DESCRIPTION

Title of the Invention: ANTICANCER AGENT

[Technical Field]

[0001]

The present invention relates to a method of treating cancer by a combination of a CBP/catenin inhibitor and an immune checkpoint inhibitor, and a therapeutic drug for cancer.

[Background Art]

[0002]

Recently, immune checkpoint inhibitors have been actively developed in cancer therapy, among which anti-CTLA-4 antibody and anti-PD-1 antibody are commercially available as therapeutic drugs for cancer.

PD-1 (programmed death 1) binds to PD-L1 and PD-L2, which are ligands thereof expressed on antigen-presenting cells, and transmits an inhibitory signal to cytotoxic T cells (CD8 positive T cells) to negatively regulate the activation state of cytotoxic T cells. It is known that cancer cells forcedly expressing PD-L1 attenuate the cytotoxic activity of antigen-specific CD8-positive T cells and induce apoptosis. It is known that PD-L1 is expressed in many cancer cells, and its prognosis becomes poorer as the expression becomes higher. An anti-PD-1 antibody nivolumab blocks the transmission of inhibitory signals, maintains the activation state of cytotoxic T cells, and attacks cancer cells.

When CTLA (cytotoxic T lymphocyte associated antigen)-4 and co-stimulatory molecule CD80 (B7-1) or CD86 (B7-2) in antigen presenting cells bind to each other, the activation of cytotoxic T cells is suppressed by the antigen presenting cells. An anti-CTLA-4 antibody, ipilimumab, activates and proliferates cytotoxic T cells by inhibiting the binding (see Fig. 1).

As for PD-L1, cells with decreased expression of PD-L1 have been reported to show features of various cancer stem cells (tumorigenicity, resting phase, ALDH activity, etc.) in the study of cancer stem cells of biliary tract cancer (non-

patent document 1). Many of the cancer stem cells are negative for HLA class I expression and are not recognized by cytotoxic T cells.

[0003]

On the other hand, it is considered that, in many of the cancer cells, β-catenin is activated by Wnt signal, due to which the cell proliferation regulatory mechanism is collapsed and cell carcinogenesis progresses. As such, inhibitors of the Wnt signal pathway have been studied as anticancer agents; however, none have been put to practical use.

The present inventors have shown that PRI-724, which is a CBP/catenin inhibitor under clinical development as an anticancer agent, is clearly less toxic in human than conventional Wnt inhibitors having other mechanisms (non-patent document 2). As the mechanism thereof, it is considered that, following the inhibition of the binding between CBP and catenin, P300 with high similarity to CBP binds instead of CBP, and such change suppresses cancer proliferation and induces differentiation (non-patent document 3).

Recently, it has been shown that the main pathway of bile duct cancer is proliferation of tumor by Wnt-β-catenin pathway, and that a CBP/catenin inhibitor, ICG-001, has a strong proliferation inhibitory effect in animal model (non-patent document 4).

Conventional Wnt inhibitors block signals based on mechanisms for inhibiting the production of Wnt ligand, blocking the function of receptor, promoting degradation of catenin and the like. Because of such mechanisms, toxicity problems occur in preclinical studies and clinical trials, and the development has mostly been discontinued. This is because the Wnt pathway is considered essential for life support. On the other hand, CBP/catenin inhibitor can be assumed to be highly safe since it shows effects by changing the switch from CBP to P300 rather than blocking the signal.

Furthermore, β-catenin is also known to suppress

activation of T cells by suppressing differentiation of T cells (non-patent document 5). Therefore, CBP/β-catenin inhibitor is considered to promote differentiation of T cells and activate T cells.

Patients for whom anti-PD-1 antibody and anti-CTLA-4 antibody are clinically markedly effective are limited. The reason therefor is because, in cancer patients, the number of CD-8 positive cells and the expression of β-catenin are correlated completely opposite, and it was shown that tissues with high expression of β-catenin contain a small number of T cells and, conversely, tissues containing many T cells show low expression, and the tissues containing many T cells show a remarkable effect (non-patent document 6).

[Document List]

[non-patent documents]

[0004]

non-patent document 1: Cancer Sci (105) 667-674, 2014

non-patent document 2: J Clin Oncol 31, 2013 (suppl; abstr 2501)

non-patent document 3: The EMBO Journal 2013, 1-13

non-patent document 4: J Clin Invest. 2015 Mar 2; 125(3):1269-85. doi: 10.1172/JCI76452

non-patent document 5: J Immunol 2011; 186:784-790

non-patent document 6: Nature. 2015 May 11. doi: 10.1038/nature14404.

[SUMMARY OF THE INVENTION]

[Problems to be Solved by the Invention]

[0005]

Patients for whom anti-PD-1 antibody and anti-CTLA-4 antibody are clinically markedly effective are limited, and combined use with various conventionally-used therapeutic drugs for cancer has been tried. However, satisfactory effects are not necessarily obtained.

[Means of Solving the Problems]

[0006]

The present invention has found that a superior treatment effect will be obtained by using an immune checkpoint inhibitor such as PD-1-PD-L1 inhibitor and the like and a CBP/catenin inhibitor in combination, which resulted in the completion of the present invention.

That is, the gist of the present invention is as follows.

(1) A therapeutic drug for cancer, comprising a CBP/catenin inhibitor and an immune checkpoint inhibitor in combination.

(2) The therapeutic drug for cancer of the above-mentioned (1), wherein the immune checkpoint inhibitor is one or more kinds of immune checkpoint inhibitors selected from PD-1 antagonist, PD-L1 antagonist, PD-L2 antagonist, CTLA-4 antagonist, KIR antagonist, CD137 antagonist, LAG3 antagonist and OX40 antagonist.

(3) The therapeutic drug for cancer of the above-mentioned (1), wherein the immune checkpoint inhibitor is one or more kinds of immune checkpoint inhibitors selected from anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CTLA-4 antibody, anti-KIR antibody, anti-LAG3 antibody and anti-OX40 antibody.

(4) The therapeutic drug for cancer of the above-mentioned (1) - (3), wherein the CBP/catenin inhibitor is an α-helix mimetic compound having a CBP/catenin inhibitory activity.

(5) The therapeutic drug for cancer of the above-mentioned (4), wherein the aforementioned α-helix mimetic compound is one or more kinds of any α-helix mimetic compounds described in WO 2003/031448, WO 2004/093828, WO 2005/116032, WO 2009/148192, WO 2010/044485, WO 2010/128685, WO 2012/115286, and/or WO 2015/098853.

(6) The therapeutic drug for cancer of the above-mentioned (1) - (4), wherein the CBP/catenin inhibitor is one or more kinds selected from the following compounds:
(6S,9aS)-N-benzyl-6-[(4-hydroxyphenyl)methyl]-8-(naphthalen-1-ylmethyl)-4,7-dioxo-3,6,9,9a-tetrahydro-2H-pyrazino[1,2-a]pyrimidine-1-carboxamide (ICG-001), 4-(((6S,9S,9aS)-1-(benzylcarbamoyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-

ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazin-6-yl)methyl)phenyl dihydrogen phosphate, (6S,9S,9aS)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, and (6S,9aS)-N-benzyl-8-((6-(3-(4-ethylpiperazin-1-yl)azetidin-1-yl)pyridin-2-yl)methyl)-6-((2-fluoro-4-hydroxyphenyl)methyl)-4,7-dioxo-2-(prop-2-en-1-yl)-octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide

(7) A method of treating cancer, comprising administering an effective amount of a CBP/catenin inhibitor and an effective amount of an immune checkpoint inhibitor to a subject in need thereof.

(8) The method of the above-mentioned (7), wherein the immune checkpoint inhibitor is one or more kinds of immune checkpoint inhibitors selected from PD-1 antagonist, PD-L1 antagonist, PD-L2 antagonist, CTLA-4 antagonist, KIR antagonist, CD137 antagonist, LAG3 antagonist and OX40 antagonist.

(9) The method of the above-mentioned (7), wherein the immune checkpoint inhibitor is one or more kinds of immune checkpoint inhibitors selected from anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CTLA-4 antibody, anti-KIR antibody, anti-LAG3 antibody and anti-OX40 antibody.

(10) The method of the above-mentioned (7) - (9), wherein the CBP/catenin inhibitor is an α-helix mimetic compound having a CBP/catenin inhibitory activity.

(11) The method of the above-mentioned (10), wherein the aforementioned α-helix mimetic compound is one or more kinds of any α-helix mimetic compounds described in WO 2003/031448, WO 2004/093828, WO 2005/116032, WO 2009/148192, WO 2010/044485, WO 2010/128685, WO 2012/115286, and/or WO 2015/098853.

(12) The method of the above-mentioned (7) - (10), wherein the CBP/catenin inhibitor is one or more kinds selected from the following compounds:

(6S,9aS)-N-benzyl-6-[(4-hydroxyphenyl)methyl]-8-(naphthalen-1-ylmethyl)-4,7-dioxo-3,6,9,9a-tetrahydro-2H-pyrazino[1,2-

a]pyrimidine-1-carboxamide (ICG-001), 4-(((6S,9S,9aS)-1-(benzylcarbamoyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazin-6-yl)methyl)phenyl dihydrogen phosphate, (6S,9S,9aS)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, and (6S,9aS)-N-benzyl-8-((6-(3-(4-ethylpiperazin-1-yl)azetidin-1-yl)pyridin-2-yl)methyl)-6-((2-fluoro-4-hydroxyphenyl)methyl)-4,7-dioxo-2-(prop-2-en-1-yl)-octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide.

[Effect of the Invention]

[0007]

Rather than a simple additive effect of a cancer treatment effect of an immune checkpoint inhibitor and a cancer treatment effect of a CBP/β-catenin inhibitor, a CBP/β-catenin inhibitor promotes activation and migration to cancer tissues of cytotoxic T cells and enhances a cancer treatment effect, as well as suppresses a cancer cell proliferation effect of CBP/β-catenin. A CBP/β-catenin inhibitor promotes differentiation of cancer stem cells and increases antigenicity, but may suppress functions of cytotoxic T cells through expression of PD-L1; however, an immune checkpoint inhibitor increases a cancer treatment effect of a CBP/β-catenin inhibitor by inhibiting binding of PD-1 and PD-L1.

[Brief Description of the Drawings]

[0008]

Fig. 1 is a schematic drawing showing the action mechanism of the immune checkpoint inhibitor.

Fig. 2 is a schematic drawing showing the action mechanism of the CBP/β-catenin inhibitor.

Fig. 3 shows disappearance of breast cancer stem cells by the CBP/β-catenin inhibitor in Example 1.

Fig. 4 shows suppression of the proliferation of colorectal cancer by the CBP/β-catenin inhibitor in Example 3.

Fig. 5 shows reduction of IL-10 by the CBP/β-catenin

inhibitor in Example 4.

[Description of Embodiments]

[0009]

The present invention provides a therapeutic drug for cancer, comprising a CBP/catenin inhibitor and an immune checkpoint inhibitor in combination. A detailed explanation is given below.

(CBP/catenin inhibitor)

β-catenin functions as a mediator of Wnt signal transduction, binds to a transcription factor Tcf/Lef (T cell factor/Lymphocyte enhancing factor), promotes expression of various genes (cyclin D1, c-Myc etc.) involved in Wnt signal transduction, and controls proliferation and differentiation of cells (He et al., 1998 Science 281 1509-1512: Kolligs et al., 1999 Mol. Cell. Biol. 19, 5696-5706: Crawford et al., 1999, Oncogene 18, 2883-2891: Shtutman et al., 1999, Proc. Natl. Acad. Sci. USA., 11, 5522-5527: Tetsu and McCormick, 1999 Nature, 398, 422-426) (see Fig. 2).

CBP (cyclic AMP response element binding protein (CREB) binding protein) directly interacts with β-catenin in the CREB binding domain, and promotes transcription activation of Tcf/Lef (Ken-Ichi Takemaru and Randall T. Moon, 2000 J. Cell. Biol., 149, 2, 249-254).

CBP/catenin inhibitor is not particularly limited as long as it inhibits interaction between CBP and catenin, particularly β-catenin, and an embodiment in which binding of β-catenin and CBP is inhibited, as a result of which gene expression by β-catenin complex is suppressed is preferable.

Inhibition of CBP/β-catenin can be measured by binding assay (radiobinding assay etc.) known per se, a reporter assay method and the like. Preferably, it can be confirmed by measuring gene expression of Wnt signal transduction by the reporter assay method described in WO 2009/148192.

The CBP/catenin inhibitor of the present invention is not particularly limited as long as it is as defined above. It is

preferably an α-helix mimetic compound having a CBP/catenin inhibitory activity, and examples thereof include α-helix mimetic compounds, pharmaceutically acceptable salts thereof and the like described in WO 2003/031448, WO 2004/093828, WO 2005/116032, WO 2009/148192, WO 2010/044485, WO 2010/128685, WO 2012/115286 and the like.

Preferably, (6S,9aS)-N-benzyl-6-[(4-hydroxyphenyl)methyl]-8-(naphthalen-1-ylmethyl)-4,7-dioxo-3,6,9,9a-tetrahydro-2H-pyrazino[1,2-a]pyrimidine-1-carboxamide (ICG-001), 4-(((6S,9S,9aS)-1-(benzylcarbamoyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazin-6-yl)methyl)phenyl dihydrogen phosphate (compound 1), and (6S,9S,9aS)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide (compound 2) and the like can be mentioned.

[0010]

As the CBP/catenin inhibitor of the present invention, a compound described in WO 2015/098853 having a Wnt Pathway modulating action, a pharmaceutically acceptable salt thereof and the like can also be mentioned.

Preferably, (6S,9aS)-N-benzyl-8-((6-(3-(4-ethylpiperazin-1-yl)azetidin-1-yl)pyridin-2-yl)methyl)-6-((2-fluoro-4-hydroxyphenyl)methyl)-4,7-dioxo-2-(prop-2-en-1-yl)-octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide (compound A) and the like can be mentioned.

[0011]

The CBP/catenin inhibitor can be administered as pharmaceutical preparations (e.g., injection, capsule, tablet, powder, granule and the like) formulated by a conventional method. For example, it is administered at a dose of about 0.01 - 1000 mg/kg (body weight) per day, preferably about 0.1 - 500 mg/kg (body weight) per day, based on the amount of the active ingredient, once or in several portions. The dose, administration method and administration frequency can be

appropriately changed according to the symptom, age and the like. For example, when formulated into an injection, carriers such as distilled water, saline and the like can be used and, when formulated into capsule, tablet, powder or granule, excipients such as starch, lactose, sucrose, calcium carbonate and the like, binders such as starch paste solution, gum arabic, gelatin, sodium alginate, carboxymethylcellulose, hydroxypropylcellulose and the like, lubricants such as magnesium stearate, talc and the like, and disintegrants such as starch, agar, crystalline cellulose, calcium carbonate, sodium hydrogen carbonate, sodium alginate and the like, and the like may be used. The content percentage of the active ingredient in the preparation can be varied within 1 - 99 wt%. For example, when the form of a tablet, capsule, granule, powder or the like is taken, 5 - 80 wt% of the active ingredient is preferably contained. In the case of an injection, 1 - 10 wt% of the active ingredient is preferably contained.

[0012]

(immune checkpoint inhibitor)

As an immune checkpoint inhibitor, for example, a blocker of a T cell inhibitory receptor can be mentioned. A blocker of a T cell inhibitory receptor is generally a molecule that specifically binds to an extracellular domain of a T cell inhibitory receptor or an extracellular domain of a T cell inhibitory receptor ligand and prevents, for example, activation of a T cell inhibitory receptor by blocking the binding thereof with their ligands such as CD80, CD86, PD-L1, PD-L2 and the like. Specifically, PD-1 antagonist (anti-PD-1 antibody such as nivolumab, pembrolizumab and the like, etc.), PD-L1 antagonist (anti-PD-L1 antibody such as pidilizumab, MPDL-3280A, MEDI4736, MSB0010718C, MEDI0680 and the like, etc.), PD-L2 antagonist (anti-PD-L2 antibody etc.), CTLA-4 antagonist (anti-CTLA-4 antibody such as ipilimumab, tremelimumab and the like, etc.), KIR antagonist (anti-killer cell immunoglobulin-

like receptor antibody (anti-KIR antibody) such as lirilumab and the like, etc.), CD137 antagonist (anti-CD137 antibody such as urelumab, PF-05082566 and the like, etc.), LAG3 antagonist (anti-lymphocyte activation factor 3 antibody (anti-LAG3 antibody) such as BMS-986016 and the like, etc.) and OX40 antagonist (anti-OX40 antibody such as MEDI6469 and the like) can be mentioned. An anti-PD-1 antibody, anti-PD-L1 antibody or anti-CTLA-4 antibody is preferable.

An immune checkpoint inhibitor can be prepared as a pharmaceutical composition by a conventional method. In many cases, the pharmaceutical composition further contains one or more buffers (e.g., neutral, saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose, or dextran), mannitol, protein, polypeptide, or amino acids such as glycine and the like, antioxidants (e.g., ascorbic acid, sodium disulfite, butylhydroxytoluene, butylhydroxyanisole and the like), bacteriostatic agent, chelating agents such as EDTA, glutathione and the like, a solute that makes the composition isotonic, hypotonic or slightly hypertonic with the blood of the recipient, suspending agent, thickener, preservative, flavor, sweetener and/or coloration compound as necessary.

[0013]

(therapeutic drug for cancer, treatment method of cancer)

The present invention provides a method of treating patients suffering from cancer, comprising administering a therapeutic drug for cancer containing a combination of a CBP/catenin inhibitor and an immune checkpoint inhibitor to a patient. The method described in the present specification is directed to a cancer treatment, for example, leukemia and solid tumor (e.g., melanoma, cancer, sarcoma, lymphoma and the like). Specifically, renal cancer, renal cell carcinoma, bladder cancer, urothelial cancer, urogenital tumor, lung cancer, lung squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, malignant melanoma, uveal melanoma, eye melanoma, gastric cancer, esophagus cancer, glioblastoma, gliosarcoma,

metastatic brain tumor, liver cancer, metastatic liver cancer, hepatoma, hepatocyte epithelial cancer, large intestine cancer, colon cancer, pancreatic cancer, metastatic pancreatic cancer, metastatic head squamous cell carcinoma, breast cancer, metastatic breast cancer, malignant pleural mesothelioma, metastatic neck squamous cell carcinoma, metastatic nasopharyngeal carcinoma, HPV-16 positive solid cancer, myelofibrosis, primary myelofibrosis (PMF) and myelofibrosis transferred from Polycythemia vera (PV) or Essential thrombocythemia (ET), primary myelofibrosis, recurrent epithelial ovarian cancer, fallopian tube cancer, peritoneal cancer, metastatic sarcoma, hormone resistant prostate cancer, adrenal cortex cancer, non-Hodgkin's lymphoma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin lymphoma, acute myeloid leukemia, chronic myeloid leukemia, chronic lymphocytic lymphoma, small lymphocytic lymphoma, T-cell leukemia, T-cell lymphoma, follicular lymphoma, myelodysplastic syndrome and the like can be mentioned.

In these cancer patients, it is particularly effective for cancer with activated β-catenin.

[0014]

A therapeutic drug for cancer of the present invention, comprising a CBP/catenin inhibitor and an immune checkpoint inhibitor in combination may be formulated each separately or together by a method appropriate for desired administration, for example, oral, transnasal, mucous membrane, rectal, vaginal, topical, intravenously, intraperitoneal, intradermal, subcutaneous, and intramuscular administration and the like.

In the present invention, determination of the dose and the timing of the administration of a therapeutically effective amount of a therapeutic drug for cancer containing a combination of a CBP/catenin inhibitor and an immune checkpoint inhibitor is sufficiently within the knowledge of those of ordinary skill in the art. For example, the initial effective amount can be assumed from the cell culture or other in vitro

assay. The dose can be set to create a circulation concentration or tissue concentration, such as IC50 concentration and the like, determined by cell culture assay in an animal model.

For the object of the invention, the administration method is selected relying on the condition under treatment and the therapeutic drug. A CBP/catenin inhibitor and an immune checkpoint inhibitor can be administered by various methods. Preferable examples of the method include, but are not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular and systemic administrations and, in some cases, direct injection into a particular organ or tumor and the like. A CBP/catenin inhibitor and an immune checkpoint inhibitor can be administered through a single pathway, or simultaneous several pathways.

A CBP/catenin inhibitor and an immune checkpoint inhibitor may be administered once per day, twice - several times per day, or further, plural times per day, depending on, among other things, the treatment indication and the judgment of the prescribing physician.

The amounts of the CBP/catenin inhibitor and immune checkpoint inhibitor necessary for affording a treatment effect can be empirically determined according to conventional procedures for a particular object. Generally, cells are given in a pharmacologically effective dose when administered for the object of treatment. The "pharmacologically effective amount" or "pharmacologically effective dose" refers to an amount sufficient for producing a desired physiological effect or capable of achieving a desired result, such as reducing or removing one or more symptoms or indications of a disorder or disease and the like, to treat a particular disorder or disease state.

The therapeutic drug for cancer of the present invention, which is a combination of a CBP/catenin inhibitor and an immune checkpoint inhibitor, can be combined with other cancer

treatments, for example, surgical resection, radiation therapy, chemotherapy, immunotherapy, and supporting therapy (e.g., analgesic, diuretic, antidiuretic, antiviral drug, antibiotic, nutritional supplement, anemia treatment, blood coagulation treatment, bone treatment, and psychopathological and psychological treatments) and the like.

While the present invention is explained in more detail in the following by showing Examples, these do not limit the scope of the present invention.

[Examples]

[0015]

Example 1

To a side population fraction (cancer stem cells were considered to have been concentrated) obtained by two-dimensional cell sorting of human breast cancer cell line (MDA-MB-231) with Hoechst Blue and Hoechst Red was added 0.3 μM CBP/catenin inhibitor (compound 2) in the culture medium. After 4 days, the side population fraction almost disappeared (when untreated with medicament, 1.6% SP cells were detected, whereas 0% was detected in compound 2 treatment group), and the cancer stem cells were considered to have differentiated (Fig. 3).

[0016]

Treatment Example 1

To a patient with malignant melanoma unable to remove tumors surgically were given nivolumab by intravenous drip infusion at 2 mg/kg (body weight)/time at 3-week intervals, and CBP/β-catenin inhibitor (compound 1) at 30 mg/m^2/day by intravenous drip infusion.

[0017]

Treatment Example 2

To a patient with malignant melanoma unable to remove tumors surgically were given ipilimumab by intravenous drip infusion at 3 mg/kg (body weight)/time at 3-week intervals, and CBP/β-catenin inhibitor (compound 1) at 100 mg/m^2/day by

intravenous drip infusion.

[0018]

Example 2

Effect of combined use of CBP/catenin inhibitor (compound A) and anti-mouse PD-1 (CD279) antibody in animal model serially transplanted with subcultured spontaneous breast cancer of transgenic mouse (MMTV-Wnt-1)

Spontaneous breast cancer of transgenic mouse (MMTV-Wnt-1), in which Wnt-1 had been locally expressed in mammary gland epithelial cells, was harvested, and serially transplanted to the background line mouse (C57BL/6J) with trocar. When the serially transplanted tumor became about 1.5 g, it was isolated, processed into fragments of about 30 mg, and subcutaneously transplanted to the side of the body of 5 mice (C57BL/6J) per each group. Engraftment of the tumor was confirmed, and compound A (50 mg/kg, twice per day, 21 days, oral administration) and anti-mouse PD-1 antibody (Clone: RMP1-14, BioXCell) (10 mg/kg, twice per week, 3 weeks, intraperitoneal administration) were each administered singly or in combination.

With the day of the start of administration as day 0, the major axis and the minor axis of the tumor developed in each mouse were measured by Digimatic Caliper (Mitsutoyo) on days 4, 7, 11, 14, 18, and 21.

The tumor volume was calculated according to the following formula.

tumor volume TV (mm^3)=tumor major axis (mm)×tumor minor $axis^2$ (mm^2)/2

The results of TV are summarized in Table 1. The combined use of compound A and anti-mouse PD-1 antibody showed an antitumor effect superior in statistical significance ($*p<0.05$) as compared to single administration of each (Repeated measures ANOVA followed by Dunnett's type multiple comparison).

[0019]

Table 1

Mean±SEM (mm^3)	Day 0	Day 4	Day 7	Day 11	Day 14	Day 18	Day 21
control group	74.3±6.9	110.7±10.3	149.7±17.2	212.1±27.9	241.3±46.0	458.8±96.8	529.3±94.9
compound A group	75.3±9.2	83.2±8.5	90.5±22.3	123.1±40.5	140.4±45.1	221.6±47.9	237.7±41.2
anti-mouse PD-1 antibody group	73.8±9.6	105.6±7.4	126.8±15.2	176.4±15.5	273.7±42.8	430.1±61.0	502.3±99.1
compound A+anti-mouse PD-1 antibody group	73.3±8.4	56.9±9.8	69.3±19.0	64.6±10.2	71.5±15.1	94.8±22.9(*)	111.3±25.9(*)

[0020]

Example 3

Effect of combined use of CBP/catenin inhibitor (compound 1) and anti-mouse PD-L1 antibody in animal model of BALB/c mouse transplanted with colorectal cancer cells

Colorectal cancer cells, CT26 cells, were subcutaneously transplanted to the right abdomen of female BALB/c mice. After breeding to an average tumor size of 80 mm^3, they were grouped into 10 mice per group according to the following.

group 1: vehicle group

group 2: anti-mouse PD-L1 antibody single administration group

group 3: compound 1 single administration group

group 4: compound 1 and anti-mouse PD-L1 antibody combined use administration group

Compound 1 (80 mg/kg, once per day, 21 days, intraperitoneal administration) and anti-mouse PD-L1 antibody (catalog No.: BE0101-100 MG, BioXCell) (2 mg/kg, twice per week, 2 weeks, intraperitoneal administration) were each administered singly or in combination.

With the day of the start of administration as day 0, the major axis and the minor axis of the tumor developed in each mouse were measured on days 3, 6, 9, 12 and 15.

The tumor volume was calculated according to the following formula.

tumor volume (mm^3)=tumor major axis (mm)×tumor minor axis^2 (mm^2)/2

The results of the tumor volume are summarized in Fig. 4. The combined use of compound 1 and anti-mouse PD-L1 antibody showed an antitumor effect superior in statistical significance (*$p<0.01$) as compared to single administration of each (Repeated measurement Two-Way ANOVA).

[0021]

Example 4

IL-10 production suppressive action of CBP/catenin inhibitor (compound 1)

624 mel cells and 928 mel cells were used, which are cell lines (melanoma) established from human malignant melanoma patients by US NIH. These cells have been activated and have IL-10 production ability.

In a culture medium (10% FCS-containing RPMI1640, penicillin and streptomycin added), respective melanoma cells were seeded in a 6-well plate at a concentration of 1×10^5 cells/2 ml/well. After seeding, compound 1 was added to a given concentration and the cells were cultured for 24 hr. After 24 hr, the culture supernatant was recovered, and the concentration of IL-10 in the supernatant was measured. For the measurement of IL-10 concentration, Human IL10 OptEIA ELISA set (BD, #555157) was used.

The results are shown in Fig. 5.

When compound 1 was added at 1 μM, an influence on the proliferation was confirmed in any cell. When compound 1 was added at 0.2 μM, an influence on the proliferation was not confirmed in any cell. Since production of IL-10 was suppressed even under condition (0.2 μM) free of an influence on the proliferation, compound 1 was found to have an IL-10 production suppressive action.

Patients for whom anti-PD-1 antibody and anti-CTLA-4 antibody, which are immune checkpoint inhibitors, are clinically markedly effective are limited. It is known that the effect thereof depends on the number of CD-8 positive T cells in cancer patients. Particularly, the number of CD-8 positive T cells and the expression of β-catenin are correlated completely opposite, and tissues with high expression of β-catenin contain a small number of T cells and, conversely, tissues containing many T cells show low expression.

A CBP/catenin inhibitor shows a higher effect in a tissue where expression of β-catenin is high. On the other hand, it can increase the number of CD-8 positive T cells by its IL-10 production suppressive action. It was found therefore that a CBP/catenin inhibitor affords a higher anti-cancer effect when

used in combination with an immune checkpoint inhibitor.

[0022]

This application is based on a patent application No. 2015-121479 filed in Japan (filing date: June 16, 2015), the contents of which are incorporated in full herein.

Claims

1. A therapeutic drug for cancer, comprising a CBP/catenin inhibitor and an immune checkpoint inhibitor in combination, wherein the CBP/catenin inhibitor is (6S,9aS)-N-benzyl-8-((6-(3-(4-ethylpiperazin-1-yl)azetidin-1-yl)pyridin-2-yl)methyl)-6-((2-fluoro-4-hydroxyphenyl)methyl)-4,7-dioxo-2-(prop-2-en-1-yl)-octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, and the immune checkpoint inhibitor is an anti-PD-1 antibody.

2. Use of the combination of a CBP/catenin inhibitor and an immune checkpoint inhibitor in the manufacture of a therapeutic drug for cancer, wherein the CBP/catenin inhibitor is (6S,9aS)-N-benzyl-8-((6-(3-(4-ethylpiperazin-1-yl)azetidin-1-yl)pyridin-2-yl)methyl)-6-((2-fluoro-4-hydroxyphenyl)methyl)-4,7-dioxo-2-(prop-2-en-1-yl)-octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, and the immune checkpoint inhibitor is an anti-PD-1 antibody.