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(54) BIOMARKERS

(57) An object of the present invention is to provide biomarkers for predicting response to chemoradiotherapy for cancer and predicting prognosis of a patient with cancer, as well as methods of measuring such biomarkers. The response to chemoradiotherapy for cancer in a vertebrate animal can be predicted by measuring concentrations of a soluble interleukin-6 receptor, MIP-1β, and an activated plasminogen activator inhibitor in the blood obtained from that individual with cancer before treatment with chemoradiotherapy, and prognosis of the same vertebrate animal can be determined by measuring a concentration of soluble interleukin-6 receptor.

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Description

Cross reference to related application

[0001] This application claims the benefit of Japanese Patent Application No. 2010-83198 filed on March 31, 2010, the entire disclosure of which is hereby incorporated by reference.

Technical field

[0002] The present invention relates to biomarkers for determining whether or not chemoradiotherapy is applicable to a patient with cancer.

Background art

[0003] Neoadjuvant chemoradiotherapy in patients with adenocarcinoma or squamous cell carcinoma has been reported to improve survival rate in the patients compared with surgery alone (see, for example, Thomas N. et al., New England Journal of Medicine, 1996 Aug, 15: 462-467 and Val Gebski et al., Lancet Oncol., 2007 Mar, 8(3): 226-234). It is, however, known that some patients have a good response to chemoradiotherapy for cancer but others not. Discrimination of these patients before initiation of the treatment allows better choice of therapy suitable for each patient.

[0004] Therefore, an object of the present invention is to provide biomarkers for predicting response to chemoradiotherapy for squamous cell carcinoma and markers for predicting prognosis of a patient with squamous cell carcinoma who has received chemoradiotherapy.

Disclosure of the invention

[0005] More specifically, a biomarker according to the present invention is a biomarker for predicting response to chemoradiotherapy for squamous cell carcinoma selected from the group consisting of a soluble interleukin-6 receptor, a macrophage inflammatory protein 1β, and an activated plasminogen activator inhibitor.

[0006] A biomarker according to the present invention is a biomarker for predicting prognosis of a patient with squamous cell carcinoma, the patient having received chemoradiotherapy, the biomarker being a soluble interleukin-6 receptor.

[0007] In the aforementioned biomarker for predicting response to chemoradiotherapy and the biomarker for predicting prognosis of a patient with squamous cell carcinoma, the patient having received chemoradiotherapy, the squamous cell carcinoma is preferably head and neck squamous cell carcinoma or esophageal squamous cell carcinoma.

[0008] In addition, in the marker for predicting prognosis of a patient with squamous cell carcinoma, the patient having received chemoradiotherapy, the chemoradiotherapy is more preferably preoperative chemoradiotherapy.

[0009] A method for measuring a biomarker according to the present invention comprising measuring concentrations of one or more biomarker(s) selected from the group consisting of a soluble interleukin-6 receptor, a macrophage inflammatory protein 1β, and an activated plasminogen activator inhibitor in the blood obtained before treatment with chemoradiotherapy.

[0010] In the method of measuring the concentration of the biomarker, the blood has been preferably obtained from a patient with squamous cell carcinoma. In addition, it is more preferable that the concentration of the biomarker is measured by using an antibody specific to the biomarker. It is most preferable that the squamous cell carcinoma is head and neck squamous cell carcinoma or esophageal squamous cell carcinoma.

Brief description of the drawings

[0011]

Fig. 1 is a graph showing survival rates of patients for up to 9 years from the beginning of treatment in a group of patients with esophageal squamous cell carcinoma with grade 3 effects of preoperative chemoradiotherapy and a group of patients with esophageal squamous cell carcinoma with grade 1 or 2 effects, in an example of the present invention;

Fig. 2 shows plots of blood concentration of a soluble interleukin-6 receptor (sIL6R) measured using a fluorescent bead-based array system in each of groups of patients with the grade 1, 2, and 3 effects of preoperative chemoradiotherapy in an example of the present invention;

Fig. 3 is a distribution pattern showing blood concentration of a macrophage inflammatory protein 1β (MIP-1β) measured using a fluorescent bead-based array system in each of groups of patients with the grade 1, 2, and 3 effects of preoperative chemoradiotherapy in an example of the present invention;

Fig. 4 is a distribution pattern showing blood concentration of an activated plasminogen activator inhibitor (PAI-1) measured using a fluorescent bead-based array system in each of groups of patients with the grade 1, 2, and 3 effects of preoperative chemoradiotherapy in an example of the present invention;

Fig. 5 is a box plot showing blood concentration of a soluble interleukin-6 receptor (sIL6R) measured using sandwich ELISA in each of groups of patients with the grade 1, 2, and 3 effects of preoperative chemoradiotherapy in an example of the present invention;

Fig. 6 is a graph showing survival rates from the beginning of treatment in patients with esophageal squamous cell carcinoma divided into groups on the basis of the threshold of 30 ng/ml of the blood sIL6R concentration measured using a fluorescent bead-based array system in an example of the present invention; and

Fig. 7 is a graph showing survival rates from the beginning of treatment in patients with esophageal squamous cell carcinoma divided into groups on the basis of a threshold of 30 ng/ml of the blood sIL6R concentration measured using sandwich ELISA in an example of the present invention.

Mode for carrying out the invention

[0012] Embodiments of the present invention that were completed based on the aforementioned findings are described below in detail in reference to Examples.

[0013] Unless otherwise noted in embodiments and examples, all procedures used are as described in standard protocols such as J. Sambrook, E. F. Fritsch & T. Maniatis (Ed.), Molecular cloning, a laboratory manual (3rd edition), Cold Spring Harbor Press, Cold Spring Harbor, New York (2001); F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl (Ed.), Current Protocols in Molecular Biology, John Wiley & Sons Ltd., with or without modifications or changes. In addition, unless otherwise noted, a commercial reagent kit or a measurement instrument, if any, is used as described according to protocols attached thereto.

[0014] The above and further objects, features, advantages, and ideas of the present invention are apparent to those skilled in the art from consideration of the detailed description of this specification. Furthermore, those skilled in the art can easily reproduce the present invention from these descriptions. The mode(s) and specific example(s) described below represent a preferable embodiment of the present invention, which is given for the purpose of illustration or description. The present invention is not limited thereto. It is obvious to those skilled in the art that various modifications may be made according to the descriptions of the present specification without departing from the spirit and scope of the present invention disclosed herein.

== Biomarkers ==

[0015] In this specification, a biomarker for predicting response to chemoradiotherapy (also referred to as a predictive marker of response) for cancer comprises a biomarker (marker of response) for identifying patients with cancer who will respond to chemoradiotherapy (responder group), and a biomarker (marker of non-response) for identifying patients with cancer who will not respond to chemoradiotherapy (non-responder group). In addition, a biomarker for predicting prognosis of patients with squamous cell carcinoma who have received chemoradiotherapy (also referred to as a predictive marker of prognosis) is for discriminating patients with good prognosis and patients with poor prognosis, in a group of patients who underwent surgical resection of cancer after chemoradiotherapy.

[0016] "Chemoradiotherapy" as used herein may be "chemoradiotherapy" performed alone or "preoperative chemoradiotherapy" and "postoperative chemoradiotherapy" performed before and after surgery, respectively. Alternatively, the chemoradiotherapy may be combined with treatment other than surgery. Although a combination of "chemotherapy" using, for example, an anticancer drug and "radiotherapy" using radiation is preferable, the "chemoradiotherapy" may be either the chemotherapy performed alone or the radiotherapy performed alone. The anticancer drug used in the chemotherapy is not limited and any anticancer drug which is well-known to those skilled in the art can be used, such as fluorouracil or CDDP. Dose and schedule of administration of the anticancer drug depend on the type of the anticancer drug and conditions of the patient. Two or more anticancer drugs may be co-administered. Intensity and duration of radiation in the radiotherapy are not specifically limited as long as they fall within a range typically used for the treatment of cancer.

[0017] The term "cancer" as used herein means neoplasms such as carcinomas originating from epithelial cells, tumors originating from non-epithelial cells, and blood cancers, and is not limited to a specific cancer staging. The cancer for which the prognosis or the response to chemoradiotherapy is predicted is preferably squamous cell carcinoma, more preferably head and neck squamous cell carcinoma or esophageal squamous cell carcinoma, and most preferably esophageal squamous cell carcinoma. Examples of the head and neck squamous cell carcinoma include nasal cavity cancer, maxillary cancer, maxillary sinus cancer, tongue cancer, carcinoma of the mouth floor, gingival carcinoma, buccal mucosa cancer, nasopharyngeal carcinoma, oropharyngeal cancer, hypopharyngeal cancer, and laryngeal cancer. Examples of the esophageal squamous cell carcinoma include upper esophageal cancer, middle esophageal cancer, and lower esophageal cancer. Epithelium of oral mucosa and epithelium of esophageal mucosa are epithelial tissues of the same type from the developmental and histological viewpoint.

[0018] The predictive marker of response according to the present invention is a soluble interleukin-6 receptor (also referred to as sIL6R), a macrophage inflammatory protein 1β (also referred to as MIP-1β), or an activated plasminogen activator inhibitor (also referred to as PAI-1). By measuring the amount of a predictive marker of response in the blood obtained from a vertebrate animal suffering from a cancer before treatment with chemoradiotherapy, the response of the animal to the chemoradiotherapy for the cancer can be predicted.

[0019] The predictive marker of prognosis according to the present invention is the soluble interleukin-6 receptor (sIL6R). Thus, the soluble interleukin-6 receptor can be used effectively as the predictive marker of response as well as the predictive marker of prognosis.

== Measurement of the biomarker ==

[0020] An animal for which the biomarker is to be measured may be a human or any vertebrate animal, as long as it has at least one biomarker according to the present invention. The animal is preferably a mammal such as a human, a mouse, a rat, a dog, a cat, a horse, a sheep, a rabbit, a pig, and a monkey. It is most preferable that the animal is a human. The age and sex of the vertebrate animal are not specifically limited. The following description is made for a human patient as an example.

[0021] The blood is preferably pretreated before being subjected to measurement of the biomarker. For example, the serum or plasma is preferably separated from the blood on standing or by centrifugation and the separated serum or plasma is used for the measurement.

[0022] As to the measurement of the biomarker according to the present invention, only a biomarker may be measured and two or more biomarkers may be measured simultaneously or in sequence. The choice of the biomarker to be measured can be appropriately determined by those skilled in the art in consideration of, for example, a method of measurement and the amount of the blood. The amount of the biomarker according to the present invention may be determined at the same time as the measurement of the content or concentration of one or more other substances.

[0023] The amount of the biomarker in the drawn blood can be determined using a known method. For example, the amount of a biomarker may be determined using an antibody specific to that biomarker using a well-known method such as ELISA (enzyme-linked immunosorbent assay) including direct competitive ELISA, indirect competitive ELISA, and sandwich ELISA, RIA (radioimmunoassay), flowmetry, immunochromatography. In this case, the antibody specific to the biomarker may be polyclonal or monoclonal and is not limited by the animal species from which the antibody is derived. The antibody may be a full-length immunoglobulin or a partial antibody. The term "partial antibody" refers to a fragment of antibody with the antigen-binding site having antigen-binding activity. Examples of the partial antibody include a Fab fragment and a $F(ab')_2$ fragment. When the antibody is labeled with a label, examples of the label include, but not limited to, fluorescent substances (e.g., FITC, rhodamine, and phalloidine), colloidal particles such as gold, fluorescent microbeads such as Luminex (registered trademark, Luminex Corporation), heavy metals (e.g., gold and platinum), chromoproteins (e.g., phycoerythrin and phycocyanin), radioisotopes (e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I and ^{131}I), enzymes (e.g., peroxidase and alkaline phosphatase), biotin and streptavidin.

[0024] An example of the measurement performed using sandwich ELISA is given below. First, antibody (antibody 1) that is specific to a biomarker is immobilized on a solid phase such as a microplate. When the serum is added to the solid phase, the biomarker in the serum binds to the antibody, producing an immune complex. After the removal of excess serum, antibody (antibody 2) that recognizes an epitope different from the epitope recognized by the antibody 1 is added to the labeled biomarker, to allow the antibody 2 to bind to the biomarker. After the removal of excess antibody 2 by washing, the amount of the label remained on the microplate is measured. A calibration curve is made in advance, which represents a relationship between the amount of the marker added to the microplate and the amount of the remaining label. This calibration curve is used to calculate the amount of the marker in the blood.

[0025] Another example is given below for the measurement performed using fluorescent bead-based array system Luminex (Hitachi Software Engineering Co., Ltd.) which is an example of flowmetry. First, an antibody (antibody 1) that is specific to a biomarker is labeled with fluorescent microbeads. When this labeled antibody is mixed with the serum, the antibody binds to the biomarker in the serum, producing an immune complex. Then, a biomarker-specific antibody (antibody 2) labeled with biotin, which recognizes an epitope different from the epitope recognized by the biomarker-specific antibody 1, is added thereto. Then the antibody 2 binds to the biomarker that has been bound with the antibody 1. When an avidin-fluorescent dye is added, the dye binds to the biotin labeling the antibody 2, producing an avidin-biotin complex. This sample is subjected to flow cytometry, which specifies the fluorescent microbeads by the wavelength of the fluorescence. The amount of the biomarker is then quantified by the strength of the fluorescence of the surface of the specified beads. With this method, two or more biomarkers can be measured simultaneously by labeling each of the antibodies (antibodies 1) that is specific to each of different biomarkers with each of fluorescent dyes having different excitation wavelengths.

== Use of the biomarker ==

[0026] Use of the biomarker according to the present invention includes, for example, following modes and aspects.

<Prediction of Response to Chemoradiotherapy>

[0027] By measuring the amount of the biomarker in the blood obtained from a vertebrate animal having cancer before treatment with the chemoradiotherapy, the animal's response to the chemoradiotherapy for cancer can be predicted.

[0028] The amount of the biomarker is preferably represented as an absolute concentration of the biomarker. The amount is, however, not limited as long as it is related to the absolute concentration of the biomarker so that the absolute concentration can be compared among the individuals using it. The amount may be a relative concentration, merely a weight per a unit volume, or raw data measured to determine the absolute concentration.

[0029] sIL6R, MIP-1β, and PAI-1 are the predictive markers of response which are useful for the prediction of the efficacy of the chemoradiotherapy. For example, the amount of the biomarker in the blood is obtained in a certain group of patients before application of the chemoradiotherapy. Thereafter, their responses to the chemoradiotherapy are evaluated, the patients are divided into a responder group with a good therapeutic response and a non-responder group with a poor therapeutic response and the ranges of the amount of the biomarker in the blood are then determined for each of the groups. To divide the patients into the groups either "with a good therapeutic response" or "with a poor therapeutic response", a criteria predetermined by those skilled in the art in consideration with a well-known technique may be used. For example, for chemoradiotherapy for esophageal squamous cell carcinoma, the grade 3 effects specified by the histopathological criteria (Classification of Esophageal Cancer, 10th edition) may be defined as a good therapeutic response, and the grade 2 or lower effects may be defined as a poor therapeutic response. The amount of the biomarker in the blood of each patient to be diagnosed may be determined and then it may be determined which range the result falls in. When the result falls in the range of the responder group, the chemoradiotherapy may be applied. When the result falls in the range of the non-responder group, or does not fall in the range of the responder group, the chemoradiotherapy may not be applied.

[0030] Alternatively, a threshold, instead of the aforementioned ranges, may be determined for the amount of the biomarker in the blood obtained before treatment with the chemoradiotherapy to predict the response. A method to determine the threshold is not specifically limited and a routine method known to those skilled in the art can be used. The threshold may be determined such that a first predetermined percentage of patients who will respond is included below the threshold and a second predetermined percentage of patients who will not respond is included at or above the threshold. The threshold is preferably determined such that the first predetermined percentage and the second predetermined percentage are both high. The percentages are preferably 50% or higher, more preferably 70% or higher, yet further preferably 90% or higher, and most preferably 100%. Setting of higher percentages for both provides higher specificity and sensitivity. This means that the patients to be diagnosed can be discriminated into the responder and non-responder groups with high accuracy, by means of determining the threshold such that both the specificity and the sensitivity become high. These values are preferably 50% or higher, more preferably 70% or higher, yet further preferably 90% or higher, and most preferably 100%. Alternatively, the threshold may be determined so that the best chi-square value for the discrimination is obtained by using a statistical software such as JMP available from SAS Institute Japan. More specifically, for example, the threshold of blood sIL6R concentration may be set from 10 to 35 ng/ml and preferably from 20 to 30 ng/ml.

[0031] More specifically, for example, the threshold of blood sIL6R concentration may be set from 10 to 35 ng/ml, and the patients whose value is smaller than the threshold may be considered as the responders and the patients whose value is equal to or larger than the threshold may be considered as the non-responders. The threshold of blood concentration is, however, preferably set from 20 to 30 ng/ml, and most preferably set at 30 ng/ml. Alternatively, the threshold of blood MIP-1β concentration may be set from 10 to 200 pg/ml, and the patients whose value is smaller than the threshold may be considered as the responders and the patients whose value is equal to or larger than the threshold may be considered as the non-responders. The threshold of blood concentration is, however, preferably set from 50 to 150 pg/ml. Furthermore, the threshold of blood PAI-1 concentration may be set from 100 to 60000 pg/ml, and the patients whose value is equal to or larger than the threshold may be considered as the responders and the patients whose value is smaller than the threshold may be considered as the non-responders. The threshold of blood concentration is, however, preferably set from 20000 to 50000 pg/ml.

[0032] In view of the accuracy of prediction of the therapeutic response, the animal from which the blood is obtained to determine a range or a threshold for the amount of the marker and the animal to be diagnosed preferably belong to the same species and suffer from the same type of cancer.

[0033] The biomarker according to the present invention may be a combination of two or more markers. The prediction of the therapeutic response using the predictive marker of response according to the present invention may be combined with other diagnostic method for cancer. For the purpose of convenience, the prediction is preferably combined with other blood markers.

<Prediction of prognosis after treatment>

[0034] By measuring the amount of the biomarker in the blood obtained from a vertebrate animal having cancer before chemoradiotherapy, the prognosis of the animal after surgical resection of the cancer can be predicted.

[0035] Prognosis of cancer patients is associated with years of survival from the beginning of treatment. For humans, prognosis may be considered good when a patient survives for 5 years from the beginning of treatment and prognosis may be considered poor when the patient dies before 5 years from the beginning of treatment.

[0036] It is noted that sIL6R is a predictive marker of prognosis with which patients with a longer prognosis and patients with a shorter prognosis can be discriminated efficiently. A range or a threshold of the blood concentration of the predictive marker of prognosis may be determined in a manner similar to the one described in the "Prediction of response to chemoradiotherapy", and prognosis may be predicted based on the range or the threshold.

Examples

[0037] [Example 1] This example shows that response to chemoradiotherapy for cancer tissue is associated with a survival rate of a patient.

[0038] At the Third Department of Surgery, Tokyo Medical University Hospital, 37 patients with advanced esophageal squamous cell carcinoma were treated with preoperative chemoradiotherapy, and esophagectomy was performed 4 weeks after the chemoradiotherapy. For the chemotherapy, fluorouracil and CDDP were used. For the radiotherapy, an electron beam from Linac (linear accelerator) was used.

[0039] The chemoradiotherapy continued for 4 weeks, and performed for the first five consecutive days for each week. The patients were administered with 350 mg/m^2 (patient's body surface area) of fluorouracil (5-FU, Kyowa Hakko Kirin Co., Ltd.) and 5 mg/m^2 (patient's body surface area) of CDDP (Nippon Kayaku Co., Ltd.) daily, and 7000 mg/m^2 and 100 mg/m^2 in total, respectively, for a total treatment period. In addition, the radiotherapy was given at 2 Gy daily fractions to a total dose of 40 Gy over the same total treatment period.

[0040] Histopathologic diagnosis of the tissue resected during the esophagectomy was performed to determine a preoperative response to the chemoradiotherapy according to the histopathological criteria (Classification of Esophageal Cancer, 10th edition, see Table 1). In addition, the aforementioned 37 patients with esophageal squamous cell carcinoma were followed up for up to 9 years from the beginning of the treatment.

Table 1

Grade		Effects	Tissue Condition
0		ineffective	no effect on cancer tissue and cancer cells
1	1a	slightly effective	cancer cells appeared to be grown account for 2/3 or more of cancer tissues
	1b		cancer cells appeared to be grown account for 1/3 or more but less than 2/3 of cancer tissue
2		moderately effective	cancer cells appeared to be grown account for less than 1/3 of cancer tissue
3		markedly effective	no cancer cells appeared to be grown

[0041] The 37 patients with esophageal squamous cell carcinoma were classified into two groups: a group of patients with the grade 3 effects to the chemoradiotherapy in the histopathologic diagnosis and a group of patients with the grade 1 or 2 effects. Table 2 below shows the gender, age, tumor location, and clinical stage of each group.

Table 2

	Grade 3 n = 7	Grade 1 + 2 n = 30(12 + 18)
Age (mean ± S.D.)	64.3 ± 7.16	60.6 ± 7.59
Gender (%) Male Female	 5 (71.4) 2 (28.6)	 27 (90) 3 (10)

(continued)

Tumor location (%)		
Ce	0	3 (10)
Te	6 (85.7)	27 (90)
Ut	1 (16.7)	5 (18.5)
Mt	2 (33.3)	15 (55.6)
Lt	3 (50)	7 (25.9)
Ae	1 (14.3)	0
Clinical stage (%)		
II	0	4 (13.3)
III	7 (100)	20 (66.7)
IV	0	6 (20)

(For the "tumor location" and the "clinical stage" in this table, see, Hayashida Y, Honda K, Osaka Y, Hara T, Umaki T, Tsuchida A, Aoki T, Hirohashi S, Yamada T. Possible prediction of chemoradiosensitivity of esophageal cancer by serum protein profiling. Clin. Cancer Res. 2005 Nov 15; 11(22): 8042-7.)

[0042] As shown in Table 2, 7 patients were classified as grade 3 and 30 patients were classified as grade 1 or 2 in the histopathologic diagnosis after the chemoradiotherapy. Fig. 1 shows a graph of survival rates of these patients for up to 9 years.

[0043] As shown in Fig. 1, the 9-year survival rate of the patients with the grade 3 effects of the chemoradiotherapy was about 80%, while the 9-year survival rate of the patients with the grade 1 or 2 effects was about 20%. In this way, the response to chemoradiotherapy is associated with the survival rate of the patients.

[0044] [Example 2] This example shows that the response to chemoradiotherapy can be predicted by using the biomarkers sIL6R, MIP-1β and PAI-1.

[0045] Three biomarkers sIL6R, MIP-1β, and PAI-1 were measured using a fluorescent bead-based array system Luminex (Hitachi Software Engineering Co., Ltd.) or sandwich ELISA on the blood obtained from the aforementioned patients with esophageal squamous cell carcinoma before the chemoradiotherapy.

== Measurement by fluorescent bead-based array system luminex ==

[0046] Extracelular Luminex Kit sIL6R (catalog No. LHR0061) available from Biosource International Inc., extracelular Luminex Kit MIP-1β (catalog No. LHC1051) available from Biosource International Inc., and PAI-1, Human, Fluorokine MAP kit (product No. LOB1359) available from R&D was used for the measurement of sIL6R, MIP-1β, and PAI-1, respectively. These analyses were contracted out to Hitachi Software Engineering Co., Ltd.

[0047] Figs. 2A and 2B show the concentration of sIL6R for the patients of the grades 1, 2, and 3. The concentration of sIL6R was significantly lower in the group of the grade 3 patients compared to the group of the grade 1 + 2 patients.

[0048] Fig. 3 shows the concentration of IP-1β for the patients of grades 1, 2, and 3. The concentration of IP-1β was significantly lower in the group of the grade 3 patients compared to the group of the grade 1 + 2 patients.

[0049] Fig. 4 shows the concentration of PAI-1 for the patients of grades 1, 2, and 3. The concentration of PAI-1 was significantly higher in the group of the grade 3 patients compared to the group of the grade 1 + 2 patients.

== Measurement by sandwich ELISA ==

[0050] Blood sIL6R concentration was measured in SRL on a contract basis by sandwich ELISA using Quantikine Human IL-6 sR Immunoassay (R&D Systems).

[0051] As shown in Fig. 5, measurements by the sandwich ELISA showed that the concentration of sIL6R was significantly lower in the responder group (grade 3) compared to the non-responder group (grades 1, 2).

[0052] Thus, the blood concentrations of sIL6R, MIP-1β, and PAI-1 are significantly different between the group (grade 3) with a good response to the chemoradiotherapy and the group (grades 1, 2) with a poor response to the chemoradiotherapy. Thus, these biomarkers can be used to predict the response to the chemoradiotherapy.

[0053] However, the distribution of the concentration of sIL6R and MIP-1β in the responder group is overlapped with the distribution in the non-responder group. Therefore, these markers are useful to identify an individual that will not respond to the chemoradiotherapy. For example, the highest concentrations of sIL6R and MIP-1β in the responder group are used as thresholds. Then the individual that will not respond can be distinguished effectively by identifying the individual whose value of concentration is larger than that threshold is identified. Since the individual whose value of concentration is smaller than the threshold is more likely to respond to the chemoradiotherapy, the chemoradiotherapy may be applied. It is, however, preferable that a decision is made depending on the situation.

[0054] On the other hand, the distribution of the concentration of PAI-1 in the non-responder group is overlapped with the distribution of PAI-1 in the responder group. Thus, this marker is useful to identify an individual that will respond well to the chemoradiotherapy. For example, the highest concentration of PAI-1 in the non-responder group is used as a threshold. The individual that will respond can be distinguished effectively by identifying the individual whose value of concentration is larger than the threshold. Since the individual whose value of concentration is smaller than the threshold is more likely not to respond to the chemoradiotherapy, the chemoradiotherapy may not be applied. It is, however, preferable that a decision is made depending on the situation.

[0055] [Example 3] This example shows that prognosis of a patient can be predicted using the biomarker according to the present invention.

[0056] For the concentration of sIL6R measured by the fluorescent bead-based array system or the sandwich ELISA in Example 2, 30 ng/ml was set as a threshold. Figs. 6 and 7 show graphs of survival rates for up to 6 and 9 years, respectively, from the beginning of the treatment in a high sIL6R group with a value of the blood concentration larger than the threshold and a low sIL6R group with a value of the blood concentration smaller than the threshold.

[0057] The measurement by the fluorescent bead-based array system indicated that 18 cases belong to the high sIL6R group and 19 cases belong to the low sIL6R group. The 6-year survival rate of about 90% in the low sIL6R group was significantly higher compared to that of about 25% in the high sIL6R group (P = 0.0012, long-rank study). The sensitivity and the specificity as a marker of response after 6 years are 77% and 89%, respectively.

[0058] The measurement by the sandwich ELISA also indicated that 18 cases belong to the high sIL6R group and 19 cases belong to the low sIL6R group. The 9-year survival rate of about 60% in the low sIL6R group was significantly higher compared to that of about 15% in the high sIL6R group (P = 0.042, long-rank study). The sensitivity and the specificity as a marker of response after 9 years are 78% and 57%, respectively.

[0059] Thus, the prognosis of a patient can be predicted using the biomarker sIL6R according to the present invention.

[0060] [Example 4] This example shows that sIL6R reflects only the response to chemoradiotherapy in patients with esophageal squamous cell carcinoma and the prognosis of such patients, and does not reflect other factors.

[0061] The Cox proportional hazards regression model was used to determine whether the age, gender, tumor location, and clinical stage were associated with the blood SIL6R concentration in the patients with esophageal squamous cell carcinoma shown in Table 2. Results are given in Tables 3 and 4.

Table 3

Univariate Analysis	n	Relative Risk	95% C.I.	p value
Age (<65/65≤)	25/12	1.308	0.5765-2.9679	0.520666
Gender (male/female)	32/5	0.3113	0.0729-1.3300	0.115234
Tumor Location (Ce, Ut/Mt, Lt, Ae)	9/28	0.6962	0.2593-1.8695	0.472447
clinical stage (0, I, II/III, IV)	19/18	3.0647	1.3369-7.0255	0.008145
sIL6R (median)	18/19	3.1881	1.3494-7.5322	0.008214

Table 4

Multivariate Analysis	n	Relative: Risk	95% C.I.	p value
Clinical Stage (0, I, II/III, IV)	19/18	2.4983	1.1016-6.5221	0.033545
sIL6R (median)	18/19	2.8715	1.2033-6.8527	0.017456

[0062] These results indicate that sIL6R is independent of the age, gender, and tumor location of the patients and is associated with the clinical stage.

Industrial applicability

[0063] The present invention provides a biomarker and a method of measuring the same for determining whether or not chemoradiotherapy is applicable to a patient with cancer.

Claims

1. A biomarker for predicting response to chemoradiotherapy for squamous cell carcinoma, the biomarker being selected from the group consisting of:

    a soluble interleukin-6 receptor, a macrophage inflammatory protein 1β, and an activated plasminogen activator inhibitor.

2. A biomarker for predicting prognosis of a patient with squamous cell carcinoma, the patient having received chemoradiotherapy, the biomarker being a soluble interleukin-6 receptor.

3. The biomarker according to Claim 1 or 2, wherein the squamous cell carcinoma is head and neck squamous cell carcinoma or esophageal squamous cell carcinoma.

4. The biomarker according to Claim 2, wherein the chemoradiotherapy is preoperative chemoradiotherapy.

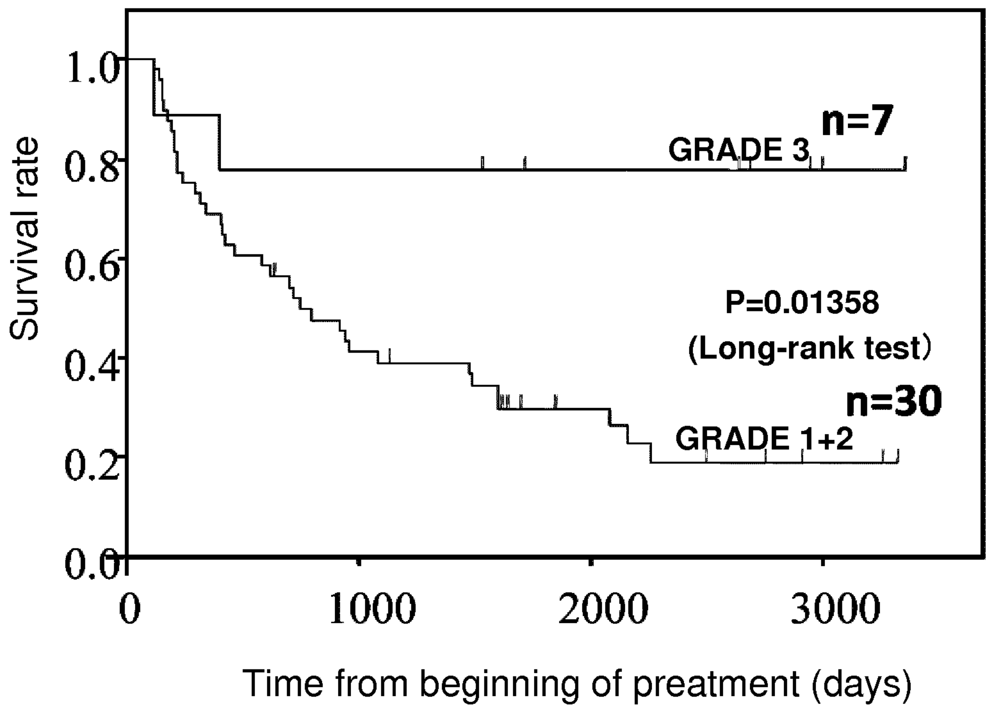
5. A method for measuring a concentration of a biomarker, comprising:

    measuring concentrations of one or more biomarker(s) according to Claim 1 in the blood obtained before treatment with chemoradiotherapy.

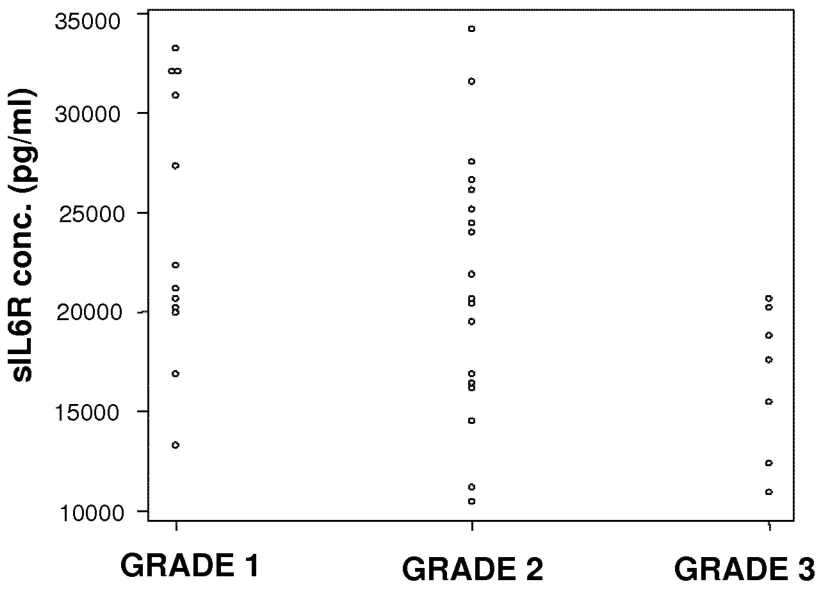
6. The method according to Claim 5, wherein the blood has been obtained from a patient with squamous cell carcinoma.

7. The method according to Claim 5 or 6, wherein the concentration of the biomarker is measured using an antibody specific to the biomarker.

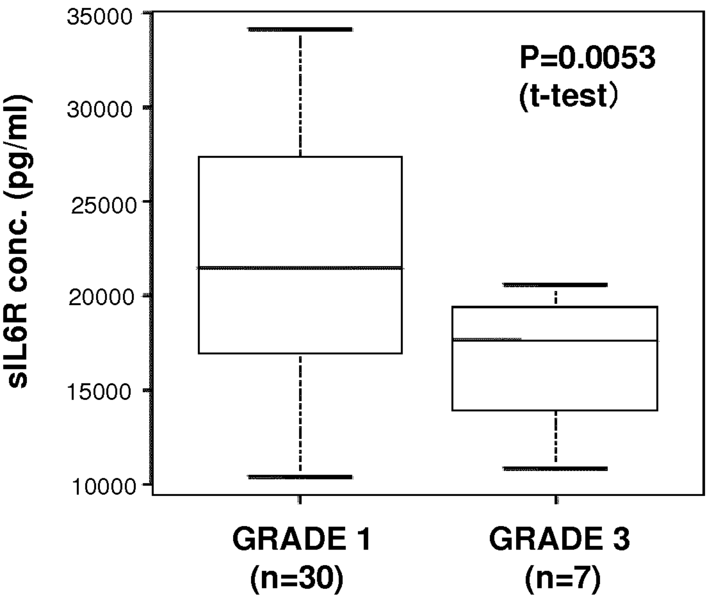
8. The method according to Claim 6 or 7, wherein the squamous cell carcinoma is head and neck squamous cell carcinoma or esophageal squamous cell carcinoma.

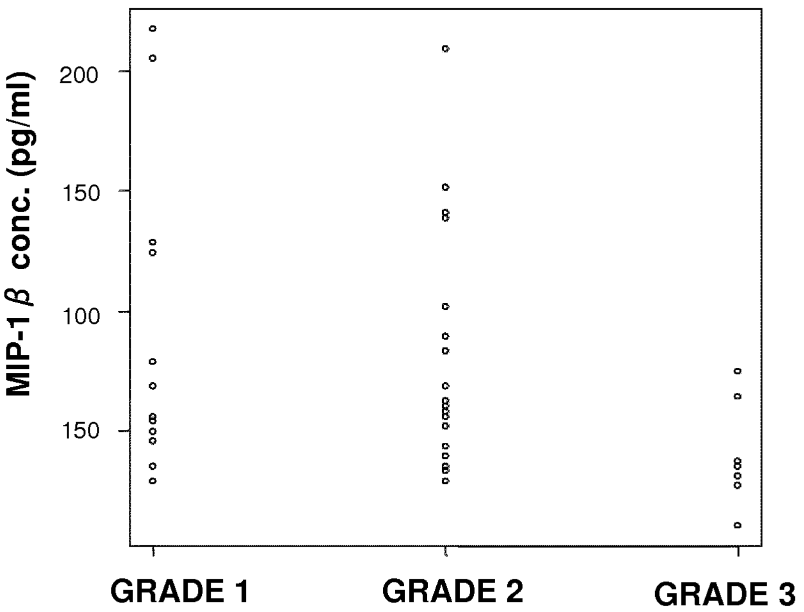

1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
Survival rate
Time from beginning of preatment (days)
GRADE 3
n=7
P=0.01358
(Long-rank test)
n=30
GRADE 1+2

A

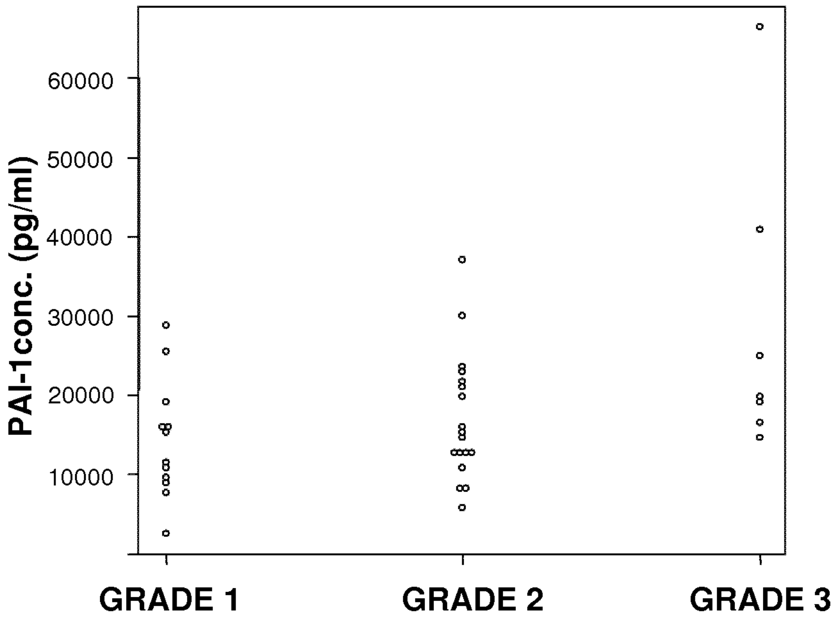

35000
30000
25000
20000
15000
10000
sIL6R conc. (pg/ml)
GRADE 1
GRADE 2
GRADE 3

B

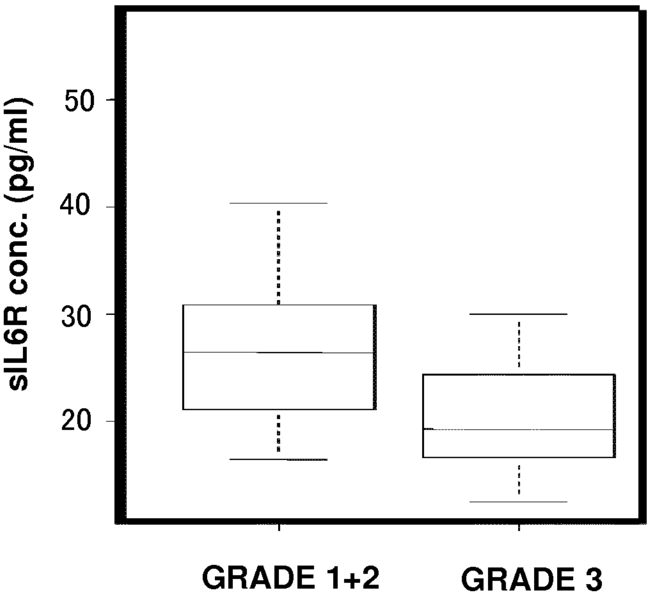

35000
30000
25000
20000
15000
10000
sIL6R conc. (pg/ml)
P=0.0053
(t-test)
GRADE 1
(n=30)
GRADE 3
(n=7)



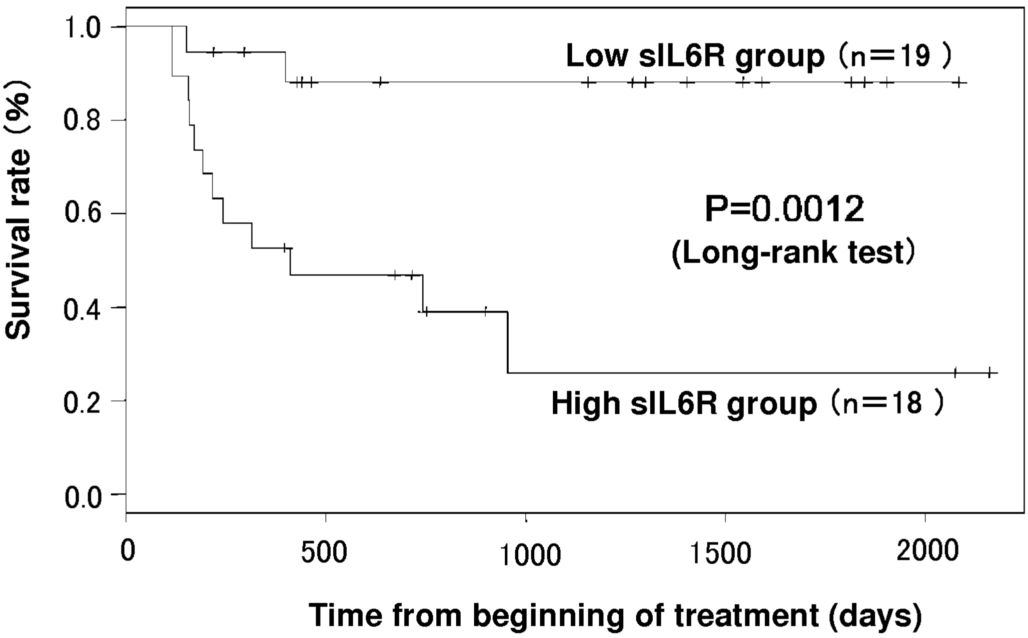
P=0.0022
(GRADE 1+2 vs. GRADE 3, t-test)

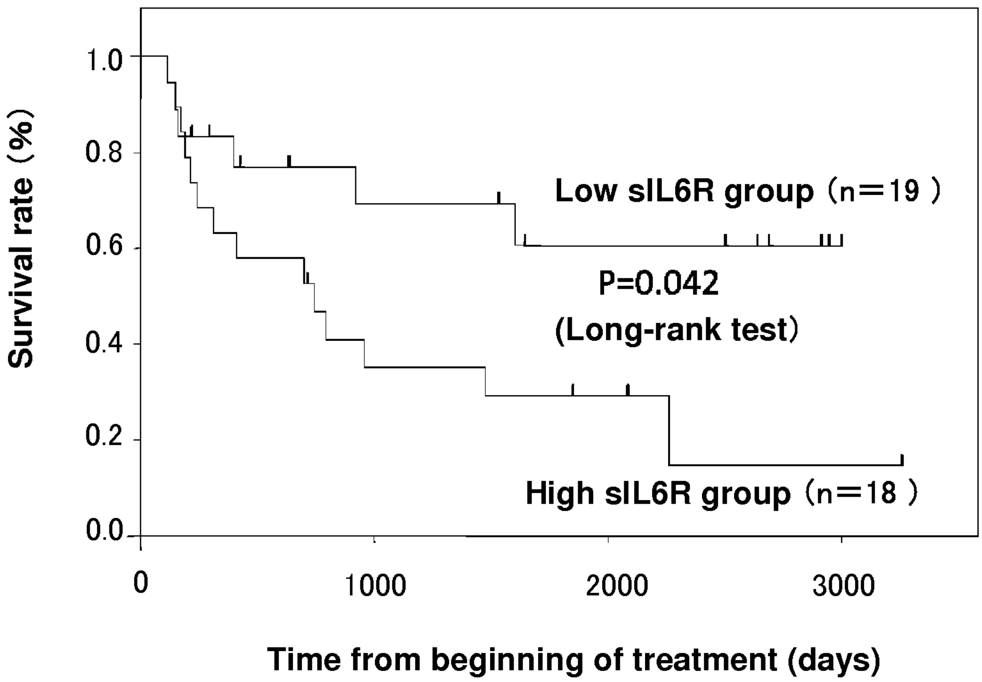


P=0.0233
(GRADE 1+2 vs. GRADE 3, U-test)



P=0.039
(GRADE 1+2 vs. GRADE 3, t-test)


Survival rate (%)
1.0
0.8
0.6
0.4
0.2
0.0
Low sIL6R group (n=19)
P=0.0012
(Long-rank test)
High sIL6R group (n=18)
0
500
1000
1500
2000
Time from beginning of treatment (days)


Survival rate (%)
1.0
0.8
0.6
0.4
0.2
0.0
Low sIL6R group (n=19)
P=0.042
(Long-rank test)
High sIL6R group (n=18)
0
1000
2000
3000
Time from beginning of treatment (days)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/058259

A. CLASSIFICATION OF SUBJECT MATTER
G01N33/574(2006.01)i, *G01N33/68*(2006.01)i, *G01N33/53*(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)
G01N33/574, G01N33/68, G01N33/53

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	1996-2011
Kokai Jitsuyo Shinan Koho	1971-2011	Toroku Jitsuyo Shinan Koho	1994-2011

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA/BIOSIS/MEDLINE (STN), JSTPlus/JMEDPlus/JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	Bayer Christine et al., PAI-1 levels predict response to fractionated irradiation in 10 human squamous cell carcinoma lines of the head and neck, Radiotherapy and oncology, 2008, Vol.86, No.3, Page.361-368 Abstract	1,3,5-8 2,4
A	Chin David et al., Novel markers for poor prognosis in head and neck cancer, Int J Cancer, 2005, Vol.113, No.5, Page.789-797 Abstract	1-8
A	Masahiro SAKAMOTO et al., "The effect of clarithromycin to prolong the survival time of patients with unresectable non-small lung cancer", Japanese Journal of Chemotherapy, 2000, vol.48, no.2, pages 112 to 116, summary	1-8

☒ 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 14 April, 2011 (14.04.11)	Date of mailing of the international search report 26 April, 2011 (26.04.11)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/058259

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Ariga, H et al., Potential biomarkers of a complete response and local control for definitive chemoradiotherapy in resectable esophageal squamous cell carcinoma, EJC supplements, 2009, Vol.7, No.2, Page.160 2031	1-8
A	Chiaki ARAI et al., "Relationship between Activation of Blood Coagulation-Fibrinolysis System by Pre-operative Radiotherapy and Molecular Markers in Patients with Oral Squamous Cell Carcinoma", Japanese Journal of Oral Diagnosis/Oral Medicine, 2006, vol.19, no.2, pages 200 to 204	1-8
A	Toyoji SATO et al., "IAP no Rinsho Oyo", The Japanese Journal of Clinical Pathology, 1988, no.Nov special extra issue, pages 101 to 107	1-8
A	OKAMURA Shinichi et al., The influence of stromal inflammatory reaction on the chemoradiation responses for advanced esophageal cancer, Proceedings of the Japanese Cancer Association, 2009, Vol.68th, Page.61 O-108	1-8
A	KUHN D J et al., Overexpression of Interleukin-2 Receptor α in a Human Squamous Cell Carcinoma of the Head and Neck Cell Line Is Associated With Increased Proliferation, Drug Resistance, and Transforming Ability, J Cell Biochem, 2003, Vol.89, No.4, Page.824-836	1-8

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- JP 2010083198 A **[0001]**

Non-patent literature cited in the description

- **THOMAS N. et al.** *New England Journal of Medicine,* August 1996, vol. 15, 462-467 **[0003]**
- **VAL GEBSKI et al.** *Lancet Oncol.,* March 2007, vol. 8 (3), 226-234 **[0003]**
- Molecular cloning, a laboratory manual. Cold Spring Harbor Press, 2001 **[0013]**
- Current Protocols in Molecular Biology. John Wiley & Sons Ltd, **[0013]**
- **HAYASHIDA Y ; HONDA K ; OSAKA Y ; HARA T ; UMAKI T ; TSUCHIDA A ; AOKI T ; HIROHASHI S ; YAMADA T.** Possible prediction of chemoradiosensitivity of esophageal cancer by serum protein profiling. *Clin. Cancer Res.,* 15 November 2005, vol. 11 (22), 8042-7 **[0041]**

专利名称(译)	生物标记物		
公开(公告)号	EP2554994A1	公开(公告)日	2013-02-06
申请号	EP2011765758	申请日	2011-03-31
[标]申请(专利权)人(译)	NAT癌症CENT		
申请(专利权)人(译)	国家癌症中心		
当前申请(专利权)人(译)	国家癌症中心		
[标]发明人	HONDA KAZUFUMI YAMADA TESSHI MAKUUCHI YOSUKE OSAKA YOSHIAKI HIROHASHI SETSUO		
发明人	HONDA, KAZUFUMI YAMADA, TESSHI MAKUUCHI, YOSUKE OSAKA, YOSHIAKI HIROHASHI, SETSUO		
IPC分类号	G01N33/574 G01N33/68 G01N33/53		
CPC分类号	G01N33/6869 G01N33/57484 G01N2333/523 G01N2333/5412 G01N2333/7155 G01N2333/8132 G01N2800/52		
优先权	2010083198 2010-03-31 JP		
其他公开文献	EP2554994B1 EP2554994A4		
外部链接	Espacenet		

摘要(译)

本发明的一个目的是提供用于预测对癌症的化学放射疗法的响应和预测患有癌症的患者的预后的生物标志物，以及测量这种生物标志物的方法。对脊椎动物癌症的化学放射疗法的反应可以通过测量在用化学放射疗法治疗之前从患有癌症的个体获得的血液中的可溶性白细胞介素-6受体，MIP-1β和活化的纤溶酶原激活物抑制剂的浓度以及预后来预测。可以通过测量可溶性白细胞介素-6受体的浓度来确定相同的脊椎动物。