NOVEL GENETICALLY ENGINEERED VACCINIA VIRUSES

BACKGROUND OF THE INVENTION

Field of the Invention

[0001]

The present invention relates to a novel genetically engineered vaccinia virus.

Description of the Related Art

[0002]

Various techniques for using viruses for cancer treatments have been recently developed. Vaccinia virus is one of the viruses used for cancer treatment. Vaccinia virus has been studied for the cancer treatment as a vector for delivering therapeutic genes to cancer cells, as an oncolytic virus that proliferates in cancer cells and destroys the cancer cells, or as a cancer vaccine that expresses tumor antigens or immunomodulatory molecules (Expert Opinion on Biological Therapy, 2011, vol. 11, p. 595-608).

[0003]

Vaccinia viruses engineered so as to have an N1L gene inactivated by the insertion of a foreign gene encoding interleukin-12 (IL-12) or interleukin-21 (IL-21) and to be deficient in the thymidine kinase (TK) gene by the insertion of the lacZ reporter gene and the firefly

luciferase gene have been reported to suppress the tumor growth or improve the survival rate in cancer-bearing mice (Patent Literature 1).

[0004]

A technique for employing, for cancer treatment, recombinant vaccinia viruses that are deficient in the function of the viral proteins vaccinia virus growth factor (VGF) and O1L and proliferate specifically in cancer cells and destroy the cancer cells has been reported. Although it is stated that a foreign gene such as a marker gene or a therapeutic gene encoding a product having cytotoxicity or the immunopotentiating effect may be introduced into a gene that is not essential to the life cycle of vaccinia virus, the introduction of a gene specifically examined in Examples is that of a marker gene, a luciferase-green fluorescent protein (GFP) fusion gene or an expression cassette of DsRed. No therapeutic gene is examined for the introduction. No suggestion is made for combining plural therapeutic genes (Patent Literature 2).

[0005]

Meanwhile, it has been reported that, in the examination of effects of recombinant proteins on isolated $CD8^+$T cells, a recombinant human interleukin-7 (IL-7) protein alone does not induce detectable levels of interferon-gamma (IFN-γ) production by $CD8^+$T cells, but a combination of the recombinant human IL-7 protein and a

recombinant human IL-12 protein synergistically enhances the production of IFN-γ (The Journal of Immunology, 1995, vol. 154, p. 5093-5102). It has been reported that an oncolytic vaccinia virus that expresses an immune-stimulating molecule may rapidly be cleared by strong immune responses. It is also stated that strong immune response could serve either as a foe or as an ally to the vaccinia virus-mediated cancer therapy (Molecular Therapy, 2005, vol. 11, No. 2, p. 180-195).

Citation List

Patent Literatures

[0006]

Patent Literature 1: WO2015/150809

Patent Literature 2: WO2015/076422

SUMMARY OF THE INVENTION

Technical Problem

[0007]

An object of the present invention is to provide a recombinant vaccinia virus (in particular, oncolytic vaccinia virus), a pharmaceutical composition and a combination kit, for treating or preventing cancer.

Solution to Problem

[0008]

As a result of considerable repetitive thinking and studies in the generation of vaccinia virus, the present inventors have generated vaccinia viruses comprising a polynucleotide encoding IL-7 or vaccinia viruses comprising a polynucleotide encoding IL-12, and vaccinia viruses comprising two polynucleotides, a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12 (Example 2); and found that 1) a vaccinia virus comprising two polynucleotides, a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12 and 2) a mixture of two vaccinia viruses, a vaccinia virus comprising a polynucleotide encoding IL-7 and a vaccinia virus comprising a polynucleotide encoding IL-12 exhibit a cytolytic effect on various human cancer cells (Example 3), thereby completing the present invention:
1) a vaccinia virus comprising two polynucleotides, a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12 could exhibit a tumor regression effect in cancer-bearing humanized mouse models (Example 6), could achieve complete remission (Example 7), and could induce acquired immunity to maintain the antitumor effect (Example 8) in syngeneic cancer-bearing mouse models. Moreover, 2) a mixture of two vaccinia viruses, a vaccinia virus comprising a polynucleotide encoding IL-7 and a vaccinia virus comprising a polynucleotide encoding IL-12 could achieve complete remission (Example 7), and could induce acquired immunity to maintain the antitumor

effect (Example 8) in syngeneic cancer-bearing mouse models.

[0009]

More specifically, the present invention may encompass, as a substance or method useful in medicine or industry, the following inventions:

[1] A vaccinia virus comprising the following (1) and (2):

(1) a polynucleotide encoding interleukin-7 (IL-7); and

(2) a polynucleotide encoding interleukin-12 (IL-12).

[2] A pharmaceutical composition selected from the following (1) or (2):

(1) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7; or

(2) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12.

[3] A combination kit comprising the following vaccinia viruses (1) and (2):

(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and

(2) a vaccinia virus comprising a polynucleotide encoding IL-12.

[4] The vaccinia virus according to [1], wherein the vaccinia virus is deficient in the function of vaccinia virus growth factor (VGF).

[5] The vaccinia virus according to [1], wherein the vaccinia virus is deficient in the function of O1L.

[6] The vaccinia virus according to [1], wherein the vaccinia virus is deficient in the functions of VGF and O1L.

[7] The vaccinia virus according to [1], wherein the vaccinia virus has a deletion in the short consensus repeat (SCR) domains in the B5R extracellular region.

[8] The vaccinia virus according to [1], wherein the vaccinia virus is deficient in the functions of VGF and O1L and has a deletion in the SCR domains in the B5R extracellular region.

[9] The vaccinia virus according to any one of [1] and [4]-[8], wherein the vaccinia virus is a LC16mO strain.

[10] A pharmaceutical composition comprising a vaccinia virus according to any one of [1] and [4]-[9] and a pharmaceutically acceptable excipient.

[11] The pharmaceutical composition according to [2] or the kit according to [3], wherein the vaccinia virus is deficient in the function of VGF.

[12] The pharmaceutical composition according to [2] or the kit according to [3], wherein the vaccinia virus is deficient in the function of O1L.

[13] The pharmaceutical composition according to [2] or the kit according to [3], wherein the vaccinia virus is deficient in the functions of VGF and O1L.

[14] The pharmaceutical composition according to [2] or the kit according to [3], wherein the vaccinia virus has a deletion in the SCR domains in the B5R extracellular region.

[15] The pharmaceutical composition according to [2] or the kit according to [3], wherein the vaccinia virus is deficient in the functions of VGF and O1L and has a deletion in the SCR domains in the B5R extracellular region.

[16] The pharmaceutical composition according to any one of [2] and [11]-[15] or the kit according to any one of [3] and [11]-[15], wherein the vaccinia virus is a LC16mO strain.

[17] The pharmaceutical composition according to any one of [2] and [11]-[16] or the kit according to any one of [3] and [11]-[16], further comprising a pharmaceutically acceptable excipient.

[18] The pharmaceutical composition or kit according to any one of [10]-[17], for preventing or treating cancer.

[19] The pharmaceutical composition or kit according to [18], wherein the cancer is malignant melanoma, lung

adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[20] A method for preventing or treating cancer, comprising the step of administering the vaccinia virus according to any one of [1] and [4]-[9] to a subject in need of the prevention or treatment for cancer.

[21] The method according to [20], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[22] The vaccinia virus according to any one of [1] and [4]-[9], for use in preventing or treating cancer.

[23] The vaccinia virus according to [22], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer,

pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[24] Use of the vaccinia virus according to any one of [1] and [4]-[9] for the manufacture of a pharmaceutical composition for preventing or treating cancer.

[25] The use according to [24], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[26] A method for preventing or treating cancer, comprising the step of administering

(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and

(2) a vaccinia virus comprising a polynucleotide encoding IL-12

to a subject in need of the prevention or treatment for cancer.

[27] The method according to [26], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma,

myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[28] A vaccinia virus selected from the following (1) or (2):

(1) a vaccinia virus comprising a polynucleotide encoding IL-7, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or

(2) a vaccinia virus comprising a polynucleotide encoding IL-12, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

[29] The vaccinia virus according to [28], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[30] Use of a vaccinia virus selected from the following (1) or (2):

(1) use of a vaccinia virus comprising a polynucleotide encoding IL-7 for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used

in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or

(2) use of a vaccinia virus comprising a polynucleotide encoding IL-12 for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

[31] The use according to [30], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

[32] Use of (1) a vaccinia virus comprising a polynucleotide encoding IL-7 and (2) a vaccinia virus comprising a polynucleotide encoding IL-12 for the manufacture of a combination kit for preventing or treating cancer.

[33] The use according to [32], wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma,

myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

Advantageous Effects of the Invention

[0010]

The vaccinia virus according to the present invention and the vaccinia viruses contained in the pharmaceutical composition and combination kit according to the present invention exhibit oncolytic activity, express IL-12 and IL-7 polypeptides encoded by polynucleotides carried by the viruses in cancer cells, and induce complete remission and acquired immunity. The vaccinia virus, pharmaceutical composition, and combination kit according to the present invention can be used for preventing or treating cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

FIG. 1 is a drawing illustrating an example of transfer vector plasmid DNAs used in the present invention, in which the upper map illustrates an example in which the BFP gene operably linked to a promoter is incorporated in the VGF gene and the lower map illustrates an example in which the BFP gene operably linked to a promoter is incorporated in the O1L gene;

[0012]

FIG. 2 is a schematic view of the genome structure of recombinant vaccinia viruses (LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-LacZ and viruses constructed in the process of generating the virus vector);

[0013]

FIG. 3 is a schematic view of the genome structure of recombinant vaccinia viruses (LC16mO ΔSCR VGF-SP-IL12/O1L-SP-LacZ, LC16mO ΔSCR VGF-SP-IL7/O1L-SP-LacZ);

[0014]

FIG. 4 is a schematic view of the genome structure of a recombinant vaccinia virus (LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7);

[0015]

FIG. 5-1 is a graph illustrating oncolytic properties of a recombinant vaccinia virus (LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7), in which the ordinate represents the cancer cell survival rate (%) and the error bars represent standard deviation;

[0016]

FIG. 5-2 is a graph illustrating oncolytic properties of a recombinant vaccinia virus (LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7), in which the ordinate represents cancer cell survival rate (%) and the error bars represents standard deviation, FIG. 5-1 and FIG. 5-2 being graphs obtained under the same experimental conditions except that the cell types measured were different;

[0017]

FIG. 5-3 is a graph illustrating oncolytic properties of a mixture of 2 recombinant vaccinia viruses (a mixture of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-LacZ and LC16mO ΔSCR VGF-SP-IL7/O1L-SP-LacZ), in which the ordinate represents cancer cell survival rate (%) and the error bars represent standard deviation;

[0018]

FIG. 5-4 is a graph illustrating oncolytic properties of a mixture of 2 recombinant vaccinia viruses (a mixture of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-LacZ and LC16mO ΔSCR VGF-SP-IL7/O1L-SP-LacZ), in which the ordinate represents cancer cell survival rate (%) and the error bars represent standard deviation, FIG. 5-3 and FIG. 5-4 being graphs obtained under the same experimental conditions except that the cell types measured were different.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0019]

The present invention is described in detail below.

[0020]

<Vaccinia virus, pharmaceutical composition to be used in combination and combination kit according to the present invention>

The present invention provides a vaccinia virus comprising the following (1) and (2):

(1) a polynucleotide encoding IL-7; and

(2) a polynucleotide encoding IL-12.

(As used herein, the vaccinia virus is also referred to as the "vaccinia virus according to the present invention".)

[0021]

The present invention also provides a pharmaceutical composition selected from the following (1) or (2):

(1) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7; or

(2) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12.

(As used herein, the pharmaceutical composition is also referred to as the "pharmaceutical composition to be used in combination according to the present invention" and a vaccinia virus comprising a polynucleotide encoding IL-7 or a vaccinia virus comprising a polynucleotide encoding IL-12 contained in the pharmaceutical composition to be used in combination according to the present invention described in (1) or (2) above is also referred to as the "vaccinia virus to be used in combination".)

[0022]

The present invention also provides a combination kit comprising the following vaccinia viruses (1) and (2):

(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and

(2) a vaccinia virus comprising a polynucleotide encoding IL-12.

(As used herein, the combination kit is also referred to as the "combination kit according to the present invention" and the vaccinia viruses contained in the combination kit according to the present invention are also referred to as the "vaccinia viruses for the combination kit".)

[0023]

The combination kit according to the present invention means one or more pharmaceutical compositions to be used to administer two vaccinia viruses: (1) a vaccinia virus comprising a polynucleotide encoding IL-7 and (2) a vaccinia virus comprising a polynucleotide encoding IL-12. When both vaccinia viruses are administered simultaneously, the combination kit can contain the two vaccinia viruses for the combination kit together in a single pharmaceutical composition such as a powder or separately in plural pharmaceutical compositions. The combination kit according to the present invention encompasses a pharmaceutical

composition containing two vaccinia viruses: a vaccinia virus comprising a polynucleotide encoding IL-7 and a vaccinia virus comprising a polynucleotide encoding IL-12. When both vaccinia viruses for the combination kit are not simultaneously administered, the combination kit contains the two vaccinia viruses for the combination kit in separate pharmaceutical compositions. For example, the combination kit comprises the two vaccinia viruses for the combination kit in separate pharmaceutical compositions in a single package or in separate pharmaceutical compositions in separate packages. The combination kit according to the present invention may comprise a pharmaceutically acceptable excipient.

[0024]

The vaccinia virus used for the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, and the vaccinia viruses for the combination kit is a virus in the genus Orthopoxvirus in the family Poxviridae. Strains of the vaccinia virus used in the present invention include, but not limited to, the strains Lister, New York City Board of Health (NYBH), Wyeth, Copenhagen, Western Reserve (WR), Modified Vaccinia Ankara (MVA), EM63, Ikeda, Dalian, Tian Tan, and the like. The strains Lister and MVA are available from American Type Culture Collection (ATCC VR-1549 and ATCC VR-1508, respectively). Furthermore, vaccinia virus strains established from these strains may be used in the

present invention. For example, the strains LC16, LC16m8, and LC16mO established from the strain Lister may be used in the present invention. The strain LC16mO is a strain generated via the strain LC16 by subculturing at low temperature the Lister strain as the parent strain. The LC16m8 strain is a strain generated by further subculturing at low temperature the strain LC16mO, having a frameshift mutation in the B5R gene, a gene encoding a viral membrane protein, and attenuated by losing the expression and the function of this protein (Tanpakushitsu kakusan koso (Protein, Nucleic acid, Enzyme), 2003, vol. 48, p. 1693-1700). The whole genome sequences of the strains Lister, LC16m8, and LC16mO are known as, for example, Accession No.AY678276.1, Accession No.AY678275.1, and Accession No.AY678277.1, respectively. Therefore, the strains LC16m8 and LC16mO can be made from the strain Lister by a known technique, such as homologous recombination or site-directed mutagenesis.

[0025]

In one embodiment, the vaccinia virus used in the present invention is the strain LC16mO.

[0026]

The vaccinia virus used in the present invention can include attenuated and/or tumor-selective vaccinia viruses. As used herein, "attenuated" means low toxicity (for example, cytolytic property) to normal cells (for example, non-tumor cells). As used herein, "tumor

selective" means toxicity to tumor cells (for example, oncolytic) higher than that to normal cells (for example, non-tumor cell). Vaccinia viruses genetically modified to be deficient in the function of a specific protein or to suppress the expression of a specific gene or protein (Expert Opinion on Biological Therapy, 2011, vol. 11, p. 595-608) may be used in the present invention. For example, in order to increase tumor selectivity of vaccinia virus, vaccinia virus deficient in the function of TK (Cancer Gene Therapy, 1999, vol. 6, p. 409-422), vaccinia virus deficient in the function of VGF (Cancer Research, 2001, vol. 61, p. 8751-8757), vaccinia virus having a modified TK gene, a modified hemagglutinin (HA) gene, and a modified F3 gene or an interrupted F3 locus (WO 2005/047458), vaccinia virus deficient in the function of VGF and O1L (WO 2015/076422), vaccinia virus in which a target sequence of a microRNA whose expression is decreased in cancer cells is inserted into the 3' noncoding region of the B5R gene (WO 2011/125469), vaccinia virus deficient in the function of VGF and TK (Cancer Research, 2001, vol. 61, p. 8751-8757), vaccinia virus deficient in the function of TK, HA, and F14.5L (Cancer Research, 2007, vol. 67, p. 10038-10046), vaccinia virus deficient in the function of TK and B18R (PLoS Medicine, 2007, vol. 4, p. e353), vaccinia virus deficient in the function of TK and ribonucleotide reductase (PLoS Pathogens, 2010, vol. 6, p. e1000984),

vaccinia virus deficient in the function of SPI-1 and SPI-2 (Cancer Research, 2005, vol. 65, p. 9991-9998), vaccinia virus deficient in the function of SPI-1, SPI-2, and TK (Gene Therapy, 2007, vol. 14, p. 638-647), or vaccinia virus having mutations in the E3L and K3L regions (WO 2005/007824) may be used. Moreover, vaccinia virus deficient in the function of O1L may be used (Journal of Virology, 2012, vol. 86, p. 2323-2336). Moreover, in hope that the clearance of virus by the neutralization effect of anti-vaccinia virus antibodies is reduced in the living body, vaccinia virus deficient in the extracellular region of B5R (Virology, 2004, vol. 325, p. 425-431) or vaccinia virus deficient in the A34R region (Molecular Therapy, 2013, vol. 21, p. 1024-1033) may be used. Moreover, in hope of the activation of immune cells by vaccinia virus, vaccinia virus deficient in interleukin-1b (IL-1b) receptor (WO 2005/030971) may be used. Such insertion of a foreign gene or deletion or mutation of a gene can be made, for example, by a known homologous recombination or site-directed mutagenesis. Moreover, vaccinia virus having a combination of such genetic modifications may be used in the present invention. As used herein, "being deficient" means that the gene region specified by this term has no function and used in a meaning including deletion of the gene region specified by this term. For example, "being deficient" may be a result of the deletion in a region

consisting of the specified gene region or the deletion in a neighboring gene region comprising the specified gene region.

[0027]

In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit is (are) deficient in the function of VGF. In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit is (are) deficient in the function of O1L. In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination or the vaccinia viruses for the combination kit is (are) deficient in the functions of VGF and O1L. The function of VGF and/or O1L may be made deficient in vaccinia virus based on the method described in WO 2015/076422.

[0028]

VGF is a protein having a high amino acid sequence homology with epidermal growth factor (EGF), binds to the epidermal growth factor receptor like EGF, and activates the signal cascade from Ras, Raf, Mitogen-activated protein kinase (MAPK)/the extracellular signal-regulated kinase (ERK) kinase (MAPK/ERK kinase, MEK), and to following ERK to promote the cell division.

[0029]

O1L maintains the activation of ERK and contributes to the cell division along with VGF.

[0030]

Being deficient in the function of VGF and/or O1L of vaccinia virus refers to loss of the expression of the gene encoding VGF and/or the gene encoding O1L or the normal function of VGF and/or O1L when expressed. The deficiency in the function of VGF and/or O1L of vaccinia virus may be caused by the deletion of all or a part of the gene encoding VGF and/or the gene encoding O1L. Moreover, the genes may be mutated by nucleotide substitution, deletion, insertion, or addition to prevent the expression of normal VGF and/or O1L. Moreover, a foreign gene may be inserted in the gene encoding VGF and/or the gene encoding O1L. In the present invention, a gene is stated to be deficient when the normal product of the gene is not expressed by mutation such as genetic substitution, deletion, insertion, or addition.

[0031]

Whether or not the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit is (are) deficient in the function of VGF and/or O1L may be determined with a known method, for example, by evaluating the function of VGF and/or O1L, testing for the presence of VGF or O1L by an immunochemical technique using an antibody against VGF or an antibody against O1L,

or determining the presence of the gene encoding VGF or the gene encoding O1L by the polymerase chain reaction (PCR).

[0032]

B5R (Accession No.AAA48316.1) is a type 1 membrane protein resides in the envelope of vaccinia virus, and serves to increase the infection efficiency when the virus infects and is transmitted to neighboring cells or other sites in the host. The extracellular region of B5R contains 4 structural domains called SCR domains (Journal of Virology, 1998, vol. 72, p. 294-302). In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit has (have) a deletion in the SCR domains in the extracellular region of B5R.

[0033]

The deletion in the SCR domains in the B5R extracellular region of vaccinia virus encompasses the deletion of a part or all of the 4 SCR domains in the B5R extracellular region and refers to the lack of expression of a gene region encoding a part or all of the 4 SCR domains in the B5R extracellular region or the lack of a part or all of the 4 SCR domains in the extracellular region in the expressed B5R protein. In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the

vaccinia viruses for the combination kit has (have) the deletion of all 4 SCR domains. In one embodiment, the 4 SCR domains deleted in the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, and the vaccinia viruses for the combination kit correspond to the region from amino acid 22 to amino acid 237 in the amino acid sequence of Accession No.AAA48316.1 described above.

[0034]

Whether or not the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit has (have) a deletion in the SCR domains in the B5R extracellular region can be determined with a known method, for example, by testing for the presence of the SCR domains by an immunochemical technique using an antibody against the SCR domains or determining the presence or the size of the gene encoding the SCR domains by PCR.

[0035]

In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit is (are) vaccinia virus deficient in the functions of VGF and O1L and having a deletion in the SCR domains in the B5R extracellular region.

[0036]

In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit is (are) vaccinia virus of the strain LC16mO deficient in the functions of VGF and O1L and having a deletion in the SCR domains in the B5R extracellular region.

[0037]

In one embodiment, the vaccinia virus according to the present invention is vaccinia virus comprising a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12 and being deficient in the functions of VGF and O1L.

[0038]

In one embodiment, the vaccinia virus according to the present invention is vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12 and being deficient in the functions of VGF and O1L.

[0039]

In one embodiment, the vaccinia virus according to the present invention is vaccinia virus comprising a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12, being deficient in the functions of VGF and O1L, and having a deletion in the SCR domains in the B5R extracellular region.

[0040]

In one embodiment, the vaccinia virus according to the present invention is vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-7 and a polynucleotide encoding IL-12, being deficient in the functions of VGF and O1L, and having a deletion in the SCR domains in the B5R extracellular region.

[0041]

In one embodiment, the vaccinia virus to be used in combination or the vaccinia viruses for the combination kit is (are) vaccinia virus comprising a polynucleotide encoding IL-7 and being deficient in the functions of VGF and O1L or vaccinia virus comprising a polynucleotide encoding IL-12 and being deficient in the functions of VGF and O1L.

[0042]

In one embodiment, the vaccinia virus to be used in combination or the vaccinia viruses for the combination kit is (are) vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-7 and being deficient in the functions of VGF and O1L or vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-12 and being deficient in the functions of VGF and O1L.

[0043]

In one embodiment, the vaccinia virus to be used in combination or the vaccinia viruses for the combination kit is (are) vaccinia virus comprising a polynucleotide

encoding IL-7, being deficient in the functions of VGF and O1L, and having a deletion in the SCR domains in the B5R extracellular region or vaccinia virus comprising a polynucleotide encoding IL-12, being deficient in the functions of VGF and O1L, and having a deletion in the SCR domains in the B5R extracellular region.

[0044]

In one embodiment, the vaccinia virus to be used in combination or the vaccinia viruses for the combination kit is (are) vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-7, being deficient in the functions of VGF and O1L and having a deletion in the SCR domain in the B5R extracellular region or vaccinia virus of the strain LC16mO comprising a polynucleotide encoding IL-12, being deficient in the functions of VGF and O1L, and having a deletion in the SCR domains in the B5R extracellular region.

[0045]

IL-7 is a secretory protein functioning as an agonist for the IL-7 receptor. It is reported that IL-7 contributes to the survival, proliferation, and differentiation of T cells, B cells, or the like (Current Drug Targets, 2006, vol. 7, p. 1571-1582). In the present invention, IL-7 encompasses IL-7 occurring naturally and modified forms having the function thereof. In one embodiment, IL-7 is human IL-7. In the present invention, human IL-7 encompasses human IL-7 occurring

naturally and modified forms having the function thereof. In one embodiment, human IL-7 is selected from the group consisting of the following (1) to (3):

(1) a polypeptide comprising the amino acid sequence set forth in Accession No. NP_000871.1 and having the function of human IL-7;

(2) a polypeptide consisting of an amino acid sequence in which 1 to 10 amino acids are deleted from, substituted in, inserted into, and/or added to the amino acid sequence set forth in Accession No. NP_000871.1 and having the function of human IL-7; and

(3) a polypeptide comprising an amino acid sequence having a 90% or more identity with the amino acid sequence set forth in Accession No. NP_000871.1 and having the function of human IL-7.

In relation with this, the function of human IL-7 refers to the effect on the survival, proliferation, and differentiation of human immune cells.

[0046]

Human IL-7 used in the present invention is preferably a polypeptide consisting of the amino acid sequence set forth in Accession No. NP_000871.1.

[0047]

IL-12 is a heterodimer of the IL-12 subunit p40 and the IL-12 subunit α. IL-12 has been reported to have the function of activating and inducing the differentiation of T cells and NK cells (Cancer Immunology Immunotherapy,

2014, vol. 63, p. 419-435). In the present invention, IL-12 encompasses IL-12 occurring naturally and modified forms having the function thereof. In one embodiment, IL-12 is human IL-12. In the present invention, human IL-12 encompasses human IL-12 occurring naturally and modified forms having the function thereof. In one embodiment, human IL-12 is selected, as a combination of the human IL-12 subunit p40 and the human IL-12 subunit α, from the group consisting of the following (1) to (3):
(1) polypeptides comprising (1-a) a polypeptide comprising the amino acid sequence set forth in Accession No. NP_002178.2, (1-b) a polypeptide consisting of an amino acid sequence in which 1 to 10 amino acids are deleted from, substituted in, inserted into, and/or added to the amino acid sequence set forth in Accession No. NP_002178.2, or (1-c) a polypeptide comprising an amino acid sequence having a 90% or more identity with the amino acid sequence set forth in Accession No. NP_002178.2; and
(2-a) a polypeptide comprising the amino acid sequence set forth in Accession No. NP_000873.2, (2-b) a polypeptide consisting of an amino acid sequence in which 1 to 10 amino acids are deleted from, substituted in, inserted into, and/or added to the amino acid sequence set forth in Accession No. NP_000873.2, or (2-c) a polypeptide comprising an amino acid sequence having a

90% or more identity with the amino acid sequence set forth in Accession No. NP_000873.2, and
having the function of human IL-12;
(2) polypeptides comprising (1-a) a polypeptide consisting of the amino acid sequence set forth in Accession No. NP_002178.2, and
(2-a) a polypeptide comprising the amino acid sequence set forth in Accession No. NP_000873.2, (2-b) a polypeptide consisting of an amino acid sequence in which 1 to 10 amino acids are deleted from, substituted in, inserted into, and/or added to the amino acid sequence set forth in Accession No. NP_000873.2, or (2-c) a polypeptide comprising an amino acid sequence having a 90% or more identity with the amino acid sequence set forth in Accession No. NP_000873.2, and
having the function of human IL-12; and
(3) a polypeptide comprising (1-a) a polypeptide comprising the amino acid sequence set forth in Accession No. NP_002178.2, (1-b) a polypeptide consisting of an amino acid sequence in which 1 to 10 amino acids are deleted from, substituted in, inserted into, and/or added to the amino acid sequence set forth in Accession No. NP_002178.2, or (1-c) a polypeptide comprising an amino acid sequence having a 90% or more identity with the amino acid sequence set forth in Accession No. NP_002178.2, and

(2-a) a polypeptide consisting of the amino acid sequence set forth in Accession No. NP_000873.2, and
having the function of human IL-12.

In relation with this, the function of human IL-12 refers to activating and/or differentiating effects on T cells or NK cells. The IL-12 subunit p40 and the IL-12 subunit α can form IL-12 by direct binding. Moreover, the IL-12 subunit p40 and the IL-12 subunit α can be conjugated via a linker.

[0048]

Human IL-12 used in the present invention is preferably a polypeptide comprising a polypeptide consisting of the amino acid sequence set forth in Accession No. NP_002178.2 and a polypeptide consisting of the amino acid sequence set forth in Accession No. NP_000873.2.

[0049]

As used herein, "identity" means the value Identity obtained by a search using the NEEDLE program (Journal of Molecular Biology, 1970, vol. 48, p. 443-453) with the default parameters. The parameters are as follows.
Gap penalty = 10
Extend penalty = 0.5
Matrix = EBLOSUM62

[0050]

The vaccinia virus according to the present invention, the vaccinia virus to be used in combination,

or the vaccinia viruses for the combination kit has (have) the oncolytic activity. Examples of methods for evaluating whether or not a test virus has the oncolytic activity include a method for evaluating decrease of the survival rate of cancer cells by the addition of the virus. Examples of cancer cells to be used for the evaluation include the malignant melanoma cell RPMI-7951 (for example, ATCC HTB-66), the lung adenocarcinoma HCC4006 (for example, ATCC CRL-2871), the lung carcinoma A549 (for example, ATCC CCL-185), the small cell lung cancer cell DMS 53 (for example, ATCC CRL-2062), the lung squamous cell carcinoma NCI-H226 (for example, ATCC CRL-5826), the kidney cancer cell Caki-1 (for example, ATCC HTB-46), the bladder cancer cell 647-V (for example, DSMZ ACC 414), the head and neck cancer cell Detroit 562 (for example, ATCC CCL-138), the breast cancer cell JIMT-1 (for example, DSMZ ACC 589), the breast cancer cell MDA-MB-231 (for example, ATCC HTB-26), the esophageal cancer cell OE33 (for example, ECACC 96070808), the glioblastoma U-87MG (for example, ECACC 89081402), the neuroblastoma GOTO (for example, JCRB JCRB0612), the myeloma RPMI 8226 (for example, ATCC CCL-155), the ovarian cancer cell SK-OV-3 (for example, ATCC HTB-77), the ovarian cancer cell OVMANA (for example, JCRB JCRB1045), the colon cancer cell RKO (for example, ATCC CRL-2577), the colorectal carcinoma HCT 116 (for example, ATCC CCL-247), the pancreatic cancer cell BxPC-3 (for example, ATCC CRL-

1687), the prostate cancer cell LNCaP clone FGC (for example, ATCC CRL-1740), the hepatocellular carcinoma JHH-4 (for example, JCRB JCRB0435), the mesothelioma NCI-H28 (for example, ATCC CRL-5820), the cervical cancer cell SiHa (for example, ATCC HTB-35), and the gastric cancer cell Kato III (for example, RIKEN BRC RCB2088). Specific examples of methods for the evaluation that can be used include the method described in Example 3 below.
[0051]

The vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit produce(s) the IL-7 and/or IL-12 polypeptide(s). Use of the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit markedly increases the antitumor effect by producing the IL-7 and IL-12 polypeptides. The production of IL-7 and IL-12 can be confirmed using a method known in the field, for example, after culturing, with cancer cells, vaccinia virus in which polynucleotides encoding the IL-7 and IL-12 polypeptides are introduced followed by measuring the IL-7 and IL-12 concentrations in the culture supernatant, by immunostaining of cells, by conducting Western blot analysis of the cell lysate, or by measuring the concentrations of IL-7 and IL-12 in the cell lysate. The concentrations of IL-7 and IL-12 can be measured using,

for example, Human IL-7 ELISA kit (RayBiotech, Inc.) and Human IL-12 p70 DuoSet ELISA (R&D Systems, Inc.), respectively. Specific examples of methods for evaluating polypeptide concentrations in the culture supernatant or cell lysate that can be used include the method described in Example 4 below. The immunostaining of cells or the Western blot analysis of the cell lysate can be conducted using commercially available antibodies against IL-7 and IL-12.

[0052]

The polynucleotides encoding IL-7 and IL-12 can be synthesized based on publicly available sequence information using a method of polynucleotide synthesis known in the field. Moreover, once the polynucleotides are obtained; then modified forms having the function of each polypeptide can be generated by introducing mutation into a predetermined site using a method known by those skilled in the art, such as site-directed mutagenesis (Current Protocols in Molecular Biology edition, 1987, John Wiley & Sons Sections 8.1-8.5).

[0053]

The polynucleotides each encoding IL-7 and IL-12 can be introduced into vaccinia virus by a known technique, such as homologous recombination or site-directed mutagenesis. For example, a plasmid (also referred to as transfer vector plasmid DNA) in which the polynucleotide(s) is (are) introduced into the nucleotide

sequence at the site desired to be introduced can be made and introduced into cells infected with vaccinia virus. The region in which the polynucleotides each encoding IL-7 and IL-12, foreign genes, are introduced is preferably a gene region that is inessential for the life cycle of vaccinia virus. For example, in a certain aspect, the region in which IL-7 and/or IL-12 is (are) introduced may be a region within the VGF gene in vaccinia virus deficient in the VGF function, a region within the O1L gene in vaccinia virus deficient in the O1 function, or a region or regions within either or both of the VGF and O1L genes in vaccinia virus deficient in both VGF and O1 functions. In the above, the foreign gene(s) can be introduced so as to be transcribed in the direction same as or opposite to that of the VGF and O1L genes.

[0054]

Methods for introducing transfer vector plasmid DNA into cells are not particularly limited, but examples of methods that can be used include the calcium phosphate method and electroporation.

[0055]

When introducing the polynucleotides each encoding IL-7 and IL-12, which are foreign genes, a suitable promoter(s) can be operably linked in the upstream of the foreign gene(s). In this way, the foreign gene(s) in the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia

viruses for the combination kit can be linked to a promoter that can promote expression in tumor cells. Examples of such a promoter include PSFJ1-10, PSFJ2-16, the p7.5K promoter, the p11K promoter, the T7.10 promoter, the CPX promoter, the HF promoter, the H6 promoter, and the T7 hybrid promoter.

[0056]

In one embodiment, the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit has (have) no drug-selection marker gene.

[0057]

The vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit may be expressed and/or proliferated by infecting host cells with the vaccinia virus(es) and culturing the infected host cells. Vaccinia virus may be expressed and/or proliferated by a method known in the field. Host cells to be used to express or proliferate the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit are not particularly limited, as long as the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit can be expressed and proliferated. Examples of such host cells include

animal cells such as BS-C-1, A549, RK13, HTK-143, Hep-2, MDCK, Vero, HeLa, CV-1, COS, BHK-21, and primary rabbit kidney cells. BS-C-1 (ATCC CCL-26), A549 (ATCC CCL-185), CV-1 (ATCC CCL-70), or RK13 (ATCC CCL-37) may be preferably used. Culture conditions for the host cells, for example, temperature, pH of the medium, and culture time, are selected as appropriate.

[0058]

Methods for producing the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, and the vaccinia viruses for the combination kit may comprise the steps of: infecting host cells with the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit; culturing the infected host cells; and expressing the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit; and optionally collecting and preferably purifying the vaccinia virus according to the present invention, the vaccinia virus to be used in combination, or the vaccinia viruses for the combination kit. Methods that can be used for the purification include DNA digestion with Benzonase, sucrose gradient centrifugation, Iodixanol density gradient centrifugation, ultrafiltration, and diafiltration.

[0059]

<Pharmaceutical composition according to the present invention>

The pharmaceutical compositions according to the present invention include a pharmaceutical composition comprising the vaccinia virus according to the present invention and a pharmaceutically acceptable excipient. The pharmaceutical compositions according to the present invention also include the pharmaceutical composition to be used in combination according to the present invention. In one embodiment, the pharmaceutical composition to be used in combination according to the present invention comprises a pharmaceutically acceptable excipient.

[0060]

The pharmaceutical compositions according to the present invention may be prepared by a method usually used in the field, using an excipient usually used in the field, that is, a pharmaceutical excipient, a pharmaceutical carrier, or the like. Examples of the dosage form of such pharmaceutical compositions include parenteral formulations such as injections and infusions and these can be administered by intravenous administration, subcutaneous administration, intratumoral administration, or the like. In the formulation, excipients, carriers, or additives suitable for these dosages form may be used as long as these are pharmaceutically acceptable.

[0061]

The effective dose varies according to the severity of the symptom or the age of the patient, the dosage form of the formulation to be used, or the titer of the virus, but, for example, approximately 10^2–10^{10} plaque-forming units (PFU) may be used as an effective dose of a single virus, as a combined effective dose of 2 viruses in a combination kit, or as a combined effective dose of 2 viruses administered in combination. Two viruses in a combination kit may be used, for example, at a dosage ratio of approximately 1:10 to 10:1, approximately 1:5 to 5:1, approximately 1:3 to 3:1, approximately 1:2 to 2:1, or about 1:1.

[0062]

<Application for preventing or treating cancer>

The pharmaceutical compositions according to the present invention can be used as a prophylactic or therapeutic agent for cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer.

[0063]

The present invention includes a pharmaceutical composition for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer, the composition comprising the vaccinia virus according to the present invention or the vaccinia virus to be used in combination.

[0064]

The present invention includes a combination kit for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer, the combination kit comprising each of the vaccinia viruses for the combination kit.

[0065]

Moreover, the present invention includes a method for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer, the method comprising the step of administering the vaccinia virus according to the present invention to a subject (for example, a patient) in need of the prevention or treatment of cancer.

[0066]

Moreover, the present invention includes a method for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer, the method comprising the step of administering the following (1) and (2) to a subject (for example, a patient) in need of the prevention or treatment of cancer:

(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and

(2) a vaccinia virus comprising a polynucleotide encoding IL-12.

The two vaccinia viruses may be administered to a subject simultaneously, separately, continuously, or at intervals.

[0067]

Moreover, the present invention includes the vaccinia virus according to the present invention, for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer.

[0068]

The present invention includes the vaccinia virus selected from the following (1) or (2), for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer,

glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer:

(1) a vaccinia virus comprising a polynucleotide encoding IL-7, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or

(2) a vaccinia virus comprising a polynucleotide encoding IL-12, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

[0069]

Furthermore, the present invention includes use of the vaccinia virus according to the present invention, for the manufacture of a pharmaceutical composition for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer.

[0070]

The present invention includes use of a vaccinia virus selected from the following (1) or (2), for the manufacture of a pharmaceutical composition for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer:

(1) use of a vaccinia virus comprising a polynucleotide encoding IL-7, for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or

(2) use of a vaccinia virus comprising a polynucleotide encoding IL-12, for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

[0071]

Furthermore, the present invention includes use of a vaccinia virus comprising a polynucleotide encoding IL-7

and a vaccinia virus comprising a polynucleotide encoding IL-12, for the manufacture of a combination kit for preventing or treating cancer, for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer and gastric cancer.

[0072]

As used herein, "for preventing" is used synonymously with "for use in preventing" and "for treating" is used synonymously with "for use in treating".

[0073]

The pharmaceutical compositions or the combination kit according to the present invention may be used in combination with various therapeutic agents having efficacy for cancer for example, a cancer selected from the group consisting of malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer

and gastric cancer. The combination use may be performed by simultaneous administration, or separate administration continuously or at the desired interval. When administered simultaneously, the pharmaceutical compositions according to the present invention may be administered as a combined drug or as formulations formulated separately.

[0074]

Cancers that the vaccinia virus according to the present invention, the pharmaceutical compositions according to the present invention, the combination kit according to the present invention, the method for preventing or treating cancer according to the present invention, or use according to the present invention is (are) applied to include metastatic cancers to an organ, for example, a lymph node, liver, or the like, besides the primary lesion.

[0075]

The present invention has been generally described, but specific Examples for reference to get further understanding of the present invention are provided below. These Examples are for the illustration purpose, but not intended to limit the present invention.

Examples

[0076]

Experiments with a commercially available kit or a reagent were conducted according to attached protocols unless otherwise specified.

[0077]

(Example 1: Construction of transfer vector plasmid DNA)

Transfer vector plasmid DNAs to be used for generating recombinant vaccinia viruses by homologous recombination were prepared as follows.

[0078]

(1) Construction of pTN-VGF-P-DsRed transfer vector plasmid DNA

The pUC19-VGF vector was prepared according to WO 2015/076422. More specifically, genomic DNA (Accession No.AY678277.1) of the strain LC16mO was used as template and the pUC19 vector (product cord: 54357) from Invitrogen was used for the preparation of the pUC19-VGF vector. The prepared pUC19-VGF vector was digested with the restriction enzyme AccI and then the ends were blunted. The transfer vector plasmid DNA was constructed by inserting a DNA fragment (SEQ ID NO: 22) containing the p7.5k promoter and a DsRed fragment in this cleavage site. The constructed plasmid DNA was named pTN-VGF-P-DsRed.

(2) Construction of pTN-VGF-SP-IL12 and pTN-VGF-SP-IL7 transfer vector plasmid DNAs

A BFP gene region was amplified with two primers (SEQ ID NO: 1 and SEQ ID NO: 2) using DNA of the pTagBFP-

N vector (FP172, Evrogen) as template. The PCR product was digested with the restriction enzymes SfiI and EcoRI and cloned into the same restriction enzyme sites in the pTK-SP-LG vector (WO 2015/076422 with the proviso that genomic DNA (Accession No.AY678277.1) of the strain LC16mO was used as template and the pUC19 vector (product cord: 54357) from Invitrogen was used; and, for the pVNC110-Luc/IRES/EGFP plasmid, pVNC110-Luc/IRES/EGFP described in WO 2011/125469 was used.) to construct pTK-SP-BFP in which BFP is linked to a synthetic vaccinia virus promoter (Journal of Virological Methods, 1997, vol. 66, p. 135-138). Next, pTK-SP-BFP was digested with the restriction enzymes SphI and EcoRI and the ends were blunted. The resulting DNA fragment was cloned into the pUC19-VGF vector at a site generated by digesting the plasmid with the restriction enzyme AccI and blunting the ends to construct pTN-VGF-SP-BFP (FIG. 1). Next, a polynucleotide encoding human IL-12 (a polynucleotide containing the human IL-12 subunit p40, an internal ribosomal entry site, and the human IL-12 subunit α; SEQ ID NO: 7) and a polynucleotide (SEQ ID NO: 8) encoding human IL-7 (each polynucleotide contains the restriction enzyme site accggtcgccacc (SEQ ID NO: 16) at the 5' side and the restriction enzyme site gctagcgaattc (SEQ ID NO: 17) at the 3 'side.) were digested with the restriction enzymes AgeI and NheI. Each of the polynucleotide fragments was cloned into the same restriction enzyme

site in pTN-VGF-SP-BFP to construct the transfer vector plasmid DNA. The constructed plasmid DNAs were named pTN-VGF-SP-IL12 and pTN-VGF-SP-IL7, respectively.

(3) Construction of pTN-O1L-SP-BFP, pTN-O1L-SP-LacZ, pTN-O1L-SP-IL12, and pTN-O1L-SP-IL7 transfer vector plasmid DNAs

In the same way as (2) above, pTK-SP-BFP was digested with the restriction enzymes SphI and EcoRI and the DNA fragment obtained by blunting the ends was cloned into the pUC19-O1L vector (WO 2015/076422 with the proviso that, like the preparation of the pUC19-VGF vector, genomic DNA (Accession No.AY678277.1) of the strain LC16mO was used as template and the pUC19 vector (product cord: 54357) from Invitrogen was used; and the O1L gene region was inserted into the XbaI site in the pUC19 vector.) at a site generated by digesting the plasmid with the restriction enzyme XbaI and blunting the ends to construct the transfer vector plasmid DNA (FIG. 1). The prepared plasmid DNA was named pTN-O1L-SP-BFP. Next, a polynucleotide (SEQ ID NO: 9) containing the Escherichia coli LacZ gene with codons optimized for human, a polynucleotide (SEQ ID NO: 7) encoding human IL-12, and a polynucleotide (SEQ ID NO: 8) encoding human IL-7 were digested with the restriction enzymes AgeI and NheI. Each of the polynucleotide fragments encoding LacZ, IL-12, or IL-7 was cloned into the same restriction enzyme sites (the AgeI and NheI sites) in the pTN-O1L-SP-

BFP vector to construct the transfer vector plasmid DNA. The constructed plasmid DNAs were named pTN-O1L-SP-LacZ, pTN-O1L-SP-IL12, and pTN-O1L-SP-IL7, respectively.

[0079]

(4) Construction of pTN-DsRed (B5R-) and pTN-B5RΔ1-4 transfer vector plasmid DNAs

The B4R gene region was amplified with two primers (SEQ ID NO: 3 and SEQ ID NO: 4) using DNA of pB5R (WO 2011/125469, with the proviso that genomic DNA (Accession No.AY678277.1) of the strain LC16mO was used as template) as template. Moreover, the DsRed gene region was amplified with two primers (SEQ ID NO: 5 and SEQ ID NO: 6) using DNA of pDsRed-Express-N1 (Clontech Laboratories, Inc.) as template. The former PCR product was digested with the restriction enzymes NotI and FspI and the latter PCR product was digested with the restriction enzymes FspI and MfeI. These two DNA fragments were cloned into pB5R digested with the restriction enzymes NotI and MfeI to construct the transfer vector plasmid DNA. The prepared plasmid DNA was named pTN-DsRed (B5R-). Meanwhile, pB5R was digested with the restriction enzymes NotI and NspI or the restriction enzymes NspI and SacI. These two DNA fragments were cloned into pB5R digested with the restriction enzymes NotI and SacI to construct the transfer vector plasmid DNA. The prepared plasmid DNA was named pTN-B5RΔ1-4. pTN-B5RΔ1-4 encodes the B5R protein with the deletion of four SCR domains. The amino

acid sequence thereof is the sequence set forth in SEQ ID NO: 18.

[0080]

(Example 2: Construction of genetically engineered vaccinia virus)

[0081]

A recombinant vaccinia virus (referred to as LC16mO VGF-SP-LucGFP/O1L-p7.5-DsRed) deficient in the functions of VGF and O1L was prepared from the vaccinia virus strain LC16mO. This recombinant vaccinia virus was sequenced with a next-generation sequencer PacBio RSII (Pacific Biosciences of California, Inc.) and the virus genome was reconstituted from the obtained sequence information using the Sprai [BMC GENOMICS. 2014 AUG 21, 15:699.] software to determine the nucleotide sequence, which is the nucleotide sequence set forth in SEQ ID NO: 21. Moreover, loop sequences were added to both ends of the nucleotide sequence and the loop sequences at both ends were the nucleotide sequences set forth in SEQ ID NOs: 19 or 20.

[0082]

(1) The recombinant vaccinia viruses having the virus genome illustrated in FIG. 2 were collected. The virus collecting procedure is specifically described below. CV1 cells (ATCC CCL-70) or RK13 cells (ATCC CCL-37) cultured to 80% confluent in 6 well dishes were infected with LC16mO VGF-SP-LucGFP/O1L-p7.5-DsRed at a

Multiplicity of infection (MOI) = 0.02-0.1 and the virus was allowed to be adsorbed at room temperature for 1 hour. pTN-O1L-SP-BFP constructed in Example 1 (3) was mixed with FuGENE® HD Transfection Reagent (Roche), added to cells according to the manual to be incorporated into the cells and the cells were cultured at 5% CO_2 and 37°C for 2-5 days. The cells were freeze-thawed, sonicated, and diluted with Opti-MEM (Invitrogen) so as to obtain single plaques by the following operation. 100 μL of the resulting diluted fluid was added to inoculate BS-C-1 cells (ATCC CCL-26) or RK13 cells cultured to sub-confluent in 6 well dishes. 2 mL of the Eagle MEM medium (NISSUI, 05900) containing 0.8% methylcellulose (Wako Pure Chemical Industries, Ltd., 136-02155), 5% fetal bovine serum, 0.225% sodium bicarbonate (Wako Pure Chemical Industries, Ltd., 195-16411), and GlutaMAX (TM) Supplement I (GIBCO, 35050-061) was added and the cells were cultured at 5% CO_2 and 37°C for 2-5 days. The medium was removed and plaques, as indicated by the BFP expression, were scraped off with the pointing end of a tip to be suspended into Opti-MEM. This operation was repeated three times or more with BS-C-1 or RK13 cells to purify plaques and collect the virus plaques (In this Example, the procedure up to this point is hereinafter referred to as the "collecting".). The plaques were suspended into Opti-MEM and sonicated. Genomic DNA was extracted from 200 μL of the sonicated solution using

High Pure Viral Nucleic Acid Kit (Roche) according to the manual and screened by PCR. PCR was performed for VGF with the two primers (SEQ ID NO: 10 and SEQ ID NO; 11), for O1L with the two primers (SEQ ID NO: 12 and SEQ ID NO: 13), and for B5R with the two primers (SEQ ID NO: 14 and SEQ ID: NO 15). Among the clones from which an expected size of PCR product was detected, a virus clone for which the correct nucleotide sequence of the PCR product was confirmed by direct sequencing (referred to as LC16mO VGF-SP-LucGFP/O1L-SP-BFP. FIG. 2) was selected and proliferated with A549 (ATCC CCL-185) or RK13 cells and then the virus titer was measured with RK13 cells. Using LC16mO VGF-SP-LucGFP/O1L-SP-BFP and pTN-DsRed (B5R-) prepared in Example 1 (4), the recombinant virus, as indicated by the DsRed expression instead of the BFP expression, was collected in a way same as that described above. The virus was named LC16mO Δ-DsRed VGF-SP-LucGFP/O1L-SP-BFP (FIG. 2).

[0083]

(2) A recombinant virus having the deletion of the 4 SCR domains in the B5R protein was collected. Specifically, using LC16mO Δ-DsRed VGF-SP-LucGFP/O1L-SP-BFP prepared in Example 2 (1) and pTN-B5RΔ1-4 constructed in Example 1 (4), the recombinant virus, as indicated by the disappearance of DsRed expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The virus was named LC16mO ΔSCR VGF-SP-

LucGFP/O1L-SP-BFP (FIG. 2). Moreover, using the prepared LC16mO ΔSCR VGF-SP-LucGFP/O1L-SP-BFP and pTN-VGF-P-DsRed constructed in Example 1 (1), the recombinant virus, as indicated by the DsRed expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The virus is named LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-BFP (FIG. 2). Next, using the obtained LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-BFP and pTN-O1L-SP-LacZ constructed in Example 1 (3), the recombinant virus, as indicated by the disappearance of BFP expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The virus was named LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-LacZ (FIG. 2).

[0084]

(3) The SCR region-deleted recombinant vaccinia viruses having the virus genome illustrated in FIG. 3 and expressing a therapeutic gene and a marker gene were collected. Specifically, using each of LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-LacZ prepared in Example 2 (2) and the transfer vector plasmid DNAs (pTN-VGF-SP-IL12 and pTN-VGF-SP-IL7) constructed in Example 1 (2), each of the recombinant viruses, as indicated by the disappearance of DsRed expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The viruses were named LC16mO ΔSCR VGF-SP-IL12/O1L-SP-LacZ (hereinafter, referred to as the "hIL12-carrying vaccinia virus".) and LC16mO ΔSCR VGF-SP-IL7/O1L-SP-LacZ

(hereinafter, referred to as the "hIL7-carrying vaccinia virus".) (FIG. 3). For purification, A549 or RK13 cells were infected with each of the recombinant viruses. The cells were cultured at 5% CO_2 and 37°C for 2-5 days and then the infected cells were harvested. The cells were freeze-thawed and sonicated. The viruses were purified by density gradient centrifugation using OptiPrep (Axis-Shield Diagnostics Ltd.). The virus titer of each virus was measured with RK13 cells.

[0085]

(4) The SCR domain-deleted recombinant vaccinia virus having the virus genome illustrated in FIG. 4 and expressing a polynucleotide encoding human IL-7 and a polynucleotide encoding human IL-12 was collected.

(4-1) Specifically, using LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-BFP prepared in Example 2 (2) and the transfer vector plasmid DNA pTN-VGF-SP-IL12 constructed in Example 1 (2), each of the recombinant viruses, as indicated by the disappearance of DsRed expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The virus was named LC16mO ΔSCR VGF-SP-IL12/O1L-SP-BFP.

[0086]

(4-2) Next, using LC16mO ΔSCR VGF-SP-IL12/O1L-SP-BFP prepared in Example 2 (4-1) and the transfer vector plasmid DNA pTN-O1L-SP-IL7 constructed in Example 1 (3), each of the recombinant viruses, as indicated by the

disappearance of BFP expression instead of the BFP expression, was collected in a way same as that in Example 2 (1). The virus was named LC16mO ΔSCR VGF-SP-IL12/O1L-SP-IL7 (hereinafter, in Examples below, also referred to as the "hIL12 and hIL7-carrying vaccinia virus".) (FIG. 4). Each recombinant virus was purified by the method in Example 2 (3) and then the virus titer of each virus was measured with RK13 cells.

[0087]

(Example 3: Oncolytic property of genetically engineered vaccinia virus)

The ability of the hIL12 and hIL7-carrying vaccinia virus prepared in Example 2 to lyse various human cancer cells (ability to kill cells) was evaluated. Moreover, the ability of a combined mixture of 2 viruses, the hIL12-carrying vaccinia virus and the hIL7-carrying vaccinia virus prepared in Example 2, to lyse various human cancer cells was similarly evaluated.

[0088]

Specifically, 100 μL each of the cells suspended at 1×10^4 cells/mL in a medium (a medium described below containing 10% fetal bovine serum (GE Healthcare) and 1% penicillin-streptomycin (Life Technologies)) was first added into 96 well plates (AGC TECHNO GLASS CO., LTD.). After culturing overnight, 1) the hIL12 and hIL7-carrying vaccinia virus and 2) a mixture combining 1:1 concentrations of the hIL12-carrying vaccinia virus and

the hIL7-carrying vaccinia virus (hereinafter, referred to as the "mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus") were each diluted with Opti-MEM (Life Technologies) at 5×10^4 PFU/mL, 5×10^5 PFU/mL, and 5×10^6 PFU/mL, respectively. 20 μL each of the virus solutions was added to each well to infect cells at MOI = 1.0, 10, or 100. As control, wells with no cells and wells to which Opti-MEM was added instead of virus (MOI = 0) were prepared. The cells were then cultured for 5 days in a CO_2 incubator set to a CO_2 concentration of 5% and at 37°C. The cell survival rate on Day 5 was measured with CellTiter-Glo Luminescent Cell Viability Assay (Promega KK.). Specifically, according to the protocol of the assay kit, 100 μL each of CellTiter-Glo Reagent was added to each well and left to stand for 30 minute, the total amount was then transferred into 96 well black plates (Corning Incorporated), and the strength of luminescence in each well was measured with EnSpire (PerkinElmer Inc.). For the calculation of the cell survival rate in each well, the value of wells in which no cells have seeded was defined as 0% survival and the value of wells in which cells have seeded and no virus was added was defined as100% survival.

[0089]

The evaluated cells were the malignant melanoma cell RPMI-7951 (ATCC HTB-66), the lung adenocarcinoma HCC4006

(ATCC CRL-2871), the lung carcinoma A549 (ATCC CCL-185), the small cell lung cancer cell DMS 53, the lung squamous cell carcinoma NCI-H226 (ATCC CRL-5826), the kidney cancer cell Caki-1 (ATCC HTB-46), the bladder cancer cell 647-V (DSMZ ACC 414), the head and neck cancer cell Detroit 562 (ATCC CCL-138), the breast cancer cell JIMT-1 (DSMZ ACC 589), the breast cancer cell MDA-MB-231 (ATCC HTB-26), the esophageal cancer cell OE33 (ECACC 96070808), the glioblastoma U-87MG (ECACC 89081402), the neuroblastoma GOTO (JCRB JCRB0612), the myeloma RPMI 8226 (ATCC CCL-155), the ovarian cancer cell SK-OV-3 (ATCC HTB-77), the ovarian cancer cell OVMANA (JCRB JCRB1045), the colon cancer cell RKO (ATCC CRL-2577), the colorectal carcinoma HCT 116, the pancreatic cancer cell BxPC-3 (ATCC CRL-1687), the prostate cancer cell LNCaP clone FGC (ATCC CRL-1740), the hepatocellular carcinoma JHH-4 (JCRB JCRB0435), the mesothelioma NCI-H28 (ATCC CRL-5820), the cervical cancer cell SiHa (ATCC HTB-35) and the gastric cancer cell Kato III (RIKEN BRC RCB2088).

[0090]

The media used were RPMI1640 medium (Sigma-Aldrich Co. LLC., R8758) for RPMI-7951, HCC4006, DMS 53, NCI-H226, Caki-1, 647-V, Detroit 562, JIMT-1, OE33, U-87MG, GOTO, RPMI8226, SK-OV-3, OVMANA, RKO, HCT 116, BxPC-3, LNCaP clone FGC, JHH-4, NCI-H28, and Kato III, DMEM medium (Sigma-Aldrich Co. LLC., D6429) for A549 and MDA-MB-231, and EMEM medium (ATCC 30-2003) for SiHa. The results

were as illustrated in FIGS. 5-1 to 5-4. In relation with this, the effects of the hIL12 and hIL7-carrying vaccinia virus were illustrated separately in FIGS. 5-1 and 5-2 and the effects of the mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus were illustrated separately in FIGS. 5-3 and 5-4.

[0091]

As a result, the hIL12 and hIL7-carrying vaccinia virus was shown to have the ability to kill cells in all examined human cancer cells (FIGS. 5-1 and 5-2). Moreover, the mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus was also shown to have the ability to kill cells in all examined human cancer cells (FIGS. 5-3 and 5-4). In FIGS. 5-1 to 5-4, the oncolytic properties at MOI = 0, 1, 10, and 100 are shown from the left in this order for each cell line.

[0092]

(Example 4: Protein production from cancer cells infected with genetically engineered vaccinia virus)

When cancer cells were infected with the hIL12 and hIL7-carrying vaccinia virus, the concentrations of the human IL-7 protein and the human IL-12 protein produced by cancer cells were measured. Furthermore, the concentrations of the human IL-7 protein and the human IL-12 protein produced by cancer cells when cancer cells were infected with the mixture of hIL12-carrying vaccinia

virus and hIL7-carrying vaccinia virus were similarly measured.

[0093]

The measurement of the human IL-7 protein was conducted as follows. Specifically, first, 100 μL of SK-OV-3 ovarian cancer cells suspended at 1×10^4 cells/mL in RPMI1640 medium containing 10% fetal bovine serum and the 1% penicillin-streptomycin was seeded into 96 well plates. After culturing overnight, 1) the hIL12 and hIL7-carrying vaccinia virus or 2) the mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus was prepared in Opti-MEM and 20 μL each was added to infect the cells at MOI = 1.0. The cells were then cultured for 24 hours in a CO_2 incubator set at a CO_2 concentration of 5% and 37°C and the culture supernatant was collected. The concentration of the protein contained in the culture supernatant was measured with the ELISA kit listed in Table 1 and EnSpire.

[0094]

The measurement of the human IL-12 protein was conducted as follows. Specifically, first, 100 μL of SK-OV-3 ovarian cancer cells suspended at 1×10^5 cells/mL in RPMI1640 medium containing 10% fetal bovine serum and the 1% penicillin-streptomycin was seeded into 96 well plates. After culturing overnight, 1) the hIL12 and hIL7-carrying vaccinia virus or 2) the mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus

was prepared in Opti-MEM and 20 μL each was added to infect the cells at MOI = 1.0. The cells were then cultured for 48 hours in a CO_2 incubator set at a CO_2 concentration of 5% and 37°C and the culture supernatant was collected. The concentration of the protein contained in the culture supernatant was measured with the ELISA kit listed in Table 1 and EnSpire.

[0095]

Table 1: ELISA kit used in Example 4

[Table 1]

Protein	ELISA kit	Provider
Human IL-7	Human IL-7 ELISA kit	RayBiotech, Inc.
Human IL-12	Human IL-12 p70 DuoSet ELISA	R&D Systems, Inc.

[0096]

As a result, it was shown that the human IL-12 protein and the human IL-7 protein were produced from the cells to which the hIL12 and hIL7-carrying vaccinia virus was added and the cells to which the mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus was added (Tables 2-1 and 2-2).

[0097]

Table 2-1: Concentration of human IL-12 protein in culture supernatant

[Table 2-1]

Genetically engineered vaccinia virus	Human IL-12 protein concentration (ng/mL)
hIL12 and hIL7-carrying vaccinia virus	31.45
Mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus	17.74

[0098]

Table 2-2: Concentration of human IL-7 protein in culture supernatant

[Table 2-2]

Genetically engineered vaccinia virus	Human IL-7 protein concentration (ng/mL)
hIL12 and hIL7-carrying vaccinia virus	0.86
Mixture of hIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus	0.60

[0099]

(Example 5: Construction of transfer vector plasmid DNA carrying polynucleotide encoding murine IL-12 and construction of recombinant vaccinia virus carrying polynucleotide encoding murine IL-12)

(1) The transfer vector plasmid DNA pTN-VGF-SP-mIL12 was constructed according to the method described in Example 1 (2). Instead of the polynucleotide (SEQ ID NO: 7) encoding human IL-12 in the method described in Example 1 (2), a polynucleotide encoding murine IL-12 (a polynucleotide containing the murine IL-12 subunit p40, an internal ribosomal entry site, and the murine IL-12

subunit α. SEQ ID NO: 23) was used and this polynucleotide fragment was cloned into pTN-VGF-SP-BFP.

(2) The transfer vector plasmid DNA pTN-O1L-SP-Luc2 was constructed according to the method described in Example 1 (3). Instead of the polynucleotide (SEQ ID NO: 9) containing the *Escherichia coli* LacZ gene in the method described in Example 1 (3), a polynucleotide (100-1752 in Accession No.DQ188840) encoding the luciferase Luc2 gene was used and this polynucleotide fragment was cloned into pTN-O1L-SP-BFP.

[0100]

(3) The recombinant virus was collected according to the method in Example 2 (2). In the method in Example 2 (2), LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-BFP and pTN-O1L-SP-Luc2 prepared in Example 5 (2) instead of pTN-O1L-SP-LacZ were used. The virus was named LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-Luc2 (hereinafter, this virus is also referred to as the "control vaccinia virus".).

[0101]

(4) The recombinant virus was collected according to the method in Example 2 (3). Instead of LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-LacZ and pTN-VGF-SP-IL12 in the method in Example 2 (3), respectively, LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-Luc2 prepared in Example 5 (3) and pTN-VGF-SP-mIL12 prepared in Example 5 (1) were used. The virus was named LC16mO ΔSCR VGF-SP-mIL12/O1L-SP-Luc2

(hereinafter, also referred to as the "mIL12-carrying vaccinia virus".).

[0102]

(5) The recombinant virus was collected according to the method in Example 2 (4-1). LC16mO ΔSCR VGF-p7.5-DsRed/O1L-SP-BFP and pTN-VGF-SP-mIL12 prepared in Example 5 (1) instead of pTN-VGF-SP-IL12 in the method in Example 2 (4-1) were used. The virus was named LC16mO ΔSCR VGF-SP-mIL12/O1L-SP-BFP.

Furthermore, the recombinant virus was collected according to the method in Example 2 (4-2). Instead of LC16mO ΔSCR VGF-SP-IL12/O1L-SP-BFP in the method in Example 2 (4-2), LC16mO ΔSCR VGF-SP-mIL12/O1L-SP-BFP prepared as described above and pTN-O1L-SP-IL7 were used. The virus was named LC16mO ΔSCR VGF-SP-mIL12/O1L-SP-IL7 (hereinafter, also referred to as the "mIL12 and hIL7-carrying vaccinia virus".).

[0103]

(Example 6: Antitumor effect of genetically engineered vaccinia virus in cancer-bearing humanized mouse)

The *in vivo* antitumor effect of the hIL12 and hIL7-carrying vaccinia virus was evaluated using humanized mice (mice in which the immune system is replaced with human immune cells by introducing human hematopoietic stem cells into a severely immunodeficient mouse) into which human cancer cells are transplanted.

[0104]

Specifically, in order to generate humanized mice, 3×10^4 hematopoietic stem cells (Lonza) derived from human umbilical cord blood were first introduced by injecting via a tail vein into NOG mice (NOD/Shi-scidIL-2RγKO Jic, female, 6 week-old, CLEA Japan, Inc.) irradiated with X-ray at a strength of 2.0 grays using an X-ray irradiation apparatus. 13 weeks after the introduction, 100 μL of the human lung cancer cell NCI-H1373 (ATCC CRL-5866) suspended at 3×10^7 cells/mL in PBS was transplanted by injecting the cells subcutaneously in the right back side of the mice. The tumor diameter was measured with a caliper after cancer cell transplantation and the mice were assigned to groups so that the mean tumor volumes of the groups (minor axis mm × minor axis mm × major axis mm × 0.52) will become 37 mm^3 to 47 mm^3. On the same day, 20 μL of the hIL12 and hIL7-carrying vaccinia virus diluted to a concentration of 1.0×10^8 PFU/mL in PBS was injected into tumor (referred to as the "hIL12 and hIL7-carrying VV treated group" in the Table). 20 μL of PBS was administered into tumor in a group, which was referred to as the vehicle (PBS) treated group. The tumor diameter of each mouse was measured with a caliper every 2-4 days, the tumor volume was calculated based on the formula above, and the percent (%) change in tumor volume on the 14th day after the virus administration was calculated by the following formula for each individual (n = 7-8):

Percent (%) change in tumor volume on 14th day after virus administration = 100 (%)× tumor volume (mm^3) on 14th day after virus administration/tumor volume (mm^3) on day of virus administration.

The tumor regression effect was determined to be positive when the mean percent (%) change in tumor volume on the 14th day after the virus administration of each group was less than 100 and a significant difference was observed (the significant difference was defined when p value < 0.05) between the tumor volume on the 14th day after the virus administration and the tumor volume on the day of the virus administration in each group when tested by the paired t-test.

In this Example, the control vaccinia virus (2×10^6 PFU/individual), the hIL12-carrying vaccinia virus (2×10^6 PFU/individual), or the hIL7-carrying vaccinia virus (2×10^6 PFU/individual) (referred to as the "control VV treated group", the "hIL12-carrying VV treated group", and the "hIL7-carrying VV treated group" in the Table.) were used with the same injection volume (20 μL) and the same dilution solution (PBS) as a virus compared with the hIL12 and hIL7-carrying vaccinia virus (2×10^6 PFU/individual).

[0105]

As a result, the hIL12 and hIL7-carrying vaccinia virus treated group exhibited a mean percent change in tumor volume on the 14th day after the virus

administration of less than 100%. Furthermore, there was a significant difference observed between the tumor volume on the 14 days after the virus administration and the tumor volume on the day of virus administration examined by the paired t-test and therefore the tumor regression effect was determined to be positive (Table 3). Thus, the administration of the hIL12 and hIL7-carrying vaccinia virus was shown to have the tumor regression effect. On the other hand, the tumor regression effect was not confirmed in the group receiving either of the hIL12-carrying vaccinia virus or the hIL7-carrying vaccinia virus (Table 3).

[0106]

Table 3: Percent (%) change in tumor volume in cancer-bearing humanized mouse with the hIL12 and hIL7-carrying vaccinia virus

[Table 3]

Experimental group	n	Percent (%) change in tumor volume on 14th day after administration Mean +/- standard error	p value (tumor volume on 14th day after virus administration and tumor volume on day of virus administration were examined by the paired t-test)
Vehicle (PBS) treated group	7	653 ± 43	<0.05
Control VV treated group	7	187 ± 39	0.09
hIL7-carrying VV treated group	8	199 ± 33	<0.05
hIL12-carrying VV treated group	8	140 ± 29	0.40
hIL12 and hIL7-carrying VV treated group	8	61 ± 6	<0.05

[0107]

(Example 7: Complete remission-inducing effect of genetically engineered vaccinia virus in syngeneic cancer-bearing mouse models)

[0108]

(1) Effect of mIL12 and hIL7-carrying vaccinia virus

The complete remission-inducing effect of the mIL12 and hIL7-carrying vaccinia virus *in vivo* was evaluated using mice subcutaneously transplanted with syngeneic murine cancer cell line (syngeneic cancer-bearing mice). Since human IL-12 is known to have no effect on murine immune cells, a genetically engineered vaccinia virus carrying a polynucleotide encoding murine IL-12 instead

of the polynucleotide encoding human IL-12 (prepared in Example 5) was used.

[0109]

Specifically, 50 μL of the murine lung cancer cell LL/2 (LLC1) (ATCC CRL-1642) (hereinafter referred to as LLC1) prepared at 4×10^6 cells/mL in PBS was first subcutaneously transplanted in the right flank of C57BL/6J mice (male, 5-7 week-old, CHARLES RIVER LABORATORIES JAPAN, INC.). The tumor volume was calculated in a way same as that in Example 6 and mice were assigned to groups so that the mean tumor volume of each group will become 50 mm^3 to 60 mm^3. On the next day, 30 μL of the mIL12 and hIL7-carrying vaccinia virus diluted to a concentration of 6.7×10^8 PFU/mL in PBS was intratumorally injected in 12 mice (2×10^7 PFU, referred to as the "mIL12 and hIL7-carrying VV treated group" in the Table.). Similar intratumoral injection of the virus was conducted 2 days and 4 days after the first administration. 30 μL of PBS instead of the virus was intratumorally administered in a group, which was referred to as the vehicle (PBS) treated group.

The tumor diameter was measured with a caliper twice a week and the tumor volume was calculated. Absence of tumor observed by palpation on 27th day after the first administration of the virus was defined as complete remission and the number of individuals achieved complete remission was counted. Groups reached a mean tumor

volume above 1,700 mm^3 during the test period were euthanized from the viewpoint of animal ethic. In this example, the control vaccinia virus, the mIL12-carrying vaccinia virus, or the hIL7-carrying vaccinia virus (each 2×10^7 PFU/dose, three doses) (respectively referred to as the "control VV treated group", the "mIL12-carrying VV treated group", and the "hIL7-carrying VV treated group" in the Table.) was used with the same injection volume (30 μL per dose) and the same dilution solution (PBS) as a virus compared with the mIL12 and hIL7-carrying vaccinia virus (2×10^7 PFU/dose, three doses).

[0110]

As a result, three individuals finally achieved complete remission in the mIL12 and hIL7-carrying vaccinia virus treated group. On the other hand, no individual achieved complete remission in the group receiving the comparison virus (Table 4-1). Thus, the administration of the mIL12 and hIL7-carrying vaccinia virus was shown to have a higher complete remission-inducing effect in comparison with the hIL7-carrying vaccinia virus or the mIL12-carrying vaccinia virus in syngeneic cancer-bearing mouse models.

[0111]

Table 4-1: The number of mice individual that achieved complete remission by administration of mIL12 and hIL7-carrying vaccinia virus

[Table 4-1]

Experimental group	Number of mouse individual achieved complete remission/Number of mouse individual examined
Vehicle (PBS) treated group	0/12
Control VV treated group	0/12
hIL7-carrying VV treated group	0/12
mIL12-carrying VV treated group	0/12
mIL12 and hIL7-carrying VV treated group	3/12

[0112]

(2) a mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus

The complete remission-inducing effect of a 1:1 mixture of the mIL12-carrying vaccinia virus and the hIL7-carrying vaccinia virus (hereinafter, referred to as the "mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus") *in vivo* was evaluated using syngeneic cancer-bearing mice.

[0113]

Experiment was conducted in the same way as (1), with the proviso that the murine lung cancer cell LLC1 suspended at 8×10^6 cells/mL was transplanted. Furthermore, instead of 30 μL (2×10^7 PFU) of the mIL12 and hIL7-carrying vaccinia virus diluted to a concentration of 6.7×10^8 PFU/mL, 30 μL (each 2×10^7 PFU/dose, three doses) of the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus (each virus was diluted to 6.7×10^8 PFU/mL in PBS) (referred

to as the "treatment group of mixture of mIL12-carrying VV and hIL7-carrying VV" in the Table.) was used. Seven mice (n = 7) were used. The control vaccinia virus (4×10^7 PFU/dose, three doses), the 1:1 mixture of the mIL12-carrying vaccinia virus and the control vaccinia virus (each 2×10^7 PFU/dose, three doses), or the 1:1 mixture of the hIL7-carrying vaccinia virus and the control vaccinia virus (each 2×10^7 PFU/dose, three doses) (respectively, referred to as the "control VV treated group", the " treatment group of mixture of mIL12-carrying VV and control VV", and the " treatment group of mixture of hIL7-carrying VV and control VV" in the Table.) was used with an injection volume of 30 μL each as a comparison virus.

[0114]

As a result, four individuals in the group receiving the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus achieved complete remission. Only one individual achieved complete remission in the group receiving the mixture of mIL12-carrying vaccinia virus and the control vaccinia virus, while no individual achieved complete remission in the groups receiving other comparison viruses (Table 4-2). Thus, the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus was shown to have higher complete remission-inducing effect in comparison with mixtures containing either of the hIL7-carrying vaccinia virus or the mIL12-

carrying vaccinia virus in a syngeneic cancer-bearing mouse model.

[0115]

Table 4-2: The number of mice individual that achieved complete remission by administration of the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus

[Table 4-2]

Experimental group	Number of mouse individual achieved complete remission/Number of mouse individual examined
Vehicle (PBS) treated group	0/7
Control VV treated group	0/7
Treatment group of mixture of hIL7-carrying VV and control VV	0/7
Treatment group of mixture of mIL12-carrying VV and control VV	1/7
Treatment group of mixture of mIL12-carrying VV and hIL7-carrying VV	4/7

[0116]

(Example 8: Acquired immunity effect of genetically engineered vaccinia virus in syngeneic cancer-bearing mouse models (tumor-rejecting effect by acquired immunity))

(1) mIL12 and hIL7-carrying vaccinia virus:

To the mice achieved complete remission as a result of treating with the mIL12 and hIL7-carrying vaccinia virus, the rechallenge experiment of the same cancer

cells was conducted to evaluate acquired immunity effect of the virus.

[0117]

Specifically, LLC1 cancer-bearing mice were first generated according to Example 7, and the mIL12 and hIL7-carrying vaccinia virus was intratumorally administered in the mice (with the proviso that the intratumoral injection of the virus was also conducted on 1st and 3rd days after the first administration in addition to 2nd and 4th days (total 5 times); referred to as the "mIL12 and hIL7-carrying VV treated group" in the Table.). The complete remission was confirmed on 23th day after the last administration of the virus. Into the individuals that still maintain the complete remission state on 51th day after the last administration and age-matched mice not inoculated with virus (control group), 50 μL of LLC1 cancer cells suspended at 8×10^6/mL in PBS was subcutaneously transplanted. The tumor volume was calculated according to Example 6 and the number of individuals that were recognized to have tumor formation by visual observation and palpation on 14th day after the LLC1 transplantation was counted to determine the ratio of the number of mouse individuals having engrafted tumor /the number of mouse individuals in which cancer cells were transplanted. In this Example, the control group and the virus treated group were tested by the Fisher's exact test and the acquired immunity effect was evaluated

to be positive when there was a significant difference (less than 5%).

[0118]

As a result, subcutaneous tumor was formed in the all cases of 10 individuals in the total 10 individuals in the control group, but 6 individuals in the total 10 individuals in the mIL12 and hIL7-carrying virus treated group had no tumor formation of rechallenged LLC1 cancer cells found in the visual observation and palpation (Table 5-1) ($P < 0.05$, Fisher's exact test). Thus, the acquired immunity effect of the administration of the mIL12 and hIL7-carrying vaccinia virus was confirmed in this Example.

[0119]

Table 5-1: Result of cancer cell rechallenge test in mice achieved complete remission

[Table 5-1]

Experimental group	Number of mouse individual having engrafted tumor /Number of mouse individual in which cancer cells were transplanted
Control group	10/10
mIL12 and hIL7-carrying VV treated group	4/10

[0120]

(2) Mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus:

To the mice achieved complete remission as a result of treating with the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus, the rechallenge experiment of the same cancer cells was conducted to evaluate acquired immunity effect of the virus.

[0121]

Specifically, the experiment was conducted in the same way as in (1). However, instead of the mice achieved complete remission by the administration of the mIL12 and hIL7-carrying vaccinia virus, the mice achieved complete remission by the administration of the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus according to Example 7 (2) (1st, 3rd, and 5th day after the group assignment, total 3 times) were used (referred to as the "treatment group of mixture of mIL12-carrying VV and hIL7-carrying VV" in the Table.). The further transplantation of the cancer cells was conducted on 74th day after the last administration of the viruses (determination of complete remission was made on 24th day after the last administration).

[0122]

As a result, subcutaneous tumor was formed in the all individuals in the total eight individuals in the control group on 14th day after further transplantation of the cancer cells, but eight individuals in the total 10 individuals in the treatment group of mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia

virus had no tumor formation of rechallenged LLC1 cancer cells found in the visual observation and palpation (Table 5-2) ($P < 0.05$, Fisher's exact test).

Thus, the acquired immunity effect of the administration of the mixture of mIL12-carrying vaccinia virus and hIL7-carrying vaccinia virus was confirmed in this Example.

[0123]

Table 5-2: Result of cancer cell rechallenge test in mice achieved complete remission

[Table 5-2]

Experimental group	Number of mouse individual having engrafted tumor/Number of mouse individual in which cancer cells were transplanted
Control group	8/8
Treatment group of mixture of mIL12-carrying VV and hIL7-carrying VV	2/10

Industrial Availability

[0124]

The vaccinia virus, the pharmaceutical composition, and the combination kit according to the present invention are useful for preventing or treating various cancers.

Free text of sequence listing

[0125]

The description of "Artificial Sequence" is stated in the numeric identifier <223> of the sequence listing.

The nucleotide sequences set forth in SEQ ID NOs: 1-6 and 10-15 are primers.

The nucleotide sequences set forth in SEQ ID NOs: 7, 8, and 9 are a polynucleotide containing the human IL-12 gene, a polynucleotide containing the human IL-7 gene, and a polynucleotide containing the Escherichia coli LacZ gene, respectively. In SEQ ID NO: 7, the nucleotide sequence of 14-1000 corresponds to the region encoding the p40 subunit of IL-12 and the nucleotide sequence of 1606-2367 corresponds to the region encoding the subunit α of IL-12.

The nucleotide sequences set forth in SEQ ID NOs: 16 and 17 are the restriction enzyme sites linked to each of the gene coding regions of SEQ ID NOs: 7-9.

The amino acid sequence set forth in SEQ ID NO: 18 is a B5R protein having the deletion of the 4 SCR domains.

The nucleotide sequences set forth in SEQ ID NOs: 19 and 20 are the sequences of loop sequences at both ends in LC16mO VGF-SP-LucGFP/O1L-p7.5-DsRed.

The nucleotide sequence set forth in SEQ ID NO: 21 is the sequence except the loop sequences at both ends in LC16mO VGF-SP-LucGFP/O1L-p7.5-DsRed.

The nucleotide sequence set forth in SEQ ID NO: 22 is a DNA fragment containing the p7.5k promoter and the DsRed fragment.

The nucleotide sequence set forth in SEQ ID NO: 23 is a polynucleotide containing the murine IL-12 gene.

What is claimed is:

1. A vaccinia virus comprising the following (1) and (2):

(1) a polynucleotide encoding interleukin-7 (IL-7); and

(2) a polynucleotide encoding interleukin-12 (IL-12).

2. A pharmaceutical composition selected from the following (1) or (2):

(1) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7; or

(2) a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7 to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12.

3. A combination kit comprising the following vaccinia viruses (1) and (2):

(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and

(2) a vaccinia virus comprising a polynucleotide encoding IL-12.

4. The vaccinia virus according to claim 1, wherein the vaccinia virus is deficient in the function of vaccinia virus growth factor (VGF).

5. The vaccinia virus according to claim 1, wherein the vaccinia virus is deficient in the function of O1L.

6. The vaccinia virus according to claim 1, wherein the vaccinia virus is deficient in the functions of VGF and O1L.

7. The vaccinia virus according to claim 1, wherein the vaccinia virus has a deletion in the short consensus repeat (SCR) domains in the B5R extracellular region.

8. The vaccinia virus according to claim 1, wherein the vaccinia virus is deficient in the functions of VGF and O1L and has a deletion in the SCR domains in the B5R extracellular region.

9. The vaccinia virus according to any one of claims 1 and 4-8, wherein the vaccinia virus is a LC16mO strain.

10. A pharmaceutical composition comprising a vaccinia virus according to any one of claims 1 and 4-9 and a pharmaceutically acceptable excipient.

11. The pharmaceutical composition according to claim 2 or the kit according to claim 3, wherein the vaccinia virus is deficient in the function of VGF.

12. The pharmaceutical composition according to claim 2 or the kit according to claim 3, wherein the vaccinia virus is deficient in the function of O1L.

13. The pharmaceutical composition according to claim 2 or the kit according to claim 3, wherein the vaccinia virus is deficient in the functions of VGF and O1L.

14. The pharmaceutical composition according to claim 2 or the kit according to claim 3, wherein the vaccinia virus has a deletion in the SCR domains in the B5R extracellular region.

15. The pharmaceutical composition according to claim 2 or the kit according to claim 3, wherein the vaccinia virus is deficient in the functions of VGF and O1L and has a deletion in the SCR domains in the B5R extracellular region.

16. The pharmaceutical composition according to any one of claims 2 and 11-15 or the kit according to any one of claims 3 and 11-15, wherein the vaccinia virus is a LC16mO strain.

17. The pharmaceutical composition according to any one of claims 2 and 11-16 or the kit according to any one of claims 3 and 11-16, further comprising a pharmaceutically acceptable excipient.

18. The pharmaceutical composition or kit according to any one of claims 10-17, for preventing or treating cancer.

19. The pharmaceutical composition or kit according to claim 18, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

20. A method for preventing or treating cancer, comprising the step of administering the vaccinia virus according to any one of claims 1 and 4-9 to a subject in need of the prevention or treatment for cancer.

21. The method according to claim 20, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer,

small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

22. The vaccinia virus according to any one of claims 1 and 4-9, for use in preventing or treating cancer.

23. The vaccinia virus according to claim 22, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

24. Use of the vaccinia virus according to any one of claims 1 and 4-9 for the manufacture of a pharmaceutical composition for preventing or treating cancer.

25. The use according to claim 24, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney

cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

26. A method for preventing or treating cancer, comprising the step of administering
(1) a vaccinia virus comprising a polynucleotide encoding IL-7; and
(2) a vaccinia virus comprising a polynucleotide encoding IL-12
to a subject in need of the prevention or treatment for cancer.

27. The method according to claim 26, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

28. A vaccinia virus selected from the following (1) or (2):

(1) a vaccinia virus comprising a polynucleotide encoding IL-7, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or
(2) a vaccinia virus comprising a polynucleotide encoding IL-12, for preventing or treating cancer in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

29. The vaccinia virus according to claim 28, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

30. Use of a vaccinia virus selected from the following (1) or (2):
(1) use of a vaccinia virus comprising a polynucleotide encoding IL-7 for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-12; or

(2) use of a vaccinia virus comprising a polynucleotide encoding IL-12 for the manufacture of a pharmaceutical composition for preventing or treating cancer to be used in combination with a pharmaceutical composition comprising a vaccinia virus comprising a polynucleotide encoding IL-7.

31. The use according to claim 30, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma, myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.

32. Use of (1) a vaccinia virus comprising a polynucleotide encoding IL-7 and (2) a vaccinia virus comprising a polynucleotide encoding IL-12 for the manufacture of a combination kit for preventing or treating cancer.

33. The use according to claim 32, wherein the cancer is malignant melanoma, lung adenocarcinoma, lung cancer, small cell lung cancer, lung squamous carcinoma, kidney cancer, bladder cancer, head and neck cancer, breast cancer, esophageal cancer, glioblastoma, neuroblastoma,

myeloma, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, mesothelioma, cervical cancer or gastric cancer.