding for VEGFR1 in a sample from the subject after the subject having been subjected to the method of treatment is decreased compared to the level of VEGFR1 or a nucleic acid coding for VEGFR1 in a sample from the subject prior to the subject having being subjected to the method of treatment, the subject is a responder to the method of treatment.

In a second embodiment of the third aspect which is also an embodiment of the first embodiment of the third aspect, the decrease is a decrease of 10% or more, of 20% or more, 40 % or more, 60% or more or 80% or more.

In a third embodiment of the third aspect which is also an embodiment of the first and the second embodiment of the third aspect, the subject is suffering from a disease or is at risk of suffering from a disease and wherein the method of treatment is a method of treatment for the disease.

The problem underlying the present invention is also solved in a fourth aspect which is also the first embodiment of the fourth aspect by a method for deciding whether a subject having undergone a first method of treatment, wherein the first method of treatment comprises administering to the subject a PKN3 inhibitor, shall be subject to a second method of treatment, wherein the second method of treatment comprises administering to the subject a PKN3 inhibitor, comprising:

- determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to a first method of treatment,

- determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after a first period of time after the first method of treatment, and

- optionally determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after a second period of time after the first method of treatment,

wherein if the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after the first or second period of time after the first method of treatment is increased compared to the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to a first method of treatment, the subject is to be subjected to the second method of treatment.

In a second embodiment of the fourth aspect which is also an embodiment of the first embodiment of the fourth aspect, the increase is 100% or more, 80% or more, 60% or more or 40% or more.

In a third embodiment of the fourth aspect which is also an embodiment of the first and the second embodiment of the fourth aspect, the subject is suffering from a disease or is at risk of suffering from a disease and wherein the method of treatment is a method of treatment for the disease.

In a fourth embodiment of the fourth aspect which is also an embodiment of the first, second and third embodiment of the fourth aspect, the first period of time after the first method of treatment is about 24 hours or 48 hours after the last administration of the PKN3 inhibitor being part of the first method of treatment.

In a fifth embodiment of the fourth aspect which is also an embodiment of the first, second and third embodiment of the fourth aspect, the second period of time after the first method of treatment starts about 1 or two months after the first period of time after the first method of treatment.

In a sixth embodiment of the fourth aspect which is also an embodiment of the first, second, third, fourth and fifth embodiment of the fourth aspect, the first method of treatment is the same as the second method of treatment, or wherein the first method of treatment is different from the second method of treatment.

The problem underlying the present invention is also solved in a fifth aspect which is also the first embodiment of the fifth aspect by a method for deciding whether a subject shall undergo a method of treatment, whereby the method of treatment comprises the administration of a

PKN3 inhibitor, comprising determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1, or determining the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, wherein if the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 200 pg/ml, the patient is amenable to the method of treatment comprising administration of a PKN3 inhibitor.

In a second embodiment of the fifth aspect which is also an embodiment of the first embodiment of the fifth aspect, if the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 400 pg/ml, preferably equal to or greater than 600 pg/ml, more preferably equal to or greater than 800 pg/ml, the patient is amenable to the method of treatment comprising administration of a PKN3 inhibitor

In an embodiment of any embodiment of the third, fourth and fifth aspect the disease is any disease as defined in any of the embodiments of any aspect of the present invention.

In an embodiment of any embodiment of the third, fourth and fifth aspect the PKN3 inhibitor is any PKN3 inhibitor as defined in any of the embodiments of any aspect of the present invention.

In an embodiment of any embodiment of the third, fourth and fifth aspect the VEGFR1 or a nucleic acid coding thereof is any VEGFR1 or a nucleic acid coding for the VEGFR1 as defined in any of the embodiments of any aspect of the present invention.

The problem underlying the present invention is also solved in a sixth aspect which is also the first embodiment of the sixth aspect by a PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease, wherein the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 200 pg/ml.

In a second embodiment of the sixth aspect which is also an embodiment of the second embodiment of the sixth aspect, the expression level of VEGFR1 or of a nucleic acid coding

for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 400 pg/ml, preferably equal to or greater than 600 pg/ml and more preferably equal to or greater than 800 pg/ml.

The present invention, in its various aspects, is based on the surprising finding that vascular endothelial growth factor receptor 1 (VEGFR1) or a nucleic acid coding for VEGFR1, i.e. VEGF receptor 1 which is also referred to as Flt-1, is a biomarker for therapies which are different from antiangiogenic therapy. More specifically, the present invention is based on the surprising finding that VEGFR1 is a biomarker in connection with methods for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor. Based on this finding it is also within the present invention that VEGFR1 or a nucleic acid coding therefor are used as a biomarker in a method for the treatment of a subject or in connection with designing a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor. Such designing may comprise, among other, at least one of the following: Identifying responders from non-responders to the method for the treatment comprising administering a PKN3 inhibitor, defining the establishment or identification of optimum drug, dose and schedule of such method for the treatment and for identifcying patients at high risk for adverse effects in connection with such method for the treatment.

As prefereably used herein, a method for the treatment means a therapy and a method for the treatment comprising administration to a subject a PKN3 inhibitor means an anti-PKN3 therapy.

As preferably used herein, a PKN3 inhibitor is a compound which addresses or targets PKN3 or a fragment thereof, whereby the targeting of PKN3 or a fragment thereof results in a therapeutic effect in a subject to which the PKN3 inhibitor is administered.

A subject as preferably used is a vertebrate, more preferably a mammal. A particular preferred subject is man.

It will be understood by a person skilled in the art that the use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker can be realized or put into practice by using or referring

to the expression level of VEGFR1 or the expression level of a nucleic acid coding for VEGFR1. Insofar, the present invention in its various aspects also relates to the use of the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor, and to the use of the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in a method for desiging or in designing a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

In accordance with the present invention, in its various aspects, either VEGFR1, i.e. the protein, or a nucleic acid which codes for VEGFR1 is used as a bioamarker or is used in the methods and prodcuts of the present invention. It will be acknowledged by a person skilled in the art that, at the level of the nucleic acid, there are a total of four different transcript variants for VEGFR1, namely VEGFR1 transcript variant 2, VEGFR1 transcript variant 1, VEGFR1 1 transcript variant 3 and VEGFR1 transcript variant 4. It is understood that VEGFR1 transcript 2 codes for soluble VEGFR1 and VEGFR1 transcript 1 codes for the membrane-bound VEGFR1. VEGFR1 transcrips 3 and 4 code for fragments of VEGFR1. It is within the present invention that any of the VEGFR1 transcript variants 1 to 4 is, in principle, suitable for use in connection with the present invention. It is also within the present invention that any of the VEGFR1 variants 1 to 4 is, in principle suitable for use in connection with the various aspects of the present invention. As preferably used herein, a VEGFR1 variant 1 is a protein encoded by VEGFR1 splice variant 1, a VEGFR1 variant 2 is a protein encoded by VEGFR1 splice variant 2, a VEGFR1 variant 3 is a protein encoded by VEGFR1 splice variant 3, and a VEGFR1 variant 4 is a protein encoded by VEGFR1 splice variant 4.

As preferably used herein in connection with any aspect of the present invention a pharmacodynamics biomarker is a biomarker whose changes after treatment are associated with target modulation by a specific agent

As preferably used herein in connection with any aspect of the present invention a predictive biomarker is a biomarker which can be used in advance of therapy to estimate response or survival of a specific patient on a specific treatment compared with another treatment.

As preferably used herein in connection with any aspect of the present invention a prognostic biomarker is a biomarker which provides information about the patient's overall disease outcome, regardless of therapy.

As preferably used herein in connection with any aspect of the present invention a surrogate biomarker is a biomarker intended to substitute for a clinical end point.

Whether a disease is a disease which can be treated, ameliorated and/or cured by a PKN3 inhibitor, can be determined by a person skilled in the art using routine methods and routine tests. For example, a person skilled in the art will use appropriate animal models, or any surrogate test system, and apply to such animal model or surrogate test system a PKN3 inhibitor and check whether the required read-out can be observed which goes along with the effect(s) of a PKN3 inhibitor. For example, such test may be the matrigel test described in international patent application WO 2008/009477, or any other tumor or cancer growth system involving PKN3 as a target molecule.

Preferaly, a disease as used in the various aspects of the present invention is one which is selected from the group comprising:
Acute Lymphoblastic Leukemia (ALL)
Acute Myeloid Leukemia (AML)
Adolescents, Cancer in
Adrenocortical Carcinoma
        Childhood
AIDS-Related Cancers
        Kaposi Sarcoma
        Lymphoma
Anal Cancer
Appendix Cancer
Astrocytomas, Childhood
        Atypical Teratoid/Rhabdoid Tumor, Childhood, Central Nervous System

B
Basal Cell Carcinoma - see Skin Cancer (Nonmelanoma)

Bile Duct Cancer, Extrahepatic

Bladder Cancer

    Childhood

Bone Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma

Brain Stem Glioma, Childhood

Brain Tumor

    Astrocytomas, Childhood

    Brain and Spinal Cord Tumors, Childhood

    Brain Stem Glioma, Childhood

    Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Childhood

    Central Nervous System Embryonal Tumors, Childhood

    Central Nervous System Germ Cell Tumors, Childhood

    Craniopharyngioma, Childhood

    Ependymoblastoma, Childhood

    Ependymoma, Childhood

    Medulloblastoma, Childhood

    Medulloepithelioma, Childhood

    Pineal Parenchymal Tumors of Intermediate Differentiation, Childhood

    Supratentorial Primitive Neuroectodermal Tumors and Pineoblastoma, Childhood

Breast Cancer

    Childhood

    Male

    Pregnancy, Breast Cancer and

Bronchial Tumors, Childhood

Burkitt Lymphoma - see Non-Hodgkin Lymphoma

C

Carcinoid Tumor

    Childhood

    Gastrointestinal

Carcinoma of Unknown Primary

    Childhood

Central Nervous System

Atypical Teratoid/Rhabdoid Tumor, Childhood

Embryonal Tumors, Childhood

Germ Cell Tumor, Childhood

Lymphoma, Primary

Cervical Cancer

Childhood

Childhood Cancers

Chordoma, Childhood

Chronic Lymphocytic Leukemia (CLL)

Chronic Myelogenous Leukemia (CML)

Chronic Myeloproliferative Disorders

Colon Cancer

Colorectal Cancer

Childhood

Craniopharyngioma, Childhood

Cutaneous T-Cell Lymphoma - see Mycosis Fungoides and Sézary Syndrome

D

Duct, Bile, Extrahepatic

Ductal Carcinoma In Situ (DCIS)

E

Embryonal Tumors, Central Nervous System, Childhood

Endometrial Cancer

Ependymoblastoma, Childhood

Ependymoma, Childhood

Esophageal Cancer

Childhood

Esthesioneuroblastoma, Childhood

Ewing Sarcoma Family of Tumors

Extracranial Germ Cell Tumor, Childhood

Extragonadal Germ Cell Tumor

Extrahepatic Bile Duct Cancer

Eye Cancer

    Intraocular Melanoma

    Retinoblastoma

F

Fibrous Histiocytoma of Bone, Malignant, and Osteosarcoma

G

Gallbladder Cancer

Gastric (Stomach) Cancer

    Childhood

Gastrointestinal Carcinoid Tumor

Gastrointestinal Stromal Tumors (GIST) - see Soft Tissue Sarcoma

Germ Cell Tumor

    Central Nervous System, Childhood

    Extracranial, Childhood

    Extragonadal

    Ovarian

Gestational Trophoblastic Tumor

Glioma - see Brain Tumor

    Childhood Brain Stem

H

Hairy Cell Leukemia

Head and Neck Cancer

    Childhood

Heart Cancer, Childhood

Hepatocellular (Liver) Cancer

Histiocytosis, Langerhans Cell

Hodgkin Lymphoma

Hypopharyngeal Cancer

I

Intraocular Melanoma

Islet Cell Tumors, Pancreatic Neuroendocrine Tumors

K

Kaposi Sarcoma

Kidney

     Renal Cell

L

Langerhans Cell Histiocytosis

Laryngeal Cancer

     Childhood

Leukemia

     Acute Lymphoblastic (ALL)

     Acute Myeloid (AML)

     Chronic Lymphocytic (CLL)

     Chronic Myelogenous (CML)

     Hairy Cell

Lip and Oral Cavity Cancer

Liver Cancer (Primary)

Lobular Carcinoma In Situ (LCIS)

Lung Cancer

     Non-Small Cell

     Small Cell

Lymphoma

     AIDS-Related

     Burkitt - see Non-Hodgkin Lymphoma

     Cutaneous T-Cell - see Mycosis Fungoides and Sézary Syndrome

     Hodgkin

     Non-Hodgkin

     Primary Central Nervous System (CNS)

M

Macroglobulinemia, Waldenström

Male Breast Cancer

Malignant Fibrous Histiocytoma of Bone and Osteosarcoma

Medulloblastoma, Childhood

Medulloepithelioma, Childhood

Melanoma

      Childhood

      Intraocular (Eye)

Merkel Cell Carcinoma

Mesothelioma, Malignant

      Childhood

Metastatic Squamous Neck Cancer with Occult Primary

Midline Tract Carcinoma Involving NUT Gene

Mouth Cancer

Multiple Endocrine Neoplasia Syndromes, Childhood

Multiple Myeloma/Plasma Cell Neoplasm

Mycosis Fungoides

Myelodysplastic Syndromes

Myelodysplastic/Myeloproliferative Neoplasms

Myelogenous Leukemia, Chronic (CML)

Myeloid Leukemia, Acute (AML)

Myeloma, Multiple

Myeloproliferative Disorders, Chronic

N

Nasal Cavity and Paranasal Sinus Cancer

Nasopharyngeal Cancer

      Childhood

Neuroblastoma

Non-Hodgkin Lymphoma

Non-Small Cell Lung Cancer

O

Oral Cancer

    Childhood

Oral Cavity Cancer, Lip and

Oropharyngeal Cancer

Osteosarcoma and Malignant Fibrous Histiocytoma of Bone

Ovarian Cancer

    Childhood

    Epithelial

    Germ Cell Tumor

    Low Malignant Potential Tumor

P

Pancreatic Cancer

    Childhood

    Pancreatic Neuroendocrine Tumors (Islet Cell Tumors)

Papillomatosis, Childhood

Paraganglioma

Paranasal Sinus and Nasal Cavity Cancer

Parathyroid Cancer

Penile Cancer

Pharyngeal Cancer

Pheochromocytoma

Pineal Parenchymal Tumors of Intermediate Differentiation, Childhood

Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Childhood

Pituitary Tumor

Plasma Cell Neoplasm/Multiple Myeloma

Pleuropulmonary Blastoma, Childhood

Pregnancy and Breast Cancer

Primary Central Nervous System (CNS) Lymphoma

Prostate Cancer

R

Rectal Cancer

Renal Cell (Kidney) Cancer

Renal Pelvis and Ureter, Transitional Cell Cancer

Retinoblastoma

Rhabdomyosarcoma, Childhood

S

Salivary Gland Cancer

    Childhood

Sarcoma

    Ewing Sarcoma Family of Tumors

    Kaposi

    Soft Tissue

    Uterine

Sézary Syndrome

Skin Cancer

    Childhood

    Melanoma

    Merkel Cell Carcinoma

    Nonmelanoma

Small Cell Lung Cancer

Small Intestine Cancer

Soft Tissue Sarcoma

Squamous Cell Carcinoma - see Skin Cancer (Nonmelanoma)

Squamous Neck Cancer with Occult Primary, Metastatic

Stomach (Gastric) Cancer

    Childhood

Supratentorial Primitive Neuroectodermal Tumors, Childhood

T

T-Cell Lymphoma, Cutaneous - see Mycosis Fungoides and Sézary Syndrome

Testicular Cancer

    Childhood

Throat Cancer

Thymoma and Thymic Carcinoma

    Childhood

Thyroid Cancer

    Childhood

Transitional Cell Cancer of the Renal Pelvis and Ureter

Trophoblastic Tumor, Gestational

U

Unknown Primary, Carcinoma of

    Childhood

Unusual Cancers of Childhood

Ureter and Renal Pelvis, Transitional Cell Cancer

Urethral Cancer

Uterine Cancer, Endometrial

Uterine Sarcoma

V

Vaginal Cancer

    Childhood

Vulvar Cancer

W

Waldenström Macroglobulinemia

Wilms Tumor

Women's Cancers

Y

Young Adults, Cancer in

as may be taken from the list of cancers provided by the National Cancer Institute at the National Institutes of Health.

In each and any aspect of the present invention the PKN3 inhibitor may be a compound which is selected from the group comprising an siRNA directed against an mRNA coding for PKN3, an antisense oligonucleotide directed against an mRNA coding for PKN3, a ribozyme directed against an mRNA coding for PKN3, an shRNA directed against an mRNA coding for PKN3, an miRNA or antagomir directed against an mRNA coding for PKN3, an aptamer directed against PKN3, a spiegelmer directed against PKN3, an antibody directed against PKN3, an anticalin directed against PKN3, a small molecule. Each and any of the above classes of compounds are known to a person skilled in the art and a PKN3 inhibitor of each any any of said classes can be generated by a person skilled in the art by applying standard technology.

The manufacture of an antibody specific for VEGFR1 in its diverse forms is known to the one skilled in the art and, for example, described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,(1988). Preferably, monoclonal antibodies may be used in connection with the present invention which may be manufactured according to the protocol of Cesar and Milstein and further developments based thereon. Antibodies as used herein, include, but are not limited to, complete antibodies, antibody fragments or derivatives such as Fab fragments, Fc fragments and single-stranded antibodies, as long as they are suitable and capable of binding to VEGFR1 as disclosed herein. Apart from monoclonal antibodies also polyclonal antibodies may be used and/or generated. The generation of polyclonal antibodies is also known to the one skilled in the art and, for example, described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, (1988). Preferably, the antibodies used for therapeutical purposes are humanized or human antibodies as defined above.

The antibodies which may be used according to the present invention may have one or several markers or labels. Such markers or labels may be useful to detect the antibody either in its diagnostic application or its therapeutic application. Preferably the markers and labels are selected from the group comprising avidine, streptavidine, biotin, gold and fluorescein and used, e. g., in ELISA methods. These and further markers as well as methods are, e. g. described in Harlow, E., and Lane, D., "Antibodies: A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, NY,(1988).

"Anticalins" are, among others, described in German patent application DE 197 42 706.

Aptamers are D-nucleic acids which are either single stranded or double stranded and which specifically interact with a target molecule. The manufacture or selection of aptamers is, e. g., described in European patent EP 0 533 838. Basically the following steps are realized. First, a mixture of nucleic acids, i. e. potential aptamers, is provided whereby each nucleic acid typically comprises a segment of several, preferably at least eight subsequent randomised nucleotides. This mixture is subsequently contacted with the target molecule whereby the nucleic acid(s) bind to the target molecule, such as based on an increased affinity towards the target or with a bigger force thereto, compared to the candidate mixture. The binding nucleic acid(s) are/is subsequently separated from the remainder of the mixture. Optionally, the thus obtained nucleic acid(s) is amplified using, e. g. polymerase chain reaction. These steps may be repeated several times giving at the end a mixture having an increased ratio of nucleic acids specifically binding to the target from which the final binding nucleic acid is then optionally selected. These specifically binding nucleic acid(s) are referred to aptamers. It is obvious that at any stage of the method for the generation or identification of the aptamers samples of the mixture of individual nucleic acids may be taken to determine the sequence thereof using standard techniques. It is within the present invention that the aptamers may be stabilized such as, e. g., by introducing defined chemical groups which are known to the one skilled in the art of generating aptamers. Such modification may for example reside in the introduction of an amino group at the 2'-position of the sugar moiety of the nucleotides. Aptamers are currently used as therapeutical agens. However, it is also within the present invention that the thus selected or generated aptamers may be used for target validation and/or as lead substance for the development of medicaments, preferably of medicaments based on small molecules. This is actually done by a competition assay whereby the specific interaction between the target molecule and the aptamer is inhibited by a candidate drug whereby upon replacement of the aptamer from the complex of target and aptamer it may be assumed that the respective drug candidate allows a specific inhibition of the interaction between target and aptamer, and if the interaction is specific, said candidate drug will, at least in principle, be suitable to block the target and thus decrease its biological availability or activity in a respective system comprising such target. The thus obtained small molecule may then be subject to further derivatisation and modification to optimise its physical, chemical, biological

and/or medical characteristics such as toxicity, specificity, biodegradability and bioavailability.

The generation or manufacture of spiegelmers which may be used or generated according to the present invention using VEGFR1 as disclosed herein, is based on a similar principle. The manufacture of spiegelmers is described in the international patent application WO 98/08856. Spiegelmers are L-nucleic acids, which means that they are composed of L-nucleotides rather than aptamers which are composed of D-nucleotides as aptamers are. Spiegelmers are characterized by the fact that they have a very high stability in biological system and, comparable to aptamers, specifically interact with the target molecule against which they are directed. In the purpose of generating spiegelmers, a heterogonous population of D-nucleic acids is created and this population is contacted with the optical antipode of the target molecule, in the present case for example with the D-enantiomer of the naturally occurring L-enantiomer of the protein kinase N beta. Subsequently, those D-nucleic acids are separated which do not interact with the optical antipode of the target molecule. However, those D-nucleic acids interacting with the optical antipode of the target molecule are separated, optionally determined and/or sequenced and subsequently the corresponding L-nucleic acids are synthesized based on the nucleic acid sequence information obtained from the D-nucleic acids. These L-nucleic acids which are identical in terms of sequence with the aforementioned D-nucleic acids interacting with the optical antipode of the target molecule, will specifically interact with the naturally occurring target molecule rather than with the optical antipode thereof. Similar to the method for the generation of aptamers it is also possible to repeat the various steps several times and thus to enrich those nucleic acids specifically interacting with the optical antipode of the target molecule.

Ribozymes are catalytically active nucleic acids which preferably consist of RNA which basically comprises two moieties. The first moiety shows a catalytic activity whereas the second moiety is responsible for the specific interaction with the target nucleic acid, in the present case the nucleic acid coding for VEGFR1 as disclosed herein. Upon interaction between the target nucleic acid and the second moiety of the ribozyme, typically by hybridisation and Watson-Crick base pairing of essentially complementary stretches of bases on the two hybridising strands, the catalytically active moiety may become active which means that it catalyses, either intramolecularly or intermolecularly, the target nucleic acid in

case the catalytic activity of the ribozyme is a phosphodiesterase activity. Subsequently, there may be a further degradation of the target nucleic acid which in the end results in the degradation of the target nucleic acid as well as the protein derived from the said target nucleic acid due to a lack of newly synthesized VEGFR1 as disclosed herein and a turn-over of prior existing VEGFR1 as disclosed herein. Ribozymes, their use and design principles are known to the one skilled in the art, and, for example described in Doherty and Doudna (Ribozym structures and mechanism. Annu ref. Biophys. Biomolstruct. 2001 ; 30 :457-75) and Lewin and Hauswirth (Ribozyme Gene Therapy: Applications for molecular medicine. 2001 7: 221-8).

Antisense oligonucleotides are as such equally known in the art as is their use for the manufacture of a medicament and as a diagnostic agent, respectively. Basically, antisense oligonucleotides hybridise based on base complementarity, with a target RNA, preferably with a mRNA, thereby activate RNase H. RNase H is activated by both phosphodiester and phosphorothioate-coupled DNA. Phosphodiester-coupled DNA, however, is rapidly degraded by cellular nucleases with the exception of phosphorothioate-coupled DNA. These resistant, non-naturally occurring DNA derivatives do not inhibit RNase H upon hybridisation with RNA. In other words, antisense polynucleotides are only effective as DNA RNA hybride complexes. Examples for this kind of antisense oligonucleotides are described, among others, in US-patent US 5,849,902 and US 5,989,912. In other words, based on the nucleic acid sequence of the target molecule which in the present case is the nucleic acid coding for VEGFR1 as disclosed herein, either from the target protein from which a respective nucleic acid sequence may in principle be deduced, or by knowing the nucleic acid sequence as such, particularly the mRNA, suitable antisense oligonucleotides may be designed base on the principle of base complementarity.

siRNA is a double stranded RNA having typically a length of about 19 to about 23 nucleotides. The sequence of one of the two RNA strands corresponds to the sequence of the target nucleic acid such as the nucleic acid coding for target molecule, to be degraded. In other words, knowing the nucleic acid sequence of the target molecule, in the present case VEGFR1 in its various forms described herein, preferably the mRNA sequence, a double stranded RNA may be designed with one of the two strands being complementary to said, e. g. mRNA of the target molecule and, upon application of said siRNA to a system containing

the gene, genomic DNA, hnRNA or mRNA coding for the target molecule, the respective target nucleic acid will be degraded and thus the level of the respective protein be reduced. The basic principles of designing, constructing and using said siRNA as medicament and diagnostic agent, respectively, is, among others, described in international patent applications WO 00/44895 and WO 01/75164.

Based on the aforementioned design principles, it is possible to generate such siRNA, antisense oligonucleotide and ribozyme, respectively, once the nucleic acid sequence coding for the target molecule is known. This is also true for precursor molecules of nucleic acid such as hnRNA, cDNA and the like, including genomic nucleic acid. Of course, also knowing the respective antisense strand may allow the design of such nucleic acid based compounds given the basic principle of base pair complementarity, preferably based on Watson-Crick base pairing. Accordingly, a further aspect of the present invention is related to specific siRNAs, ribozymes and antisense nucleotides which are directed against or specific for protein kinase N-beta. In the following, this is further illustrated by siRNA, however, this applies to antisense oligonucleotides and ribozymes as well, as will be acknowledged by the ones skilled in the art.

Such siRNA comprises preferably a length of from 15 to 25 nucleotides, whereby this means actually any length comprising 15, 16, 17, 18, 20, 21, 22, 23, 24 or 25 nucleotides. In further embodiments, the siRNA may even exhibit more nucleotides. According the design principles well known in the art, respective siRNA can be generated. Accordingly, the siRNA claimed herein comprises a stretch of preferably any nucleotide length from 15 to 25 consecutive nucleotides which is either at least partially complementary to the sense or to the antisense strand encoding the target molecule, and a second ribonucleotide strand which is at least partially complementary to the first one and thus to the antisense strand and sense strand respectively, encoding the target molecule. Any design principle known in the art of generation or manufacture of siRNA may be applied to this kind of duplex structure. The siRNA space disclosed herein comprises siRNA molecules the antisense strand of which starts with a nucleotides which corresponds to nucleotide no. 1 of a the target molecule as specified above. Further such siRNA molecules start with a nucleotide which corresponds to nucleotide no 2 of the target molecule encoding sequence as specified above, and so on. This kind of scanning over the the target molecule encoding sequence is repeated so as to provide

all possible siRNA molecules which can be directed against the target molecule. The length of any of the siRNA molecules thus generated may be any length suitable for siRNA, more particularly any length as specified above. Preferably, the various siRNA molecule of the siRNA molecule space disclosed herein, overlap except the most 5'terminal nucleotide of the antisense strand or sense strand. It is obvious that the thus obtained antisense sequences have to complemented through base pairing so as to form the at least partially double-stranded structure required for a functionally active siRNA.

MicroRNA for use in therapeutic applications are, for example, described in Soifer HS et al. (Soifer HS et al., Molecular therapy, vol. 15, no. 12, dec 2007, pages 2070-2079).

Antagomirs for use in therapeutic appications are, for example, described in Stenvang J & Kauppinen S (Stenvang J & Kauppinen S, Expert Opin. Biol. Ther. (2008) 8(1), pages 59-81)

A preferred embodiment the PKN3 inhibitor is an siRNA which is double-stranded (ds 23mer) having the following composition and structure, respectively.

5' 5u8u7c6g8a6g5c7u7a6g5c5 3'
3' a6c6g8u7c5u7a8g6g5u7a8a 5'

with unmodified ribonuclotides being as indicated and modified ribonucleotides being represented as follows:

5: 2'-O-Methyl-u,
6: 2'-O-Methyl-a,
7: 2'-O-Methyl-c,
8:2'-O-Methyl-g.

In a further preferred embodiment of the various aspects of the present invention the PKN3 inhibitor is a composition referred to in the prior art and herein as Atu027. The active pharmaceutical ingredient (API) of Atu027 is a PKN3- specific siRNA. The siRNA is a double stranded RNA molecule containing naturally occuring 2'-O-Methyl-modifications.

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The 2'-O-methyl modifications stabilize the RNA by protection from nuclease attack. The double strand (ds-23mer) is composed of the following sequences

5' 5u8u7c6g8a6g5c7u7a6g5c5 3'

3' a6c6g8u7c5u7a8g6g5u7a8a 5'

with unmodified ribonuclotides being as indicated and modified ribonucleotides being represented as follows:
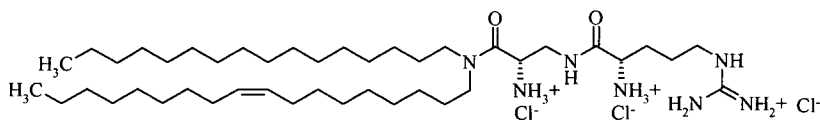
5: 2'-O-Methyl-u,

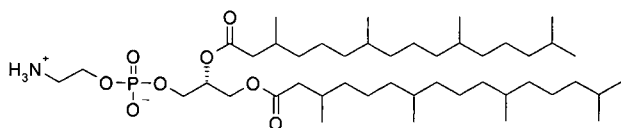6: 2'-O-Methyl-a,

7: 2'-O-Methyl-c,

8:2'-O-Methyl-g.

For production of Atu027 the PKN3- specific siRNA is formulated with positively charged liposomes composed of three lipids, i.e. the cationic AtuFect01, the neutral, fusogenic DPyPE helperlipid and the PEGylated lipid MPEG-2000-DSPE in a molar ratio of 50/49/1 to deliver the negatively charged siRNA in lipoplexed form. AtuFect01 and DPyPE helperlipid are novel excipients. The third lipid MPEG-2000-DSPE is a known excipient. The structure of the 3 lipids is presented below:

Cationic lipid AtuFect01:   (β-(L-Arginyl)-2,3-L-diaminopropionic   acid-N-palmityl-N-oleyl-amide tri-hydrochloride)
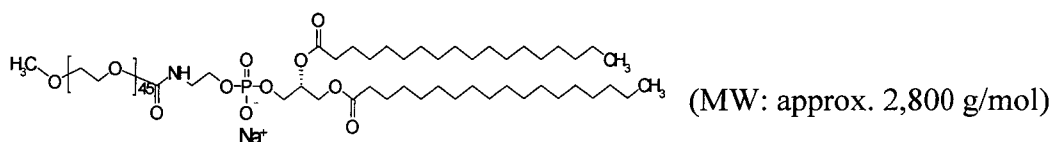

(MW: 843.6 g/mol)

Fusogenic/helperlipid DPyPE: 1,2-Diphytanoyl-sn-glycero-3-phosphoethanolamine

(MW: 804.19 g/mol)

PEG-lipid MPEG-2000-DSPE: N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-
distearoyl-sn-glycero-3-phosphoethanolamine sodium salt

(MW: approx. 2,800 g/mol)

It is within the present invention that any of the PKN3 inhibitors disclosed herein can be used in connection with each and any aspect of the present invention, including the method of the present invention and the PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease of the present invention.

It is within the present invention that any of the disease disclosed herein can be used in connection with each and any aspect of the present invention, including the method of the present invention and the PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease of the present invention.

It is also within the present invention that any of the VEGFR1 and nucleic acid coding therefor disclosed herein can be used in connection with each and any aspect of the present invention, including the method of the present invention and the PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease of the present invention.

Methods for the detection of VEGFR1 are known in the art and include, but are not limited to anti-VEGFR1 antibodies. In embodiments of the various aspects of the present invention VEGFR1 is detected by means of ELISA. However, other means are known to a person skilled in the art such as detection by mass spectrometry and the like.

In connection with the various aspects of the present invention a sample is a sample of a body fluid, whereby the body fluid is selected from the group comprising blood, plasma, liquor, urine and saliva. Preferred samples are blood samples and plasma samples.

Methods for the detection of a nucleic acid coding for VEGFR1 are also known in the art and include, but are not limited to PCR and RT-PCR.

VEGFR1 and soluble VEGFR1 (sVEGFR1) are known in the art and described, among others, in Caine GJ et al. (Caine, GJ, European Journal of Clinical Investigation (2003), 33, 883-890) or Barleon B et al. (Barleon B et al., Angionesis 4: 143-154 (2001)). Barleaon B et al. (supra) also disclose that the titre of soluble VEGFR1 is about 160 pg/ml in healthy volunteers.

The present invention is now further illustrated by the attached Figs and examples from which further feature, embodiments and advantages of the present invention may be taken.

The following list summarizes some of the SEQ ID NOs and provide an indication as to what they represent.

SEQ ID NO: 1	amino acid sequence of soluble VEGFR1 or VEGFR1 variant 2
SEQ ID NO: 2	nucleotide sequence coding for soluble VEGFR1 or VEGFR1 transcript variant 2
SEQ ID NO: 3	amino acid sequence of VEGFR1 variant 1
SEQ ID NO: 4	nucleotide sequence coding for VEGFR1 transcript variant 1
SEQ ID NO: 5	amino acid sequence of VEGFR1 variant 3
SEQ ID NO: 6	nucleotide sequence coding for VEGFR1 transcript variant 3
SEQ ID NO: 7	amino acid sequence of VEGFR1 variant 4
SEQ ID NO: 8	nucleotide sequence coding for VEGFR1 transcript variant 4
SEQ ID NO: 9	amino acid sequence of PKN3
SEQ ID NO: 10	nucleotide sequence coding for PKN3

Fig. 1   is a diagram indicating PKN3 knock down, VEGFR1 expression and VEGFR2 expression in PC-3 cells.

Fig. 2   is a diagram indicating PKN3 knock down, VEGFR1 expression and VEGFR2 expression in MDA-MB-435 cells.

Fig. 3   is a diagram indicating PKN3 knock down, VEGFR1 expression and VEGFR2 expression in SKBR3 cells.

Fig. 4   is a representation of the design of a clinical phase I study.

Fig. 5   shows the result of a biomarker analysis in plasma from humans treated with Atu027.

Fig.6   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGF.

Fig.7   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGF-B.

Fig.8   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGF-C.

Fig.9   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGF-D.

Fig.10   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGFR1 (which is also referred to as VEGFR-1).

Fig.11   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGFR2 (which is also referred to as VEGFR-2).

Fig.12   shows the result of a biomarker analysis in plasma from humans treated with Atu027: single analytes – VEGFR3 (which is also referred to as VEGFR-3).

Fig. 13   represents SEQ ID NO: 1.

Fig. 14   represents SEQ ID NO: 2.

Fig. 15   represents SEQ ID NO: 3.

Fig. 16   represents SEQ ID NO: 4.

Fig. 17   represents SEQ ID NO: 5.

Fig. 18   represents SEQ ID NO: 6.

Fig. 19   represents SEQ ID NO: 7.

Fig. 20   represents SEQ ID NO: 8.

Fig. 21   represents SEQ ID NO: 9.

Fig. 22   represents SEQ ID NO: 10.

Examples

Example 1:   Synthesis of RNAi molecule (AtuRNAi) for *in vitro* studies

The siRNA molecules (AtuRNAi) of this invention are described in Table 1. These molecules were synthesized by BioSpring GmbH (Frankfurt a. M., Germany).

TABLE 1

siRNA name	sequence 5'to 3'	SEQ ID NO
PKN3 s	agacuugaggacuuccuggacaa	
PKN3 as	uuguccaggaaguccucaagucu	
Luciferase s	aucacguacgcggaauacuucga	
Luciferase as	ucgaaguauuccgcguacgugau	

Nucleotides with 2'-O-methyl modifications are underlined; "s" stands for the sense strand; and "as" stands for the antisense strand.

The duplexes formed by "PKN3 as" and "PKN3 s", and formed by "Luciferase as" and "Luciferase s" lack 3'-overhangs and are chemically stabilized by alternating 2'-O-methyl sugar modifications on both strands, whereby unmodified nucleotides face modified ones on the opposite strand (Table 1). These duplexes are also referred to herein as PKN3 and Luciferase and were resolved in water to obtain a stock concentration of 1 μM.

Example 2: Formulation of the AtuPLEX

The AtuPLEX formulation was prepared as essentially described in Santel et al. (Santel et al. Gene Therapy (2006) 13, 1222–1234). Cationic liposomes comprising the novel cationic lipid AtuFECT01 (β-L-arginyl-2,3-L-diaminopropionic acid-N-palmityl-N-oleyl-amide   trihydrochloride),   the   neutral   phospholipid   1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine   (DPyPE)   and   the   PEGylated   lipid   N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phospho-ethanolamine sodium

salt (DSPE-PEG) in a molar ratio of 50/49/1 were prepared by lipid film re-hydration in 270 mM sterile RNase-free sucrose solution to a total lipid concentration of 4.335 mg/ml. Subsequently, the multilamellar dispersion was further processed by high-pressure homogenization using an EmulsiFlex C3 device (Avestin, Inc., Ottawa, Canada).

To generate siRNA-lipoplexes (AtuPLEX), the obtained liposomal dispersion was mixed with an equal volume of a 0.5625 mg/ml solution of siRNA in 270 mM sucrose, resulting in a calculated charge ratio of nucleic acid backbone phosphates to cationic lipid nitrogen atoms of approximately 1:4. The size of the liposome and the lipoplex dispersion and the zeta potential were measured using a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK).

The active pharmaceutical ingredient (API) of Atu027 is the PKN3-siRNA. The siRNA is a double stranded RNA molecule containing naturally occuring 2'-O-Methyl-modifications. The 2'-O-methyl modifications stabilize the RNA by protection from nuclease attack.

The double strand (ds-23mer) is composed of the following sequence
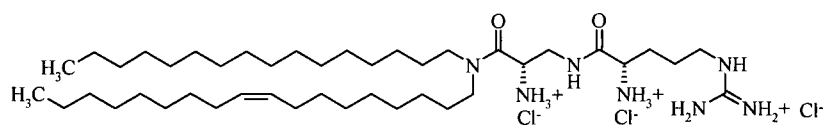
5' 5u8u7c6g8a6g5c7u7a6g5c5 3'

3' a6c6g8u7c5u7a8g6g5u7a8a 5'

Unmodified ribonuclotides: x;

Modifications: 5: 2'-O-Methyl-u, 6: 2'-O-Methyl-a, 7: 2'-O-Methyl-c, 8:2'-O-Methyl-g
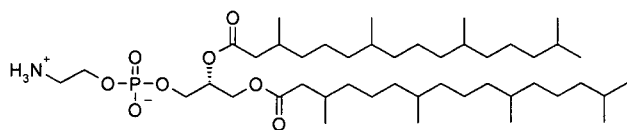
For Atu027 production PKN3-siRNA is formulated with positively charged liposomes composed of three lipids, i.e. the cationic AtuFect01, the neutral, fusogenic DPyPE helperlipid and the PEGylated lipid MPEG-2000-DSPE in a molar ratio of 50/49/1 to deliver the negatively charged siRNA in lipoplexed form. AtuFect01 and DPyPE helperlipid are novel excipients. The third lipid MPEG-2000-DSPE is a known excipient. The structure of the 3 lipids is presented below:

Cationic lipid AtuFect01:   (β-(L-arginyl)-2,3-L-diaminopropionic acid-N-palmityl-N-oleyl-amide tri-hydrochloride)
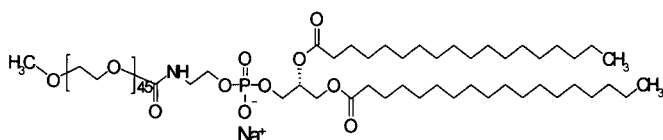

843.6 g/mol

Fusogenic/helperlipid DPyPE: 1,2-Diphytanoyl-sn-Glycero-3-Phosphoethanolamine

804.19 g/mol

PEG-lipid MPEG-2000-DSPE: N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt

approx. 2,800 g/mol

Example 3:   Expression of VEGFR-1 in response to in vitro gene silencing of human PKN3 in Human Prostate Cancer PC-3 cells

3×10^5 PC-3 cells were plated in 10-cm dishes, transfected 16 h later with 20 nM of the siRNA as described in Example 1 and 1 μg/ml liposome were prepared as described in Example 2, whereby the liposome consisted of the cationic lipid β-(L-arginyl)-2,3-L-diaminopropionic acid-*N*-palmityl-*N*-oleyl-amide tri-hydrochloride (AtuFECT01), the fusogenic lipid 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) and the PEGylated lipid MPEG-2000-DSPE in a molar ratio of 50/49/1. Cells were lysed for RNA extraction 24 h, 48 h, 72 h and 96 h after transfection, respectively.

Total RNA was prepared with the InviTrap® Spin Cell RNA Mini Kit (Invitek, Berlin, Germany). Inhibition of PKN3 mRNA expression, expression of VEGFR1 and VEGFR2 mRNA was detected by real time RT-PCR (TaqMan) analysis using 300 nM PKN3, VEGFR1 and VEGFR2 specific forward and reverse primer and 100 nM probe. The sequences of the primers in TaqMan are listed in the following table, whereby UPR means upper primer, LWR means lower primer, and PRB means probe.

TABLE 2

Name	Primer/Probe	Sequence 5' to 3'	SEQ ID NO
Human PKN3	UPR	CACTTTGGGAAGGTCCTCCTG	
	PRB	FAM-TTCAAGGGGACAGGGAAATACTACGCCA-BHQ1	
	LWR	CCTCCTGCTTCTTCAGTGCTTT	
Human VEGFR1	UPR	CCCCGATTATGTGAGAAAAGGA	
	PRB	FAM-CGACTTCCTCTGAAATGGATGGCTCCTG-BHQ1	
	LWR	CGCTCTTGGTGCTGTAGATTTTG	
Human VEGFR2	UPR	TCTGCCTACCTCACCTGTTTCC	
	PRB	FAM-ATGGAGGAGGAGGAAGTATGTGACCCCA-TAMRA	
	LWR	TGACTGATTCCTGCTGTGTTGTC	

The reaction was carried out in 25 μl and assayed on the StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions under the following conditions: 48°C for 30 min, 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. mRNA amounts are shown relative to untreated control lysed 24 h after transfection.

The results are shown in Fig.1 indicating in vitro a marked decrease in VEGFR1 but not VEGFR2 mRNA expression in response to PKN3 knock down 72 h and 96 h post-transfection in human prostate cancer PC-3 cells.

Example 4:   Expression of VEGFR-1 in response to in vitro gene silencing of human PKN3 in Human Breast Carcinoma MDA-MB-435 cells

3×10^5 MDA-MB-435 cells were plated in 10-cm dishes, transfected 16 h later with 20 nM of the siRNA as described in Example 1 and 1 µg/ml liposome were prepared as described in Example 2, whereby the liposome consisted of the cationic lipid β-(L-arginyl)-2,3-L-diaminopropionic acid-*N*-palmityl-*N*-oleyl-amide tri-hydrochloride (AtuFECT01), the fusogenic lipid 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) and the PEGylated lipid MPEG-2000-DSPE in a molar ratio of 50/49/1. Cells were lysed for RNA extraction 24 h, 48 h, 72 h and 96 h after transfection, respectively.

Total RNA was prepared with the InviTrap® Spin Cell RNA Mini Kit (Invitek, Berlin, Germany). Inhibition of PKN3 mRNA expression, expression of VEGFR1 and VEGFR2 mRNA was detected by real time RT-PCR (TaqMan) analysis as described in example 3. mRNA amounts are shown relative to untreated control lysed 24 h after transfection.

The results are shown in Fig.2 indicating in vitro a marked decrease in VEGFR1 but not VEGFR2 mRNA expression in response to PKN3 knock down at all time-points analyzed (24 h, 48 h, 72 h and 96 h post-transfection) in human breast carcinoma MDA-MB-435 cells.

Example 5:   Expression of VEGFR-1 in response to in vitro gene silencing of human PKN3 in Human Ovarian Cancer SKBR3 cells

3×10^5 SKBR3 cells were plated in 10-cm dishes, transfected 16 h later with 20 nM of the siRNA as described in Example 1 and 1 µg/ml liposome were prepared as described in Example 2, whereby the liposome consisted of the cationic lipid β-(L-arginyl)-2,3-L-diaminopropionic acid-*N*-palmityl-*N*-oleyl-amide tri-hydrochloride (AtuFECT01), the fusogenic lipid 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) and the PEGylated lipid MPEG-2000-DSPE in a molar ratio of 50/49/1. Cells were lysed for RNA extraction 24 h, 48 h and 72 h after transfection, respectively.

Total RNA was prepared with the InviTrap® Spin Cell RNA Mini Kit (Invitek, Berlin, Germany). Inhibition of PKN3 mRNA expression, expression of VEGFR1 and VEGFR2

mRNA was detected by real time RT-PCR (TaqMan) analysis as described in example 3. mRNA amounts are shown relative to untreated control lysed 24 h after transfection.

The results are shown in Fig.3 indicating in vitro a marked decrease in VEGFR1 but not VEGFR2 mRNA expression in response to PKN3 knock down 72 h post-transfection in human ovarian cancer SKBR3 cells.

Example 6:   Design of Clinical Phase I study with Atu027

The Phase I study with Atu027 "A prospective, open label, single-centre, dose finding phase I study with Atu027 (an siRNA formulation) in subjects with advanced solid cancer - Atu027-I-01" (EudraCT No. 2008-005588-32/NCT00938574) was conducted. Study design is shown in Fig. 4. Before (0 h) and 24 h after the last (24 h) repeated 4-h intravenous infusion treatment with Atu027 blood was withdrawn for biomarker analysis. EDTA plasma was prepared by centrifugation according to standard protocols and stored at -20°C.

Example 7:   Biomarker analysis in plasma of humans treated with Atu027

Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from nine patients and EDTA plasma was prepared. Samples were analyzed in a multiplexed immunoassay for concentration of respective analytes using the Luminex technology.

In Fig. 5 the ratio of post/pre treatment plasma level (24 h level divided by the 0 h value) in the individual patient is shown for the VEGF isoforms VEGF, VEGF-B, VEGF-C, VEGF-D and their receptors VEGFR1, VEGFR2 and VEGFR3. The Atu027 doses based on the siRNA content are given in the right table. In seven out of nine patients VEGFR1 level were markedly decreased after Atu027 treatment.

In addition to the Atu027 doses indicated in Fig. 5 doses of 0.036 mg/kg Atu027 (based on siRNA content) were administered to three patients whereby two patients showed a marked decrease in the ratio of soluble FLT-1 (sFLT-1) post/pretreatment.

The absolute plasma level of the single analytes VEGF, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2 and VEGFR3 are shown in graphs and tables in Fig.6, Fig.7, Fig.8, Fig.9,

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Fig.10, Fig.11 and Fig.12. Again, a marked decrease of VEGFR1 concentration after treatment could be detected (Fig.10) whereas the VEGF, VEGF-B, VEGF-C, VEGF-D, VEGFR2 and VEGFR3 level remained unchanged in most patients and slightly increased or decreased in single individuals.

The features of the present invention disclosed in the specification, the claims and/or the drawings may both separately and in any combination thereof be material for realizing the invention in various forms thereof.

Claims

1.   Use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

2.   Use according to claim 1, wherein the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 is a or the biomarker.

3.   Use of VEGFR1 or of a nucleic acid coding for VEGFR1 as a biomarker in designing a method for the treatment of a subject, wherein the method for the treatment comprises administering to the subject a PKN3 inhibitor.

4.   Use according to claim 3, wherein the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1 is a or the biomarker.

5.   Use according to any one of claims 1 to 4, wherein the VEGFR1 is selected from the group comprising VEGFR1 variant 2, VEGFR1 variant 1, VEGFR1 variant 3 and VEGFR1 variant 4.

6.   Use according to any one of claims 1 to 4, wherein the nucleic acid coding for VEGFR1 is selected from the group comprising VEGFR1 transcript variant 2, VEGFR1 transcript variant 1, VEGFR1 transcript variant 3 and VEGFR1 transcript variant 4.

7.   Use according to any one of claims 1 to 6, wherein the VEGFR1 is soluble VEGFR1 or VEGFR1 variant 2.

8.   Use according to claim 7, wherein the soluble VEGFR1 or VEGFR1 variant 2 comprises an amino acid sequence according to SEQ ID NO:1 or is encoded by a nucleotide sequence according to SEQ ID NO: 2.

9.   Use according to any one of claims 1 to 6, wherein the nucleic acid coding for VEGFR1 is a nucleic acid coding for soluble VEGFR1 or VEGFR1 variant 2.

10.   Use according to claim 9, wherein the nucleic acid coding for soluble VEGFR1 or VEGFR1 variant 2 comprises a nucleotide sequence according to SEQ ID NO:2 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 1.

11.   Use according to any one of claims 1 to 6, wherein the VEGFR1 is VEGFR1 variant 1.

12.   Use according to claim 11, wherein the VEGFR1 variant 1 comprises an amino acid sequence according to SEQ ID NO: 3 or is encoded by a nucleotide sequence according to SEQ ID NO: 4.

13.   Use according to any one of claims 1 to 6, wherein the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 1.

14.   Use according to claim 13, wherein the nucleic acid coding for VEGFR1 variant 1 comprises a nucleotide sequence according to SEQ ID NO: 4 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 3.

15.   Use according to any one of claims 1 to 6, wherein the VEGFR1 is VEGFR1 variant 3.

16.   Use according to claim 15, wherein the VEGFR1 variant 3 comprises an amino acid sequence according to SEQ ID NO: 5 or is encoded by a nucleotide sequence according to SEQ ID NO: 6.

17.   Use according to any one of claims 1 to 6, wherein the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 3.

18.   Use according to claim 17, wherein the nucleic acid coding for VEGFR1 variant 3 comprises a nucleotide sequence according to SEQ ID NO: 6 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 5.

19.   Use according to any one of claims 1 to 6, wherein the VEGFR1 is VEGFR1 variant 4.

20.   Use according to claim 19, wherein the VEGFR1 variant 4 comprises an amino acid sequence according to SEQ ID NO: 7 or is encoded by a nucleotide sequence according to SEQ ID NO: 8.

21.   Use according to any one of claims 1 to 6, wherein the nucleic acid coding for VEGFR1 is a nucleic acid coding for VEGFR1 variant 4.

22.   Use according to claim 21, wherein the nucleic acid coding for VEGFR1 variant 4 comprises a nucleotide sequence according to SEQ ID NO: 8 or a nucleotide sequence encoding an amino acid sequence according to SEQ ID NO: 7.

23.   Use according to any one of claims 1 to 22, wherein the biomarker is a pharmacodynamic biomarker.

24.   Use according to any one of claims 1 to 22, wherein the biomarker is a predictive biomarker.

25.   Use according to any one of claims 1 to 22, wherein the biomarker is a prognostic biomarker.

26.   Use according to any one of claims 1 to 22, wherein the biomarker is a surrogate biomarker.

27.   Use according to any one of claims 1 to 26, wherein the subject is suffering from or at risk of suffering from a disease, wherein the disease is a disease which can be treated, ameliorated and/or cured by a PKN3 inhibitor.

28.   Use according to claim 27, wherein the disease is selected from the group comprising tumor diseases, cancer diseases and pre-eclampsia.

29.   Use according to any one of claims 27 to 28, wherein the disease is selected from the group comprising lung cancer, pancreas cancer, liver cancer, endometrial cancer, colorectal carcinomas, gliomas, adenocarcinomas, endometrial hyperplasias, hereditary non-polyposis colorectal carcinoma, breast-ovarian cancer, prostate cancer, gastrointestinal harmatomas, lipomas, thryroid adenomas, fibrocystic disease of the breast, cerebellar dysplastic gangliocytoma, breast and thyroid malignancies, large cell carcinoma, small cell carcinoma and squamous cell carcinoma.

30.   Use according to any one of claims 27 to 28, wherein the disease is a disease is selected from the group comprising cancers, metastatic cancers and any pathological conditions involving the PI 3-kinase pathway.

31.   Use according to any one of claims 27 to 28, wherein the disease is characterized in that the cells being involved in said disease lack PTEN activity, show an increased aggressive behavior, or are cells of a late stage tumor.

32.   Use according to any one of claims 27 to 28, wherein the disease is selected from the group comprising metastatic cancers and any pathological conditions involving the PI 3-kinase pathway, whereby such pathological condition consists of endometrial cancer, colorectal carcinomas, gliomas, adenocarcinomas, endometrial hyperplasias, Cowden's syndrome, hereditary non-polyposis colorectal carcinoma, Li-Fraumene's syndrome, breast-ovarian cancer, prostate cancer, Bannayan-Zonana syndrome, LDD (Lhermitte-Duklos' syndrome) hamartoma-macrocephaly diseases including Cow disease (CD) and Bannayan-Ruvalcaba-Rily syndrome (BRR), mucocutaneous lesions such as trichilemmonmas, macrocephaly, mental retardation, gastrointestinal harmatomas, lipomas, thryroid adenomas, fibrocystic disease of the breast, cerebellar dysplastic gangliocytoma, and breast and thyroid malignancies and large cell carcinoma, small cell carcinoma and squamous cell carcinoma.

33.   Use according to any one of claims 1 to 32, wherein the PKN3 inhibitor is selected from the group comprising an siRNA directed against an mRNA coding for PKN3, an antisense oligonucleotide directed against an mRNA coding for PKN3, a ribozyme directed against an mRNA coding for PKN3, an shRNA directed against an mRNA coding for PKN3, an miRNA or antagomir directed against an mRNA coding for PKN3, an aptamer directed against PKN3,

a spiegelmer directed against PKN3, an antibody directed against PKN3, an anticalin directed against PKN3, and a small molecule.

34.   Use according to any one of claims 1 to 33, wherein the PKN3 inhibitor is an siRNA wherein the siRNA is as follows:

        5' 5u8u7c6g8a6g5c7u7a6g5c5 3'

        3' a6c6g8u7c5u7a8g6g5u7a8a 5'

with unmodified ribonuclotides being as indicated and modified ribonucleotides being represented as follows:

        5: 2'-O-Methyl-u,

        6: 2'-O-Methyl-a,

        7: 2'-O-Methyl-c,

        8:2'-O-Methyl-g.

35.   Use according to any one of claims 1 to 34, wherein PKN3 comprises an amino acid sequence according to SEQ ID NO: 9.

36.   Use according to any one of claims 1 to 34, wherein PKN3 is encoded by a nucleotide sequence according to SEQ ID NO: 10 or a nucleotide sequence coding for an amino acid sequence according to SEQ ID NO: 9.

37.   A method for deciding whether a subject is a responder to a method of treatment, wherein the method of treatment comprises administering to the subject a PKN3 inhibitor, comprising:

        - determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to the subject being subjected to the method of treatment,

        - determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after the subject having been subjected to the method of treatment;

wherein if the level of VEGFR1 or a nucleic acid coding for VEGFR1 in a sample from the subject after the subject having been subjected to the method of treatment is decreased compared to the level of VEGFR1 or a nucleic acid coding for VEGFR1 in a sample from the subject prior to the subject having being subjected to the method of treatment, the subject is a responder to the method of treatment.

38.   The method according to claim 37, wherein the decrease is a decrease of 10% or more, of 20% or more, 40 % or more, 60% or more or 80% or more.

39.   The method according to any one of claims 37 to 38, wherein the subject is suffering from a disease or is at risk of suffering from a disease and wherein the method of treatment is a method of treatment for the disease.

40.   A method for deciding whether a subject having undergone a first method of treatment, wherein the first method of treatment comprises administering to the subject a PKN3 inhibitor, shall be subject to a second method of treatment, wherein the second method of treatment comprises administering to the subject a PKN3 inhibitor, comprising:

      - determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to a first method of treatment,

      - determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after a first period of time after the first method of treatment, and

      - optionally determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after a second period of time after the first method of treatment,

wherein if the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject after the first or second period of time after the first method of treatment is increased compared to the level of VEGFR1 or of a nucleic acid coding for VEGFR1 in a sample from the subject prior to a first method of treatment, the subject is to be subjected to the second method of treatment.

41.   The method according to claim 40, wherein the increase is 100% or more, 80% or more, 60% or more or 40% or more.

42.   The method according to any one of claims 40 to 41, wherein the subject is suffering from a disease or is at risk of suffering from a disease and wherein the method of treatment is a method of treatment for the disease.

43.   The method according to any one of claims 40 to 41, wherein first period of time after the first method of treatment is about 24 hours or 48 hours after the last administration of the PKN3 inhibitor being part of the first method of treatment.

44.   The method according to any of claims 40 to 41, wherein the second period of time after the first method of treatment starts about 1 or two months after the first period of time after the first method of treatment.

45.   The method according to any one of claims 40 to 44, wherein the first method of treatment is the same as the second method of treatment, or wherein the first method of treatment is different from the second method of treatment.

46.   A method for deciding whether a subject shall undergo a method of treatment, whereby the method of treatment comprises the administration of a PKN3 inhibitor, comprising determining the level of VEGFR1 or of a nucleic acid coding for VEGFR1, or determining the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, wherein if the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 200 pg/ml, the patient is amenable to the method of treatment comprising administration of a PKN3 inhibitor.

47.   The method according to claim 46, wherein if the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 400 pg/ml, preferably equal to or greater than 600 pg/ml, more preferably equal to or greater than 800 pg/ml, the patient is amenable to the method of treatment comprising administration of a PKN3 inhibitor

48.   The method according to any one of claims 37 to 47, wherein the disease is any disease as defined in any of the preceding claims.

49.   The method according to any one of claims 37 to 48, wherein the PKN3 inhibitor is any PKN3 inhibitor as defined in any of the preceding claims.

50.   The method according to any one of claims 37 to 49, wherein the VEGFR1 or a nucleic acid coding thereof is any VEGFR1 or a nucleic acid coding for the VEGFR1 as defined in any of the preceding claims.

51.   A PKN3 inhibitor for use in a method for the treatment of a subject suffering from or being at risk of suffering from a disease, wherein the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 200 pg/ml.

52.   The PKN3 inhibitor according to claim 51, wherein the expression level of VEGFR1 or of a nucleic acid coding for VEGFR1, expressed as titre of VEGF1 in blood or plasma of the subject, is equal to or greater than 400 pg/ml, preferably equal to or greater than 600 pg/ml and more preferably equal to or greater than 800 pg/ml.

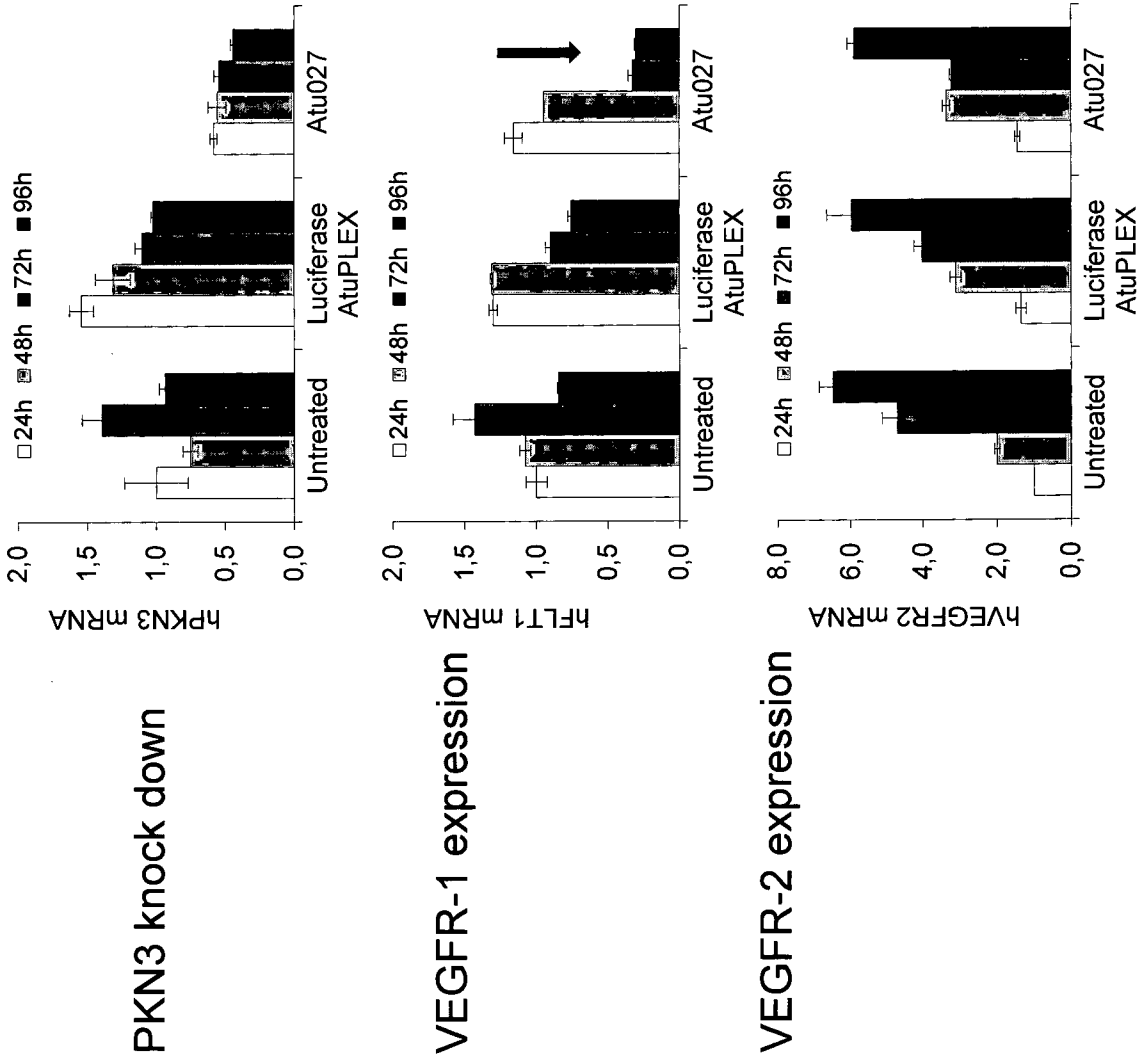**VEGFR1 biomarker in response to PKN3 knock down in PC-3 cells**

Figure 1: Human PC-3 cell line was obtained from American Type Culture Collection and cultivated according to the ATCC's recommendation. Cell line was transfected with Luciferase AtuPLEX and Atu027, respectively. Briefly, about 16 h after cell seeding siRNA-lipoplex solution diluted in 10% serum containing medium was added to the cells to achieve transfection concentration of 20 nM siRNA. After transfection (24 h, 48 h, 72 h, and 96 h), cells were lysed, total RNA was isolated and subjected to qRT-PCR. Graphs show respective mRNA level as mean of technical replicate relative to untreated @ 24 h. A decrease in VEGFR1 but not VEGFR2 expression in response to PKN3 knock down is observed.

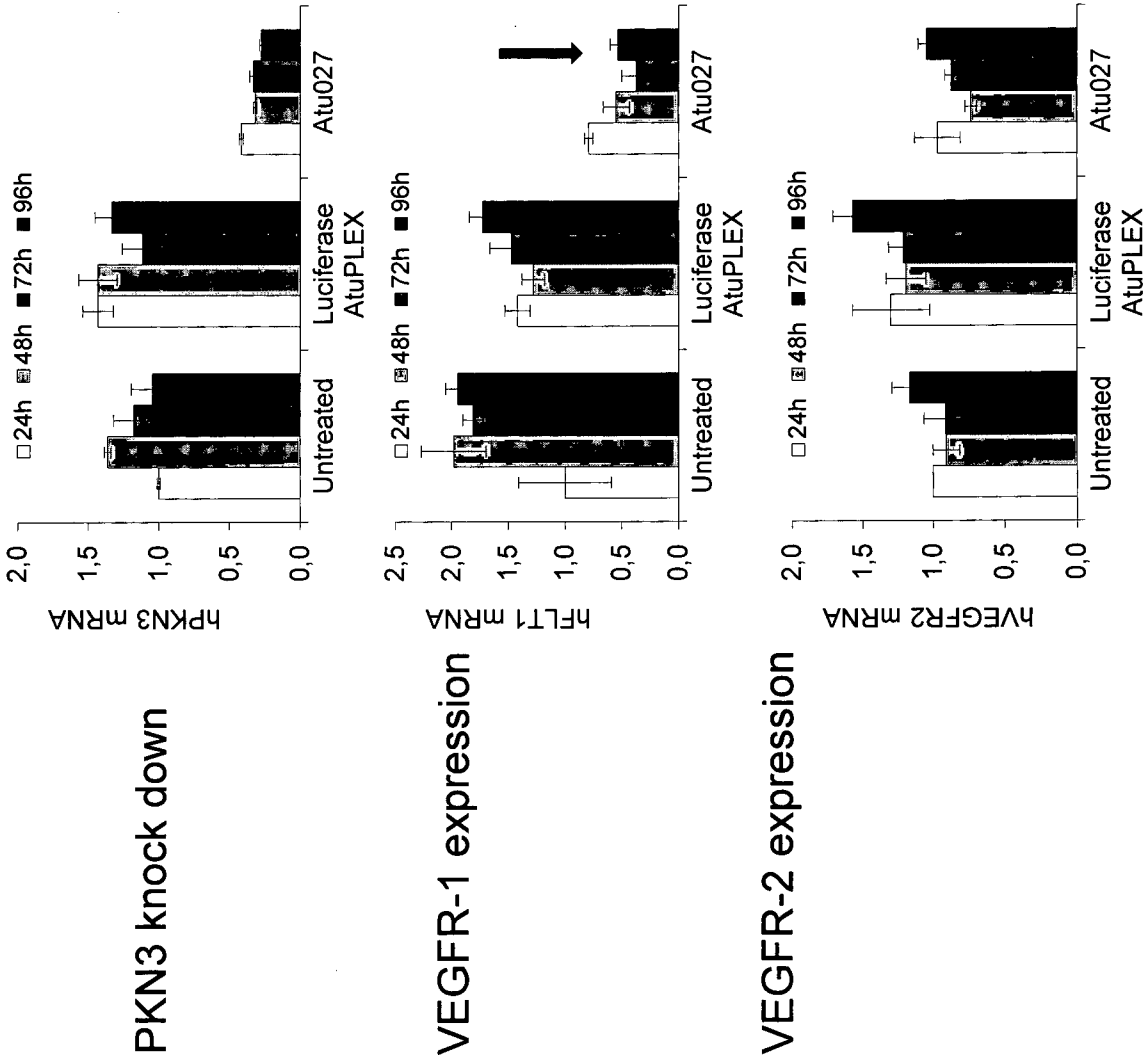# VEGFR1 biomarker in response to PKN3 knock down in MDA-MB-435 cells

Figure 2: Human MDA-MB-435 cell line was obtained from American Type Culture Collection and cultivated according to the ATCC's recommendation. Cell line was transfected with Luciferase AtuPLEX and Atu027, respectively. Briefly, about 16 h after cell seeding siRNA-lipoplex solution diluted in 10% serum containing medium was added to the cells to achieve transfection concentration of 20 nM siRNA. After transfection (24 h, 48 h, 72 h, and 96 h), cells were lysed, total RNA was isolated and subjected to qRT-PCR. Graphs show respective mRNA level as mean of technical replicate relative to untreated @ 24 h. A decrease in VEGFR1 but not VEGFR2 expression in response to PKN3 knock down is observed.

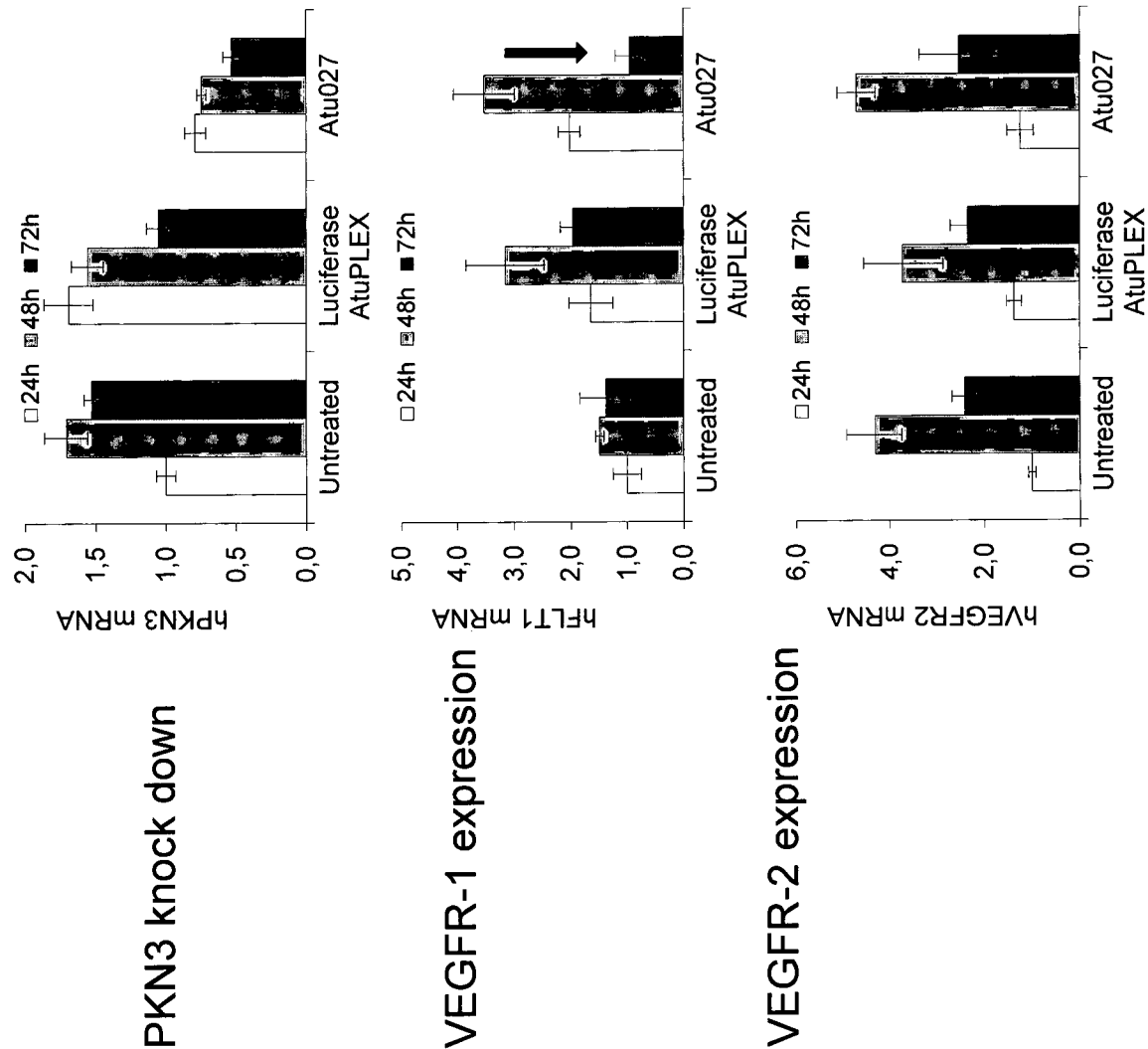**VEGFR1 biomarker in response to PKN3 knock down in SKBR3 cells**

Figure 3: Human SKBR3 cell line was obtained from American Type Culture Collection and cultivated according to the ATCC's recommendation. Cell line was transfected with Luciferase AtuPLEX and Atu027, respectively. Briefly, about 12 h after cell seeding siRNA-lipoplex solution diluted in 10% serum containing medium was added to the cells to achieve transfection concentration of 20 nM siRNA. After transfection (24 h, 48 h, 72 h, and 96 h), cells were lysed, total RNA was isolated and subjected to qRT-PCR. Graphs show respective mRNA level as mean of technical replicate relative to untreated @ 24 h. A decrease in VEGFR1 but not VEGFR2 expression in response to PKN3 knock down is observed.

Figure 4: Design of Clinical Phase I. Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn for biomarker analysis.

FU-1 = Follow-up period 1; FU-2 = Follow-up period 2

Biomarker analysis in plasma from humans treated with Atu027

Figure 5: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the ratio of post/pre treatment plasma level is shown for the VEGF isoforms and their receptors.

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGF

	2-06-001	2-06-003	2-07-002	2-07-003	2-08-001	2-08-003	x-09-001	x-09-002	x-09-003
0 h	699	10100	17400	-	-	-	1050	1110	15600
24 h	1090	7100	17600	-	-	-	1570	987	11000

- = no value

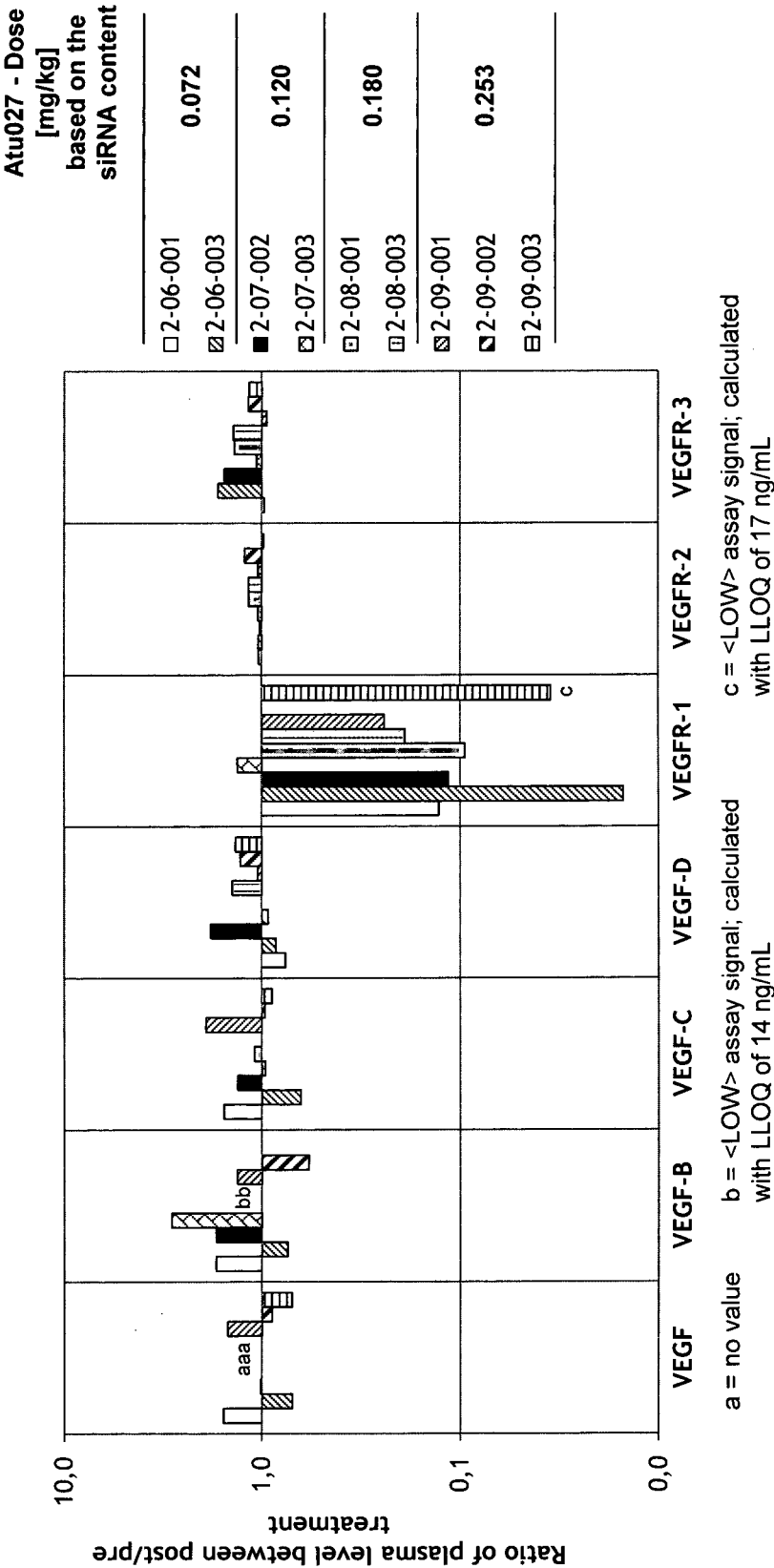Least detectable dose: 20 pg/mL

Figure 6: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGF are presented.

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGF-B

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

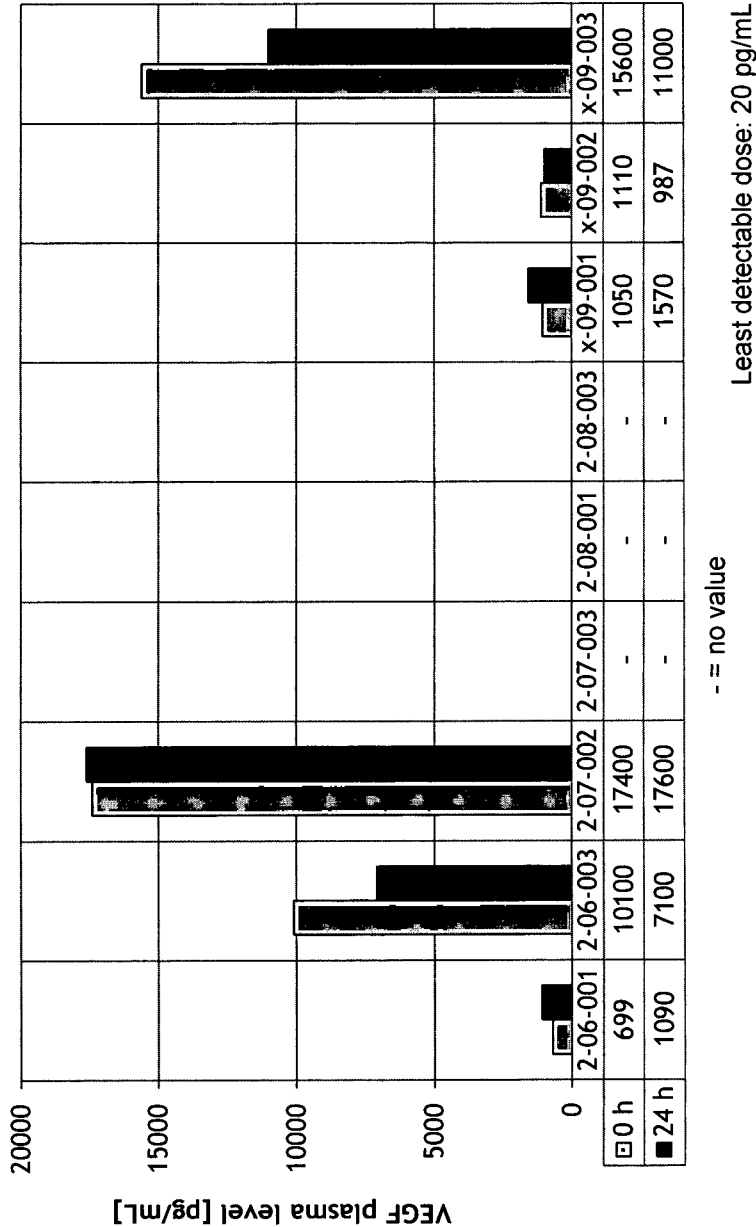Least detectable dose: 13.7 ng/mL

Figure 7: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGF-B are presented.

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGF-C

	2-06-001	2-06-003	2-07-002	2-07-003	2-08-001	2-08-003	x-09-001	x-09-002	x-09-003
0 h	11	6	8	6	17	10	10	8	4
24 h	7	9	6	6	16	10	5	8	4

VEGF-C plasma level [pg/mL]

Least detectable dose: 1.26 ng/mL

Figure 8: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGF-C are presented.

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGF-D
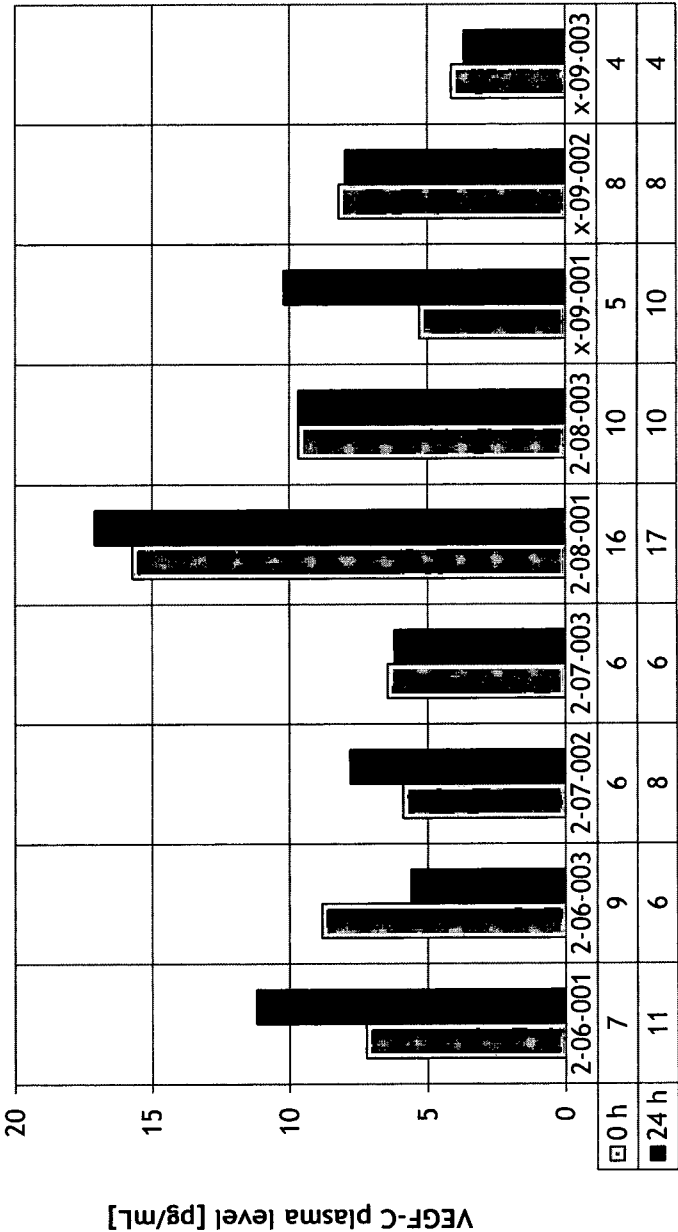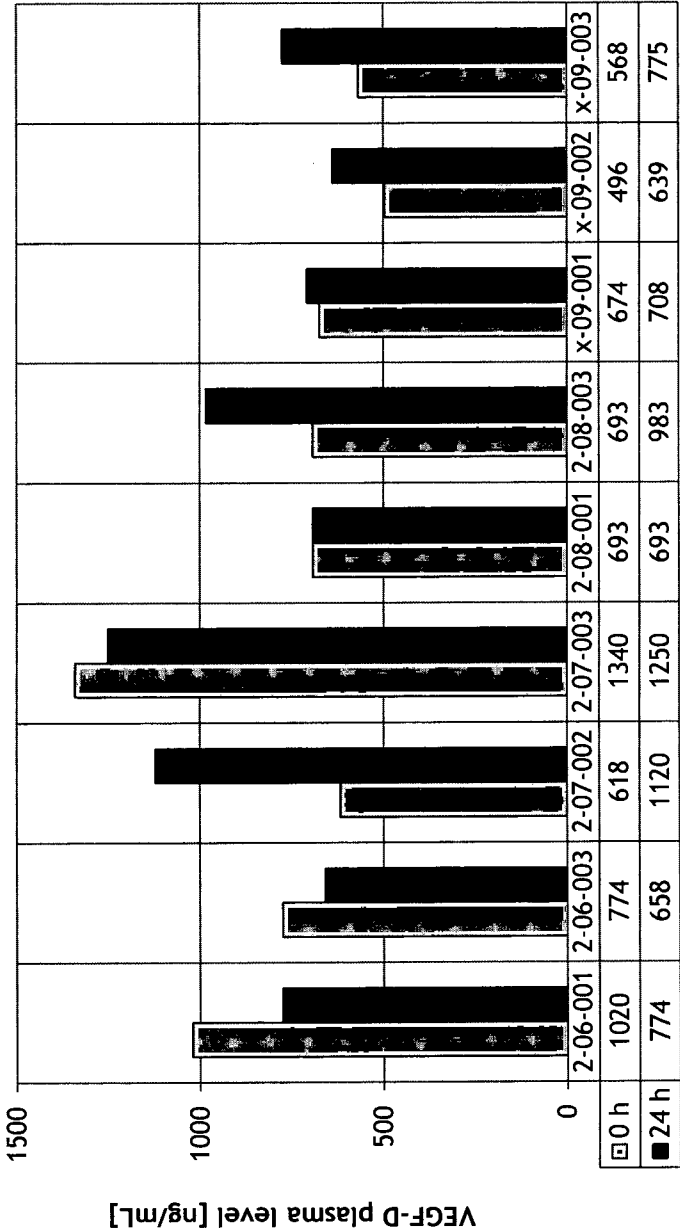

Least detectable dose: 500 pg/mL

Figure 9: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGF-D are presented.

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

	2-06-001	2-06-003	2-07-002	2-07-003	2-08-001	2-08-003	x-09-001	x-09-002	x-09-003
0 h	1020	774	618	1340	693	693	674	496	568
24 h	774	658	1120	1250	693	983	708	639	775

VEGF-D plasma level [ng/mL]

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGFR-1

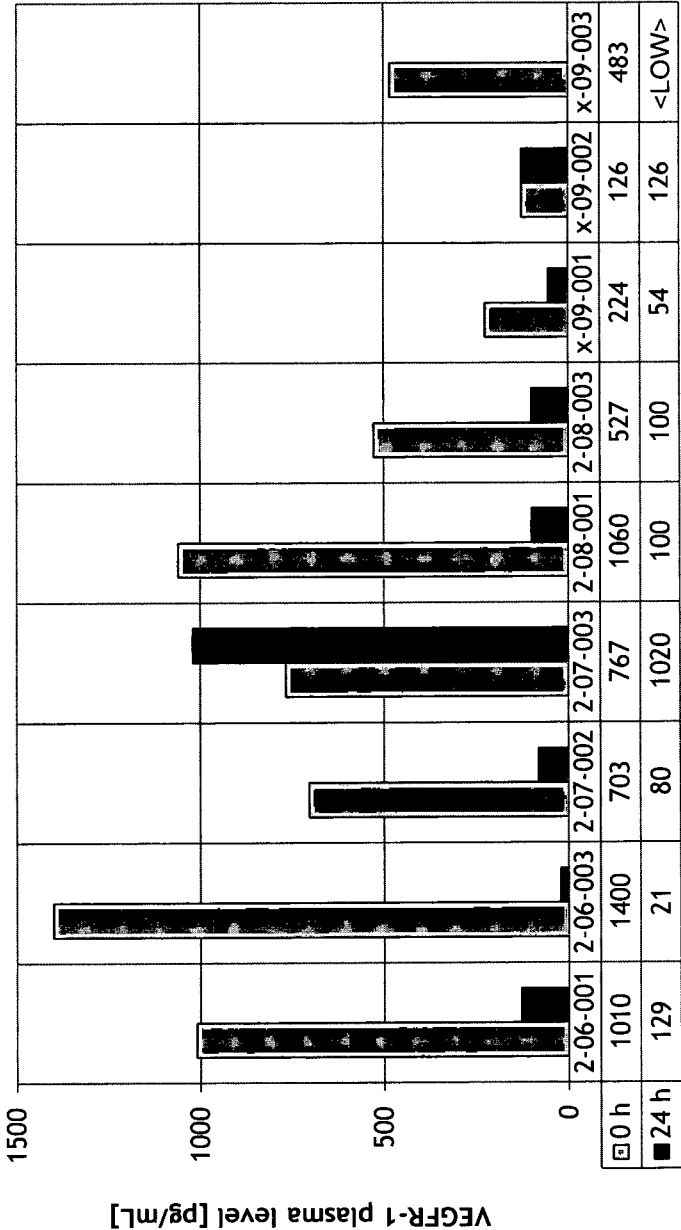|	2-06-001	2-06-003	2-07-002	2-07-003	2-08-001	2-08-003	x-09-001	x-09-002	x-09-003
0 h	1010	1400	703	767	1060	527	224	126	483
24 h	129	21	80	1020	100	100	54	126	<LOW>

Least detectable dose: 17.05 pg/mL

Figure 10: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGFR-1 are presented. In 7 out of 9 patient samples, VEGFR-1 was decreased after repeated Atu027 treatment.

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGFR-2

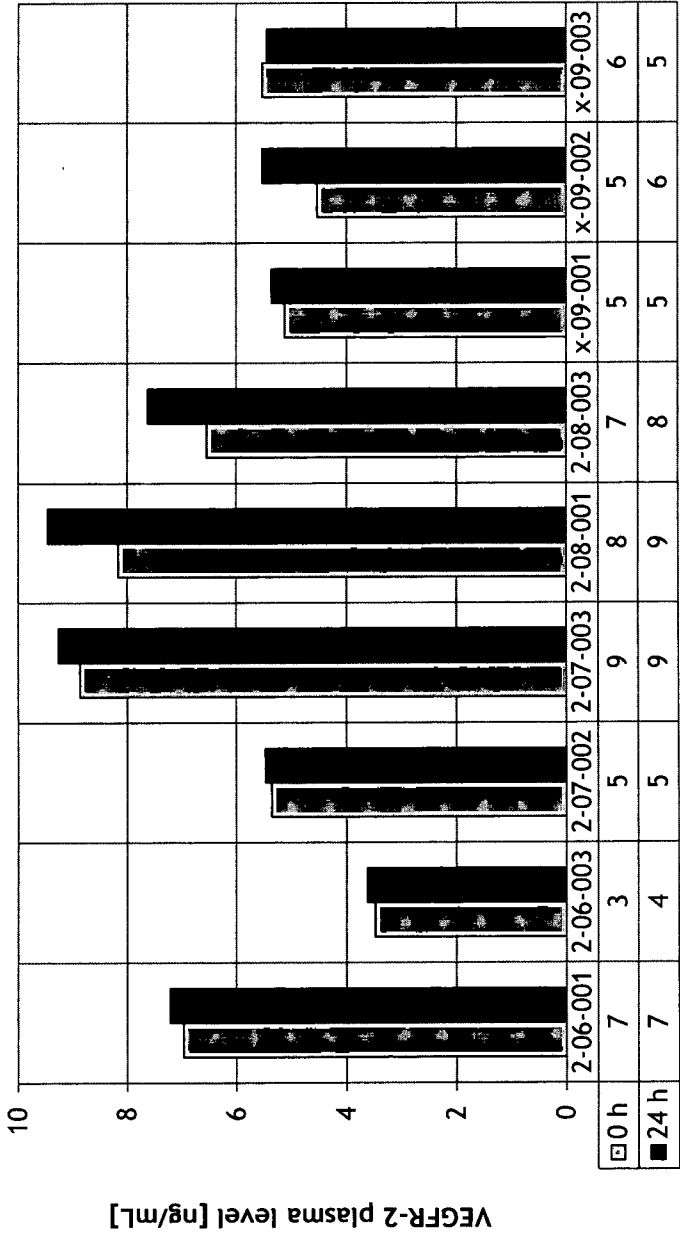| Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

Least detectable dose: 0.041 ng/mL

Figure 11: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGFR-2 is presented.

Biomarker analysis in plasma from humans treated with Atu027

Single analytes- VEGFR-3

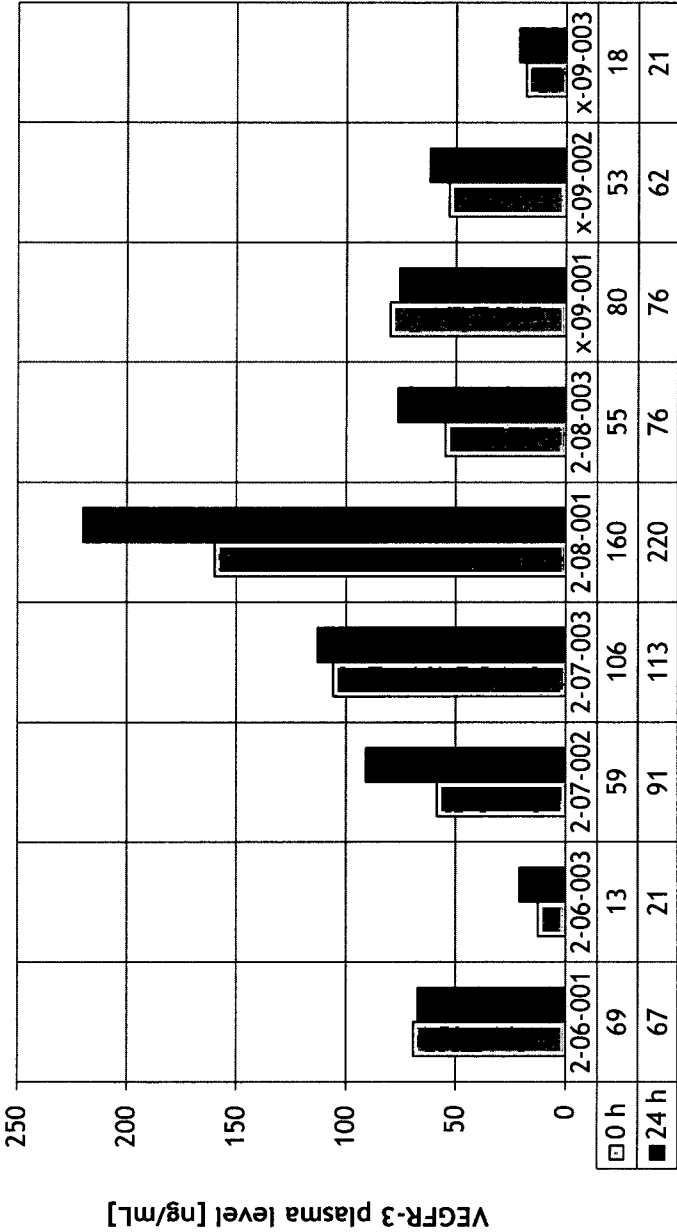**Figure 12**: Before (0 h) and 24 h after the last (24 h) repeated treatment with Atu027 blood was withdrawn from 9 patients and plasma was prepared. Samples were analyzed for biomarker analyte concentration. In the graph the absolute values for VEGFR-3 is presented.

Least detectable dose: 1.02 ng/mL

Dose level	Atu027 - Dose [mg/kg] based on the siRNA content
6	0.072
7	0.120
8	0.180
9	0.253

Fig. 13   SEQ ID NO: 1

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SNVKH

Fig. 14   SEQ ID NO: 2

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Fig. 15   SEQ ID NO: 3

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Fig. 16   SEQ ID NO: 4

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2281 aacctcagtg atcacacagt ggccatcagc agttccacca ctttagactg tcatgctaat
2341 ggtgtccccg agcctcagat cacttggttt aaaaacaacc acaaaataca acaagagcct
2401 ggaattattt taggaccagg aagcagcacg ctgtttattg aaagagtcac agaagaggat
2461 gaaggtgtct atcactgcaa agccaccaac cagaagggct ctgtggaaag ttcagcatac
2521 ctcactgttc aaggaacctc ggacaagtct aatctggagc tgatcactct aacatgcacc
2581 tgtgtggctg cgactctctt ctggctccta ttaaccctct ttatccgaaa aatgaaaagg
2641 tcttcttctg aaataaagac tgactaccta tcaattataa tggacccaga tgaagttcct
2701 ttggatgagc agtgtgagcg gctcccttat gatgccagca gtgggagtt gcccgggag
2761 agacttaaac tgggcaaatc acttggaaga ggggctttttg gaaagtggt tcaagcatca
2821 gcatttggca ttaagaaatc acctacgtgc cggactgtgg ctgtgaaaat gctgaaagag
2881 ggggccacgg ccagcgagta caaagctctg atgactgagc taaaatctt gacccacatt
2941 ggccaccatc tgaacgtggt taacctgctg ggagcctgca ccaagcaagg agggcctctg
3001 atggtgattg ttgaatactg caaatatgga aatctctcca actacctcaa gagcaaacgt
3061 gacttatttt ttctcaacaa ggatgcagca ctacacatgg agcctaagaa agaaaaaatg
3121 gagccaggcc tggaacaagg caagaaacca agactagata gcgtcaccag cagcgaaagc
```

```
3181  tttgcgagct  ccggctttca  ggaagataaa  agtctgagtg  atgttgagga  agaggaggat
3241  tctgacggtt  tctacaagga  gcccatcact  atggaagatc  tgatttctta  cagttttcaa
3301  gtggccagag  gcatggagtt  cctgtcttcc  agaaagtgca  ttcatcggga  cctggcagcg
3361  agaaacattc  ttttatctga  gaacaacgtg  gtgaagattt  gtgattttgg  ccttgcccgg
3421  gatatttata  agaaccccga  ttatgtgaga  aaaggagata  ctcgacttcc  tctgaaatgg
3481  atggctcctg  aatctatctt  tgacaaaatc  tacagcacca  agagcgacgt  gtggtcttac
3541  ggagtattgc  tgtgggaaat  cttctcctta  ggtgggtctc  catacccagg  agtacaaatg
3601  gatgaggact  tttgcagtcg  cctgagggaa  ggcatgagga  tgagagctcc  tgagtactct
3661  actcctgaaa  tctatcagat  catgctggac  tgctggcaca  gagacccaaa  agaaaggcca
3721  agatttgcag  aacttgtgga  aaaactaggt  gatttgcttc  aagcaaatgt  acaacaggat
3781  ggtaaagact  acatcccaat  caatgccata  ctgacaggaa  atagtgggtt  tacatactca
3841  actcctgcct  tctctgagga  cttcttcaag  gaaagtattt  cagctccgaa  gtttaattca
3901  ggaagctctg  atgatgtcag  atacgtaaat  gctttcaagt  tcatgagcct  ggaaagaatc
3961  aaaacctttg  aagaactttt  accgaatgcc  acctccatgt  ttgatgacta  ccaggggcgac
4021  agcagcactc  tgttggcctc  tcccatgctg  aagcgcttca  cctggactga  cagcaaaccc
4081  aaggcctcgc  tcaagattga  cttgagagta  accagtaaaa  gtaaggagtc  ggggctgtct
4141  gatgtcagca  ggcccagttt  ctgccattcc  agctgtgggc  acgtcagcga  aggcaagcgc
4201  aggttcacct  acgaccacgc  tgagctggaa  aggaaaatcg  cgtgctgctc  ccgcccccca
4261  gactacaact  cggtggtcct  gtactccacc  ccacccatct  agagtttgac  acgaagcctt
4321  atttctagaa  gcacatgtgt  atttataccc  caggaaact  agcttttgcc  agtattatgc
4381  atatataagt  ttacaccttt  atctttccat  gggagccagc  tgcttttttgt  gattttttta
4441  atagtgcttt  tttttttttg  actaacaaga  atgtaactcc  agatagagaa  atagtgacaa
4501  gtgaagaaca  ctactgctaa  atcctcatgt  tactcagtgt  tagagaaatc  cttcctaaac
4561  ccaatgactt  ccctgctcca  acccccgcca  cctcagggca  cgcaggacca  gtttgattga
4621  ggagctgcac  tgatcaccca  atgcatcacg  taccccactg  ggccagccct  gcagcccaaa
4681  acccagggca  acaagcccgt  tagccccagg  gatcactggc  tggcctgagc  aacatctcgg
4741  gagtcctcta  gcaggcctaa  gacatgtgag  gaggaaaagg  aaaaaaagca  aaaagcaagg
4801  gagaaaagag  aaaccgggag  aaggcatgag  aaagaatttg  agacgcacca  tgtgggcacg
4861  gagggggacg  gggctcagca  atgccatttc  agtggcttcc  cagctctgac  ccttctacat
4921  ttgagggccc  agccaggagc  agatggacag  cgatgagggg  acattttctg  gattctggga
4981  ggcaagaaaa  ggacaaatat  cttttttgga  actaaagcaa  attttagaac  tttacctatg
5041  gaagtggttc  tatgtccatt  ctcattcgtg  gcatgttttg  atttgtagca  ctgagggtgg
5101  cactcaactc  tgagcccata  cttttggctc  ctctagtaag  atgcactgaa  aacttagcca
5161  gagttaggtt  gtctccaggc  catgatggcc  ttacactgaa  aatgtcacat  tctattttgg
5221  gtattaatat  atagtccaga  cacttaactc  aatttcttgg  tattattctg  ttttgcacag
5281  ttagttgtga  aagaaagctg  agaagaatga  aaatgcagtc  ctgaggagag  gagttttctc
5341  catatcaaaa  cgagggctga  tggaggaaaa  aggtcaataa  ggtcaaggga  aaaccccgtc
5401  tctataccaa  ccaaaccaat  tcaccaacac  agttgggacc  caaaacacag  gaagtcagtc
5461  acgtttcctt  ttcatttaat  ggggattcca  ctatctcaca  ctaatctgaa  aggatgtgga
5521  agagcattag  ctggcgcata  ttaagcactt  taagctcctt  gagtaaaaag  gtggtatgta
5581  atttatgcaa  ggtatttctc  cagttgggac  tcaggatatt  agttaatgag  ccatcactag
5641  aagaaaagcc  cattttcaac  tgctttgaaa  cttgcctggg  gtctgagcat  gatgggaata
5701  gggagacagg  gtaggaaagg  gcgcctactc  ttcagggtct  aaagatcaag  tgggccttgg
5761  atcgctaagc  tggctctgtt  tgatgctatt  tatgcaagtt  agggtctatg  tatttatgat
5821  gtctgcacct  tctgcagcca  gtcagaagct  ggagaggcaa  cagtggattg  ctgcttcttg
5881  gggagaagag  tatgcttcct  tttatccatg  taatttaact  gtagaacctg  agctctaagt
5941  aaccgaagaa  tgtatgcctc  tgttcttatg  tgccacatcc  ttgtttaaag  gctctctgta
6001  tgaagagatg  ggaccgtcat  cagcacattc  cctagtgagc  ctactggctc  ctggcagcgg
6061  cttttgtgga  agactcacta  gccagaagag  aggagtggga  cagtcctctc  caccaagatc
6121  taaatccaaa  caaaagcagg  ctagagccag  aagagaggac  aaatctttgt  tcttcctctt
6181  ctttacatac  gcaaccacc  tgtgacagct  ggcaatttta  taaatcaggt  aactggaagg
6241  aggttaaaca  cagaaaaaag  aagacctcag  tcaattctct  actttttttt  tttttttccaa
6301  atcagataat  agcccagcaa  atagtgataa  caaataaaac  cttagctatt  catgtcttga
6361  tttcaataat  taattcttaa  tcattaagag  accataataa  atactccttt  tcaagagaaa
6421  agcaaaacca  ttagaattgt  tactcagctc  cttcaaactc  aggtttgtag  catacatgag
6481  tccatccatc  agtcaaagaa  tggttccatc  tggagtctta  atgtagaaag  aaaaatggag
6541  acttgtaata  atgagctagt  tacaaagtgc  ttgttcatta  aaatagcact  gaaaattgaa
6601  acatgaatta  actgataata  ttccaatcat  ttgccattta  tgacaaaaat  ggttggcact
6661  aacaaagaac  gagcacttcc  tttcagagtt  tctgagataa  tgtacgtgga  acagtctggg
6721  tggaatgggg  ctgaaaccat  gtgcaagtct  gtgtcttgtc  agtccaagaa  gtgacaccga
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6781 gatgttaatt ttagggaccc gtgccttgtt tcctagccca caagaatgca aacatcaaac
6841 agatactcgc tagcctcatt taaattgatt aaaggaggag tgcatctttg gccgacagtg
6901 gtgtaactgt atgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgggt gtatgtgtgt
6961 tttgtgcata actatttaag gaaactggaa ttttaaagtt acttttatac aaaccaagaa
7021 tatatgctac agatataaga cagacatggt ttggtcctat atttctagtc atgatgaatg
7081 tattttgtat accatcttca tataataaac ttccaaaaac aca
```

Fig. 17   SEQ ID NO: 5

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQ

AGQTLHLQCRGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHT

GFYSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRV

TSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKT

NYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRA

SVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFI

TVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGYSLII

KDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYPLGSRQ

ILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILDADSNMGNRIESITQR

MAIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNISFYITDVPNGFHVNLEKM

PTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIM

NVSLQDSGTYACRARNVYTGEEILQKKEITIRDQEAPYLLRNLSDHTVAISSSTTLDC

HANGVPEPQITWFKNNHKIQQEPELYTSTSPSSSSSSPLSSSSSSSSSSSS

Fig. 18   SEQ ID NO: 6

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   1 atcgaggtcc gcgggaggct cggagcgcgc caggcggaca ctcctctcgg ctcctccccg
  61 gcagcggcgg cggctcggag cgggctccgg ggctcgggtg cagcggccag cgggcgcctg
 121 gcggcgagga ttacccgggg aagtggttgt ctcctggctg gagccgcgag acgggcgctc
 181 agggcgcggg gccggcggcg gcgaacgaga ggacggactc tggcggccgg tcgttggcc
 241 gcggggagcg cgggcaccgg gcgagcaggc cgcgtcgcgc tcaccatggt cagctactgg
 301 gacaccgggg tcctgctgtg cgcgctgctc agctgtctgc ttctcacagg atctagttca
 361 ggttcaaaat taaaagatcc tgaactgagt ttaaaaggca cccagcacat catgcaagca
 421 ggccagacac tgcatctcca atgcaggggg gaagcagccc ataaatggtc tttgcctgaa
 481 atggtgagta aggaaagcga aaggctgagc ataactaaat ctgcctgtgg aagaaatggc
 541 aaacaattct gcagtacttt aaccttgaac acagctcaag caaaccacac tggcttctac
 601 agctgcaaat atctagctgt acctacttca aagaagaagg aaacagaatc tgcaatctat
 661 atatttatta gtgatacagg tagacctttc gtagagatgt acagtgaaat ccccgaaatt
 721 atacacatga ctgaaggaag ggagctcgtc attccctgcc gggttacgtc acctaacatc
 781 actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc
 841 tgggacagta gaaagggctt catcatatca aatgcaacgt acaaagaaat agggcttctg
 901 acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa
 961 accaatacaa tcatagatgt ccaaataagc acaccacgcc cagtcaaatt acttagaggc
1021 catactcttg tcctcaattg tactgctacc actcccttga acacgagagt tcaaatgacc
1081 tggagttacc ctgatgaaaa aaataagaga gcttccgtaa ggcgacgaat tgaccaaagc
1141 aattcccatg ccaacatatt ctacagtgtt cttactattg acaaaatgca gaacaaagac
1201 aaaggacttt atacttgtcg tgtaaggagt ggaccatcat tcaaatctgt aacacctca
1261 gtgcatatat atgataaagc attcatcact gtgaaacatc gaaaacagca ggtgcttgaa
1321 accgtagctg gcaagcggtc ttaccggctc tctatgaaag tgaaggcatt ccctcgccg
1381 gaagttgtat ggttaaaaga tgggttacct gcgactgaga atctgctcg ctatttgact
1441 cgtggctact cgttaattat caaggacgta actgaagagg atgcagggaa ttatacaatc
1501 ttgctgagca taaaacagtc aaatgtgttt aaaaacctca ctgccactct aattgtcaat
1561 gtgaaacccc agatttacga aaaggccgtg tcatcgtttc cagaccggc tctctaccca
1621 ctgggcagca gacaaatcct gacttgtacc gcatatggta tccctcaacc tacaatcaag
1681 tggttctggc acccctgtaa ccataatcat tccgaagcaa ggtgtgactt ttgttccaat
1741 aatgaagagt cctttatcct ggatgctgac agcaacatgg aaacagaat tgagagcatc
1801 actcagcgca tggcaataat agaaggaaag aataagatgg ctagcacctt ggttgtggct
1861 gactctagaa tttctggaat ctacatttgc atagcttcca ataaagttgg gactgtggga
1921 agaaacataa gctttatat cacagatgtg ccaaatgggt ttcatgttaa cttggaaaaa
1981 atgccgacgg aaggagagga cctgaaactg tcttgcacag ttaacaagtt cttatacaga
2041 gacgttactt ggatttttact gcggacagtt aataacagaa caatgcacta cagtattagc
2101 aagcaaaaaa tggccatcac taaggagcac tccatcactc ttaatcttac catcatgaat
2161 gtttccctgc aagattcagg cacctatgcc tgcagagcca ggaatgtata cacaggggaa
2221 gaaatcctcc agaagaaaga aattacaatc agagatcagg aagcaccata cctcctgcga
2281 aacctcagtg atcacacagt ggccatcagc agttccacca ctttagactg tcatgctaat
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2341 ggtgtccccg agcctcagat cacttggttt aaaaacaacc acaaaataca acaagagcct
2401 gaactgtata catcaacgtc accatcgtca tcgtcatcat caccattgtc atcatcatca
2461 tcatcgtcat catcatcatc atcatagcta tcatcattat catcatcatc atcatcatca
2521 tcatagctac catttattga aaactattat gtgtcaactt caaagaactt atcctttagt
2581 tggagagcca agacaatcat aacaataaca aatggccggg catggtggct cacgcctgta
2641 atcccagcac tttgggaggc caaggcaggt ggatcatttg aggtcaggag ttcaagacca
2701 gcctgaccaa gatggtgaaa tgctgtctct attaaaaata caaaattagc caggcatggt
2761 ggctcatgcc tgtaatgcca gctactcggg aggctgagac aggagaatca cttgaaccca
2821 ggaggcagag gttgcaggga gccgagatcg tgtactgcac tccagcctgg gcaacaagag
2881 cgaaactccg tctcaaaaaa caaataaata aataaataaa taaacagaca aaattcactt
2941 tttattctat taaacttaac atacatgc
```

Fig. 19   SEQ ID NO: 7

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQ

AGQTLHLQCRGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHT

GFYSCKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRV

TSPNITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKT

NYLTHRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRA

SVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVSGPSFKSVNTSVHIYDKAFI

TVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGYSLII

KDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYPLGSRQ

ILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILDADSNMGNRIESITQR

MAIIEGKNKLPPANSSFMLPPTSFSSNYFHFLP

Fig. 20　　　SEQ ID NO: 8

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   1 atcgaggtcc gcgggaggct cggagcgcgc caggcggaca ctcctctcgg ctcctccccg
  61 gcagcggcgg cggctcggag cgggctccgg ggctcgggtg cagcggccag cgggcgcctg
 121 gcggcgagga ttacccgggg aagtggttgt ctcctggctg gagccgcgag acgggcgctc
 181 agggcgcggg gccggcggcg gcgaacgaga ggacggactc tggcggccgg gtcgttggcc
 241 gcggggagcg cgggcaccgg gcgagcaggc cgcgtcgcgc tcaccatggt cagctactgg
 301 gacaccgggg tcctgctgtg cgcgctgctc agctgtctgc ttctcacagg atctagttca
 361 ggttcaaaat taaaagatcc tgaactgagt ttaaaaggca cccagcacat catgcaagca
 421 ggccagacac tgcatctcca atgcaggggg gaagcagccc ataaatggtc tttgcctgaa
 481 atggtgagta aggaaagcga aaggctgagc ataactaaat ctgcctgtgg aagaaatggc
 541 aaacaattct gcagtacttt aaccttgaac acagctcaag caaaccacac tggcttctac
 601 agctgcaaat atctagctgt acctacttca aagaagaagg aaacagaatc tgcaatctat
 661 atatttatta gtgatacagg tagacctttc gtagagatgt acagtgaaat ccccgaaatt
 721 atacacatga ctgaaggaag ggagctcgtc attccctgcc gggttacgtc acctaacatc
 781 actgttactt taaaaaagtt tccacttgac actttgatcc ctgatggaaa acgcataatc
 841 tgggacagta gaaagggctt catcatatca aatgcaacgt acaaagaaat agggcttctg
 901 acctgtgaag caacagtcaa tgggcatttg tataagacaa actatctcac acatcgacaa
 961 accaatacaa tcatagatgt ccaaataagc acaccacgcc cagtcaaatt acttagaggc
1021 catactcttg tcctcaattg tactgctacc actcccttga acacgagagt tcaaatgacc
1081 tggagttacc ctgatgaaaa aaataagaga gcttccgtaa ggcgacgaat tgaccaaagc
1141 aattcccatg ccaacatatt ctacagtgtt cttactattg acaaaatgca gaacaaagac
1201 aaaggacttt atacttgtcg tgtaaggagt ggaccatcat tcaaatctgt aacacctca
1261 gtgcatatat atgataaagc attcatcact gtgaaacatc gaaaacagca ggtgcttgaa
1321 accgtagctg gcaagcggtc ttaccggctc tctatgaaag tgaaggcatt tccctcgccg
1381 gaagttgtat ggttaaaaga tgggttacct gcgactgaga aatctgctcg ctatttgact
1441 cgtggctact cgttaattat caaggacgta actgaagagg atgcagggaa ttatacaatc
1501 ttgctgagca taaaacagtc aaatgtgttt aaaaacctca ctgccactct aattgtcaat
1561 gtgaaacccc agatttacga aaaggccgtg tcatcgtttc cagacccggc tctctaccca
1621 ctgggcagca gacaaatcct gacttgtacc gcatatggta tccctcaacc tacaatcaag
1681 tggttctggc acccctgtaa ccataatcat tccgaagcaa ggtgtgactt ttgttccaat
1741 aatgaagagt cctttatcct ggatgctgac agcaacatgg gaaacagaat tgagagcatc
1801 actcagcgca tggcaataat agaaggaaag aataagcttc caccagctaa cagttctttc
1861 atgttgccac ctacaagctt ctcttccaac tacttccatt ccttccgtg a
```

Fig. 21   SEQ ID NO: 9

Met Glu Glu Gly Ala Pro Arg Gln Pro Gly Pro Ser Gln Trp Pro Pro

Glu Asp Glu Lys Glu Val Ile Arg Arg Ala Ile Gln Lys Glu Leu Lys

Ile Lys Glu Gly Val Glu Asn Leu Arg Arg Val Ala Thr Asp Arg Arg

His Leu Gly His Val Gln Gln Leu Leu Arg Ser Ser Asn Arg Arg Leu

Glu Gln Leu His Gly Glu Leu Arg Glu Leu His Ala Arg Ile Leu Leu

Pro Gly Pro Gly Pro Gly Pro Ala Glu Pro Val Ala Ser Gly Pro Arg

Pro Trp Ala Glu Gln Leu Arg Ala Arg His Leu Glu Ala Leu Arg Arg

Gln Leu His Val Glu Leu Lys Val Lys Gln Gly Ala Glu Asn Met Thr

His Thr Cys Ala Ser Gly Thr Pro Lys Glu Arg Lys Leu Leu Ala Ala

Ala Gln Gln Met Leu Arg Asp Ser Gln Leu Lys Val Ala Leu Leu Arg

Met Lys Ile Ser Ser Leu Glu Ala Ser Gly Ser Pro Glu Pro Gly Pro

Glu Leu Leu Ala Glu Glu Leu Gln His Arg Leu His Val Glu Ala Ala

Val Ala Glu Gly Ala Lys Asn Val Val Lys Leu Leu Ser Ser Arg Arg

Thr Gln Asp Arg Lys Ala Leu Ala Glu Ala Gln Ala Gln Leu Gln Glu

Ser Ser Gln Lys Leu Asp Leu Leu Arg Leu Ala Leu Glu Gln Leu Leu

Glu Gln Leu Pro Pro Ala His Pro Leu Arg Ser Arg Val Thr Arg Glu

Leu Arg Ala Ala Val Pro Gly Tyr Pro Gln Pro Ser Gly Thr Pro Val

Lys Pro Thr Ala Leu Thr Gly Thr Leu Gln Val Arg Leu Leu Gly Cys

Glu Gln Leu Leu Thr Ala Val Pro Gly Arg Ser Pro Ala Ala Ala Leu

Ala Ser Ser Pro Ser Glu Gly Trp Leu Arg Thr Lys Ala Lys His Gln

Arg Gly Arg Gly Glu Leu Ala Ser Glu Val Leu Ala Val Leu Lys Val

Asp Asn Arg Val Val Gly Gln Thr Gly Trp Gly Gln Val Ala Glu Gln

Ser Trp Asp Gln Thr Phe Val Ile Pro Leu Glu Arg Ala Arg Glu Leu

Glu Ile Gly Val His Trp Arg Asp Trp Arg Gln Leu Cys Gly Val Ala

Phe Leu Arg Leu Glu Asp Phe Leu Asp Asn Ala Cys His Gln Leu Ser

Leu Ser Leu Val Pro Gln Gly Leu Leu Phe Ala Gln Val Thr Phe Cys

Asp Pro Val Ile Glu Arg Arg Pro Arg Leu Gln Arg Gln Glu Arg Ile

Phe Ser Lys Arg Arg Gly Gln Asp Phe Leu Arg Arg Ser Gln Met Asn

Leu Gly Met Ala Ala Trp Gly Arg Leu Val Met Asn Leu Leu Pro Pro

Cys Ser Ser Pro Ser Thr Ile Ser Pro Pro Lys Gly Cys Pro Arg Thr

Pro Thr Thr Leu Arg Glu Ala Ser Asp Pro Ala Thr Pro Ser Asn Phe

Leu Pro Lys Lys Thr Pro Leu Gly Glu Glu Met Thr Pro Pro Pro Lys

Pro Pro Arg Leu Tyr Leu Pro Gln Glu Pro Thr Ser Glu Glu Thr Pro

Arg Thr Lys Arg Pro His Met Glu Pro Arg Thr Arg Arg Gly Pro Ser

Pro Pro Ala Ser Pro Thr Arg Lys Pro Pro Arg Leu Gln Asp Phe Arg

Cys Leu Ala Val Leu Gly Arg Gly His Phe Gly Lys Val Leu Leu Val

Gln Phe Lys Gly Thr Gly Lys Tyr Tyr Ala Ile Lys Ala Leu Lys Lys

Gln Glu Val Leu Ser Arg Asp Glu Ile Glu Ser Leu Tyr Cys Glu Lys

Arg Ile Leu Glu Ala Val Gly Cys Thr Gly His Pro Phe Leu Leu Ser

Leu Leu Val Cys Phe Gln Thr Ser Ser His Ala Arg Phe Val Thr Glu

Phe Val Pro Gly Gly Asp Leu Met Met Gln Ile His Glu Asp Val Phe

Pro Glu Pro Gln Ala Arg Phe Tyr Val Ala Cys Val Val Leu Gly Leu

Gln Phe Leu His Glu Lys Lys Ile Ile Tyr Arg Asp Leu Lys Leu Asp

Asn Leu Leu Leu Asp Ala Gln Gly Phe Leu Lys Ile Ala Asp Phe Gly

Leu Cys Lys Glu Gly Ile Gly Phe Gly Asp Arg Thr Ser Thr Phe Cys

Gly Thr Pro Glu Phe Leu Ala Pro Glu Val Leu Thr Gln Glu Ala Tyr

Thr Gln Ala Val Asp Trp Trp Ala Leu Gly Val Leu Leu Tyr Glu Met

Leu Val Gly Glu Cys Pro Phe Pro Gly Asp Thr Glu Glu Glu Val Phe

Asp Cys Ile Val Asn Met Asp Ala Pro Tyr Pro Gly Phe Leu Ser Val

Gln Gly Leu Glu Phe Ile Gln Lys Leu Leu Gln Lys Cys Pro Glu Lys

Arg Leu Gly Ala Gly Glu Gln Asp Ala Glu Glu Ile Lys Val Gln Pro

Phe Phe Arg Thr Thr Asn Trp Gln Ala Leu Leu Ala Arg Thr Ile Gln

Pro Pro Phe Val Pro Thr Leu Cys Gly Pro Ala Asp Leu Arg Tyr Phe

Glu Gly Glu Phe Thr Gly Leu Pro Pro Ala Leu Thr Pro Pro Ala Pro

His Ser Leu Leu Thr Ala Arg Gln Gln Ala Ala Phe Arg Asp Phe Asp

Phe Val Ser Glu Arg Phe Leu Glu Pro

Fig. 22   SEQ ID NO: 10

atggaggagg gggcgccgcg gcagcctggg ccgagccagt ggcccccaga ggatgagaag   60

gaggtgatcc gccgggccat ccagaaagag ctgaagatca aggaggggggt ggagaacctg   120

cggcgcgtgg ccacagaccg ccgccacttg ggccatgtgc agcagctgct gcggtcctcc   180

aaccgccgcc tggagcagct gcatggcgag ctgcgggagc tgcacgcccg aatcctgctg   240

cccggcccctg ggcctggccc agctgagcct gtggcctcag accccggcc gtgggcagag   300

cagctcaggg ctcggcacct agaggctctc cggaggcagc tgcatgtgga gctgaaggtg   360

aaacaggggg ctgagaacat gacccacacg tgcgccagtg gcacccccaa ggagaggaag   420

ctccttgcag ctgcccagca gatgctgcgg gacagccagc tgaaggtggc cctgctgcgg   480

atgaagatca gcagcctgga ggccagtggg tccccggagc cagggcctga gctactggcg   540

gaggagctac agcatcgact gcacgttgag gcagcggtgg ctgagggcgc caagaacgtg   600

gtgaaactgc ttagtagccg gagaacacag gaccgcaagg cactggctga ggcccaggcc   660

cagctacagg agtcctctca gaaactggac ctcctgcgcc tggccttgga gcagctgctg   720

gagcaactgc ctcctgccca ccctttgcgc agcagagtga cccgagagtt gcgggctgcg   780

gtgcctggat accccagcc ttcagggaca cctgtgaagc ccaccgccct aacagggaca   840

ctgcaggtcc gcctcctggg ctgtgaacag ttgctgacag ccgtgcctgg gcgctcccca   900

gcggccgcac tggccagcag cccctccgag ggctggcttc ggaccaaggc caagcaccag   960

cgtggccgag gcgagcttgc cagtgaggtg ctggctgtgc taaaggtgga caaccgtgtt   1020

gtggggcaga cgggctgggg gcaggtggcc gaacagtcct gggaccagac ctttgtcatc   1080

ccactggagc gagcccgtga gctggagatt ggggtacact ggcgggactg gcggcagcta   1140

tgtggcgtgg ccttcctgag acttgaagac ttcctggaca atgcctgtca ccaactgtcc   1200

ctcagcctgg taccgcaggg actgcttttt gcccaggtga ccttctgcga tcctgtcatt   1260

gagaggcggc cccggctgca gaggcaggaa cgcatcttct ctaaacgcag aggccaggac   1320

ttcctgaggc gttcgcagat gaacctcggc atggcggcct gggggcgcct cgtcatgaac   1380

ctgctgcccc cctgcagctc cccgagcaca atcagccccc ctaaaggatg ccctcggacc   1440

ccaacaacac tgcgagaggc ctctgaccct gccactccca gtaatttcct gcccaagaag   1500

accccttgg gtgaagagat gacaccccca cccaagcccc cacgcctcta cctcccccag　1560

gagccaacat ccgaggagac tccgcgcacc aaacgtcccc atatggagcc taggactcga　1620

cgtgggccat ctccaccagc ctcccccacc aggaaacccc ctcggcttca ggacttccgc　1680

tgcttagctg tgctgggccg gggacacttt gggaaggtcc tcctggtcca gttcaagggg　1740

acagggaaat actacgccat caaagcactg aagaagcagg aggtgctcag ccgggacgag　1800

atagagagcc tgtactgcga gaagcggatc ctggaggctg tgggctgcac agggcaccct　1860

ttcctgctct ccctccttgt ctgcttccag acctccagcc atgcccgctt tgtgactgag　1920

tttgtgcctg gtggtgacct catgatgcag atccacgagg atgtcttccc cgagccccag　1980

gcccgcttct acgtggcttg tgttgtcctg gggctgcagt tcttacacga gaagaagatc　2040

atttacaggg acctgaagtt ggataacctt ctgctggatg cccagggatt cctgaagatc　2100

gcagactttg gactctgcaa ggaagggatc ggcttcgggg accggactag caccttctgt　2160

ggcaccccgg agttcctggc tcccgaggtg ctgacccagg aggcatacac acaggccgtc　2220

gactggtggg cgctgggtgt gctgctctac gagatgctgg tgggtgagtg cccgttccca　2280

ggggacacag aggaagaggt gtttgactgc atcgtcaaca tggacgcccc ctaccccggc 2340

tttctgtcgg tgcaagggct tgagttcatt cagaagctcc tccagaagtg cccggagaag 2400

cgcctcgggg caggtgagca ggatgccgag gagatcaagg tccagccatt cttcaggacc 2460

accaactggc aagccctgct cgcccgcacc atccagcccc ccttcgtgcc taccctgtgt 2520

ggccctgcgg acctgcgcta ctttgagggc gagttcacag ggctgccgcc tgccctgacc 2580

ccacctgcac cccacagcct cctcactgcc cgccaacagg ccgccttccg ggacttcgac 2640

tttgtgtcag agcgattcct ggaaccctga   2670

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/00   G01N33/50
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)

A61K 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	US 2004/220128 A1 (PAVCO PAMELA [US] ET AL) 4 November 2004 (2004-11-04) the whole document In particular: See example 11. ----- -/--	1-52

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

| X | See patent family annex. |

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Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2013	07/08/2013

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page 1 of 2

INTERNATIONAL SEARCH REPORT

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MANUELA ALEKU ET AL: "Atu027, a Liposomal Small Interfering RNA Formulation Targeting Protein kinase N3, Inhibits Cancer Progression", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 68, no. 23, 1 December 2008 (2008-12-01), pages 9788-9798, XP002637553, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-08-2428	51,52
A	the whole document In particular: Title; Abstract; Materials and methods section. -----	1-50
X	STRUMBERG DIRK ET AL: "Phase I clinical development of Atu027, a siRNA formulation targeting PKN3 in patients with advanced solid tumors", INTERNATIONAL JOURNAL OF CLINICAL PHARMACOLOGY AND THERAPEUTICS, vol. 50, no. 1, January 2012 (2012-01), pages 76-78, XP55073385, ISSN: 0946-1965 Retrieved from the Internet: URL:http://meeting.ascopubs.org/cgi/conten t/abstract/30/15_suppl/e13597?sid=bbf7a4e2 -0ada-4e67-91e8-19bd2f503bb1>	51,52
A	the whole document In particular: Title; Results section. -----	1-50
X,P	"Antimetastatic activity of Atu027, a liposomal small interfering RNA formulation, targeting protein kinase N3 (PKN3): Final results of a phase I study in patients with advanced solid tumors.", , vol. 30, no. 15 20 May 2012 (2012-05-20), XP55073401, Retrieved from the Internet: URL:http://meeting.ascopubs.org/cgi/conten t/abstract/30/15_suppl/e13597?sid=bbf7a4e2 -0ada-4e67-91e8-19bd2f503bb1 [retrieved on 2013-07-29] the whole document -----	1-52

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004220128 A1	04-11-2004	NONE	