desired in view of reduction of side effects.

[0005]

Therefore, development of a drug having high inhibitory activity with respect to exon 18 and exon 21 mutant EGFR, as well as high selectivity with respect to mutant EGFR compared with wild-type EGFR, is expected to enable inhibition of growth of lung cancer cells having mutant EGFR with a dose lower than the dose causing the side effects in the skin or digestive tract, thereby contributing to life prolongation or increase in QOL of patients with mutant EGFR-positive cancers for which treatment methods have not been established. Further, a drug having high inhibitory activity with respect to T790M, which is acquired resistance mutation against treatments using EGFR inhibitors, is expected to reduce expression frequency of acquired resistance during the treatments using EGFR inhibitors against exon 18 or exon 21 mutant EGFR, which is *de novo* mutation; and is therefore expected to contribute to the life prolongation of cancer patients.

Citation List

Patent Literature

[0006]

PTL 1: WO2015/175632A1

PTL 2: WO2015/025936A1

Non-Patent Literature

[0007]

NPL 1: Nat. Rev. Cancer, Vol. 6, pp. 803-812 (2006)

NPL 2: Nature Medicine, Vol. 19, pp. 1389-1400 (2013)

NPL 3: Nat. Rev. Cancer, Vol. 7, pp. 169-181 (2007)

NPL 4: Lancet Oncol. Vol. 13, e. 23-31 (2012)

Summary

Technical Problem

[0008]

An object of the present invention is to provide an antitumor agent that does not cause inhibition of wild-type EGFR and thus causes smaller side effects, serving as an inhibitor that can ensure high selectivity with respect to exon 18 and/or exon 21 mutant EGFR for which the therapeutic effects of the previously known EGFR inhibitors are insufficient.

Solution to Problem

[0009]

The inventors of the present invention conducted extensive research, and found that exon 18 and/or exon 21 mutant EGFR is an appropriate target in treating cancers, and that EGFR inhibitors conventionally used for the treatments have inferior selectivity between exon 18 and/or exon 21 mutant EGFR. Further, the inventors also confirmed that the compound of the present invention exerts superior selectivity and tumor growth inhibitory effects with respect to exon 18 and/or exon 21 mutant EGFR. With this finding, the inventors accomplished the present invention.

[0009a]

In one aspect, the present disclosure refers to the use of an antitumor agentin the manufacture of a medicamentfor treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting 5 of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue, the antitumor agent comprising (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof.

In another aspect, the present disclosure refers to (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof for use in the treatment of a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

In yet another aspect, the present disclosure refers to the use of (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in the manufacture of a medicament for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation ofexon 21, wherein X represents an arbitrary amino-acid residue.

In a further aspect,the present disclosure refers to the use of (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof for the production of a pharmaceutical agent for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

In a further aspect, the present disclosure refers to a pharmaceutical composition comprising (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof and a pharmaceutically acceptable carrier, for use in treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

In a further aspect, the present disclosure refers to an *ex vivo* method for predicting therapeutic effects of chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in a malignant tumor patient, the method comprising steps (1) and (2) below:

(1) a step of detecting the presence or absence of mutation of EGFR gene contained in a biological sample obtained from the patient; and

(2) a step of predicting that the chemotherapy is highly likely to exhibit sufficient therapeutic effects with respect to the

patient when the results of the detection in step (1) found that the EGFR gene has at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

In a still further aspect, the present disclosure refers to the use of(S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in the manufacture of a medicament for treating malignant tumor, wherein the medicament is to be administered to a malignant tumor patient who was predicted highly likely to sufficiently respond to the chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof, after a biological sample is to be obtained from the patient in a first step to detect the presence or absence of mutation of EGFR;

and wherein in a second step, chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof is predicted to highly likely to exhibit sufficient therapeutic effects with respect to the patient when the results of the detection in the first step found that the EGFR gene has at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[0010]

The present disclosure encompasses the following examples.

[0011]

Item 1.

An antitumor agent for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X

represents an arbitrary amino-acid residue, the antitumor agent comprising (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof.

[0012]

Item 2.

The antitumor agent according to Item 1, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

The previously known EGFR inhibitors have low selectivity with respect to exon 18 and exon 21 mutant EGFR, compared with wild-type EGFR; therefore, the difference between the dosage for ensuring the antitumor effects and the dosage causing the side effects (skin disorders, digestive tract disorders, etc.) derived from wild-type EGFR inhibition was small. Accordingly, the previously known EGFR inhibitors have difficulty in exerting sufficient therapeutic effects. In contrast, since the antitumor agent of the present invention has high selectivity with respect to exon 18 and exon 21 mutant EGFR, it is possible to increase the dosage without causing side effects derived from wild-type EGFR inhibition. Therefore, the antitumor agent of the present invention exerts superior therapeutic effects for a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0046]

In addition, the antitumor agent of the present invention exhibited high inhibitory activity with respect to exon 18 and exon 21 mutant EGFR in the presence of T790M mutation, which is acquired resistance mutation in the exon 20 region. Therefore, the antitumor agent of the present invention exerts superior therapeutic effects with respect to a malignant tumor patient whose response to the existing drug is low due to acquired resistance mutation caused by the use of existing antitumor agent.

[0047]

Further, the antitumor agent of the present invention is also useful in terms of reducing expression frequency of acquired resistance during the treatments using EGFR inhibitors against exon 18 or exon 21 mutant EGFR, which is *de novo* mutation, because of its high inhibitory activity against exon 18 and exon 21 mutant EGFR even under the presence of T790M mutation, which is acquired resistance mutation in the exon 20 region.

Brief Description of Drawings

[0048]

Fig. 1 illustrates the tumor volume (which may be hereinafter referred to as "TV") of mouse models subcutaneously transplanted with G719A mutant EGFR-expressing cell line to measure the antitumor effect of compound A.

Fig. 2 illustrates the body weight change during a dosing period of compounds of mouse models subcutaneously transplanted with G719A mutant EGFR-expressing cell line to measure the toxicity of compound A.

Fig. 3 illustrates the tumor volume of mouse models subcutaneously transplanted with G719A+T790M mutant EGFR-expressing cell line to measure the antitumor effect of compound A.

Fig. 4 illustrates the body weight change during a dosing period of compounds of mouse models subcutaneously transplanted with G719A+T790M mutant EGFR-expressing cell line to measure the toxicity of compound A.

Description of Embodiments

[0049]

Preferable examples of various definitions in the scope of the present invention used in this specification are explained below in detail.

[0050]

In this specification, "EGFR" refers to a human epidermal growth factor receptor protein, and is also referred to as ErbB-1 or HER1.

[0051]

In this specification, "wild-type EGFR" refers to EGFR free of somatic mutation, which is a protein comprising the amino acid sequence represented by SEQ ID NO: 1 (GenBank accession number: NP_005219.2).

More preferably, the exon 21 mutation is L861X, which is point mutation in which leucine encoded by codon 861 in the exon 21 region is substituted with an arbitrary amino acid. More specifically, L861Q, which is point mutation in which leucine encoded by codon 861 in the exon 21 region is substituted with glutamine, is preferable.

[0056]

In the present invention, "exon 18 and/or exon 21 mutation" encompasses "exon 18 mutation," "exon 21 mutation," and "exon 18 and exon 21 mutation."

[0057]

In the present invention, "point mutation" refers to mutation causing substitution, insertion, or deletion of one or more (e.g., about 1 to 10, preferably about 1 to 5, more preferably about 1, 2, or 3) amino-acid residues; and may include in-frame insertion and/or deletion mutation as nucleic acid.

[0058]

"EGFR having exon 18 and/or exon 21 mutation" encompasses "EGFR having exon 18 mutation," "EGFR having exon 21 mutation," and "EGFR having exon 18 and exon 21 mutation".

[0059]

In this specification, the "EGFR having exon 18 mutation" refers to EGFR having at least one exon 18 mutation. The EGFR may have two or more different exon 18 mutations, and preferably has a single exon 18 mutation. Further, the EGFR may also have a mutation other than exon 18 mutation (such as exon 19 deletion mutation, L858R mutation, or L790M mutation).

[0060]

In this specification, the "EGFR having exon 21 mutation" refers to EGFR having at least one exon 21 mutation. The EGFR may have two or more different exon 21 mutations, and preferably has a single exon 21 mutation. Further, the EGFR may also have a mutation other than exon 21 mutation (such as exon 19 deletion mutation, L858R mutation, or L790M mutation).

[0061]

medium used for suspending the cells. The solutions were then individually added to each well of the culture plate of the cells, and the cells were incubated in a 5% CO_2 gas-containing incubator at 37°C for 6 hours. After incubation, the cells were immobilized using 20% neutral buffered formalin (Wako Pure Chemical Industries, Ltd.), and blocked by an Odyssey (trademark) blocking buffer (PBS) (M&S TechnoSystems Inc.). The cells were then reacted with a primary antibody (EGFR Monoclonal Antibody (R19/48MIX) #AHR5062) (Thermo Fisher Scientific Inc.) that was diluted with an Odyssey (trademark) blocking buffer (PBS) to 1/200, and a Phospho-EGFR Receptor (Tyr1068) Antibody #2234L (CST)), followed by subjecting the cells to permeation at 4°C overnight. The following day, the cells were reacted with a secondary antibody (IRDye 800CW Goat aRabbit #926-32211 and IRDye 680RD Goat aMouse #926-68070 (M&S TechnoSystems Inc.)) diluted with an Odyssey (trademark) blocking buffer (PBS) to 1/800, and subjected to permeation at room temperature for 1 hour. The fluorescence intensity (which may be hereinafter referred to as "FI") was detected with an Odyssey Infrared Imaging System (LI-COR Bioscience) at a fluorescence wavelength of 800 nm and 700 nm.

[0104]

The value obtained by deducting the FI of a well without the cells from the FI detected at a fluorescence wavelength of 800 nm or 700 nm is referred to as FI (800, EGFR)-Blank (for 800 nm) and FI (700, p-EGFR)-Blank (for 700 nm). The value obtained by dividing FI (700, p-EGFR)-Blank of each well by FI (800, EGFR)-Blank was determined to be FI (p-EGFR/EGFR). The phosphorylated EGFR inhibitory rate was calculated using the following formula to determine the concentration of the test compounds at which phosphorylated EGFR was inhibited by 50% (IC_{50} (μM)). Table 1 illustrates the results.

[0105]

Phosphorylated EGFR Rate (%) = T/C × 100

T: FI (p-EGFR/EGFR) of a well to which a test compound was added.

C: FI (p-EGFR/EGFR) of a well to which a test compound was not

together with a Super PiggyBac Transposase expression vector, by electroporation using an Amaxa (trademark) Cell Line Nucleofector (trademark) Kit V, followed by selection using puromycin (SIGMA). Ba/F3 cells expressing wild-type EGFR (which may be hereinafter referred to as "Ba/F3—EGFR_WT") exhibited mIL-3-independent growth in the presence of 50 ng/mL EGF (R&D Systems); and Ba/F3 cells expressing exon 18 or exon 21 active mutant EGFR (which may be hereinafter referred to as "Ba/F3-EGFR G719A," and "Ba/F3-EGFR L861Q") exhibited mIL-3-independent growth in the absence of EGF.

[0109]

To evaluate a cell growth inhibitory effect, Ba/F3—EGFR_WT cells were suspended in an RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 ng/mL EGF; and the cell suspension was seeded in each well of a 96-well flat-bottom microplate such that the cell count per well was 30,000. Ba/F3-EGFR G719A cells and Ba/F3-EGFR L861Q cells were suspended in respective RPMI-1640 mediums containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin; and the cell suspensions were individually seeded in each well of a 96-well flat-bottom microplate such that the cell count per well was 15,000. Subsequently, compound A, gefitinib, erlotinib, afatinib, and osimertinib (gefitinib, erlotinib, afatinib, and osimertinib may be each hereinafter referred to as a "comparative compound") were individually dissolved in DMSO, and diluted with DMSO or the medium used for suspending the cells. The solutions were then added to each well of the culture plate of the cells, and the cells were incubated in a 5% CO_2 gas-containing incubator at 37°C for 3 days. The cell count after incubation was measured by a CellTiter-Glo (trademark) Luminescent Cell Viability Assay (Promega Corporation) in accordance with the manufacturer's recommended protocol. The growth rate was calculated using the following formula to determine the concentration of each test compound for 50% inhibition (IC_{50} (μM)).

[0110]

Growth Rate (%) = T/C × 100

introduced. Ba/F3 cells were maintained in an RPMI-1640 medium (Thermo Fisher Scientific Inc.) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin/100 μg/mL streptomycin (Thermo Fisher Scientific Inc.), and 1 ng/mL mouse interleukin-3 (mIL-3) (CST); and a PB-CMV-MCS-EF1-GFP+Puro vector or PB-CMV-MCS-EF1-RFP+Puro vector into which a human EGFR gene (wild-type (WT), G719A+T790M, or L861Q+T790M) was encoded was introduced into the cells, together with a Super PiggyBac Transposase expression vector, by electroporation using an Amaxa (trademark) Cell Line Nucleofector (trademark) Kit V, followed by selection using puromycin (SIGMA). Ba/F3 cells expressing G719A+T790M mutant EGFR or L861Q+T790M mutant EGFR (which may be hereinafter referred to as "Ba/F3-EGFR G719A+T790M," and "Ba/F3-EGFR L861Q+T790M") exhibited mIL-3-independent growth in the absence of EGF.

[0124]

To evaluate a cell growth inhibitory effect, Ba/F3-EGFR G719A+T790M cells and Ba/F3-EGFR L861Q+T790M cells were individually suspended in an RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin; and the cell suspensions were individually seeded in each well of a 96-well flat-bottom microplate such that the cell count per well was 15,000. Subsequently, compound A, gefitinib, erlotinib, afatinib, and osimertinib (gefitinib, erlotinib, afatinib, and osimertinib may be each hereinafter referred to as a "comparative compound") were individually dissolved in DMSO, and diluted with DMSO or the medium used for suspending the cells. These solutions were then added to each well of the culture plate of the cells, and incubated in a 5% CO_2 gas-containing incubator at 37°C for 3 days. The cell count after incubation was measured using a CellTiter-Glo (trademark) Luminescent Cell Viability Assay (Promega Corporation), in accordance with the manufacturer's recommended protocol. The growth rate was calculated using the following formula to determine the concentration of each test compound for 50% inhibition (IC_{50} (μM)).

[0125]

Growth Rate (%) = T/C × 100

T: the luminescence intensity of a well to which a test compound was added.

C: the luminescence intensity of a well to which the test compound was not added.

[0126]

Additionally, the IC_{50} ratio between wild-type EGFR and G719A+T790M mutant EGFR, or between wild-type EGFR and L861Q+T790M mutant EGFR was calculated using the following formula. Table 3 illustrates the results.

[0127]

IC_{50} Ratio = IC_{50} (WT)/IC_{50} (G719A+T790M or L861Q+T790M)

[0128]

As is clear from Table 3, compound A exhibited selective inhibitory activity against G719A+T790M mutation and L861Q+T790M mutation.

[0129]

Table 3

	IC_{50} (nM)		IC_{50} Ratio	
	G719A+T790M	L861Q+T790M	WT/ G719A+T790M	WT/ L861Q+T790M
Compound A of the Present Invention	15.8	37.5	37.9	15.9
Osimertinib	89.4	36.4	4.2	10.4
Afatinib	68.2	168.7	0.3	0.1
Erlotinib	8030.0	6424.2	0.1	0.1

[0130]

Test Example 5: In Vivo Drug Efficacy Test

Evaluation of Antitumor Effect on Mouse Model Subcutaneously Transplanted with G719A+T790M Mutant EGFR-Expressing Cell Line

The evaluation of mouse models subcutaneously transplanted with G719A+T790M mutant EGFR-expressing cell line was performed using NIH-3T3 cells, which are mouse fibroblast cell line into which a human EGFR gene was introduced. NIH-3T3 cells were maintained in a D-MEM (high-glucose) medium (Wako Pure Chemical Industries, Ltd.) containing 10% newborn calf serum

CLAIMS

[Claim 1]

Use of an antitumor agent in the manufacture of a medicament for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue, the antitumor agent comprising (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof.

[Claim 2]

The use according to claim 1, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[Claim 3]

The use according to claim 1 or 2, wherein the exon 21 mutation is L861Q.

[Claim 4]

The use according to any one of claims 1 to 3, wherein the EGFR further has T790M mutation.

[Claim 5]

(S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof for use in the treatment of a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 6]

Use of (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-

dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in the manufacture of a medicament for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 7]

The use according to claim 6, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[Claim 8]

The use according to claim 6 or 7, wherein the exon 21 mutation is L861Q.

[Claim 9]

The use according to any one of claims 6 to 8, wherein the EGFR further has T790M mutation.

[Claim 10]

Use of (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof for the production of a pharmaceutical agent for treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 11]

The use according to claim 10, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[Claim 12]

The use according to claim 10 or 11, wherein the exon 21 mutation is L861Q.

[Claim 13]

The use according to any one of claims 10 to 12, wherein the EGFR further has T790M mutation.

[Claim 14]

A pharmaceutical composition comprising (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof and a pharmaceutically acceptable carrier, for use in treating a malignant tumor patient expressing EGFR having at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 15]

An *ex vivo* method for predicting therapeutic effects of chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in a malignant tumor patient, the method comprising steps (1) and (2) below:

(1) a step of detecting the presence or absence of mutation of EGFR gene contained in a biological sample obtained from the patient; and

(2) a step of predicting that the chemotherapy is highly likely to exhibit sufficient therapeutic effects with respect to the patient when the results of the detection in step (1) found that the EGFR gene has at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 16]

The method according to claim 15, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[Claim 17]

The method according to claim 15 or 16, wherein the exon 21 mutation is L861Q.

[Claim 18]

The method according to any one of claims 15 to 17, wherein the EGFR further has T790M mutation.

[Claim 19]

Use of(S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof in the manufacture of a medicament for treating malignant tumor, wherein the medicament is to be administered to a malignant tumor patient who was predicted highly likely to sufficiently respond to the chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof, after a biological sample is to be obtained from the patient in a first step to detect the presence or absence of mutation of EGFR;

and wherein in a second step, chemotherapy using an antitumor agent comprising, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof is predicted to highly likely to exhibit sufficient therapeutic effects with respect to the patient when the results of the detection in the first step found that the EGFR gene has at least one mutation selected from the group consisting of G719X mutation of exon 18, E709X mutation of exon 18, and L861X mutation of exon 21, wherein X represents an arbitrary amino-acid residue.

[Claim 20]

The use according to claim 19, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[Claim 21]

The use according to claim 19 or 20, wherein the exon 21 mutation is L861Q.

[Claim 22]

The use according to any one of claims 19 to 21, wherein the EGFR further has T790M mutation.