[Non-patent Document 25] Zheng, J., Liu, P. & Yang, X. YB-1 immunization combined with regulatory T-cell depletion induces specific T-cell responses that protect against neuroblastoma in the early stage. Acta biochimica et biophysica Sinica 44, 1006-1014, doi:10.1093/abbs/gms089 (2012).

[Non-patent Document 26] D'Arena, G. et al. Regulatory T-cell number is increased in chronic lymphocytic leukemia patients and correlates with progressive disease. Leukemia research 35, 363-368, doi:10.1016/j.leukres.2010.08.010 (2011).

[Non-patent Document 27] French, J. D. et al. Tumor-associated lymphocytes and increased FoxP3$^+$ regulatory T cell frequency correlate with more aggressive papillary thyroid cancer. The Journal of clinical endocrinology and metabolism 95, 2325-2333, doi:10.1210/jc.2009-2564 (2010).

[Non-patent Document 28] Khan, A. R., Dovedi, S. J., Wilkinson, R. W. & Pritchard, D. I. Tumor infiltrating regulatory T cells: tractable targets for immunotherapy. International reviews of immunology 29, 461-484, doi:10.3109/08830185.2010.508854 (2010).

[Non-patent Document 29] Yang, Z. Z., Novak, A. J., Ziesmer, S. C., Witzig, T. E. & Ansell, S. M. Attenuation of CD8$^+$ T-cell function by CD4$^+$CD25$^+$ regulatory T cells in B-cell non-Hodgkin's lymphoma. Cancer research 66, 10145-10152, doi:10.1158/0008-5472.CAN-06-1822 (2006).

[Non-patent Document 30] Yang, Z. Z., Novak, A. J., Stenson, M. J., Witzig, T. E. & Ansell, S. M. Intratumoral CD4$^+$CD25$^+$ regulatory T-cell-mediated suppression of infiltrating CD4$^+$ T cells in B-cell non-Hodgkin lymphoma. Blood 107, 3639-3646, doi:10.1182/blood-2005-08-3376 (2006).

Summary

[Problems to be solved by the Disclosure]

The present disclosure was achieved in view of the above circumstances. An objective of the present disclosure is to provide antigen-binding molecules that exert superior antitumor effects by damaging cells having immune response-suppressing functions, and pharmaceutical compositions comprising the antigen-binding molecule as an active ingredient, or methods for treating cancer using the pharmaceutical compositions.

[Means for Solving the Problems]

The present inventors discovered that antigen-binding molecules comprising a domain that binds to a molecule expressed on the surface of cells having immune response-suppressing functions and a domain that binds to a T-cell receptor (TCR) complex, damage cells having immune response-suppressing functions and exert more superior antitumor activity than

conventional antigen-binding molecules that bind to molecules expressed on the surfaces of regulatory T cells and exhausted T cells and also have ADCC activity. Furthermore, the present inventors discovered pharmaceutical compositions that can treat various carcinomas by having the antigen-binding molecule as an active ingredient. In one aspect, the present disclosure refers to a bispecific antibody that inhibits immune response-suppressing activity of a regulatory T cell, comprising: (i) a domain that binds to a molecule expressed on the surface of a regulatory T cell; and (ii) a CD3-binding domain, which further comprises an Fc region of an antibody in which an amino acid(s) forming the Fc region is mutated, wherein the Fc region mutant has reduced Fcγ receptor-binding activity by said mutation, wherein the molecule expressed on the surface of a regulatory T cell is CTLA4 or OX40. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the bispecific antibody of the present disclosure as an active ingredient. In still another aspect, the present disclosure refers to a pharmaceutical composition for use in inhibiting an immune response-suppressing activity, comprising the bispecific antibody of the present disclosure as an active ingredient.

Furthermore, the present disclosure provides the following:

[1] an antigen-binding molecule that inhibits immune response-suppressing activity of a cell having an immune response-suppressing function, comprising:

(i) a domain that binds to a molecule expressed on the surface of a cell having an immune response-suppressing function; and

(ii) a T cell receptor complex-binding domain;

[2] the antigen-binding molecule of [1], wherein the cell having immune response-suppressing function is a regulatory T cell or an exhausted T cell;

[3] the antigen-binding molecule of [1], wherein the molecule expressed on the surface of a cell having an immune response-suppressing function is any molecule selected from among CTLA4, PD1, TIM3, LAG3, CD244 (2B4), CD160, GARP, OX40, CD137 (4-1BB), CD25, VISTA, BTLA, TNFR25, CD57, KLRG1, CCR2, CCR5, CCR6, CD39, CD73, CD4, CD18, CD49b, CD1d, CD5, CD21, TIM1, CD19, CD20, CD23, CD24, CD38, CD93, IgM, B220(CD45R), CD317, PD-L1, CD11b, Ly6G, ICAM-1, FAP, PDGFR, Podoplanin, and TIGIT;

[4] the antigen-binding molecule of [3], wherein the molecule expressed on the surface of a cell having an immune response-suppressing function is any molecule selected from among CTLA4, TIM3, LAG3, CD137 (4-1BB), CD25, CCR5, CCR6, CD38, TIGIT, and OX40;

[5] the antigen-binding molecule of any one of [1] to [4], wherein the T cell receptor complex-binding domain is a T cell receptor-binding domain;

[6] the antigen-binding molecule of any one of [1] to [4], wherein the T cell receptor complex-binding domain is a CD3-binding domain;

[7] the antigen-binding molecule of any one of [1] to [6], which further comprises an

FcRn-binding domain;

[8] the antigen-binding molecule of [7], wherein the FcRn-binding domain is an Fc region of an antibody;

[9] the antigen-binding molecule of [8], wherein the FcRn-binding domain is an Fc region of an antibody having reduced Fcγ receptor-binding activity;

[10] the antigen-binding molecule of any one of [1] to [9], which is a multispecific antibody;

[11] a pharmaceutical composition comprising the antigen-binding molecule of any one of [1] to [10] as an active ingredient;

[12] a pharmaceutical composition for inhibiting an immune response-suppressing activity, comprising the antigen-binding molecule of any one of [1] to [10] as an active ingredient;

[13] the pharmaceutical composition of [12], wherein the inhibition of an immune response-suppressing activity is inhibition caused by a T cell;

[14] the pharmaceutical composition of [13], wherein the inhibition caused by a T cell is inhibition resulting from cytotoxic activity of the T cell;

[15] the pharmaceutical composition of any one of [12] to [14], wherein the immune response-suppressing activity is an activity by a regulatory T cell or an exhausted T cell;

[16] the pharmaceutical composition of any one of [12] to [14], wherein the immune response-suppressing activity is an activity of a cell expressing any molecule selected from among CTLA4, PD1, TIM3, LAG3, CD244 (2B4), CD160, GARP, OX40, CD137 (4-1BB), CD25, VISTA, BTLA, TNFR25, CD57, KLRG1, CCR2, CCR5, CCR6, CD39, CD73, CD4, CD18, CD49b, CD1d, CD5, CD21, TIM1, CD19, CD20, CD23, CD24, CD38, CD93, IgM, B220(CD45R), CD317, PD-L1, CD11b, Ly6G, ICAM-1, FAP, PDGFR, Podoplanin, and TIGIT;

[17] the pharmaceutical composition of any one of [11] to [16], which is a therapeutic agent for cancer;

[18] the pharmaceutical composition of claim 17, which is a therapeutic agent any cancer selected from among ovarian cancer, gastric cancer, esophageal cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, breast cancer, malignant melanoma, non-small-cell lung cancer, cervical cancer, glioblastoma, prostate cancer, neuroblastoma, chronic lymphocytic leukemia, papillary thyroid cancer, colorectal cancer, Hodgkin's lymphoma, and endometriosis;

[19] Also disclosed herein is a method for inducing cytotoxicity, suppressing cell proliferation, activating immunity against a cancer cell or a cancer cell-comprising tumor tissue , or treating or preventing cancer, which comprises the step of administering the antigen-binding molecule of any one of [1] to [10] or the pharmaceutical composition of any one of [11] to [18];

[20] the antigen-binding molecule of any one of [1] to [10] or the pharmaceutical composition of any one of [11] to [18] for use in inducing cytotoxicity, suppressing cell proliferation, activating immunity against a cancer cell or a cancer cell-comprising tumor tissue, or treating or preventing

cancer;

[21] use of the antigen-binding molecule of any one of [1] to [10] in the production of the pharmaceutical composition of any one of [11] to [18]; and

[22] a method for producing the pharmaceutical composition of any one of [11] to [18], which comprises the step of using the antigen-binding molecule of any one of [1] to [10].

Furthermore, the present disclosure relates to methods for treating or preventing cancer, or for inhibiting suppression of an immune response, which comprise administering an antigen-binding molecule as described or a pharmaceutical composition as described to patients in need of treatment. The present disclosure also relates to a kit for use in the methods as described, which comprises an antigen-binding molecule as described. The present disclosure also relates to the use of an antigen-binding molecule as described in producing a pharmaceutical composition for inhibiting immune response-suppression. Furthermore, the present disclosure relates to antigen-binding molecules as described or pharmaceutical compositions as described for use in the methods of the present disclosure.

Brief Description of the Drawings

Fig. 1 presents a schematic diagram showing cytotoxic activity against cancer cells by bispecific antibodies that target a cancer antigen expressed on cancer cells and CD3 expressed on T cells.

Fig. 2-1 presents a schematic diagram showing crosslinking between T cells caused by crosslinking between CD3s on different T cells *via* an anti-CD3 antibody.

Fig. 2-2 presents a schematic diagram showing crosslinking between CD3s on the same T cell *via* an anti-CD3 antibody.

Fig. 3 presents a schematic diagram showing that an anti-CTLA4/CD3 bispecific antibody causes crosslinking between CTLA4 and CD3 on the same regulatory T cell, and therefore, does not cause intercellular crosslinking between an effector T cell and a regulatory T cell. This schematic diagram shows a regulatory T cell as the target cell and CTLA4 as the antigen, but the target cell and antigen are not limited thereto.

Fig. 4 presents a schematic diagram showing intercellular crosslinking caused by crosslinking between CD3 on an effector T cell and CTLA4 on a regulatory T cell *via* an anti-CTLA4/CD3 bispecific antibody. This schematic diagram shows a regulatory T cell as the target cell and CTLA4 as the antigen, but the target cell and antigen are not limited thereto.

Fig. 5 presents a graph showing the ADCC activity of the anti-mouse CTLA4 antibody hUH02hUL01-mFa55 on mouse CTLA4-expressing cells.

Fig. 6 presents a graph showing the cytotoxic activity of the anti-mouse CTLA4/anti-mouse CD3 bispecific antibody (hUH02UL01/2C11-F760) on mouse

CTLA4-expressing cells.

Fig. 7-1 presents a schematic diagram showing crosslinking between a mouse CTLA4-bound bead and a mouse CD3-bound bead by an anti-mouse CTLA4/anti-mouse CD3 bispecific antibody.

Fig. 7-2 presents a graph showing the experiment results of crosslinking between mouse CTLA4-bound beads and mouse CD3-bound beads *via* anti-mouse CTLA4/anti-mouse CD3 bispecific antibodies (N = 3). The graph shows the mean values and standard deviations for each of the measurement results.

Fig. 7-3 presents a graph showing the experiment results of crosslinking between mouse

CLAIMS

1. A bispecific antibody that inhibits immune response-suppressing activity of a regulatory T cell, comprising:
(i) a domain that binds to a molecule expressed on the surface of a regulatory T cell; and
(ii) a CD3-binding domain,
which further comprises an Fc region of an antibody in which an amino acid(s) forming the Fc region is mutated, wherein the Fc region mutant has reduced Fcγ receptor-binding activity by said mutation,
wherein the molecule expressed on the surface of a regulatory T cell is CTLA4 or OX40.

2. The antigen-binding molecule of claim 1, wherein the molecule expressed on the surface of a regulatory T cell is CTLA4.

3. A pharmaceutical composition comprising the bispecific antibody of claim 1 or 2 as an active ingredient.

4. A pharmaceutical composition for use in inhibiting an immune response-suppressing activity, comprising the bispecific antibody of claim 1 or 2 as an active ingredient.

5. The pharmaceutical composition of claim 4, wherein the immune response-suppressing activity is an activity of a regulatory T cell.

6. The pharmaceutical composition of any one of claims 3 to 5 for use in treating cancer.

7. The pharmaceutical composition of claim 6, wherein the cancer is selected from the group consisting of ovarian cancer, gastric cancer, esophageal cancer, pancreatic cancer, renal cell carcinoma, hepatocellular carcinoma, breast cancer, malignant melanoma, non-small-cell lung cancer, cervical cancer, glioblastoma, prostate cancer, neuroblastoma, chronic lymphocytic leukemia, papillary thyroid cancer, colorectal cancer, Hodgkin's lymphoma, and endometriosis.