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(54) Benævnelse: **EXON-18- OG/ELLER EXON-21-MUTANT EGFR-SELEKTIV HÆMMER**

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DESCRIPTION

Description

Technical Field

[0001] The present invention relates to an antitumor agent against cancers, comprising an exon 18 and/or exon 21 mutant epidermal growth factor receptor (hereinafter also referred to as "EGFR").

Background Art

[0002] EGFR is a receptor-type tyrosine kinase, exerts its physiological function in normal tissue by being bound to Epidermal Growth Factor (hereinafter also referred to as EGF), which is a ligand, and contributes to growth and apoptosis inhibition in epithelial tissues (NPL 1). Further, somatic mutation of EGFR gene has been known as a cancer-causing gene; for example, EGFR in which codons 746 to 750 in exon 19 are deleted (hereinafter also referred to as "exon 19 deletion mutation") and EGFR in which leucine encoded by codon 858 in exon 21 is mutated to arginine (hereinafter also referred to as "L858R mutation") constantly induces EGF-independent kinase activity, and contributes to the growth and survival of cancer cells (NPL 2). These mutations are observed, for example, in 30 to 50% of non-small-cell lung cancer in East Asia. The mutations are also observed in about 10% of non-small-cell lung cancer in Europe and the United States, and are regarded as one of the causes of cancers (NPL 3).

[0003] Therefore, research and development of EGFR inhibitor as an antitumor agent have actively been conducted, and introduced into the treatment of various EGFR mutation-positive lung cancers (NPL 2 and NPL 4). Gefitinib, erlotinib, and afatinib have been used as a therapeutic agent against exon 19 deletion mutant and L858R mutant EGFR-positive lung cancers. Exon 19 deletion mutation and L858R mutation account for 90% of EGFR mutation. Further, occurrence of acquired resistance in the process of the treatment using these agents has been known, and 50% thereof is caused by resistance mutation EGFR in which codon 790 of exon 20 is changed from threonine to methionine (hereinafter also referred to as "T790M mutation"). To treat lung cancers having this mutation, osimertinib has been used as a therapeutic agent. Therefore, treatments using EGFR inhibitors are in the process of being established for lung cancer patients having major EGFR mutations.

[0004] On the other hand, at present, no treatments using EGFF inhibitors have been

established with respect to some rare EGFR mutations, such as point mutation or deletion mutation of exon 18, point mutation of exon 21, or the like; and there are reports that the drug sensitivity of these mutant EGFR varies depending on the mutation type (NPL 4). For example, the sensitivity of the lung cancer having point mutation in which glycine encoded by codon 719 of exon 18 is substituted with an arbitrary amino acid (hereinafter also referred to as "G719X mutation") or the lung cancer in which leucine encoded by codon 861 of exon 21 is substituted with glutamine (hereinafter also referred to as "L861Q mutation") with respect to gefitinib, erlotinib, and afatinib is lower than those of exon 19 deletion mutation and L858R mutation, which are drug-sensitive mutations. Further, there are reports of skin disorders and digestive tract disorders as common side effects by the administration of therapeutic doses of afatinib, gefitinib, and erlotinib. It is widely thought that these side effects are attributable to inhibition of the function of wild-type EGFR expressed in normal tissues, such as skin or digestive tract, by the therapeutic agent (NPL 1); the development of an inhibitor characterized by lower inhibitory activity with respect to wild-type EGFR of normal tissues, compared with mutant EGFR expressed in tumor tissues, has been 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.

[0006] WO 2015/025936 A1 describes a compound which has an EGFR-inhibiting activity and also a cell proliferation-inhibiting effect, and a medicine which has an EGFR-inhibiting activity. Cancer Science, Vol. 107, pp. 1179-1186 (2016) describes epidermal growth factor receptor mutations in lung cancer and perspectives for individualized treatment strategy. Lancet Oncol. Vol. 16, PP. 830-838 (2012) describes the clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon EGFR mutations. Internet<URL:https://www.haigan.gr.jp/uploads/files/photos/1329.p df>(2016) (accessed 18 October 2018) describes guidelines of examination about EGFR gene mutation in lung cancer patients.

Citation List

Patent Literature

[0007]

PTL 1: WO2015/175632A1

PTL 2: WO2015/025936A1

Non-Patent Literature

[0008]

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)

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NPL 6: Lancet Oncol. Vol. 16, PP. 830-838 (2012)

NPL 7: Internet<URL:https://www.haigan.gr.jp/uploads/files/photos/1329.p df>(2016) (accessed 18 October 2018)

Summary of Invention

Technical Problem

[0009] 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

[0010] 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.

[0011] In a first aspect, the present invention provides 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 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.

[0012] In a first embodiment of the first aspect, the present invention provides 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 as claimed, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[0013] In a second embodiment of the first aspect, the present invention provides 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 as claimed, wherein the exon 21 mutation is L861Q.

[0014] In a third embodiment of the first aspect, the present invention provides 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 as claimed, wherein the EGFR further has T790M mutation.

[0015] In a fourth embodiment of the first aspect, the present invention provides 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 as claimed, wherein 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 in the form of a pharmaceutical composition.

[0016] In a second aspect, the present invention provides a 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. (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. (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.

[0017] In a first embodiment of the second aspect, the present invention provides the method as claimed, wherein the exon 18 mutation is at least one mutation selected from the group consisting of G719A, G719S, G719C, E709K and E709A.

[0018] In a second embodiment of the second aspect, the present invention provides the method as claimed, wherein the exon 21 mutation is L861Q.

[0019] In a third embodiment of the second aspect, the present invention provides the method as claimed, wherein the EGFR further has T790M mutation.

[0020] In a fourth embodiment of the second aspect, the present invention provides 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 as claimed, wherein the patient has undergone the prediction as claimed.

Advantageous Effects of Invention

[0021] The antitumor agent of the present invention exerts high selectivity with respect to exon 18 and/or exon 21 mutant EGFR. Therefore, the antitumor agent for use in the present invention is useful in view of providing an antitumor agent that exerts superior therapeutic effects for a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation, for which the therapeutic effects of the previously known EGFR inhibitors are insufficient.

[0022] The present invention is also useful in terms of providing a method for treating a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0023] 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 for use in 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 for use in the present invention exerts superior therapeutic effects for a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0024] In addition, the antitumor agent for use in 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 for use in 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.

[0025] Further, the antitumor agent for use in 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

[0026]

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

[0027] The invention is as set out in the claims.

[0028] Preferable examples of various definitions in the scope of the present invention used in this specification are explained below in detail.

[0029] In this specification, "EGFR" refers to a human epidermal growth factor receptor protein, and is also referred to as ErbB-1 or HER1.

[0030] 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).

[0031] In this specification, "exon 18" refers to 688-728 region in the amino acid sequence of wild-type EGFR (SEQ ID NO: 1).

[0032] In this specification, "exon 18 mutation" refers to point mutation in amino acid in the exon 18 region of wild-type EGFR (SEQ ID NO: 1). Preferable exon 18 mutation is point mutation or deletion mutation with 1 amino acid substitution in the exon 18 region. More preferably, the exon 18 mutation is E709X, which is point mutation in which glutamic acid encoded by codon 709 of exon 18 is substituted with an arbitrary amino acid; or G719X, which is point mutation in which glycine encoded by codon 719 of exon 18 is substituted with an arbitrary amino acid. More specifically, preferable examples of E709X include E709K, which is point mutation in which glutamic acid encoded by codon 709 in the exon 18 region is substituted with lysine; and E709A, which is point mutation in which glutamic acid encoded by codon 709 in the exon 18 region is substituted with alanine. Preferable examples of G719X include G719A, which is point mutation in which glycine encoded by codon 719 in the exon 18 region is substituted with alanine; G719S, which is point mutation in which glycine encoded by codon 719 in the exon 18 region is substituted with serine; and G719C, which is point mutation in which glycine encoded by codon 719 in the exon 18 region is substituted with cysteine. Among these, G719A is particularly preferable.

[0033] In the present invention, "exon 21" refers to 824-875 region in the amino acid sequence of wild-type EGFR (SEQ ID NO: 1).

[0034] In this specification, "exon 21 mutation" refers to point mutation in amino acid in the exon 21 region of wild-type EGFR (SEQ ID NO: 1). Preferable exon 21 mutation is point mutation with 1 amino acid substitution in the exon 21 region. 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.

[0035] 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."

[0036] 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.

[0037] "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".

[0038] 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).

[0039] 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).

[0040] Further, EGFR having exon 18 and/or exon 21 mutation may further have T790M mutation. T790M is acquired resistance mutation in the exon 20 region. T790M is known to be generated by the use of existing EGFR inhibitors. The acquisition of T790M often decreases the effects of existing drug with respect to malignant tumor patients.

[0041] In the present invention, specifically, the EGFR having exon 18 and/or exon 21 mutation further having T790M mutation is preferably one of EGFR having E709X and/or G719X mutation in the exon 18 region further having T790M mutation, and EGFR having L861X mutation in the exon 21 region further having T790M mutation. In the present invention, more specifically, the EGFR having exon 18 and/or exon 21 mutation further having T790M mutation is preferably one of EGFR having E709K or E709A mutation further having T790M mutation, EGFR having G719A, G719S or G719C mutation further having T790M mutation, and EGFR having L861Q mutation further having T790M mutation. Among these, EGFR having G719A mutation further having T790M mutation and EGFR having L861Q mutation further having T790M mutation are particularly preferable.

[0042] In the present invention, the method for detecting exon 18 and/or exon 21 mutation of EGFR expressed by a malignant tumor patient is not particularly limited insofar as the method is capable of detecting the mutations, and any known detection methods may be used.

[0043] The sample used in the detection of exon 18 and/or exon 21 mutation is not particularly limited as long as the sample is a biological sample isolated from a malignant tumor patient, in particular, a sample obtained from a malignant tumor patient, and contains malignant tumor cells. Examples of biological samples include body fluids (e.g., blood, urine, etc.), tissues, the extracts thereof, and the cultures of obtained tissues. The method for obtaining a biological sample can be suitably selected depending on the type of biological sample.

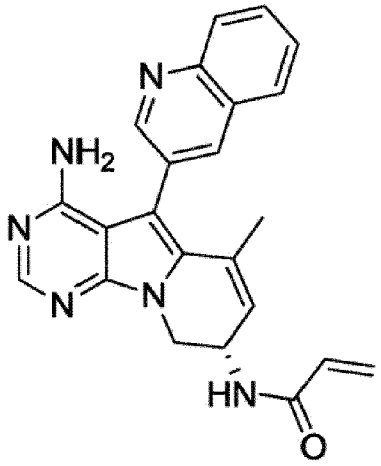
[0044] The biological sample is prepared by being appropriately treated according to the measurement method. Further, the reagent comprising primer or probe used for the detection may be prepared by a conventional method according to the measurement method therefor.

[0045] In one embodiment of the present invention, a step of detecting the presence of exon 18 and/or exon 21 mutation of EGFR expressed by a malignant tumor patient may be

performed before the administration of an antitumor agent to a malignant tumor patient.

[0046] A malignant tumor may include two or more different kinds of malignant tumor cells. Further, two or more malignant tumors may be generated in a single patient. Therefore, a single patient may have different mutations in the same amino acid position of EGFR (for example, the exon 18 mutation is G719A, G719S and G719C exon 18 mutation; E709K and E709A exon 18 mutation) at the same time.

[0047] The antitumor agent for use in the present invention as claimed comprises, as an active ingredient, (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide (Compound (A)) or a salt thereof. Compound (A) is represented by the following chemical formula.



[0048] The method for producing the compound for use in the present invention is explained below.

[0049] Compound A for use in the present invention may be produced, for example, through the production method disclosed in WO2015/025936A1, the methods described in the Examples, and the like. However, the production method of the compound for use in the present invention is not limited to these reaction examples.

[0050] When Compound A for use in the present invention has isomers such as optical isomers, stereoisomers, and tautomers, any of the isomers and mixtures thereof are included within the scope of the compound for use in the present invention, unless otherwise specified. For example, when Compound A for use in the present invention has optical isomers, racemic mixtures and the optical isomers separated from a racemic mixture are also included within the scope of the compound for use in the present invention, unless otherwise specified.

[0051] The salts of Compound A refer to any pharmaceutically acceptable salts; examples include base addition salts and acid addition salts.

[0052] Examples of base addition salts include alkali metal salts such as sodium salts and

potassium salts; alkaline earth metal salts such as calcium salts and magnesium salts; ammonium salts; and organic amine salts such as trimethylamine salts, triethylamine salts, dicyclohexylamine salts, ethanolamine salts, diethanolamine salts, triethanolamine salts, procaine salts, and N,N'-dibenzylethylenediamine salts.

[0053] Examples of acid addition salts include inorganic acid salts such as hydrochlorides, sulfates, nitrates, phosphates, and perchlorates; organic acid salts such as acetates, formates, maleates, fumarates, tartrates, citrates, ascorbates, and trifluoroacetates; and sulfonates such as methanesulfonates, isethionates, benzenesulfonates, and p-toluenesulfonates.

[0054] The compound for use in the present invention and salts thereof also encompass prodrugs thereof. A prodrug refers to a compound that can be converted to the compound for use in the present invention or a salt thereof through a reaction with an enzyme, gastric acid, or the like, under physiological conditions *in vivo,* i.e., a compound that can be converted to the compound for use in the present invention or a salt thereof by enzymatic oxidation, reduction, hydrolysis, or the like; or a compound that can be converted to the compound for use in the present invention or a salt thereof by hydrolysis or the like with gastric acid or the like. Further, the prodrug may be compounds that can be converted to the compound for use in the present invention or a salt thereof under physiological conditions, such as those described in "Iyakuhin no Kaihatsu [Development of Pharmaceuticals]," Vol. 7, Molecular Design, published in 1990 by Hirokawa Shoten Co., pp. 163-198.

Description of Diseases

[0055] Specific examples of tumors targeted in the present invention include, but are not particularly limited to, head and neck cancer, gastrointestinal cancer (esophageal cancer, stomach cancer, duodenal cancer, liver cancer, biliary cancer (e.g., gallbladder and bile duct cancer), pancreatic cancer, colorectal cancer (e.g., colon cancer, and rectal cancer), etc.), lung cancer (e.g., non-small-cell lung cancer, small-cell lung cancer, and mesothelioma), breast cancer, genital cancer (ovarian cancer, uterine cancer (e.g., cervical cancer, and endometrial cancer), etc.), urological cancer (e.g., kidney cancer, bladder cancer, prostate cancer, and testicular tumor), hematopoietic tumor (e.g., leukemia, malignant lymphoma, and multiple myeloma), osteosarcoma, soft-tissue sarcoma, skin cancer, brain tumor, and the like. Preferable examples include lung cancer, breast cancer, head and neck cancer, brain tumor, uterine cancer, digestive organ cancer, hematopoietic tumor, or skin cancer. Lung cancer is particularly preferable.

[0056] When Compound A or a salt thereof are used as a pharmaceutical agent, a pharmaceutical carrier can be added, if required, thereby forming a suitable dosage form according to prevention and treatment purposes. Examples of the dosage form include oral preparations, injections, suppositories, ointments, patches, and the like. Oral preparations are preferable. Such dosage forms can be formed by methods conventionally known to persons skilled in the art.

[0057] In one embodiment, the antitumor agent for use in the present invention as claimed is provided as a pharmaceutical composition comprising Compound A or a salt thereof, and a pharmaceutically acceptable carrier.

[0058] As the pharmaceutically acceptable carrier, various conventional organic or inorganic carrier materials used as preparation materials may be blended as an excipient, binder, disintegrant, lubricant, or colorant in solid preparations; or as a solvent, solubilizing agent, suspending agent, isotonizing agent, buffer, or soothing agent in liquid preparations. Moreover, pharmaceutical preparation additives, such as antiseptics, antioxidants, colorants, sweeteners, and stabilizers, may also be used, if required.

[0059] Oral solid preparations are prepared as follows. After an excipient is added optionally with an excipient, binder, disintegrant, lubricant, colorant, taste-masking or flavoring agent, etc., to the compound for use in the present invention, the resulting mixture is formulated into tablets, coated tablets, granules, powders, capsules, or the like by ordinary methods.

[0060] Examples of excipients include lactose, sucrose, D-mannitol, glucose, starch, calcium carbonate, kaolin, microcrystalline cellulose, and silicic acid anhydride. Examples of binders include water, ethanol, 1-propanol, 2-propanol, simple syrup, liquid glucose, liquid α-starch, liquid gelatin, D-mannitol, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl starch, methyl cellulose, ethyl cellulose, shellac, calcium phosphate, polyvinylpyrrolidone, and the like. Examples of disintegrators include dry starch, sodium alginate, powdered agar, sodium hydrogen carbonate, calcium carbonate, sodium lauryl sulfate, stearic acid monoglyceride, lactose, and the like. Examples of lubricants include purified talc, sodium stearate, magnesium stearate, borax, polyethylene glycol, and the like. Examples of colorants include titanium oxide, iron oxide, and the like. Examples of taste-masking or flavoring agents include sucrose, bitter orange peel, citric acid, tartaric acid, and the like.

[0061] When a liquid preparation for oral administration is prepared, a taste-masking agent, a buffer, a stabilizer, a flavoring agent, and the like may be added to the compound for use in the present invention; and the resulting mixture may be formulated into an oral liquid preparation, syrup, elixir, etc., according to an ordinary method.

[0062] When an injection agent is prepared, a pH regulator, a buffer, a stabilizer, an isotonizing agent, a local anesthetic, and the like, may be added to the compound for use in the present invention; and the mixture may be formulated into a subcutaneous, intramuscular, or intravenous injection according to an ordinary method.

[0063] Examples of the pH adjuster and the buffer used herein include sodium citrate, sodium acetate, and sodium phosphate. Examples of the stabilizer include sodium pyrosulfite, EDTA, thioglycolic acid, and thiolactic acid. Examples of the local anesthetic include procaine hydrochloride and lidocaine hydrochloride. Examples of the tonicity agent include sodium chloride, glucose, D-mannitol, and glycerol.

[0064] When a suppository is prepared, pharmaceutically acceptable carriers known in the related field, such as polyethylene glycol, lanolin, cacao butter, and fatty acid triglyceride; and as necessary, surfactants such as Tween 80 (registered trademark), may be added to Compound A, and the resulting mixture may be formulated into a suppository according to an ordinary method.

[0065] When an ointment is prepared, a commonly used base, stabilizer, wetting agent, preservative, and the like, may be blended into Compound A, as necessary; and the obtained mixture is mixed and formulated into an ointment according to an ordinary method.

[0066] Examples of the base include liquid paraffin, white petrolatum, white beeswax, octyl dodecyl alcohol, and paraffin.

[0067] Examples of the preservative include methyl paraoxybenzoate, ethyl paraoxybenzoate, and propyl paraoxybenzoate.

[0068] When a patch is prepared, the above-described ointment, cream, gel, paste, or the like, may be applied to an ordinary substrate according to an ordinary method.

[0069] Examples of substrates include woven fabrics or nonwoven fabrics comprising cotton, staple fibers, or chemical fibers; and films or foam sheets of soft vinyl chloride, polyethylene, polyurethane, etc., may also be used.

[0070] The amount of Compound A to be incorporated in each of such dosage unit forms depends on the condition of the patient to whom the compound is administered, the dosage form thereof, etc. In general, in the case of an oral agent, the amount of the compound is preferably 0.05 to 1000 mg per dosage unit form. In the case of an injection, the amount of the compound is preferably 0.01 to 500 mg per dosage unit form; and in the case of a suppository, the amount of the compound is preferably 1 to 1000 mg per dosage unit form.

[0071] Further, the daily dose of the medicine in such a dosage form depends on the condition, body weight, age, sex, etc., of the patient, and cannot be generalized or limited. Usually, the daily dose for an adult (body weight: 50 kg) of Compound A may generally be 0.05 to 5000 mg, and preferably 0.1 to 1000 mg; and is preferably administered in one dose, or in two to three divided doses, per day.

[0072] Also described is a method for treating a malignant tumor patient, comprising the step of administering Compound A or a salt thereof to a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0073] Also described is Compound A or a salt thereof for treating a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0074] Also described is use of Compound A or a salt thereof for treating a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0075] Also described is use of Compound A or a salt thereof for the production of a pharmaceutical agent for treating a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation.

[0076] Also described is a pharmaceutical composition for treating a malignant tumor patient expressing EGFR having exon 18 and/or exon 21 mutation, the pharmaceutical composition comprising Compound A or a salt thereof, and a pharmaceutically acceptable carrier.

[0077] Also described is a method for predicting therapeutic effects of chemotherapy using an antitumor agent comprising, as an active ingredient, Compound A or a salt thereof in a malignant tumor patient, the method comprising steps (1) and (2) below:

1. (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. (2) a step of predicting that the chemotherapy is highly likely to exhibit sufficient therapeutic effects to the patient when the results of the detection in step (1) found that the EGFR gene has exon 18 and/or exon 21 mutation.

[0078] Also described is a method for treating a malignant tumor patient, comprising steps (1) to (3) below:

1. (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. (2) a step of predicting that 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 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 exon 18 and/or exon 21 mutation; and
3. (3) a step of administering (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamide or a salt thereof 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, in step (2).

[0079] The base sequence of EGFR gene is publicly known. The GenBank accession number of the base sequence of cDNA is NM_005228.4.

[0080] The "therapeutic effects" can be evaluated by tumor shrinkage effects, relapse-suppressing effects, life-prolonging effects, and the like. The relapse-suppressing effects may be shown as degree of the extension of non-relapse period or the degree of the improvement in relapse rate; and the life-prolonging effects may be shown as the degree of the entire survival time or the degree of the extension of the median of progression-free survival, or the like. The "sufficient therapeutic effects" of the chemotherapy using an antitumor agent comprising, as an active ingredient, Compound A or a salt thereof means, for example, that superior therapeutic effects are obtained by the administration of the antitumor agent comprising, as an active ingredient, Compound A or a salt thereof, such as extension of survival time, suppression of relapse, and the like, compared with non-administration.

Examples

[0081] The following describes the present invention in more detail with reference to the following Test Examples. However, the present invention is not limited to these Examples (Test Examples).

Test Example 1: In Vitro Drug Efficacy Test

Evaluation Results of Intracellular Phosphorylation in Mutant EGFR Forced Expression System Using HEK293 Cells (Inhibitory Activity)

[0082] The intracellular target inhibitory activity of compounds was evaluated based on the following as an index: intracellular EGFR phosphorylation in a mutant EGFR forced expression system using Jump-In (trademark) Grip (trademark) HEK293 cells (Thermo Fisher Scientific Inc.) (which may be hereinafter referred to as "HEK293 cells").

[0083] HEK293 cells were maintained in D-MEM with GlutaMAX (trademark)-I (high-glucose) (Thermo Fisher Scientific Inc.) that contained 10% dialyzed FBS and 100 U/mL penicillin/100 ug/mL streptomycin (Thermo Fisher Scientific Inc.); and a pJTI™ R4 DEST CMV pA vector to which a human EGFR gene (G719A, G719S, G719C, E709K, E709A, L861Q, G719A+T790M, or L861Q+T790M; the symbol "+" indicates that both mutations are contained) was encoded was introduced into HEK293 cells, together with Opti-MEM (trademark) I (Thermo Fisher Scientific Inc.), using a ViaFect (trademark) Transfection Reagent (Promega Corporation).

[0084] HEK293 cells expressing mutant human EGFR were seeded in each well of a 384-well flat-bottom microplate such that the cell count per well was 10,000, and incubated in a 5% CO_2 gas-containing incubator at 37°C for 1 day. Compound A, erlotinib, afatinib, and osimertinib (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 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.

[0085] 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.

$$\text{Phosphorylated EGFR Inhibitory Rate (\%)} = T/C \times 100$$

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 added.

[0086] As is clear from Table 1, compound A exhibited high inhibitory activity against intracellular phosphorylation of exon 18 or exon 21 mutant EGFR; and the activity was higher than that of erlotinib or osimertinib, and was equivalent to that of afatinib. The inhibitory activity of compound A against exon 18 or exon 21 mutant EGFR was higher than that of afatinib in the presence of T790M mutation, which is an acquired resistant mutation.

Table 1

	Compound A	Osimertinib	Afatinib	Erlotinib
G719A	20.5	237.0	20.0	>1000
G719S	22.7	251.9	16.9	539.7
G719C	9.3	102.0	15.3	98.3
E709K	86.0	424.2	14.0	451.4
E709A	103.5	548.9	19.2	478.1
L861Q	40.5	143.1	24.7	608.1

	Compound A	Osimertinib	Afatinib	Erlotinib
G719A+ T790M	31.3	132.1	110.2	>1000
L861Q+ T790M	81.8	132.4	177.6	>1000

Test Example 2

Evaluation of Cell Growth Inhibitory Effect Against Wild-Type EGFR- and Mutant EGFR-Expressing Cell Line (In Vitro)

[0087] The inhibitory activity of compounds against wild-type EGFR and mutant EGFR was evaluated using Ba/F3 cells, which are mouse B-lymphocyte precursor cell line to which a human EGFR gene was 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 ug/mL streptomycin (Thermo Fisher Scientific Inc.), and 1 ng/mL mouse interleukin-3 (mIL-3) (CST). 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, or L861Q) 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 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.

[0088] 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 ug/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 ug/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 inhibition rate was calculated using the following formula to determine the

concentration of each test compound for 50% inhibition (IC_{50} (μM)).

Growth Inhibitory 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 a test compound was not added.

[0089] The IC_{50} ratio between wild-type EGFR and G719A mutant EGFR, or between wild-type EGFR and L861Q mutant EGFR was determined using the following formula. Table 2 illustrates the results.

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

[0090] As is clear from Table 2, compound A exhibited selective inhibitory activity against G719A mutation and L861Q mutation.

Table 2

	IC_{50} (nM)			IC_{50} Ratio	
	WT	G719A	L861Q	WT/G719A	WT/L861Q
Compound A	597.3	9.0	19.0	66.4	31.4
Osimertinib	378.7	209.0	47.0	1.8	8.1
Afatinib	17.7	3.3	2.3	5.3	7.6
Erlotinib	778.3	336.3	353.0	2.3	2.2

Test Example 3: In Vivo Drug Efficacy Test

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

[0091] The evaluation on mouse models subcutaneously transplanted with G719A mutant EGFR-expressing cell line was performed using NIH-3T3 cells, which are mouse fibroblast cell line to 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 (NBCS), 1,500 mg/L sodium hydrogen carbonate, and 100 U/mL penicillin/100 ug/mL streptomycin (Thermo Fisher Scientific Inc.); and a PB-CMV-MCS-EF1-RFP+Puro vector into which a human EGFR gene (G719A) was encoded was introduced to the cells, together with a Super PiggyBac Transposase expression vector, by electroporation using an Amaxa (trademark) Cell Line Nucleofector (trademark) Kit R, followed by selection using puromycin (SIGMA). NIH-3T3 cells expressing exon 18 mutant EGFR (which may be hereinafter referred

to as "NIH3T3-EGFR G719A") exhibited growth in the absence of EGF under 1% NBCS conditions.

[0092] In evaluation using mouse models subcutaneously transplanted with G719A mutant EGFR-expressing cell line, nude mice were subcutaneously transplanted with NIH3T3-EGFR G719A cells into which a mutant human EGFR was introduced. At the point at which the tumor volume of the tumor engrafted in the mice grew to about 100 to 200 mm^3, the mice were allocated into groups, 5 or 6 mice for each group, by stratified randomization such that the average tumor volume between the groups was uniform. The mice were then orally administered compound A, afatinib, or osimertinib once daily for 14 consecutive days.

[0093] The dose of afatinib was 20 mg/kg/day, which is the maximum tolerated dose (the maximum dose at which the weight loss during a dosing period is less than 20%), for 14 days, the dosing period of this test. The dose of osimertinib was 25 mg/kg/day, which is a clinically efficacious dose. For compound A, three types of doses were set: 200 mg/kg/day (maximum tolerated dose), 100 mg/kg/day, and 50 mg/kg/day. The maximum tolerated dose was determined in accordance with the "Guidelines Involving Experimental Neoplasia Proposals in Mice and Rats" of the National Cancer Institute (NCI), from a humanitarian perspective.

[0094] To compare the changes in growth of tumor over time due to administration of the individual test compounds, the tumor volume (which may be hereinafter referred to as "TV") was used as an index. For a toxicity index, the body weight was measured over time, and the body weight change (which may be hereinafter referred to as "BWC (%)") from the day on which the mice were divided into groups was calculated in accordance with the following formula.

BWC (%) = (the body weight measured on body weight measurement day)/(the body weight on the day mice were divided into groups)

[0095] When the difference in the average TV between the control group and the group administered with a test compound on the final evaluation day was statistically significant (Dunnett's test, $p<0.05$), and the value of treatment/control (T/C) calculated using the following formula was less than 100, the test compound was determined to be effective. Such a case is indicated by the symbol "*" in the figures.

T/C (%) = (the average TV of the group administered with a test compound)/(the average TV of the control group) × 100

[0096] As is clear from the results illustrated in Fig. 1, compound A for use in the present invention exhibited a remarkable antitumor effect on G719A mutant EGFR-expressing cell line subcutaneously transplanted into nude mice, accompanied by tumor regression. The effect was also higher than that of afatinib or osimertinib. The mice did not show symptoms, such as serious weight loss, as illustrated in Fig. 2, abnormal feces, or abnormal skin.

Test Example 4: In Vitro Drug Efficacy Test

Evaluation of Cell Growth Inhibitory Effect on T790M Mutant EGFR-Expressing Cell Line (In Vitro)

[0097] The inhibitory activity of a compound on T790M mutant EGFR was evaluated using Ba/F3 cells, which are mouse B-lymphocyte precursor cell line into which a human EGFR gene was 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 ug/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.

[0098] 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 ug/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 inhibition rate was calculated using the following formula to determine the concentration of each test compound for 50% inhibition (IC_{50} (μM)).

$$\text{Growth Inhibition Rate (\%)} = T/C \times 100$$

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.

[0099] 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.

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

[0100] As is clear from Table 3, compound A exhibited selective inhibitory activity against G719A+T790M mutation and L861Q+T790M mutation.

Table 3

	IC_{50} (nM)		IC_{50} Ratio	
	G719A+T790M	L861Q+T790M	WT/ G719A+T790M	WT/ L861Q+T790M
Compound A	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

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

[0101] 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 (NBCS), 1,500 mg/L sodium hydrogen carbonate, and 100 U/mL penicillin/100 ug/mL streptomycin (Thermo Fisher Scientific Inc.); and a PB-CMV-MCS-EF1-RFP+Puro vector into which a human EGFR gene (G719A+T790M) was encoded was introduced to the cells, together with a Super PiggyBac Transposase expression vector, by electroporation using an Amaxa (trademark) Cell Line Nucleofector (trademark) Kit R, followed by selection using puromycin (SIGMA). NIH-3T3 cells expressing exon 18 mutant EGFR (which may be hereinafter referred to as "NIH3T3-EGFR G719A+T790M") exhibited growth in the absence of EGF under 1% NBCS conditions.

[0102] In evaluation using mouse models subcutaneously transplanted with G719A+T790M mutant EGFR-expressing cell line, nude mice were subcutaneously transplanted with NIH3T3-EGFR G719A+T790M cells into which mutant human EGFR was introduced. At the point at which the tumor volume of the tumor engrafted in the mice grew to about 100 to 300 mm^3, the

mice were allocated into groups, 5 mice for each group, by stratified randomization such that the average tumor volume between the groups was uniform. The mice were then orally administered compound A for use in the present invention or afatinib once daily on consecutive days.

[0103] The dose of afatinib was 20 mg/kg/day, which is the maximum tolerated dose (the maximum dose at which the weight loss during a dosing period is less than 20%), for 14 days, the dosing period of this test. For compound A for use in the present invention, three types of doses were set: 200 mg/kg/day (maximum tolerated dose), 100 mg/kg/day, and 50 mg/kg/day. The maximum tolerated dose was determined in accordance with the "Guidelines Involving Experimental Neoplasia Proposals in Mice and Rats" of the National Cancer Institute (NCI), from a humanitarian perspective.

[0104] To compare the changes in growth of tumor over time due to administration of the individual test compounds, the tumor volume (which may be hereinafter referred to as "TV") was used as an index. Fig. 3 illustrates changes in TV over time. For a toxicity index, the body weight was measured over time, and the body weight change (which may be hereinafter referred to as "BWC (%)") from the day on which the mice were divided into groups was calculated in accordance with the following formula. Fig. 4 illustrates changes in body weight over time.

BWC (%) = (the body weight measured on body weight measurement day)/(the body weight on the day mice were divided into groups)

[0105] A Dunnett's test was performed using the average TV on the final evaluation day as an index. When the difference in the average TV between the control group and the group administered with a test compound was statistically significant (p<0.05), and the value of treatment/control (T/C) calculated using the following formula was less than 100, the test compound was determined to be effective. Such a case is indicated by the symbol "*" in Fig. 3 and Table 4 (*: p<0.05, **: p<0.01, ***: p<0.001). Additionally, when the difference in the average TV between the group administered with compound A for use in the present invention and the group administered with afatinib was statistically significant (p<0.05), and the T/C value of the group administered with compound A for use in the present invention was smaller than the T/C value of the group administered with afatinib, compound A was determined to have a higher antitumor effect than afatinib. Such a case is indicated by the symbol "#" in Fig. 3 and Table 4 (#: p<0.05,##: p<0.01, ###: p<0.001).

T/C (%) = (the average TV of the group administered with a test compound)/(the average TV of the control group) × 100

[0106] As is clear from the results illustrated in Fig. 3, compound A for use in the present invention exhibited a significant antitumor effect on G719A+T790M mutant EGFR-expressing cell line subcutaneously transplanted into nude mice. Additionally, as shown in Table 4, the effect was higher than that of afatinib, without symptoms such as serious weight loss (as

illustrated in Fig. 4), abnormal feces, or abnormal skin in mice.

Table 4

Compound	Dose (mg/kg)	T/C (%)	*p* value vs	
			Control	Afatinib
Control	-	100.0	-	-
Compound	50	47.7	***	N.S.
Compound	100	36.4	***	##
Compound	200	28.0	***	###
Afatinib	20	57.4	***	-
N.S.: No Significant Difference ***: $p<0.001$ (Dunnett's test vs Control Group) ##: $p<0.01$ (Dunnett's test vs Afatinib Group) ###: $p<0.001$ (Dunnett's test vs Afatinib Group)				

REFERENCES CITED IN THE DESCRIPTION

Cited references

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EXON-18- OG/ELLER EXON-21-MUTANT EGFR-SELEKTIV HÆMMER

PATENTKRAV

1. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf til anvendelse i behandling af en patient med en malign tumor, der udtrykker EGFR med mindst én mutation valgt fra gruppen bestående af G719X-mutation af exon-18, E709X-mutation af exon-18, og L861X-mutation af exon-21, hvor X repræsenterer en vilkårlig aminosyrerest.

2. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf til anvendelse ifølge krav 1, hvor exon-18-mutationen er mindst én mutation valgt fra gruppen bestående af G719A, G719S, G719C, E709K og E709A.

3. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf til anvendelse ifølge krav 1 eller 2, hvor exon-21-mutationen er L861Q.

4. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf til anvendelse ifølge et hvilket som helst af krav 1 til 3, hvor EGFR'en endvidere har T790M-mutation.

5. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf til anvendelse ifølge et hvilket som helst af krav 1-4, hvor S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf er i form af en farmaceutisk sammensætning.

6. Fremgangsmåde til forudsigelse af terapeutiske virkninger af kemoterapi ved hjælp af et antitumormiddel, der, som en aktiv bestanddel, omfatter (S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf hos en patient med en malign tumor, hvilken fremgangsmåde omfatter nedenstående trin (1) og (2):

(1) et trin med påvisning af tilstedeværelse eller fravær af mutation af EGFR-gen indeholdt i en biologisk prøve opnået fra patienten; og

(2) et trin med forudsigelse af, at kemoterapien med stor sandsynlighed vil have tilstrækkelige terapeutiske virkninger i forhold til patienten, når resultaterne af påvisningen i trin (1) viser, at EGFR-genet har mindst én mutation valgt fra gruppen bestående af G719X-mutation af exon-18, E709X-mutation af exon-18 og L861X-mutation af exon-21, hvor X repræsenterer en vilkårlig aminosyrerest.

7. Fremgangsmåde ifølge krav 6, hvor exon-18-mutationen er mindst én mutation valgt fra gruppen bestående af G719A, G719S, G719C, E709K og E709A.

8. Fremgangsmåde ifølge krav 6 eller 7, hvor exon-21-mutationen er L861Q.

9. Fremgangsmåde ifølge et hvilket som helst af krav 6 til 7, hvor EGFR'en endvidere har T790M-mutation.

10. S)-N-(4-amino-6-methyl-5-(quinolin-3-yl)-8,9-dihydropyrimido[5,4-b]indolizin-8-yl)acrylamid eller et salt deraf, til anvendelse ifølge et hvilket som helst af krav 1-5, hvor patienten har været omfattet af forudsigelsen ifølge et hvilket som helst af krav 6-9.

DRAWINGS

Drawing

Fig. 1

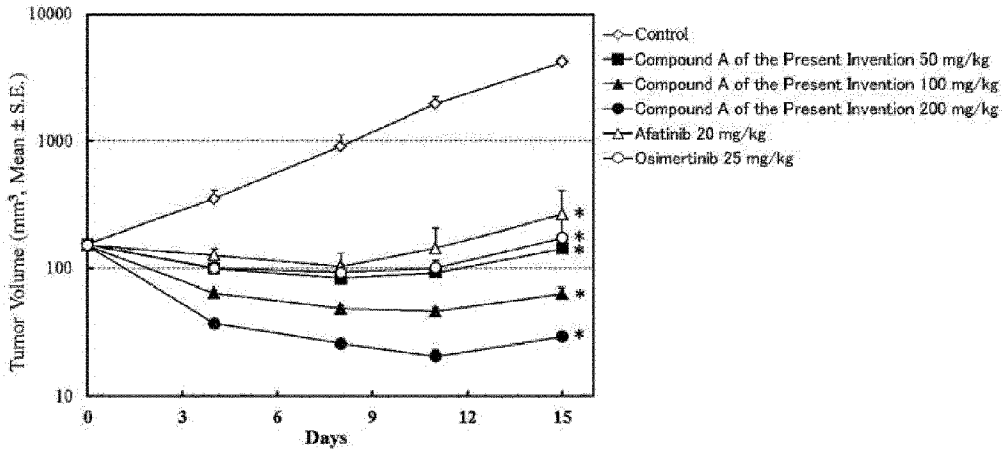


Fig. 2

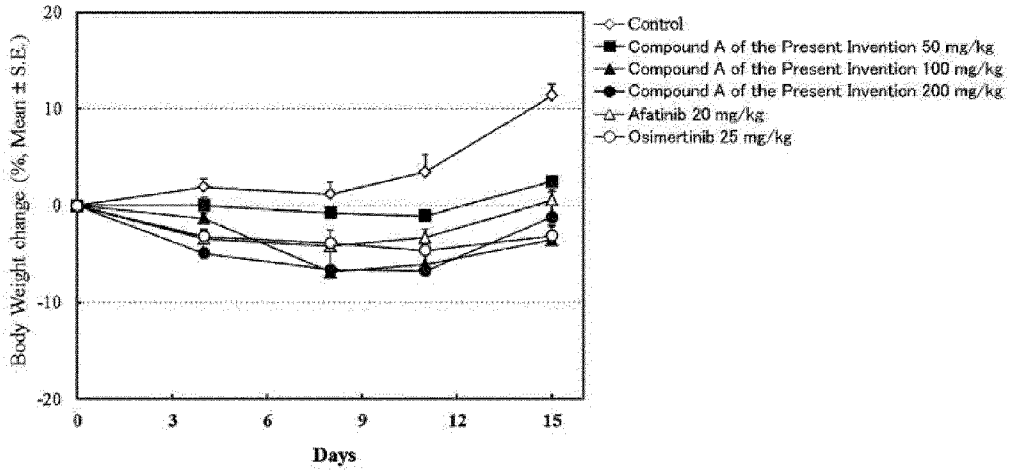
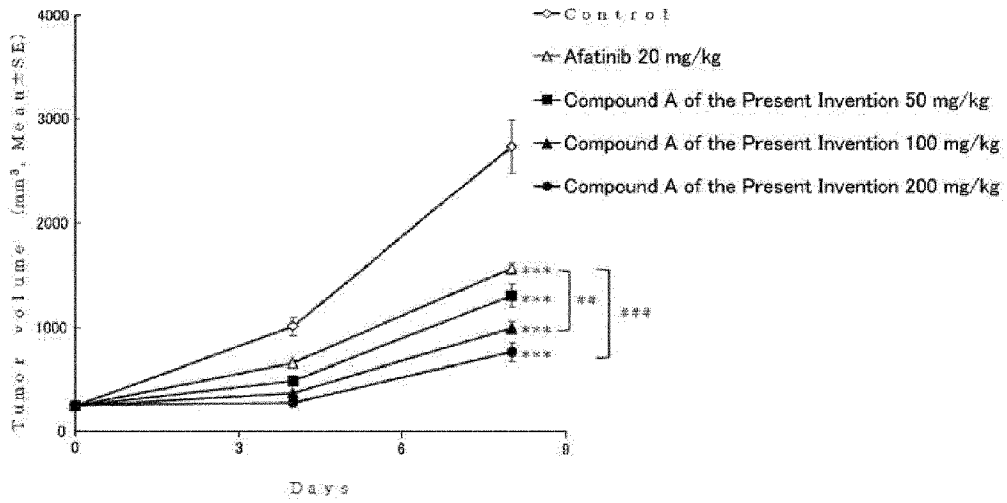

20
10
0
-10
-20
Body Weight change (%, Mean ± S.E.)
0
3
6
9
12
15
Days
Control
Compound A of the Present Invention 50 mg/kg
Compound A of the Present Invention 100 mg/kg
Compound A of the Present Invention 200 mg/kg
Afatinib 20 mg/kg
Osimertinib 25 mg/kg

Fig. 3


Control
Afatinib 20 mg/kg
Compound A of the Present Invention 50 mg/kg
Compound A of the Present Invention 100 mg/kg
Compound A of the Present Invention 200 mg/kg
Tumor volume (mm³, Mean±SE)
4000
3000
2000
1000
0
0
3
6
9
Days
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**
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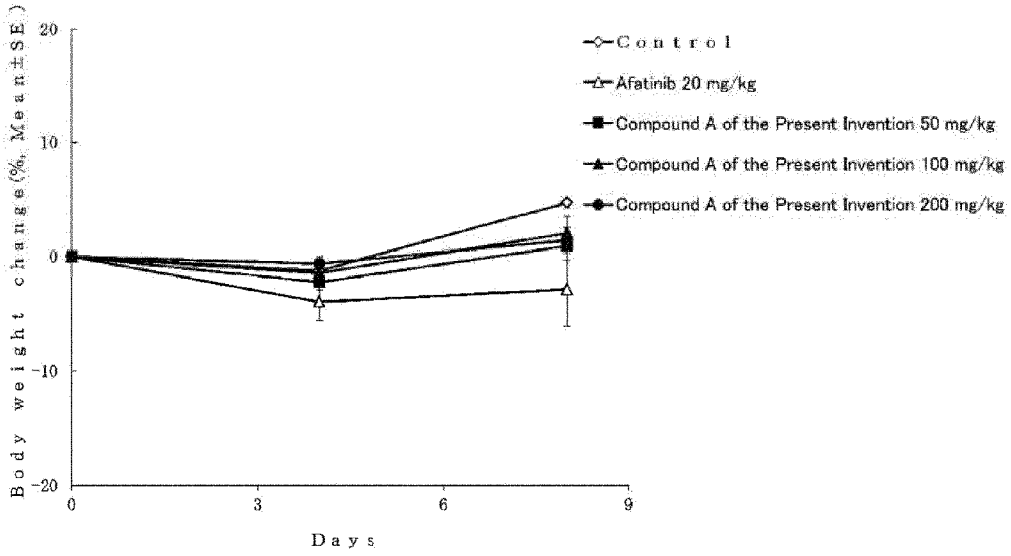
Fig. 4


Body weight change(%, Mean±SE)
20
10
0
-10
-20
0
3
6
9
Days
Control
Afatinib 20 mg/kg
Compound A of the Present Invention 50 mg/kg
Compound A of the Present Invention 100 mg/kg
Compound A of the Present Invention 200 mg/kg

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.