

(19)



(11)

EP 3 364 188 A1

(12)

EUROPEAN PATENT APPLICATION
published in accordance with Art. 153(4) EPC

(43) Date of publication:

22.08.2018 Bulletin 2018/34

(51) Int Cl.:

G01N 33/53 (2006.01) **G01N 33/50** (2006.01)
G01N 33/574 (2006.01) **A61K 45/00** (2006.01)
A61P 13/12 (2006.01) **A61P 35/00** (2006.01)

(21) Application number: **16855381.6**

(22) Date of filing: **11.10.2016**

(86) International application number:

PCT/JP2016/080084

(87) International publication number:

WO 2017/065127 (20.04.2017 Gazette 2017/16)

(84) Designated Contracting States:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

Designated Extension States:

BA ME

Designated Validation States:

MA MD

(72) Inventors:

- **NAKAIGAWA, Noboru**
Yokohama-shi
Kanagawa 236-0004 (JP)
- **UENO, Daiki**
Yokohama-shi
Kanagawa 236-0004 (JP)
- **YAO, Masahiro**
Yokohama-shi
Kanagawa 236-0004 (JP)

(30) Priority: **14.10.2015 JP 2015202614**

(71) Applicant: **Public University Corporation
Yokohama City
University
Yokohama-shi, Kanagawa 236-0027 (JP)**

(74) Representative: **Keirstead, Tanis Evelyne et al
Mewburn Ellis LLP
City Tower
40 Basinghall Street
London EC2V 5DE (GB)**

(54) **BLOOD BIOMARKER FOR USE IN EVALUATION OF EFFECT OF DRUG THERAPY ON KIDNEY CANCER**

(57) Disclosed is a means whereby the efficacy of a pharmacotherapy drug for kidney cancer can be easily determined by a blood test. A method of assisting the evaluation of the effect of a drug therapy for the treatment of kidney cancer according to the present invention comprises measuring the PARK7 level in a blood sample taken from a patient with kidney cancer who receives the drug therapy for the treatment of kidney cancer. An increased PARK7 level indicates that the drug therapy is not effective. Moreover, by using the blood PARK7 level as an indicator, the efficacy of a candidate substance for a therapeutic agent for kidney cancer can also be determined.

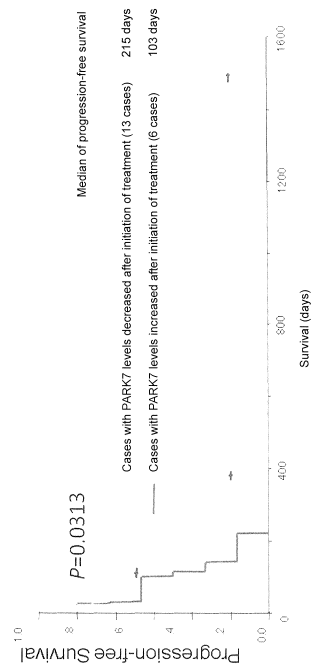


Fig.1

EP 3 364 188 A1

Description

TECHNICAL FIELD

5 **[0001]** The present invention relates to a blood biomarker for evaluation of the effect of a drug therapy for kidney cancer.

BACKGROUND ART

10 **[0002]** Incidence of kidney cancer in Japan is 7 to 8 in every one hundred thousand people, and is rising year after year. Surgical resection is the gold standard treatment of kidney cancer. However, in approximately 30% of all patients with kidney cancer, kidney cancer will metastasize to different sites, which is called advanced cancer. Drug therapy or immunotherapy is applied to patients with advanced cancer in whom surgery is not feasible.

15 **[0003]** The pharmacotherapy drugs currently available for patients with kidney cancer are molecularly targeted drugs, which are categorized into tyrosine kinase inhibitors (TKIs) and mTOR inhibitors. The efficacy of such pharmacotherapy drugs for kidney cancer have been determined by the rate of tumor shrinkage examined with images obtained by CT scanning or the like. However, some drugs rarely cause tumor shrinkage, which makes the evaluation of the effects of the drugs difficult. For example, in TKI therapy, the response rate (tumor shrinkage rate) is not as outstanding as that from conventional anticancer drug therapy, and a rate of tumor shrinkage of 30% or higher, which is calculated based on CT imaging, is associated with only approximately 10 to 30% of the cases of kidney cancer treated by that therapy. 20 Moreover, even in patients on whom the drug therapy is effective, several months are often needed to allow any tumor shrinkage to be detected by CT scanning. Therefore, currently, administration of that type of pharmacotherapy drugs is continued until a clear response is observed by CT imaging.

25 **[0004]** TKI therapy causes various side effects different from those induced by conventional anticancer drugs, including hypertension, diarrhea, general malaise, hand-foot syndrome and the like, which is considered problematic. If the therapeutic effect of a drug therapy on a patient can be determined early after the start of treatment, then the treatment which has evaluated as an ineffective therapy practically producing no positive effects on the patient is not continued, which can result in improved prognosis and/or QOL of patients. However, disadvantageously, evaluation of the effect by CT scanning takes longer time as described above. Furthermore, frequent CT scanning is difficult from the viewpoint of radiation exposure, which may result in missing the timing to change a pharmacotherapy drug. If a specific blood test method (blood biomarker) that reflects the pathology of kidney cancer can be established, more frequent examination is possible without the risk of radiation exposure and evaluation of the effect at the required timing is possible even at an institution equipped with no CT scanner. However, currently such a blood biomarker does not exist.

30 **[0005]** The *DJ-1* gene was isolated as a novel oncogene by Ariga *et al.*, at Hokkaido University in 1997, which is cooperatively involved in neoplastic transformation of cells. Subsequently, the same gene was isolated as *PARK7*, a causative gene for familial Parkinson's disease, in 2003 and is currently called *PARK7/DJ1*. The *PARK7/DJ1* gene is reportedly involved in protection from oxidative stress due to reactive oxygen, transcriptional regulation, and regulation of protease activity and mitochondrial function, as well as its relationship with breast cancer and non-small cell lung cancer is also indicated (Non-Patent Documents 1 and 2). Two reports, one by Sitaram *et al.*, in 2009 (Non-Patent Document 3) and the other by Baumunk *et al.*, in 2013 (Non-Patent Document 4), are related to the relationship of 40 *PARK7/DJ1* and kidney cancer (renal carcinoma).

[0006] In Non-Patent Document 3, expression of *PARK7/DJ-1*, c-Myc and hTERT was analyzed by RT-PCR. The authors reported that expression of *PARK7/DJ-1* was increased in order of normal tissue (n=49) < papillary renal cell carcinoma tissue (n=23) < renal clear cell carcinoma tissue (n=153) and renal cell carcinoma (RCC) tissue, and concluded that any correlation between the expression of *DJ-1*, c-Myc and hTERT genes and the prognosis was not observed.

45 **[0007]** In Non-Patent Document 4, expression of *PDK-1* and *PARK7/DJ1* was analyzed in 91 RCC cases by RT-PCR. The authors reported that expression of *PARK7/DJ1* was detected in both normal renal tissue and RCC tissue but there was no difference in expression level between them, and concluded that any clinical or pathological correlation was not observed.

50 **[0008]** As described above, correlation between the progression or activity of kidney cancer and the expression of *PARK7* in the cancer tissue has been negatively reported, and, furthermore, neither correlation between blood *PARK7* level and the therapeutic effect of a drug therapy for kidney cancer nor usefulness of *PARK7* as a blood biomarker for evaluation of the effect of a drug therapy for kidney cancer is recognized at all.

PRIOR ART DOCUMENTS

55

[Non-Patent Documents]

[0009]

Non-Patent Document 1: Le Naour et al., Clin. Cancer Res. 7, 3328-3335, 2001.

Non-Patent Document 2: MacKeigan et al., Cancer Res. 63, 6928-6934, 2003.

Non-Patent Document 3: Sitaram et al., Int. J. Cancer, 125, 783-790, 2009.

Non-Patent Document 4: Baumunk et al., World J Urol, 31, 1191-1196, 2013.

5

SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

10 **[0010]** An object of the present invention is to provide a means whereby the efficacy of a pharmacotherapy drug for kidney cancer can be easily determined by a blood test.

MEANS FOR SOLVING THE PROBLEMS

15 **[0011]** The inventors intensively made an effort to screen such a protein that its concentration in blood is decreased by TKI therapy in a responder group, in which long-term progression-free survival was obtained, and increased by the same therapy in a non-responder (progression) group, to find that PARK7 was a potent blood biomarker for evaluating the efficacy of TKIs and mTOR inhibitors. The inventors further found that, because the pathology (activity) of kidney cancer was reflected by the level of PARK7 in blood, the blood PARK7 level could be used for evaluation of the therapeutic effects of not only molecularly targeted therapeutic agents such as TKIs and mTOR inhibitors but also various phar-

20 macotherapy drugs for kidney cancer including a novel therapeutic agent for kidney cancer that will be put into practical use in future, and also for evaluation of the therapeutic effect of a candidate substance for use as the novel therapeutic agent. Thus, the present invention has been completed.

[0012] That is, the present invention provides a method of assisting the evaluation of the effect of a drug therapy, the method comprising measuring a PARK7 level in a blood sample taken from a patient with kidney cancer who receives the drug therapy for the treatment of kidney cancer, wherein an increased PARK7 level indicates that the drug therapy is not effective. The present invention also provides use of PARK7 as a blood biomarker for evaluation of the effect of a drug therapy for kidney cancer. The present invention further provides a blood biomarker composed of PARK7 for evaluation of the effect of a drug therapy for kidney cancer. The present invention still further provides a method of

25 assisting the evaluation of the effect of a candidate substance for a therapeutic agent for kidney cancer, the method comprising measuring a PARK7 level in blood samples taken from a test subject with kidney cancer before and after administration of the candidate substance, wherein the candidate substance is determined to potentially be effective in the treatment of kidney cancer when the PARK7 level in the blood sample taken after the administration is decreased relative to the PARK7 level in the blood sample taken before the administration.

35

EFFECT OF THE INVENTION

[0013] According to the present invention, a blood biomarker capable of evaluating the effect of a drug therapy for kidney cancer was provided for the first time. The blood PARK7 level of a patient who receives a drug therapy is useful

40 as data for use in evaluation of the therapeutic effect of a pharmacotherapy drug being used and serves as a great aid for evaluation of the effect of the drug therapy by a physician. Because the therapeutic effect can be determined by the blood PARK7 level at a very early stage after the start of treatment, the effect of the drug therapy can be determined earlier by the blood PARK7 level than by examining whether tumor has been shrunk or not by CT scanning. Moreover, according to the present invention, because the therapeutic effect can be determined with a small volume of blood

45 sample, more frequent monitoring is possible without concerns about radiation exposure, which is also advantageous for quick evaluation of the therapeutic effect. Since a blood test can be easily performed even at an institution equipped with no CT scanner, the present invention is highly convenient. The present invention enables early detection of loss of therapeutic effects and allows one to take countermeasures against it, such as changing the therapeutic agent, as well as the present invention enables the evaluation of therapeutic effects at appropriate timing. Thus, the number of patients

50 with kidney cancer who continue a drug therapy practically producing no therapeutic effects is decreased, from which improvement of prognosis in patients with kidney cancer as well as improvement of QOL and a positive effect on medical economy can be expected. Because the blood PARK7 level reflects the activity of kidney cancer, it can be used for evaluation of the effect of a candidate substance for a therapeutic agent for kidney cancer, as well as for evaluation of the effects of various pharmacotherapy drugs. By using the blood PARK7 level for evaluation of the effect of a candidate

55 substance for a therapeutic agent, in the clinical study of a new therapeutic agent for human kidney cancer, for example, the effect of the corresponding new therapeutic agent can be quickly evaluated. The present invention also contributes to the development of a new therapeutic agent for kidney cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014]

5 Fig. 1 shows the result of measuring the progression-free survival rate in kidney cancer patient groups with decreased blood PARK7 levels and with increased blood PARK7 levels at two weeks after the start of TKI administration.
Fig. 2 shows the results of measuring the change in blood PARK7 level by ELISA and of examining the activity of kidney cancer by PET/CT in four patients with kidney cancer, to whom an mTOR inhibitor has started to be administered.

MODE FOR CARRYING OUT THE INVENTION

15 **[0015]** The amino acid sequence shown in SEQ ID NO: 2 is the amino acid sequence of human PARK7. Multiple mRNA sequences are registered in the database at NCBI as transcription variants 1, 2, and XI (accession Nos. NM_007262.4, NM_001123377.1, and XM_005263424.2, respectively), any of which encodes a PARK7 protein with an identical amino acid sequence. The base sequence registered under the accession No. NM_007262.4 is shown in SEQ ID NO: 1 as an exemplary mRNA sequence from the *PARK7* gene.

[0016] The target patient of the present invention is a patient with kidney cancer (particularly, advanced kidney cancer, such as metastatic kidney cancer and unresectable kidney cancer) who receives a drug therapy, typically a human patient.

20 **[0017]** The term "kidney cancer" in the present invention has the same meaning as "renal cell carcinoma", including renal clear cell carcinoma, papillary renal cell carcinoma, chromophobe renal cell carcinoma, collecting duct carcinoma, and the like.

[0018] The term "drug therapy" includes therapy with various agents that exert therapeutic effects on kidney cancer, and also includes, for example, immunotherapy with cytokines or the like. The medicine used in the drug therapy is not particularly limited, and includes various agents such as small molecule compounds, molecularly targeted drugs, nucleic acid therapeutics, antibody therapeutics, peptide therapeutics, and the like. Pharmacotherapy drugs for kidney cancer currently in practical use are molecularly targeted drugs, which are broadly classified into tyrosine kinase inhibitors (TKIs), such as sunitinib and sorafenib, which inhibit vascularization, and mTOR protein inhibitors, which suppress cancer growth mainly by inhibiting the enzyme that regulates cancer growth. Any pharmacotherapy drug belonging to either category can be evaluated for its effect according to the present invention because the change in blood PARK7 level reflects the effect of a medicine being used, as described in Examples below. Moreover, recently, immune checkpoint inhibitors such as anti-PD-1 antibody and anti-PD-L1 antibody gain attention in the field of cancer therapy. In Japan and in foreign countries, immune checkpoint inhibitors are in clinical trials as therapeutic agents for various cancers such as kidney cancer, malignant melanoma, and non-small cell lung cancer, and some of them have already been approved also in Japan. They are expected to also be approved and practically used in future as therapeutic agents for kidney cancer. Such new therapeutic agents for kidney cancer as immune checkpoint inhibitors will also be subjects of the evaluation of therapeutic effects according to the present invention. Because the blood PARK7 level reflects the activity of kidney cancer and, thus, the change in the activity of kidney cancer can be evaluated by the change in blood PARK7 level, the present invention based on the blood PARK7 level is not limited to the above indicated examples but can be used for evaluation of the effects of various pharmacotherapy drugs for kidney cancer.

[0019] The blood sample includes a whole blood sample, a plasma sample, and a serum sample. A blood sample used in the present invention may be, for example, a plasma sample or a serum sample. Or, the blood sample may be a plasma sample.

45 **[0020]** The collection of blood samples is continued during the period in which the patient receives drug administration. For the comparison purpose, blood samples are preferably collected prior to and/or at the start of drug therapy. Blood samples are usually collected every a few or several weeks, but the timing of blood sampling may be changed appropriately according to the conditions of a patient, symptoms, and the like.

[0021] The PARK7 level of a sample can be determined by measuring the abundance of the PARK7 protein or its fragment in the sample. The means of measuring the PARK7 level in a sample is not particularly limited as long as it is a means capable of measuring polypeptides. Examples of the means of measuring polypeptides include immunoassay, mass spectrometry, and the like, and immunoassay is preferably used in the present invention because it does not need a large-scale machinery and equipment and its measuring operations are easy to perform. Polyclonal and monoclonal antibodies that can detect PARK7 are known and are also commercially available. Moreover, because the amino acid sequence of PARK7 and the base sequence encoding the same are known as described above, an anti-PARK7 antibody or an antigen-binding fragment thereof that specifically recognizes PARK7 may be prepared by a commonly used method such as the hybridoma method.

55 **[0022]** An anti-PARK7 polyclonal antibody can be obtained by, for example, immunizing a non-human animal with the full-length PARK7 protein or its fragment comprising an appropriate partial region together with an adjuvant as necessary,

collecting blood from the non-human animal to obtain an antiserum, and purifying the polyclonal antibody of interest in the antiserum. The immunization is typically carried out multiple times over several weeks to raise the antibody titer in the immunized animal. The antibody in the antiserum can be purified, for example, by fractionation based on ammonium sulfate precipitation or anion chromatography, affinity column purification, and the like.

5 [0023] An anti-PARK7 monoclonal antibody can be obtained by, for example, immunizing a non-human animal with the full-length PARK7 protein or an appropriate fragment of the protein, collecting antibody-producing cells such as splenocytes or lymphocytes from the animal, preparing hybridomas by the fusion of those cells with myeloma cells, screening a hybridoma clone capable of producing an antibody that binds to PARK7, and culturing the hybridoma clone and collecting the culture supernatant to obtain the antibody of interest.

10 [0024] The full-length PARK7 protein or a fragment thereof used for the immunization of a non-human animal can be produced based on the information including the known base sequence and amino acid sequence of PARK7, which are also indicated in the Sequence Listing, by commonly used methods such as chemical synthesis, genetic engineering technique, and the like.

15 [0025] Specific examples of the chemical synthesis method include the Fmoc method (the fluorenylmethyloxycarbonyl method), tBoc method (the t-butyloxycarbonyl method), and the like. Moreover, the synthesis may also be carried out using various commercially available peptide synthesizers by a commonly used method. In the case of chemical synthesis, a desired polypeptide can be synthesized based only on its amino acid sequence.

20 [0026] In cases where the full-length PARK7 protein or its fragment is produced by genetic engineering technique, it may be produced by, for example, amplifying a desired region of the *PARK7* cDNA from a human cDNA library, integrating the amplified region into an appropriate vector, expressing a polypeptide corresponding to the amplified region in an appropriate expression system, and recovering the expressed polypeptide. The vectors and various expression systems (bacterial expression system, yeast cell expression system, mammalian cell expression system, insect cell expression system, cell-free expression system, and the like) to be used are also well known, and various vectors, host cells, reagents, and kits are commercially available. Therefore, those skilled in the art can select and use appropriate ones.

25 Human-derived cultured cells are also commercially available or distributed and can be easily obtained.

30 [0027] The "antigen-binding fragment" may be any antibody fragment as long as it retains the binding activity to the antigen (antigen-antibody reactivity) of the original antibody. Specific examples of the antigen-binding fragment include, but are not limited to, Fab, F(ab')₂, scFv, and the like. Fab and F(ab')₂ fragments can be obtained, as known well, by treating a monoclonal antibody with a protein degradation enzyme such as papain or pepsin. The production method for scFv (single chain fragment of variable region, single-chain antibody) is also well known. A scFv fragment can be obtained, for example, by extracting mRNA from the hybridoma produced as described above to prepare single-stranded cDNA; carrying out PCR using immunoglobulin H chain-specific and L chain-specific primers to amplify the immunoglobulin H chain gene and L chain gene from the single-stranded cDNA; connecting the amplified fragments by a linker; adding thereto appropriate restriction enzyme sites; introducing it into a plasmid vector; transforming *E. coli* bacteria with the plasmid vector to express the scFv fragment; and recovering the scFv fragment from the *E. coli* bacteria.

35 [0028] The immunoassay *per se* is a well-known commonly used method. Immunoassays are categorized based on the format of reaction into sandwich immunoassay, competitive immunoassay, agglutination immunoassay, Western blot, and the like, while immunoassays are categorized based on the label to be used into enzyme immunoassay, radioimmunoassay, fluorescent immunoassay, and the like. In the present invention, any immunoassay method capable of quantitative detection of the protein of interest may be used. For example, a sandwich method such as, but not limited to, sandwich ELISA may be preferably used.

40 [0029] In the sandwich method, an anti-PARK7 antibody that binds to PARK7 is immobilized on a solid phase (immobilized antibody), and allowed to react with a sample. After washing, a labeled anti-PARK7 antibody which can bind to PARK7 in parallel with the immobilized antibody (typically, an anti-PARK7 antibody that binds to PARK7 at a different site from that of the immobilized antibody) is allowed to react with the solid phase. After washing, the labeled antibody bound to the solid phase is measured. Both the immobilized antibody and the labeled antibody may be polyclonal or monoclonal antibodies. Antigen-binding fragments of the respective antibodies may also be used instead of the antibodies.

45 [0030] The measurement of the labeled antibody can be carried out by measuring the signal from the labeling substance. The method of measuring the signal is appropriately selected depending on the type of the labeling substance. In the case of an enzyme label, for example, a substrate for the enzyme is added to the reaction system, and the intensity of color or luminescence produced by the enzyme reaction may be measured with an absorptiometer or a luminometer. The amount of PARK7 in a blood sample can be determined as follows: standard samples containing various known concentrations of PARK7 are immunologically assayed using an anti-PARK7 antibody or an antigen-binding fragment thereof, and the correlation between the amount of signal from the label and the concentrations of PARK7 in the standard samples is plotted to generate a standard curve, and the same procedure is performed on a blood sample containing an unknown concentration of PARK7 to determine the amount of signal from the label, and the determined value of signal is applied to the standard curve to obtain the concentration of PARK7. The measured value is simply required to

be compared with a previously measured value from the same patient, and therefore, the calculation of an absolute concentration value is not essential. The change in PARK7 level may also be evaluated by the comparison between detected signal values.

5 [0031] When monoclonal antibodies are used as both an immobilized antibody and a labeled antibody, whether the combination of monoclonal antibodies is preferable or not can be easily examined by actually performing an immunoassay. Optionally, the recognition sites of the antibodies may be identified to determine if they recognize different epitopes. The identification of antibody recognition sites can be performed by a commonly used method well known in the art. Briefly, for example, the corresponding antigen, PARK7, is partially digested with a protein degradation enzyme such as trypsin, and a solution containing the partial digests is filtered through an affinity column of an immobilized antibody, which is to
10 be examined for its recognition site. Thereafter, the digests are allowed to bind to the antibody, and the bound digests are then eluted from the column and analyzed by a conventional mass spectrometry, and thereby the recognition site of the antibody can be identified.

[0032] The blood PARK7 level varies depending on the size of a kidney cancer, and a larger tumor size is associated with a higher blood level of PARK7, while the blood level of PARK7 is not very different between normal subjects and patients with a small tumor size. Thus, it is difficult to set up an absolute cut-off value that can be commonly applied to overall patients with kidney cancer. However, a change in blood PARK7 level is observed regardless of the size of a kidney cancer, where the level of PARK7 in blood is maintained low when a drug therapy is effective, and is increased when the drug therapy is ineffective or loses its effect. Therefore, in the present invention, whether the PARK7 level has been increased or not is determined by examining the change in PARK7 level in an individual and comparing the
15 measured value with a previously measured value from the identical patient.

[0033] Whether the PARK7 level has been increased or not is determined by comparing the measured value at least with the last measurement value. That is, it can be determined mainly by comparison with the last measurement value, and additionally by considering the tendency of change from the second last measurement or an earlier measurement as necessary. If the blood PARK7 level is decreased or maintained low, a medicine being used can be determined to be therapeutically effective and, therefore, the use of the same medicine may be continued with monitoring the progress. If the blood PARK7 level is sharply increased, it may indicate the possibility of rapid progression of kidney cancer, and it is desirable to immediately replace a medicine being used with another type of medicine. If the blood PARK7 level tends to be gradually increased, a medicine being used may be immediately replaced with another type of medicine, or replacement of the medicine may be considered after performing the next measurement earlier than scheduled and reconfirming the increasing tendency. If the blood PARK7 level is not particularly changed from the level at the start of therapy with the medicine, the medicine can be less effective. In such a case, the next measurement may be performed
20 earlier than scheduled as necessary, and then the replacement of the medicine being used may be considered, taking the extent of side effects into account.

[0034] Because the blood PARK7 level reflects the activity of kidney cancer, the effect of a candidate substance for a therapeutic agent for kidney cancer can be evaluated by using the blood PARK7 level as an indicator. In the evaluation of the effect of a candidate substance, the candidate substance is administered to a test subject with kidney cancer. The test subject is a human or non-human mammal. If the test subject is a human, it is primarily envisioned that evaluation of the effect of the candidate substance is carried out in clinical trials. The non-human mammal may be, for example, a kidney cancer model to which renal carcinoma cells have been transplanted. Blood samples are collected from the test subject before and after administration of the candidate substance, and the PARK7 level is measured in these blood
25 samples. The blood sample may be collected once or multiple times after the administration. If the blood PARK7 level after the administration is decreased relative to the blood PARK7 level before the administration, the candidate substance can be determined to potentially be effective for the treatment of kidney cancer.

45 EXAMPLES

[0035] The present invention will be more specifically described below by way of examples. However, the present invention is not limited to the examples below.

50 1. Correlation between the change in blood concentration of PARK7 and the prognosis in patients with kidney cancer being treated with a TKI

[0036] From 19 patients with advanced renal cell carcinoma who had been receiving administration of a TKI (tyrosine kinase inhibitor) as a therapeutic agent for kidney cancer, blood samples were collected at the start of dosing and at week 2 after the first dosing. Plasma was separated therefrom, and the PARK7 level in the plasma was measured. A commercially available sandwich ELISA kit (Cyclex Co., Ltd) was used for the measurement, and the color developed by the reaction between HRP enzyme label and its substrate substance was detected by a luminometer. The PARK7 standard attached in the kit was used to prepare reference samples for generation of a standard curve. The concentration
55

of PARK7 in the plasma samples were calculated by applying measured values to the standard curve. When the change in plasma PARK7 level was evaluated by comparing the calculated PARK7 concentrations or comparing the values detected by the luminometer, the plasma PARK7 level at week 2 after the first dosing relative to that at the start of TKI administration was decreased in 13 cases, and it was increased in 6 cases. Then, the progression-free survival in the groups of cases with decreased levels and of cases with increased levels was investigated.

[0037] The result is shown in Fig. 1. A significant difference in progression-free survival was observed between the 13 cases with PARK7 levels decreased as compared with that at the start of treatment and the 6 cases with PARK7 levels increased as compared with that at the start of treatment, and the progression-free survival rate was significantly higher in the group of cases with decreased PARK7 levels than in the other group ($P=0.0313$). The median of progression-free survival was 215 days in the group of cases with decreased levels, while it was 103 days in the group of cases with increased levels. Accordingly, it was confirmed that the blood PARK7 level enabled the evaluation of the therapeutic effect of a drug therapy for kidney cancer at a stage as early as week 2 after the start of treatment.

2. Correlation between the change in blood concentration of PARK7 and the prognosis in patients with kidney cancer being treated with an mTOR inhibitor (Temozolimumus)

[0038] A change in blood PARK7 concentration was observed during treatment in four patients with kidney cancer who began to receive administration of mTOR inhibitor. The blood PARK7 concentration was measured as a concentration of plasma PARK7 with the commercially available ELISA kit in the same manner as in 1. described above, and the change in blood PARK7 level was evaluated by comparing values detected by a luminometer.

[0039] The therapeutic effect of the mTOR inhibitor was clinically evaluated by FDG PET/CT. Kidney cancer progression was evaluated by FDG PET/CT, in parallel with the measurement of blood PARK7 concentration. FDG is an agent composed of glucose and a positron-emitting nuclide introduced thereto, and it has almost the same behavior in the body as glucose. Since glucose is highly accumulated in tumor regions when a cancer is highly active, the activity of the cancer can be evaluated by examining the accumulation of FDG.

[0040] The result is shown in Fig. 2. The SUVmax values indicated below the respective images are semi-quantified values of FDG accumulation. In all of the three cases (Case Nos. 1 to 3) in whom the concentration of PARK7 in blood was decreased during the mTOR inhibitor therapy, the evaluation by PET/CT demonstrated a decreased accumulation of FDG, indicating a decreased activity of the cancer, that is, the achievement of therapeutic effects. On the other hand, in one case (Case No. 4) in whom the concentration of PARK7 was increased, no change in accumulation of FDG was observed, indicating the ineffectiveness of the therapy. Accordingly, it was suggested that the therapeutic effect can be determined based on the change in blood PARK7 concentration, also for an mTOR inhibitor-type therapeutic agent for kidney cancer.

EP 3 364 188 A1

SEQUENCE LISTING

<110> Public University Corporation Yokohama City University

5 <120> Blood biomarker for evaluation of effect of drug therapy for renal carcinoma

<130> PF576-PCT

10 <160> 2

<170> PatentIn version 3.5

<210> 1

<211> 979

15 <212> DNA

<213> Homo sapiens

<220>

20 <221> CDS

<222> (164)..(733)

<400> 1

tgagtctgcg cagtgtgggg ctgagggagg ccggacggcg cgcgtgctgt ctggcgtgcg 60

25 ttcattttca gcctggtgtg gggtagtggtg tacccaacgg gccggggcgc cgcgtccgca

ggaagaggcg cggggtgcag gcttgtaaac atataacata aaa atg gct tcc aaa 175
Met Ala Ser Lys
1

30 aga gct ctg gtc atc ctg gct aaa gga gca gag gaa atg gag acg gtc 223
Arg Ala Leu Val Ile Leu Ala Lys Gly Ala Glu Glu Met Glu Thr Val
5 10 15 20

atc cct gta gat gtc atg agg cga gct ggg att aag gtc acc gtt gca 271
Ile Pro Val Asp Val Met Arg Arg Ala Gly Ile Lys Val Thr Val Ala
35 25 30 35

ggc ctg gct gga aaa gac cca gta cag tgt agc cgt gat gtg gtc att 319
Gly Leu Ala Gly Lys Asp Pro Val Gln Cys Ser Arg Asp Val Val Ile
40 40 45 50

40 tgt cct gat gcc agc ctt gaa gat gca aaa aaa gag gga cca tat gat 367
Cys Pro Asp Ala Ser Leu Glu Asp Ala Lys Lys Glu Gly Pro Tyr Asp
55 60 65

45 gtg gtg gtt cta cca gga ggt aat ctg ggc gca cag aat tta tct gag 415
Val Val Val Leu Pro Gly Gly Asn Leu Gly Ala Gln Asn Leu Ser Glu
70 75 80

tct gct gct gtg aag gag ata ctg aag gag cag gaa aac cgg aag ggc 463
Ser Ala Ala Val Lys Glu Ile Leu Lys Glu Gln Glu Asn Arg Lys Gly
50 85 90 95 100

ctg ata gcc gcc atc tgt gca ggt cct act gct ctg ttg gct cat gaa 511
Leu Ile Ala Ala Ile Cys Ala Gly Pro Thr Ala Leu Leu Ala His Glu
105 110 115

55 ata ggt ttt gga agt aaa gtt aca aca cac cct ctt gct aaa gac aaa 559
Ile Gly Phe Gly Ser Lys Val Thr Thr His Pro Leu Ala Lys Asp Lys
120 125 130

EP 3 364 188 A1

atg atg aat gga ggt cat tac acc tac tct gag aat cgt gtg gaa aaa 607
 Met Met Asn Gly Gly His Tyr Thr Tyr Ser Glu Asn Arg Val Glu Lys
 135 140 145

5 gac ggc ctg att ctt aca agc cgg ggg cct ggg acc agc ttc gag ttt 655
 Asp Gly Leu Ile Leu Thr Ser Arg Gly Pro Gly Thr Ser Phe Glu Phe
 150 155 160

10 gcg ctt gca att gtt gaa gcc ctg aat ggc aag gag gtg gcg gct caa 703
 Ala Leu Ala Ile Val Glu Ala Leu Asn Gly Lys Glu Val Ala Ala Gln
 165 170 175 180

gtg aag gct cca ctt gtt ctt aaa gac tag agcagcgaac tgcgacgatc 753
 Val Lys Ala Pro Leu Val Leu Lys Asp
 185

15 acttagagaa acaggccgtt aggaatccat tctcactgtg ttcgctctaa acaaaacagt 813

ggtaggttaa tgtgttcaga agtcgctgtc cttactactt ttgcggaagt atggaagtca 873

20 caactacaca gagatttctc agcctacaaa ttgtgtctat acatttctaa gccttgtttg 933

cagaataaac agggcattta gcaactaaa aaaaaaaaaa aaaaaa 979

<210> 2
 <211> 189
 <212> PRT
 <213> Homo sapiens

<400> 2

30 Met Ala Ser Lys Arg Ala Leu Val Ile Leu Ala Lys Gly Ala Glu Glu
 1 5 10 15

35 Met Glu Thr Val Ile Pro Val Asp Val Met Arg Arg Ala Gly Ile Lys
 20 25 30

Val Thr Val Ala Gly Leu Ala Gly Lys Asp Pro Val Gln Cys Ser Arg
 35 40 45

40 Asp Val Val Ile Cys Pro Asp Ala Ser Leu Glu Asp Ala Lys Lys Glu
 50 55 60

45 Gly Pro Tyr Asp Val Val Val Leu Pro Gly Gly Asn Leu Gly Ala Gln
 65 70 75 80

50 Asn Leu Ser Glu Ser Ala Ala Val Lys Glu Ile Leu Lys Glu Gln Glu
 85 90 95

Asn Arg Lys Gly Leu Ile Ala Ala Ile Cys Ala Gly Pro Thr Ala Leu
 100 105 110

55 Leu Ala His Glu Ile Gly Phe Gly Ser Lys Val Thr Thr His Pro Leu
 115 120 125

EP 3 364 188 A1

Ala Lys Asp Lys Met Met Asn Gly Gly His Tyr Thr Tyr Ser Glu Asn
130 135 140

5 Arg Val Glu Lys Asp Gly Leu Ile Leu Thr Ser Arg Gly Pro Gly Thr
145 150 155 160

10 Ser Phe Glu Phe Ala Leu Ala Ile Val Glu Ala Leu Asn Gly Lys Glu
165 170 175

15 Val Ala Ala Gln Val Lys Ala Pro Leu Val Leu Lys Asp
180 185

Claims

- 20 1. A method of assisting the evaluation of the effect of a drug therapy, the method comprising measuring a PARK7 level in a blood sample taken from a patient with kidney cancer who receives the drug therapy for the treatment of kidney cancer, wherein an increased PARK7 level indicates that the drug therapy is not effective.
- 25 2. The method according to claim 1, wherein whether the PARK7 level has been increased or not is determined by comparing the measured value at least with the last measurement value in the patient.
3. The method according to claim 1 or 2, wherein the blood sample is a plasma or serum sample.
- 30 4. Use of PARK7 as a blood biomarker for evaluation of the effect of a drug therapy for kidney cancer.
- 35 5. A blood biomarker composed of PARK7 for evaluation of the effect of a drug therapy for kidney cancer.
- 40 6. A method of assisting the evaluation of the effect of a candidate substance for a therapeutic agent for kidney cancer, the method comprising measuring a PARK7 level in blood samples taken from a test subject with kidney cancer before and after administration of the candidate substance, wherein the candidate substance is determined to potentially be effective in the treatment of kidney cancer when the PARK7 level in the blood sample taken after the administration is decreased relative to the PARK7 level in the blood sample taken before the administration.

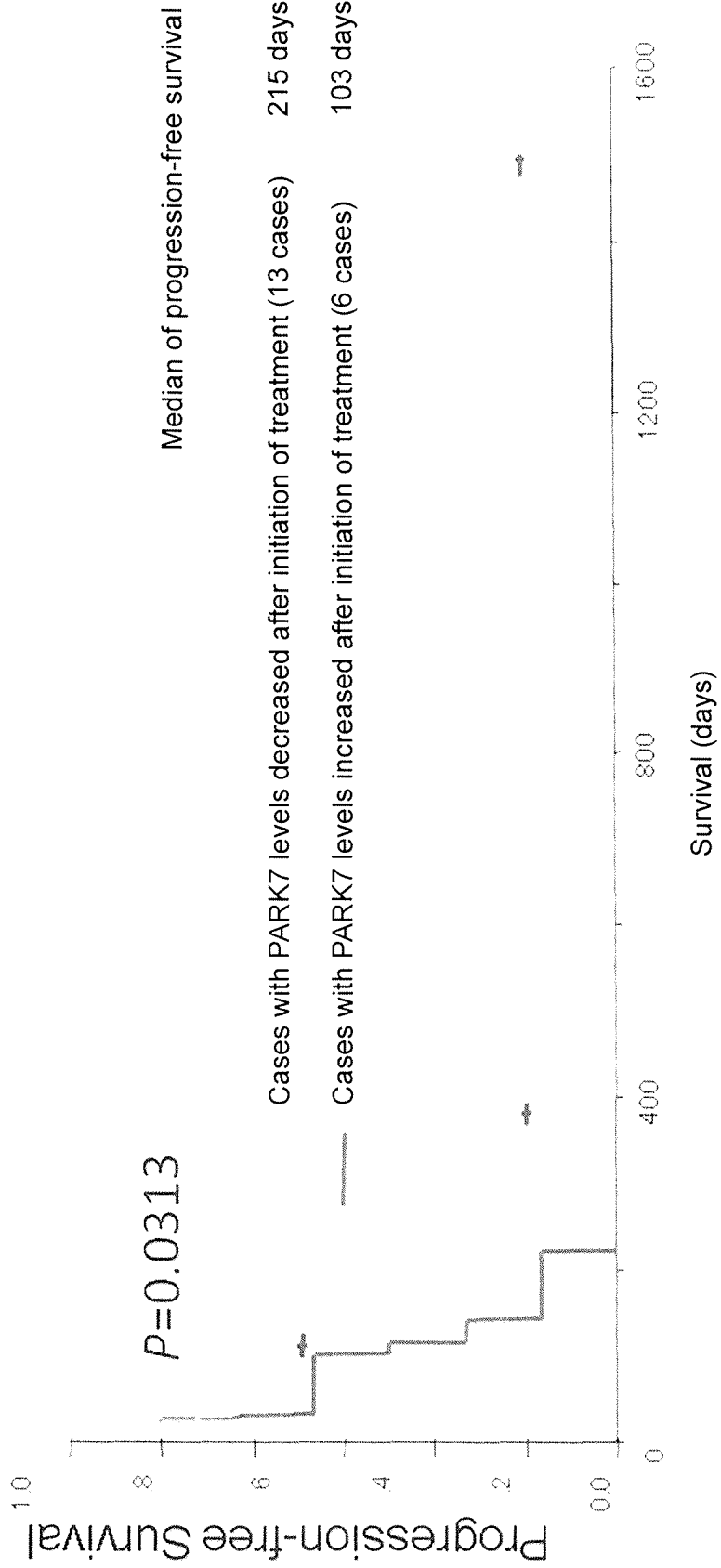


Fig.1

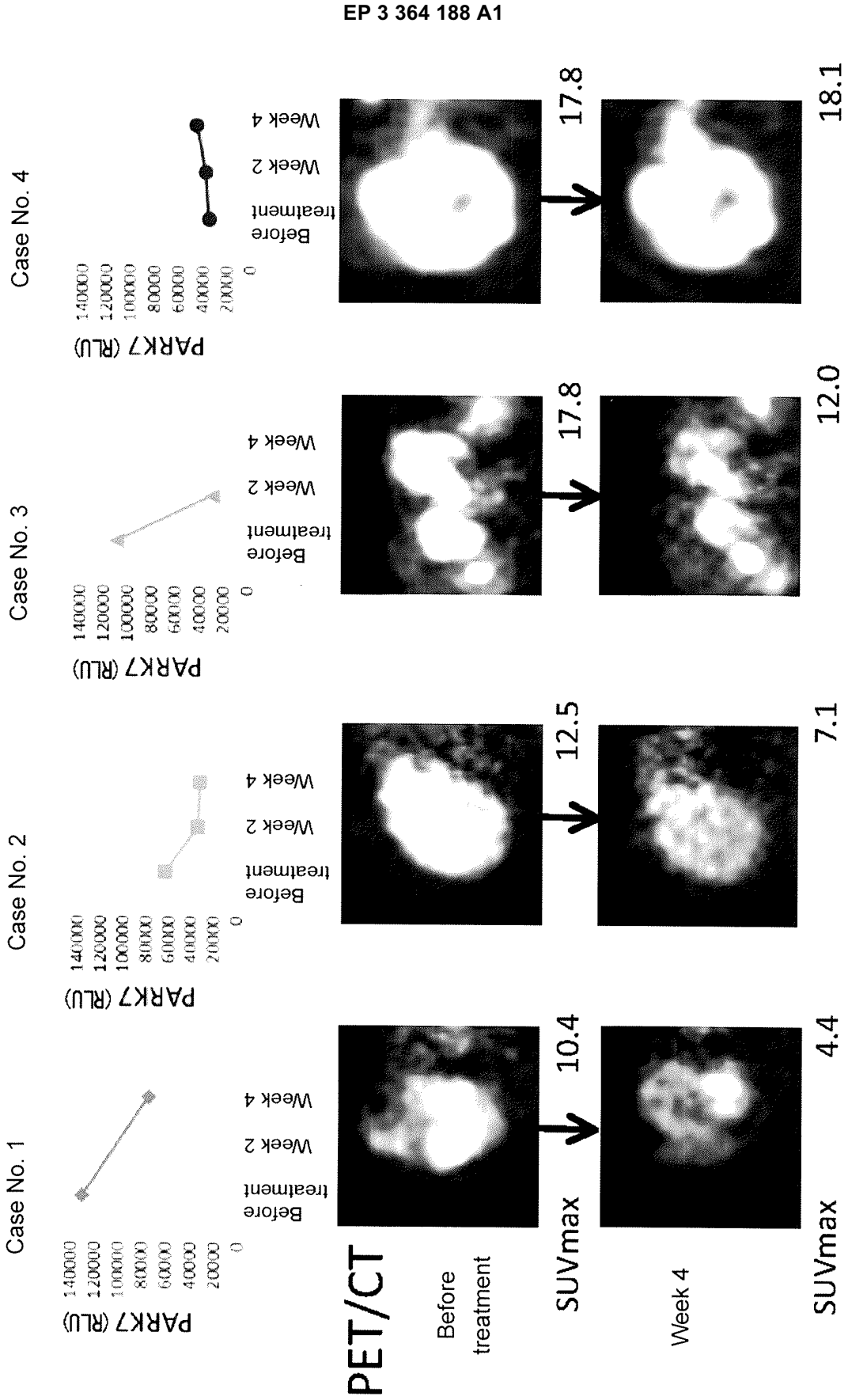


Fig.2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2016/080084

A. CLASSIFICATION OF SUBJECT MATTER

G01N33/53(2006.01)i, G01N33/50(2006.01)i, G01N33/574(2006.01)i, A61K45/00(2006.01)n, A61P13/12(2006.01)n, A61P35/00(2006.01)n

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)

G01N33/53, G01N33/50, G01N33/574, A61K45/00, A61P13/12, A61P35/00

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

Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2016
Kokai Jitsuyo Shinan Koho 1971-2016 Toroku Jitsuyo Shinan Koho 1994-2016

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

JSTPlus/JMEDPlus/JST7580 (JDreamIII), CAPplus/MEDLINE/EMBASE/BIOSIS (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A/X	JP 2015-519876 A (Berg LLC), 16 July 2015 (16.07.2015), entire text; particularly, paragraphs [0026], [0032], [0102], [0339], [0408], [0409], [0418], [0427], [0428], [0437] to [0439] & US 2013/0259847 A1 entire text; particularly, paragraphs [0036], [0048], [0175], [0528], [0620], [0621], [0630], [0639], [0640], [0649] to [0651] & WO 2013/151577 A1 & EP 2834366 A1	1-3, 6/4, 5
A	JP 2009-511028 A (Laboratorios Salvat S.A.), 19 March 2009 (19.03.2009), entire text & US 2009/0209431 A1 & WO 2007/042256 A1 & EP 1775590 A1	1-6

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search
09 December 2016 (09.12.16)

Date of mailing of the international search report
20 December 2016 (20.12.16)

Name and mailing address of the ISA/
Japan Patent Office
3-4-3, Kasumigaseki, Chiyoda-ku,
Tokyo 100-8915, Japan

Authorized officer

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2016/080084

5
10
15
20
25
30
35
40
45
50
55

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2015-513370 A (The Johns Hopkins University), 11 May 2015 (11.05.2015), entire text & US 2015/0031048 A1 & WO 2013/138509 A1 & EP 2825893 A1	1-6
A	JP 2008-542742 A (Cambridge Enterprise Ltd.), 27 November 2008 (27.11.2008), entire text & US 2008/0220530 A1 & WO 2006/129131 A2 & EP 1889088 A2	1-6
A	JP 2014-517300 A (Metanomics Health GmbH), 17 July 2014 (17.07.2014), entire text & WO 2012/164026 A1 & EP 2715361 A1	1-6

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **LE NAOUR et al.** *Clin. Cancer Res.*, 2001, vol. 7, 3328-3335 [0009]
- **MACKEIGAN et al.** *Cancer Res.*, 2003, vol. 63, 6928-6934 [0009]
- **SITARAM et al.** *Int. J. Cancer*, 2009, vol. 125, 783-790 [0009]
- **BAUMUNK et al.** *World J Urol*, 2013, vol. 31, 1191-1196 [0009]

专利名称(译)	血液生物标志物用于评估药物治疗对肾癌的影响		
公开(公告)号	EP3364188A4	公开(公告)日	2019-06-26
申请号	EP2016855381	申请日	2016-10-11
[标]申请(专利权)人(译)	公立大学法人横浜市立大学		
申请(专利权)人(译)	公立大学CORPORATION横浜市立大学		
当前申请(专利权)人(译)	公立大学CORPORATION横浜市立大学		
[标]发明人	NAKAIGAWA NOBORU UENO DAIKI YAO MASAHIRO		
发明人	NAKAIGAWA, NOBORU UENO, DAIKI YAO, MASAHIRO		
IPC分类号	G01N33/53 G01N33/50 G01N33/574 A61K45/00 A61P13/12 A61P35/00		
CPC分类号	A61K45/00 G01N33/50 G01N33/53 G01N33/574 G01N33/57438 G01N2333/98		
优先权	2015202614 2015-10-14 JP		
其他公开文献	EP3364188A1		
外部链接	Espacenet		

摘要(译)

公开了一种通过血液测试可以容易地确定用于肾癌的药物疗法药物的功效的方法。根据本发明的辅助评价药物疗法治疗肾癌的效果的方法包括测量取自用于治疗肾脏的药物疗法的肾癌患者的血液样品中的PARK7水平。癌症。PARK7水平增加表明药物治疗无效。此外，通过使用血液PARK7水平作为指标，还可以确定候选物质对肾癌治疗剂的功效。