problems may occur, such as transplant rejection, or immunological elimination caused by recognition of non-self *in vivo*. Accordingly, in order to overcome these drawbacks, there is a need for an alternative to making allogeneic immune cells into a cell banking while allowing the allogeneic immune cells to be recognized as self.

Disclosure

The present inventors have synthesized guide RNAs that target a gene encoding MHC I cell membrane receptor and a gene encoding MHC II cell membrane receptor in a cell. In addition, the present inventors have prepared a cell, in which expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited, using a composition for inhibiting gene expression which comprises, as active ingredients, the guide RNA and an RNA-guided endonuclease, wherein HLA-E may be introduced thereto so that *in vivo* immunological elimination to the cell is prevented.

Aspects of the invention are defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

Thus, there is disclosed a guide RNA that complementarily binds to a nucleic acid sequence encoding β2-microglobulin (B2M), the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 17, and SEQ ID NO: 26; a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DQ, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 87, and SEQ ID NO: 90; a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DP, the guide RNA comprising the nucleic acid sequence of SEQ ID NO: 123 or SEQ ID NO: 129; and a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-

DR, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 186, SEQ ID NO: 188, and SEQ ID NO: 225.

Thus it may be possible to provide guide RNAs that target a gene encoding MHC I cell membrane receptor and a gene encoding MHC II cell membrane receptor, and to provide a cell transformed using the guide RNA.

In addition, there is provided a composition for inhibiting gene expression comprising as active ingredients a guide RNA or a nucleotide sequence encoding the guide RNA, and an RNA-guided endonuclease or a nucleotide sequence encoding the RNA-guided endonuclease.

In addition, there is provided a transformed cell in which expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited.

In addition, there is provided a pharmaceutical composition for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, comprising the transformed cell as an active ingredient; and a method for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, comprising administering the composition to a subject.

In addition, there is provided a use of a transformed cell for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, wherein expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited in the transformed cell, and the transformed cell expresses a peptide antigen, such as G-peptide, bound to a modified MHC I cell membrane receptor on the cell membrane surface.

Advantageous Effects

It is possible to prepare a cell in which a gene encoding MHC I cell

membrane receptor and a gene encoding MHC II cell membrane receptor are modified, by using a gene expression inhibition system using a guide RNA according to the present disclosure. In addition, it is possible to additionally introduce, into the cell, HLA-E to which a peptide antigen such as G-peptide is bound. A cell transformed as described above can effectively show its therapeutic efficacy even *in vivo*, and is not eliminated by an *in vivo* immune response. Therefore, it is expected that a composition comprising the cell as an active ingredient can be usefully used for the treatment of cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease.

Brief Description of Drawings

Fig. 1 illustrates results obtained by analyzing, with flow cytometry, HLA-ABC negative cells in cells prepared by using a B2M-targeted gRNA.

Fig. 2 illustrates results obtained by analyzing, with flow cytometry, HLA-DR negative cells in cells prepared by using an HLA-DRA-targeted gRNA.

Fig. 3 illustrates results obtained by analyzing, with flow cytometry, HLA-DQ negative cells in cells prepared by using an HLA-DQA-targeted gRNA.

Fig. 4 illustrates results obtained by analyzing, with flow cytometry, HLA-DP negative cells in cells prepared by using an HLA-DPA-targeted gRNA.

Fig. 5 illustrates production rates of HLA-ABC negative cell line depending on B2M-targeted gRNAs.

Fig. 6 illustrates production rates of HLA-DR negative cell line depending on DRA-targeted gRNAs.

Fig. 7 illustrates production rates of HLA-DQ negative cell line depending on DQA-targeted gRNAs.

Fig. 8 illustrates production rates of HLA-DP negative cell line depending on DPA-targeted gRNAs.

Fig. 9 illustrates mutation in a nucleic acid encoding B2M in cell lines prepared with B2M-targeted gRNAs.

Fig. 10 illustrates mutation in a nucleic acid encoding HLA-DRA in cell lines prepared with HLA-DRA-targeted gRNAs.

Fig. 11 illustrates mutation in a nucleic acid encoding HLA-DQA in cell lines prepared with HLA-DQA-targeted gRNAs.

Fig. 12 illustrates mutation in a nucleic acid encoding HLA-DPA in cell lines prepared with HLA-DPA-targeted gRNAs.

Fig. 13 illustrates HLA-I positive NK-92MI cell line and HLA-I negative NK-92MI cell line after cell separation.

Fig. 14 illustrates evaluation results for cell-killing capacity of the HLA-I positive NK-92MI cell line and the HLA-I negative NK-92MI cell line.

Fig. 15 illustrates results obtained by transforming CD4 T cells, CD8 T cells, and NK cells using gRNAs and then performing analysis with flow cytometry.

Fig. 16 illustrates deletion efficiency for targets in single gRNA-transformed cells and multiple gRNA-transformed cells.

Fig. 17 compares cell growth rate among single gRNA-transformed cells, multiple gRNA-transformed cells, and control group cells.

Fig. 18 compares cytokine production capacity between HLA-I positive T cells and HLA-I negative T cells.

Fig. 19 compares cytokine production capacity between HLA-I positive NK cells and HLA-I negative NK cells.

Fig. 20 illustrates evaluation results for cell-killing capacity of NK cells against HLA-I positive Raji cell line and HLA-I negative Raji cell line.

Fig. 21 illustrates a schematic diagram of HLA-E loaded with G-peptide and a structure of a protein for expressing the same.

Fig. 22 illustrates results obtained by analyzing HLA-E expressed in K562 cell line through transduction.

Fig. 23 illustrates evaluation results for cell-killing capacity of NK cells against K562 cell line (K562 G-B2M-HLA-E) expressing HLA-E and control group K562 cell line (K562).

In an exemplary technique, there is provided a guide RNA that complementarily binds to a nucleic acid sequence encoding β2-microglobulin (B2M), the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 17, and SEQ ID NO: 26.

As used herein, the term "B2M" refers to β2-microglobulin protein that is a component of MHC I. B2M is essential for expression of MHC I cell membrane receptor on the cell surface; and when B2M is removed or modified, expression of the MHC I cell membrane receptor on the cell surface is difficult to occur. Thus, the function of the MHC I cell membrane receptor may be removed by modifying the gene of B2M.

The guide RNA that complementarily binds to a nucleic acid sequence

encoding B2M may be any one selected from the group consisting of SEQ ID NOs: 1 to 58, and may specifically be any one selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 17, and SEQ ID NO: 26.

In addition, in an exemplary technique, there is provided a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DQ, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 87, and SEQ ID NO: 90.

As used herein, the term "HLA" refers to a human leukocyte antigen that is a product of MHC gene. HLA is composed of HLA I and HLA II. HLA I may include HLA-A, HLA-B, and HLA-C; and HLA II may include HLA-DQ, HLA-DP, and HLA-DR.

As used herein, the term "HLA-DQ" refers to an αβ heterodimer constituting MHC II. DQ consists of HLA-DQA1 and HLA-DQB1. The α subunit is encoded by HLA-DQA1 gene, and the β subunit is encoded by HLA-DQB1 gene. Expression of MHC II cell membrane receptor may be inhibited by modifying the gene of DQ.

The guide RNA that complementarily binds to a nucleic acid sequence encoding DQ may be any one selected from the group consisting of SEQ ID NOs: 59 to 116, and may specifically be any one selected from the group consisting of SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 87, and SEQ ID NO: 90.

In another exemplary technique, there is provided a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DP, the guide RNA comprising the nucleic acid sequence of SEQ ID NO: 123 or SEQ ID NO: 129.

As used herein, the term "HLA-DP" refers to an encoded MHC II cell surface receptor that consists of DPα subunit and DPβ subunit. DPα is encoded by HLA-DPA1, and DPβ is encoded by HLA-DPB1. Expression of MHC II cell membrane receptor may be inhibited by modifying the gene of DP.

The guide RNA that complementarily binds to a nucleic acid sequence encoding DP may be any one selected from the group consisting of SEQ ID NOs: 117 to 175, and may specifically be SEQ ID NO: 123 or SEQ ID NO: 129.

In addition, in yet another exemplary technique, there is provided a guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DR, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 186, SEQ ID NO: 188, and SEQ ID NO: 225.

As used herein, the term "HLA-DR" refers to an MHC II cell surface receptor, specifically an αβ heterodimer that constitutes the MHC II cell surface receptor. Each subunit of HLA-DR contains two extracellular domains, a membrane-spanning domain and a cytoplasmic tail. Expression of MHC II cell membrane receptor may be inhibited by modifying the gene of DR.

The guide RNA that complementarily binds to a nucleic acid sequence encoding DR may be any one selected from the group consisting of SEQ ID NOs: 176 to 234, and may preferably be any one selected from the group consisting of SEQ ID NO: 186, SEQ ID NO: 188, and SEQ ID NO: 225.

As used herein, the term "guide RNA (gRNA)" refers to an RNA molecule that specifically recognizes a target DNA and forms a complex with a nuclease, thereby guiding the nuclease to the target DNA.

The guide RNA may be a guide RNA derived from a prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) system.

The guide RNA may contain a non-naturally occurring chimeric crRNA sequence, and the crRNA sequence may contain a variable targeting domain capable of hybridizing to a target sequence.

In addition, the guide RNA contains a complementary sequence for each of B2M, HLA-DQ, HLA-DP, and HLA-DR genes. After being delivered into a cell, the guide RNA is capable of recognizing the target sequence and forming a complex

with an RNA-guided endonuclease.

In yet another exemplary technique, there is provided a composition for inhibiting gene expression, comprising as active ingredients, the guide RNA or a nucleotide sequence encoding the guide RNA, and an RNA-guided endonuclease or a nucleotide sequence encoding the RNA-guided endonuclease.

The RNA-guided endonuclease may be delivered in the form of mRNA or protein, or may be delivered to a target cell by transformation using a vector loaded with DNA encoding the same. When an endonuclease in the form of protein is used, the endonuclease may function as an RNP complex obtained by forming a complex with the guide RNA.

As used herein, the term "RNP complex" refers to a complex that comprises, as active ingredients, the guide RNA and the RNA-guided endonuclease, wherein the complex is capable of recognizing and binding to a target sequence, thereby selectively nicking or cleaving the target sequence. The RNA complex may be, for example, a Cas9-gRNA complex but is not limited thereto.

In an exemplary technique, the RNA-guided endonuclease may be any one selected from the group consisting of Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas 13a, Cas 13b, Cas 13c, Cas 13d, Cpf1, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, and Csf4, and may specifically be Cas9.

In an exemplary technique, there is provided a transformed cell in which expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited.

As used herein, the term "expression inhibition" means modification on a nucleotide sequence which causes a decrease in the function of a target gene, and

preferably means that expression of a target gene is made undetectable or the target gene is expressed to a meaningless level, due to such expression inhibition.

In an exemplary technique, the transformed cell may express a peptide antigen on the cell membrane surface. Examples of the peptide antigen include, but are not limited to, signal peptides of HLA-A, HLA-B, HLA-C, and HLA-G, and the peptide antigen is specifically a signal peptide (G-peptide) of HLA-G. The peptide antigen may be bound to modified MHC I cell membrane receptor.

In an exemplary technique, the modified MHC I cell membrane receptor has a structure in which HLA-E and B2M are linked. Specifically, the C-terminus of B2M may be linked, via a first linker, to the N-terminus of α1 of HLA-E and the C-terminus of G-peptide may be linked, via a second linker, to the N-terminus of B2M in the modified MHC I cell membrane receptor. The modified MHC I cell membrane receptor may have a structure in which HLA-G and B2M are linked.

In an exemplary technique, G-peptide may have the sequence of SEQ ID NO: 236; HLA-E may have the sequence of SEQ ID NO: 240; B2M may have the sequence of SEQ ID NO: 237; and the first linker may be $(G_4S)_n$ (n is an integer of 1 to 5) and may have the sequence of SEQ ID NO: 238. The second linker may be $(G_4S)_n$ (n is an integer of 2 to 6) and may have the sequence of SEQ ID NO: 241.

In an exemplary technique, modification of a gene encoding the MHC I cell membrane receptor may be performed using the guide RNA (for example, SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 17, or SEQ ID NO: 26) that complementarily binds to a nucleic acid sequence encoding B2M. Specifically, the modification of MHC I may be performed by single deletion using a single guide RNA.

In an exemplary technique, modification of DQ, DP, and DR genes encoding the MHC II cell membrane receptor may be performed using the guide RNA (for example, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 87, or SEQ ID NO: 90) that complementarily binds to a nucleic acid sequence encoding DQ, the guide RNA (for example, SEQ ID NO: 123 or SEQ ID NO: 129) that complementarily binds to

a nucleic acid sequence encoding DP, and the guide RNA (for example, SEQ ID NO: 186, SEQ ID NO: 188, or SEQ ID NO: 225) that complementarily binds to a nucleic acid sequence encoding DR. The modification of MHC II is performed together with the modification of MHC I, which may be performed by multiplex deletion using a multiple guide RNA (such as containing all of SEQ ID NO: 1, SEQ ID NO: 64, SEQ ID NO: 129, and SEQ ID NO: 188).

In an exemplary technique, the transformed cell may be a therapeutic allogeneic cell. As used herein, the term "therapeutic allogeneic cell" refers to a non-autologous allogeneic cell to be injected into a subject for the purpose of suppressing progression of, treating, or alleviating symptoms of a disease, and examples thereof include, but are not limited to, immune cells and stem cells.

As used herein, the term "immune cell" refers to a cell involved in immune responses of the human body, and examples thereof include NK cells, T cells, B cells, dendritic cells, and macrophages.

In an exemplary technique, the immune cell may be an NK cell or T cell.

As used herein, the term "stem cell" refers to a pluripotent cell capable of being differentiated into various cells. Examples of the stem cell may include human embryonic stem cells, bone marrow stem cells, mesenchymal stem cells, human nerve stem cells, oral mucosal cells, and the like. Specifically, the stem cell may be a mesenchymal stem cell.

In addition, in an exemplary technique, there is provided a pharmaceutical composition for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, comprising the transformed cell as an active ingredient.

In an exemplary technique, the cancer may be any one selected from the group consisting of chronic lymphocytic leukemia (CLL), B-cell acute lymphocytic leukemia (B-ALL), acute lymphoblastic leukemia, acute myeloid leukemia,

lymphoma, non-Hodgkin's lymphoma (NHL), multiple myeloma, blood cancer, gastric cancer, liver cancer, pancreatic cancer, colorectal cancer, lung cancer, breast cancer, ovarian cancer, skin cancer, melanoma, sarcoma, prostate cancer, esophageal cancer, hepatocellular carcinoma, astrocytoma, mesothelioma, head and neck cancer, and medulloblastoma.

In an exemplary technique, the infectious disease may be any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, Epstein Barr virus (EBV) infection, viral respiratory disease, and influenza.

As used herein, the term "degenerative disease" refers to a pathological condition in which a tissue loses its original function due to irreversible quantitative loss of the tissue. Examples of the degenerative disease include, but are not limited to, brain neurological disease, ischemic disease, skin damage, bone disease, and degenerative arthritis.

As used herein, the term "hereditary disease" refers to a pathological condition that occurs due to a mutation that is harmful to a gene or chromosome. Examples of the hereditary disease include, but are not limited to, hemophilia, albinism, Fabry disease, Hunter syndrome, and glycogen storage disorder.

As used herein, the term "immune disease" refers to any pathological condition in which a tissue is damaged due to an excessive or undesired immune response. Accordingly, the term "immune disease" has the same meaning as "hyperactive immune disease", and the term "composition for preventing or treating an immune disease" has the same meaning as "immunosuppressant".

Examples of the immune disease include, but are not limited to, graft-versus-host disease, graft rejection, chronic inflammatory disease, inflammatory pain, neuropathic pain, chronic obstructive pulmonary disease (COPD), and autoimmune disease.

The term "autoimmune disease" refers to a pathological condition that occurs when immune cells fail to distinguish self from a foreign substance and thus attack the self. Examples of the autoimmune disease may include, but are not limited to, rheumatoid arthritis, systemic lupus erythematosis, Hashimoto's thyroiditis, Grave's disease, multiple sclerosis, scleroderma, myasthenia gravis, type I diabetes, allergic encephalomyelitis, glomerulonephritis, vitiligo, Behcet's disease, Crohn's disease, ankylosing spondylitis, thrombocytopenic purpura, pemphigus vulgaris, autoimmune hemolytic anemia, adrenoleukodystrophy (ALD), and systemic lupus erythematosus (SLE).

In an exemplary technique, there is provided a method for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, comprising administering the pharmaceutical composition to a subject.

In an exemplary technique, the administration may be performed via any one route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, intraperitoneal, intraarteriolar, intraventricular, intralesional, intrathecal, topical, and combinations thereof.

In another exemplary technique, there is provided a use of a transformed cell for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, wherein expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited in the transformed cell, and the transformed cell expresses G-peptide bound to modified MHC I cell membrane receptor on the cell membrane surface.

In yet another exemplary technique, there is provided a kit for modifying a gene for MHC I cell membrane receptor and a gene for MHC II cell membrane receptor, the kit comprising the guide RNA or a nucleotide sequence encoding a guide RNA, and an RNA-guided endonuclease or a nucleotide sequence encoding the RNA-guided endonuclease.

Mode for the Invention

Hereinafter, the present invention will be described in more detail by way of the following examples. However, the following examples are only for illustrating the present invention, and the scope of the present invention is not limited thereto.

Example 1. Synthesis and selection of gRNA targeting HLA

Example 1.1. Search and synthesis of gRNA sequence

In order to search for a gRNA sequence, the complete nucleotide sequences of genes provided by NCBI (https://www.ncbi.nlm.nih.gov/) were used. As design tools for gRNAs, web-based systems, CHOPCHOP (http://chopchop.cbu.uib.no/), E-CRISP (http://www.e-crisp.org/E-CRISP/designcrispr.html), CRISPR-ERA (http://crispr-era.stanford.edu/), RGEN Tools (http://www.rgenome.net/cas-designer/) were used. Among the designed gRNAs, about 60 gRNAs, which were most suitable for gene knock-out, were obtained per each desired target. Based on these sequences, gRNAs were synthesized using the GeneArt Precision gRNA Synthesis Kit (Thermo Fisher Scientific, A29377) according to the manufacturer's instructions.

Claims

[Claim 1]

A guide RNA that complementarily binds to a nucleic acid sequence encoding β2-microglobulin (B2M), the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 17, and SEQ ID NO: 26.

[Claim 2]

A guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DQ, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 87, and SEQ ID NO: 90.

[Claim 3]

A guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DP, the guide RNA comprising the nucleic acid sequence of SEQ ID NO: 123 or SEQ ID NO: 129.

[Claim 4]

A guide RNA that complementarily binds to a nucleic acid sequence encoding HLA-DR, the guide RNA comprising any one nucleic acid sequence selected from the group consisting of SEQ ID NO: 186, SEQ ID NO: 188, and SEQ ID NO: 225.

[Claim 5]

A composition for inhibiting gene expression comprising as active ingredients:

the guide RNA of any one of claims 1 to 4 or a nucleotide sequence encoding the guide RNA; and

an RNA-guided endonuclease or a nucleotide sequence encoding the RNA-guided endonuclease.

[Claim 6]

The composition of claim 5, wherein the RNA-guided endonuclease is any one selected from the group consisting of Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas 13a, Cas 13b, Cas 13c, Cas 13d, Cpf1, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, and Csf4.

[Claim 7]

A transformed cell in which expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited.

[Claim 8]

The transformed cell of claim 7, wherein the transformed cell expresses a peptide antigen on the cell membrane surface.

[Claim 9]

The transformed cell of claim 8, wherein the peptide antigen is G-peptide.

[Claim 10]

The transformed cell of claim 9, wherein the G-peptide is bound to a modified MHC I cell membrane receptor.

[Claim 11]

The transformed cell of claim 10, wherein the modified MHC I cell membrane receptor has a structure in which HLA-E and B2M are linked.

[Claim 12]

The transformed cell of claim 11, wherein the C-terminus of the B2M is linked, via a first linker, to the N-terminus of α1 of the HLA-E, and the C-terminus of the G-peptide is linked, via a second linker, to the N-terminus of the B2M in the modified MHC I cell membrane receptor.

[Claim 13]

The transformed cell of claim 12, wherein the G-peptide has the sequence of SEQ ID NO: 236.

[Claim 14]

The transformed cell of claim 12, wherein the HLA-E has the sequence of SEQ ID NO: 240.

[Claim 15]

The transformed cell of claim 12, wherein the B2M has the sequence of SEQ ID NO: 237.

[Claim 16]

The transformed cell of claim 12, wherein the first linker has the sequence of SEQ ID NO: 238.

[Claim 17]

The transformed cell of claim 12, wherein the second linker has the sequence of SEQ ID NO: 241.

[Claim 18]

The transformed cell of claim 7, wherein modification in a gene encoding the MHC I cell membrane receptor is performed using the guide RNA of claim 1.

[Claim 19]

The transformed cell of claim 7, wherein modification in DQ, DP, and DR genes encoding the MHC II cell membrane receptor is performed using the guide RNAs of claims 2, 3, and 4, respectively.

[Claim 20]

The transformed cell of claim 7, wherein the transformed cell is a therapeutic allogeneic cell.

[Claim 21]

The transformed cell of claim 20, wherein the therapeutic allogeneic cell is an immune cell or stem cell.

[Claim 22]

The transformed cell of claim 21, wherein the immune cell is an NK cell or T cell.

[Claim 23]

A pharmaceutical composition for use in the treatment of cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, comprising as an active ingredient the transformed cell of claim 7.

[Claim 24]

The pharmaceutical composition of claim 23, wherein the cancer is any one selected from the group consisting of chronic lymphocytic leukemia (CLL), B-cell acute lymphocytic leukemia (B-ALL), acute lymphoblastic leukemia, acute myeloid leukemia, lymphoma, non-Hodgkin's lymphoma (NHL), multiple myeloma, blood cancer, gastric cancer, liver cancer, pancreatic cancer, colorectal cancer, lung cancer, breast cancer, ovarian cancer, skin cancer, melanoma, sarcoma, prostate cancer, esophageal cancer, hepatocellular carcinoma, astrocytoma, mesothelioma, head and neck cancer, and medulloblastoma.

[Claim 25]

The pharmaceutical composition of claim 23, wherein the infectious disease is any one selected from the group consisting of hepatitis B, hepatitis C, human papilloma virus (HPV) infection, cytomegalovirus infection, Epstein Barr virus (EBV) infection, viral respiratory disease, and influenza.

[Claim 26]

The pharmaceutical composition of any one of claims 23 to 25, wherein the pharmaceutical composition is to be administered via any one route selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, intraperitoneal, intraarteriolar, intraventricular, intralesional, intrathecal, topical, and combinations thereof.

[Claim 27]

A use of a transformed cell in the manufacture of a pharmaceutical composition for treating cancer, an infectious disease, a degenerative disease, a hereditary disease, or an immune disease, wherein expression of MHC I cell membrane receptor and MHC II cell membrane receptor is inhibited in the transformed cell, and the transformed cell expresses a peptide antigen bound to a modified MHC I cell membrane receptor on the cell membrane surface.

[Claim 28]

The use of claim 27 wherein the pharmaceutical composition is to be administered via any one route selected from the group consisting of intravenous,

intramuscular, intradermal, subcutaneous, intraperitoneal, intraarteriolar, intraventricular, intralesional, intrathecal, topical, and combinations thereof.