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(71) Applicants: **MOGAM INSTITUTE FOR BIOMEDICAL RESEARCH** [KR/KR]; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **GREEN CROSS CORPORATION** [KR/KR]; 107, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR).

(72) Inventors: **EUN, So-Young**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **OH, Miyoung**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **PARK, Hye-Young**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **LEE, Mijung**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **YOON, Aerin**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **YUM, Hye In**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **NAM, Hyemi**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **LEE, Eunhee**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR). **WON, Jongwha**; 93, Ihyeon-ro 30beon-gil, Giheung-gu, Yongin-si, Gyeonggi-do 16924 (KR).

(74) Agent: **FIRSTLAW P.C.**; 60 Mabang-Ro, Seocho-Ku, Seoul 06775 (KR).

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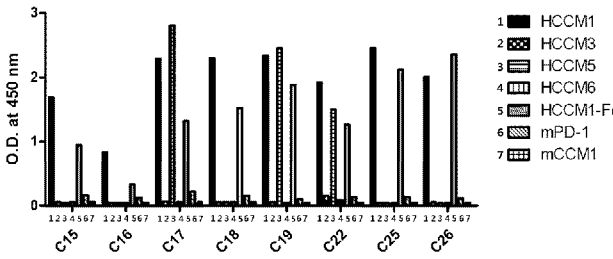
(54) Title: ANTI-CEACAM1 ANTIBODY AND USE THEREOF



(57) Abstract: The present invention provides anti-CEACAM1 antibodies with improved binding abilities specific to CEACAM1, and a use thereof. Anti-CEACAM1 antibodies according to the present invention exhibit superior binding abilities specific to CEACAM1, and also activate the anti-cancer immune functions of cytotoxic T cells and natural killer cells, and thus, each one of them can be effectively used as an anti-cancer agent and a composition for treating cancer.

Description

Title of Invention: ANTI-CEACAM1 ANTIBODY AND USE THEREOF

Technical Field

[1] The present invention relates to an anti-CEACAM1 antibody that specifically binds to CEACAM1, and a use thereof.

Background Art

[2] Carcinoembryonic antigen-related cell adhesion molecule 1 (hereinafter, referred to as CEACAM1), a transmembrane glycoprotein, belongs to the carcinoembryonic antigen (CEA) family. Among CEA family members, CEACAM1, CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7 and CEACAM8 are expressed in humans. More importantly, CEACAM1 is the only member of CEA family expressed in lymphocyte populations including activated T cells and natural killer cells. CEACAM1 has been reported highly expressed in cancer cells. In addition, low levels of CEACAM1 expression has also been observed in epithelial cells, endothelial cells, and myeloid cells. On the surface of lymphocytes, CEACAM1 plays a role in the regulation of immune responses. Specifically, CEACAM1 has turned out to be an inhibitory receptor for activated T cells including those contained within the human intestinal epithelium (Gray-Owen & Bloomberg, *Nat. Rev.* Immunol. 2006; 6:433-446; Morales *et al., J. Immunol*, 1999; 163: 1363-1370).

[3] In particular, CEACAM1 is recognized as an immune checkpoint molecule similar to PD-1 and CTLA-4, playing a crucial role in modulating T cell activation. The immune checkpoint pathways protect tissues from immune-mediated damages under non-inflammatory physiological conditions. When CEACAM1 is activated on T lymphocytes, mainly upon CEACAM1-CEACAM1 trans-homophilic engagement, CEACAM1 signals to inhibit TCR-mediated inflammatory pathways by recruiting phosphatases to its own cytoplasmic ITIM motif (Chen et al., *J.* Immunol. 2008; 180: 6085-6093). Thus, suppression of immune checkpoint pathways in the context of cancer has emerged as a promising anti-cancer treatment strategy.

[4] Studies on several human tumor types have reported that tumors can avoid immunity by inducing CEACAM1. In addition, in preclinical animal tumor models, it has been shown that blocking CEACAM1 interactions using monoclonal antibodies (mAbs) can enhance immune responses against tumors, promoting tumor suppression (Ortenberg et al., *Mol. Cancer Ther.* 2012; 11(6):1300-1310).

[5] One of the biggest issues with conventional anti-cancer drugs is that treatment renders detrimental side effects as compared to their limited anti-cancer efficacies with

high recurrence rates. On the other hand, a recently spotlighted approach, so-called immune checkpoint blockade, eliminates cancer by reactivating tumor-reactive exhausted T cells instead of directly killing cancer cells. This type of approach seems relatively safe and effective because it utilizes host immune functions to eliminate cancer with being able to keep irrelevant normal cells untouched. In the case of PD-1-targeting nivolumab from Bristol-Myers Squibb, the toxicity profile is in the manageable range as compared to those of conventional anti-cancer drugs, while its anti-cancer effects are dramatically higher than those of conventional drugs. In a phase III study of head-to-head comparison between nivolumab and dacarbazine, a standard chemotherapeutic agent, in treating metastatic melanoma patients, published in 2015, for example, nivolumab showed 40% objective response rate (95% CI, 33.3 to 47.0) compared to 13.9% ORR (95% CI, 9.5 to 19.4) by dacarbazine. The median progression-free survival was 5.1 months in the nivolumab group versus 2.2 months in the dacarbazine group. Toxicity profiling also convinced superiority of nivolumab to dacarbazine (Robert et al., *New Engl. J. Med.* 2015; 372:320-330).

[6] Meanwhile, CEACAM1-blocking antibody acts on CEACAM1 expressed on the surface of cytotoxic T cells and of natural killer cells, interacting with CEACAM1 molecules overexpressed on tumor cells. Therefore, in case of CEACAM1-overexpressing tumors, CEACAM1-targeting antibody is expected to block CEACAM1-CEACAM1 homophilic suppressive interaction between T/NK cell and tumor cell, thereby reactivating anti-tumor T/NK cell responses. The CEACAM1-targeting antibody currently under development (a phase 1 clinical trial was prematurely terminated in February 2017) recognizes CEACAM3 and CEACAM5 in addition to CEACAM1. Such an off-target recognition property of this BMS clone may be due to its epitope sequences in N-domain that are highly homologous among human CEACAM1, CEACAM3 and CEACAM5.

Disclosure of Invention

Technical Problem

[7] Therefore, in order to develop an anti-CEACAM1 antibody that specifically binds to CEACAM1, the present inventors have endeavored to find that an anti-CEACAM1 antibody binds to the N-domain of CEACAM1 and does not cross-react with CEACAM3, CEACAM5, CEACAM6 or CEACAM8, and have completed the present invention.

Solution to Problem

[8] In accordance with one object of the present invention, there is provided an anti-CEACAM1 antibody or a fragment thereof comprising: light chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 1

to 8; light chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 9 to 16; light chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 17 to 29; heavy chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 30 to 38; heavy chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 39 to 46; and heavy chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 47 to 55.

[9] In accordance with another object of the present invention, there is provided an anti-CEACAM1 antibody or a fragment thereof comprising: light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[10] Further, in accordance with another object of the present invention, there is provided an anti-cancer agent comprising the anti-CEACAM1 antibody or a fragment thereof described above as an active ingredient.

[11] Furthermore, in accordance with another object of a present invention, there is provided an anti-cancer adjuvant comprising the anti-CEACAM1 antibody or the fragment thereof described above as an active ingredient.

[12] Also, in accordance with another object of the present invention, there is provided a composition for treating cancer comprising the anti-cancer adjuvant described above and a cell therapeutic agent.

[13] Moreover, in accordance with another object of the present invention, there is provided a method for treating cancer comprising administering to a subject lymphocytes contacted with the anti-CEACAM1 antibody or a fragment thereof described above.

[14] In addition, in accordance with another object of the present invention, there is provided a method for inhibiting proliferation of CEACAM1-expressing tumor cells, which comprises contacting the CEACAM1-expressing tumor cells with the anti-CEACAM1 antibody or a fragment thereof.

Advantageous Effects of Invention

[15] An anti-CEACAM1 antibody according to the present invention specifically binds to CEACAM1, and thereby activates the anti-cancer immune functions of cytotoxic T cells and natural killer cells, and thus, it can be effectively used as an anti-cancer agent

and a composition for treating cancer.

Brief Description of Drawings

[16] Fig. 1 is a schematic diagram of the structure of recombinant CEACAMl prepared according to one embodiment.

[17] Fig. 2 shows the binding ability of anti-CEACAM1 antibodies depending on the constitutive domains of recombinant CEACAM1.

[18] Fig. 3 illustrates comparative results of the binding of C25 to recombinant CEACAMl depending on the concentrations of C25.

[19] Fig. 4 demonstrates the binding of anti-CEACAM1 antibodies to CEACAM1 depending on the concentrations of anti-CEACAM1 antibodies.

[20] Fig. 5 depicts the binding ability of CEACAM1 expressed on the surface of CEACAM1-Jurkat T cell line depending on the concentrations of C25 and C25-derived antibody clones:

[21] (a) represents the binding ability to CEACAM1 expressed on the surface of a CEACAM1-Jurkat T cell line depending on the concentrations of C25; and (b) represents the binding ability to CEACAM1 expressed on the surface of a CEACAM1-Jurkat T cell line depending on the concentrations of C25-derived antibody clones.

[22] Fig. 6 is a table showing the affinity of anti-CEACAM1 antibodies to CEACAM1. The affinity was obtained by the kinetic speed constant K_{on} and K_{off} and the equilibrium dissociation constant K_D.

[23] Fig. 7 is a photograph showing the isoelectric point of C25. The first, second and third lanes are the results with IEF-Markers 3-10, huIgG4, and C25, respectively.

[24] Fig. 8 shows the activation of the Jurkat E6.1 T cell line by C25 along with anti-CD3 (OKT3; 0.1 ㎍/ml) antibody through CD69, CD25 and Ki67 marker expressions. The concentrations of C25 and huIgG4 were 10 ㎍/ml.

[25] Fig. 9 is a graph showing the number of the Jurkat E6.1 T cells activated by C25 along with OKT3 (0.1 ㎍/ml) and secretion levels of IL-2. The concentrations of C25 and huIgG4 were 10 ㎍/ml:

[26] (a) represents the number of Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of Jurkat E6.1 T cells activated by C25.

[27] Fig. 10 illustrates the activation of Jurkat E6.1 T cell line by C25 along with OKT3 (0.1 ㎍/ml) through CD69, CD25 and Ki67 marker expression. The concentrations of C25 and huIgG4 were 25 ㎍/ml.

[28] Fig. 11 is a graph showing the number of the Jurkat E6.1 T cells activated by C25 along with OKT3 (0.1 ㎍/ml) and secretion levels of IL-2. The concentrations of C25

and huIgG4 were 25 μg/ml:

[29] (a) represents the number of Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of Jurkat E6.1 T cells activated by C25 along with OKT3. The concentration of OKT3 was 0.1 μg/ml

[30] Fig. 12 demonstrates the activation of CEACAM1-overexpressing Jurkat E6.1 T cell line by C25 along with OKT3 through CD69, CD25 and Ki67 marker expression. The concentration of OKT3 was 0.1 μg/ml.

[31] Fig. 13 is a graph showing the number of the CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 and secretion levels of IL-2. The concentrations of OKT3 and C25 or control Ab were 0.1 μg/ml and 10 μg/ml, respectively:

[32] (a) represents the number of CEACAM1-overexpressing Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3.

[33] Fig. 14 is a graph showing the number of the CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3 and secretion levels of IL-2. The concentrations of OKT3 and C25 or control Ab were 0.1 μg/ml and 25 μg/ml, respectively:

[34] (a) represents the number of CEACAM1-overexpressing Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3.

[35] Fig. 15 depicts the activation of CEACAM1-overexpressing Jurkat E6.1 T cell line by C25 along with OKT3 through CD69, CD25 and Ki67 marker expression. The concentration of OKT3 was 1 μg/ml.

[36] Fig. 16 is a graph showing the number of the CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3 and secretion levels of IL-2. The concentrations of OKT3 and C25 or control Ab were 0.1 μg/ml and 10 μg/ml, respectively:

[37] (a) represents the number of CEACAM1-overexpressing Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3.

[38] Fig. 17 is a graph showing the number of the CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3 and secretion levels of IL-2. The concentrations of OKT3and C25 or control Ab were 1 μg/ml and 25 μg/ml, respectively:

[39] (a) represents the number of CEACAM1-overexpressing Jurkat E6.1 T cells proliferated by C25 along with OKT3; (b) shows the number of activated T cells expressing CD69 and Ki67 markers; and (c) shows the IL-2 secretion level of

CEACAM1-overexpressing Jurkat E6.1 T cells activated by C25 along with OKT3.

[40] Fig. 18 is a graph showing T cell activation by C25 treatment with the aid of TCR-induced NFAT activation. The concentrations of OKT3 and C25 or control Ab were 0.05 μg/ml and 10 μg/ml, respectively:

[41] (a) shows the measurement results of TCR-induced NFAT activation by treating Jurkat-GFP/NFAT-luc cells, which do not express CEACAM1, with C25; and (b) is the measurement results of TCR-induced NFAT activation by treatment of Jurkat-CCM1/NFAT-luc cells overexpressing CEACAM1 with C25.

[42] Fig. 19 is a result of T cell activation by C25 treatment with the aid of TCR-induced NFAT activation. The concentrations of OKT3 and C25 or control Ab were 0.1 μg/ml and 10 μg/ml, respectively:

[43] (a) shows the measurement results of TCR-induced NFAT activation by treatment of Jurkat-GFP/NFAT-luc cells, which do not express CEACAM1, with C25; and (b) is the measurement results of TCR-induced NFAT activation by treatment of Jurkat-CCM1/NFAT-luc cells overexpressing CEACAM1 with C25.

[44] Fig. 20 is a graph showing the increase in NFAT luciferase activity of T cells by anti-CEACAM1 antibodies including C25 in comparison along with the control. The concentrations of OKT3 and anti-CEACAM or control Ab were 0.1 μg/ml and 10 μg/ml, respectively:

[45] Fig. 21 provides photographs showing staining results with C25 to evaluate the degree of CEACAM1 expressions in normal tissues of a human and a monkey.

[46] Fig. 22 provides photographs showing staining results with huIgG4 in normal tissues of a human and a monkey.

[47] Fig. 23 demonstrates the cross reactivity of anti-CEACAM1 antibodies with CEACAM family proteins.

[48] Fig. 24 shows the cross reactivity of anti-CEACAM1 antibodies with CEACAM family proteins.

[49] Fig. 25 shows that C25 (a) and C25-derived anti-CEACAM1 antibody clones (b) do not cross-react with CEACAM3, CEACAM5, CEACAM6 or CEACAM8 expressed on the cell surface.

[50] Fig. 26 depicts that C25 (a) and C25-derived anti-CEACAM1 antibody clones (b) bind not only to human CEACAM1 but also to monkey CEACAM1by examining the binding ability to a protein expressed on the cell surface.

[51] Fig. 27 illustrates C25-mediated enhancement effect on the anti-cancer activity of CEACAM1$^+$ TALL-104 T cells against CEACAM1$^+$ cancer cells:

[52] (a) shows the survival rates of cancer cells when TALL-104 T cells and CEACAM1$^+$ MNK45 cancer cells were co-cultured in the presence of C25 at various Effector:Target (E:T) ratios; and (b) shows the survival rates of cancer cells when

TALL-104 T cells and CEACAM1- MNK1 cancer cells were co-cultured in the presence of C25 at various E:T ratios.

[53] Fig. 28 is a graph showing C25-mediated enhancement effect on the anti-cancer activity of CEACAM1+ NK92MI NK cells against CEACAM1+ cancer cells:

[54] (a) shows the survival rates of cancer cells when CEACAM1+ NK92MI NK cells and CEACAM1+ MNK45 cancer cells were co-cultured in the presence of C25 at various E:T ratios; and (b) shows the survival rates of cancer cells when CEACAM1+ NK92MI NK cells and CEACAM1+ MNK1 cancer cells were co-cultured in the presence of C25 at various E:T ratios.

[55] Fig. 29 is a graph showing the enhancement levels on the anti-cancer activity of CEACAM1+ TALL-104 cells activated by C25 and C25-derived antibody clones against CEACAM1+ cancer cells:

[56] The cancer cell death by TALL-104 cells promoted by C25 and C25-derived antibody clones was shown as the cancer cell survival rates compared to those by the control antibody when CEACAM1+ TALL-104 T cells were co-cultured with CEACAM1+ cancer cells (MKN45) at an E:T ratio of 1: 1.

Best Mode for Carrying out the Invention

[57] Hereinafter, the present invention is described in detail.

[58] The present invention provides an anti-CEACAM1 antibody or a fragment thereof comprising: light chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 1 to 8; light chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 9 to 16; light chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 17 to 29; heavy chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 30 to 38; heavy chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 39 to 46; and heavy chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 47 to 55.

[59] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[60] Also, the antibody or the fragment thereof described above may comprise a light

chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 56, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[61] Further, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 106, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[62] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 18, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[63] Furthermore, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 58, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[64] In addition, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 107, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[65] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 19, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[66] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 60, and a heavy chain variable domain comprising the amino acid sequence rep-

resented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[67] Moreover, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 108, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[68] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 20, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[69] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 62, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[70] Moreover, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 109, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[71] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[72] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Es-

pecially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[73] The antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 110, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[74] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 21, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[75] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 66, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[76] Further, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 111, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[77] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 11, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 22, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[78] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 68, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[79] Furthermore, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 112, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[80] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 48.

[81] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 88. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[82] Moreover, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 110, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 122.

[83] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 31, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[84] The antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 90. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[85] Also, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 110, and a

heavy chain comprising the amino acid sequence represented by SEQ ID NO: 123.

[86] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 2, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 12, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 24, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 32, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 40, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 49.

[87] Further, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 72, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 92. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[88] Furthermore, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 114, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 124.

[89] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 3, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 13, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 25, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 33, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 41, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 50.

[90] Moreover, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 74, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 94. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[91] The antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 115, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 125.

[92] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above

may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 4, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 14, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 34, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 42, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 51.

[93] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 76, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 96. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[94] Further, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 116, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 126.

[95] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 5, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 15, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 26, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 35, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 43, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 52.

[96] Furthermore, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 78, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 98. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[97] Moreover, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 117, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 127.

[98] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 6, light chain CDR2 comprising the amino acid sequence represented by

SEQ ID NO: 16, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 27, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 36, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 44, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 53.

[99] The antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 80, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 100. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[100] Also, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 118, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 128.

[101] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 7, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 14, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 28, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 37, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 45, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 54.

[102] Further, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 82, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 102. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[103] Furthermore, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 119, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 129.

[104] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 8, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 15, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 29, heavy chain CDR1 comprising the amino acid sequence represented

by SEQ ID NO: 38, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 46, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 55.

[105] Moreover, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 84, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 104. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[106] The antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 120, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 130.

[107] Specifically, the anti-CEACAM1 antibody or the fragment thereof described above may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[108] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 70, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[109] Further, the antibody or the fragment thereof described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 113, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[110] The term "antibody" as used herein refers to an immune protein that binds to an antigen and interferes with the action of the antigen or eliminates the antigen. There are five types of antibodies, IgM, IgD, IgG, IgA and IgE, each of which contains a heavy chain produced from heavy chain constant region genes μ, δ, γ, α, and ε. In an antibody technology, IgG is mainly used. Four kinds of isotypes of IgG are IgG1, IgG2, IgG3 and IgG4, and their structures and functional characteristics may be different.

[111] Also, the IgG has a Y-shaped, highly stable structure (molecular weight, about 150 kDa) composed of two heavy chain proteins (about 50 kDa) and two light chain proteins (about 25 kDa). The light and heavy chains of an antibody are divided into constant regions in which the amino acid sequences are identical among antibodies and variable regions in which the amino acid sequences are different among antibodies. A heavy chain constant region contains the CH1, H (hinge), CH2, and CH3 domains. Each domain is composed of two β-sheets and is linked by a disulfide bond in the molecule. Two variable regions of heavy and light chains are combined to form an antigen binding site. The antigen binding site is present on two arms of an antibody, one at each arm, and such portion that can bind to an antigen is called Fab (antibody binding fragment), and a portion that cannot bind to an antigen is called Fc (crystalizable fragment). The Fab and Fc are connected by a flexible hinge region.

[112] Also, the term "CDR" as used herein refers to a hypervariable region which is a site having a different amino acid sequence for each antibody in the heavy chain and light chain variable regions of the antibody, and refers to an antigen-binding site. With regard to a stereostructure of an antibody, the CDR forms a loop shape on the surface of the antibody, and a framework region (FR) is present under the loop to structurally support the CDR. There are three loop structures in each of the heavy chain and the light chain, and these six loop structures are combined with each other to directly contact an antigen.

[113] Also, the antibody fragment may be one selected from the group consisting of Fab, scFv, $F(ab)_2$, and Fv. An antibody fragment refers to antigen binding domains which excludes the Fc region, which serves an effector function to transfer binding stimuli with an antigen to cells or complements, etc., and may include 3rd generation antibody fragments such as a single domain antibody or a minibody, etc.

[114] In addition, the antibody fragments have good permeability into tissues and tumors since they have small sizes compared to a full structure IgG. They have has an advantage of low production cost since they can be produced in bacteria, and can be used when the function of transferring binding stimuli with an antigen to cells or complements is not desired since they have no Fc. Antibody fragments are suitable for *in* vivo diagnosis due to their short half-life in the human body. However, when some basic, acidic or neutral amino acids among the amino acids constituting the antibody are replaced with each other, the isoelectric point (pI) can be changed. The change in the isoelectric point of the antibody can induce changes such as a decrease in toxic side effects *in* vivo or an increase in the water solubility of the antibody, and thus, in the case of a therapeutic antibody, a full structure IgG can be used considering its affinity and the structural form.

[115] Also, the light chain variable domain of the anti-CEACAM1 antibody or a fragment

thereof of the present invention may have a light chain variable domain sequence comprising the amino acid sequence represented by SEQ ID NOS: 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82 or 84, or it may have homology of 97%, 98% or 99% with the above light chain variable domain sequence.

[116] Further, the heavy chain variable domain of the anti-CEACAM1 antibody or a fragment thereof of the present invention may have a heavy chain variable domain sequence comprising the amino acid sequence represented by SEQ ID NOS: 86, 88, 90, 92, 94, 96, 98, 102 or 104, or it may have homology of 97%, 98% or 99% with the above heavy chain variable domain sequence.

[117] Furthermore, the anti-CEACAM1 antibody or a fragment thereof of the present invention may have homology of 97%, 98% or 99% with a light chain variable domain sequence comprising the amino acid sequence represented by SEQ ID NOS: 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82 or 84, and a heavy chain variable domain sequence comprising the amino acid sequence represented by SEQ ID NOS: 86, 88, 90, 92, 94, 96, 98, 102 or 104.

[118] Moreover, the anti-CEACAM1 antibody refers to an antibody that binds to CEACAM1. As used herein, the term "C25" is an embodiment of the anti-CEACAM1 antibody. The C25 may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[119] Also, the antibody or the fragment thereof described above may comprise a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 70, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86. Herein, if the CDR1, CDR2, and CDR3 of the heavy chain variable domain are identical, the framework portion can be modified. Especially, the amino acid sequences of some of the framework portion can be modified to produce humanized antibodies.

[120] The CD25 described above may comprise a light chain comprising the amino acid sequence represented by SEQ ID NO: 113, and a heavy chain comprising the amino acid sequence represented by SEQ ID NO: 121.

[121] A variant of C25 may comprise light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, 10 or 11, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, 18, 19, 20, 21 or 22, heavy chain

CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30 or 31, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47 or 48. Specifically, in one embodiment of the present invention, the variants of C25 were shown in Table 1 as 1-19, 1-23, 3-07, 3-27, 4R9, 4R20, 4R26, 4R9_H2-2 and 4R9_H4-n20.

[122] Also, the anti-CEACAM1 antibody or the fragment thereof described above recognizes the N-domain and the B-domain of CEACAM1 as an epitope. Also, the antibody does not cross-react with CEACAM3, CEACAM5, CEACAM6 or CAECAM8.

[123] Further, the anti-CEACAM1 antibody of the present invention can be easily prepared by a known monoclonal antibody preparation technique. Methods for preparing monoclonal antibodies can be implemented by preparing hybridomas using B lymphocytes from immunized animals, or by using phage display techniques, but are not limited thereto.

[124] The present invention also provides an anti-cancer agent comprising the anti-CEACAM1 antibody or a fragment thereof as an active ingredient.

[125] The anti-cancer agent of the present invention comprising the anti-CEACAM1 antibody or the fragment thereof as an active ingredient can be used for treating cancers or tumors overexpressing CEACAM1. Specifically, when a T cell receptor (TCR) of a cytotoxic T cell, which plays a role in removing cancer cells, recognizes an epitope of cancer or a tumor cell, the LCK(lymphocyte-specific protein tyrosine kinase) protein bound to the CD4 (cluster of differentiation 4), one component of the TCR phosphorylates CD3ζ (cluster of differentiation 3ζ), another component of the TCR. When ZAP70 (Zeta-chain-associated protein kinase 70) protein is bound to the phosphorylated CD3ζ, the terminal portion of ZAP70 protein is phosphorylated again by LCK protein, thereby activating T cell inflammatory pathways including RAS-MAPK (Ras-MAP kinase) signal transduction, and thus, T cells are activated.

[126] However, in the case of cancer cells or tumor cells overexpressing the CEACAM1, SHP1(Src homology region 2 domain-containing phosphatase-1) protein is bound to the CEACAM1 ITIM (immunoreceptor tyrosine-based inhibition motif) portion which is phosphorylated by the LCK protein bound to the end of CD4 of the TCR due to the CEACAM1-CEACAM1 interaction. Also, the terminal of CD3ζ is dephosphorylated as well as ZAP70 by the SHP1 protein, and thus, TCR downstram signaling pathways including RAS-MAPK pathway is not activated and, as a result, T cells are not activated.

[127] Thus, the anti-CEACAM1 antibody or the fragment thereof can be used as an anti-cancer agent by blocking the CEACAM1-CEACAM1 interaction in advance through

binding to CEACAM1 expressed in cytotoxic T cells, natural killer cells and cancer cells.

[128] Also, the term "anti-cancer" as used herein encompasses "prevention" and "treatment." Herein, "prevention" refers to all actions of preventing cancer proliferation and delaying the progress of cancer by administration of the anti-cancer agent, and "treatment" refers to all actions of improving or ameliorating the symptoms of cancer by administration of the antibody of the present invention.

[129] Also, the term "cancer" as used herein, may be selected from the group consisting of gastric cancer, thyroid cancer, pancreatic cancer, melanoma, lung cancer and myeloma, but, is not limited thereto. It may include solid cancer and blood cancer and is not particularly limited as long as it has CEACAM1 as a receptor and its immune checkpoint pathway is abnormal. Also, the present invention provides an anti-cancer adjuvant comprising the anti-CEACAM1 antibody or a fragment thereof as an active ingredient.

[130] In addition, the present invention provides a composition for treating cancer comprising the anti-cancer adjuvant described above and a cell therapeutic agent. The cell therapy agent may include cytotoxic T cells or natural killer cells.

[131] Also, the term "cell therapeutic agent" as used herein refers to a drug used for the purpose of prevention or treatment through a series of actions that change the biological characteristics by proliferating and selecting living autologous, allogenic, and xenogenic cells *in vitro* to restore the function of cells and tissues. Specifically, it may be cytotoxic T cells or natural killer cells.

[132] Also, provided is a method for treating cancer using the anti-CEACAM1 antibody or a fragment thereof of the present invention. Specifically, the method may comprise administering to a subject lymphocytes contacted with the anti-CEACAM1 antibody or the fragment thereof. The lymphocytes are a kind of leukocytes, which account for about 25% of all leukocytes, and may be natural killer cells, T cells and B cells. Also, the lymphocytes may be obtained from a subject. Preferably, the lymphocytes may include at least one of cytotoxic T cells and natural killer cells. The cancer is as described above.

[133] Also, the term "subject" as used herein refers to a person who is in a state where a disease can be alleviated, suppressed or treated by administering the anti-cancer adjuvant of the present invention, or is suffering from a disease.

[134] Also, the term "contacting" as used herein also refers to mixing the anti-CEACAM1 antibody or a fragment thereof with cells expressing CEACAM1.

[135] The term "administration" as used herein refers to introduction of an effective amount of a substance into a subject by an appropriate method, and the administration of a composition comprising the anti-CEACAM1 antibody or the fragment thereof of the present invention may be carried out via a general administration route which

allows the substance to reach target tissues. Specifically, the administration may be parenteral administration (i.e., intravenous, subcutaneous, intraperitoneal or local administration, etc.) depending on the intended use, and preferably, it may be intravenous administration. In some cases of administration to solid tumors, local administration may be preferable in terms of rapid and easy access of the antibodies. The dosage varies depending on the patient's weight, age, sex, health condition, diet, the administration time, the administration method, the excretion rate, and the severity of a disease. The single dose may be about 0.001 to 10 mg/kg, which may be administered by a daily or weekly basis. The effective amount may be adjusted according to the discretion of a physician treating the patient.

[136] A composition for treating cancer according to the present invention may be administered in a pharmaceutically effective amount to treat cancer cells or their metastasis or to inhibit cancer growth. The dosage may vary depending on the type of cancer, patient's age and weight, the nature and severity of symptoms, the type of current treatment, the number of treatments, the type and route of administration, etc., and can be easily determined by experts in the art.

[137] As for the composition of the present invention, the pharmacological or physiological components described above may be administered concurrently or sequentially, or may be administered in combination with an additional conventional therapeutic agent sequentially or concurrently. Such administration may be single or multiple administrations. It is important to take into account all of the above factors and administer the amount which leads to a maximum effect with a minimal amount without side effects, which can be easily determined by those skilled in the art.

[138] Also, the present invention provides a method for inhibiting proliferation of CEACAM1-expressing tumor cells using the anti-CEACAM1 antibody or a fragment thereof. Specifically, it may comprise contacting the CEACAM1-expressing tumor cells with the anti-CEACAM1 antibody or the fragment thereof.

Mode for the Invention

[139] Hereinafter, the present invention is explained in detail by Examples. The following Examples are intended to further illustrate the present invention without limiting its scope.

[140] **Example 1. Anti-**CEACAM1 antibody (C25)

[141] **Example 1.1 Preparation of anti-**CEACAM1 antibody

[142] The antibody fragment genes inserted in the pComb3X vector (Addgene; Cat. No. 63891) in the form of a single-chain variable fragment (scFv) were subjected to perform PCR to obtain the light chain variable region genes represented by SEQ ID NOS: 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83 or 85, and the heavy chain

variable region gene represented by SEQ ID NOS: 87, 89, 91, 93, 95, 97, 99, 101, 103 or 105, which include the sequences recognized by each restriction enzyme. The heavy chain genes were treated with NotI and ApaI restriction enzymes and the light chain genes were treated with NotI and BamHI restriction enzymes.

[143] The heavy and light chain genes were inserted into the pcIW vector (Promega; Cat. No. E1731) digested with the same restriction enzymes as for the heavy or light chain genes. Then, vectors containing both the heavy chain transcription unit and the light chain transcription unit were selected using restriction enzymes. The selected vectors were extracted using QIAGEN Plasmid Plus Midi Kit (QIAGEN; Cat. No. 12943), and the base sequences of the antibodies were finally identified by base sequence analysis using some of the extracted DNA. The amino acid sequences of the antibodies were analyzed based on the above base sequences. The amino acid sequences and base sequences of the analyzed antibodies are shown in Table 1 and Table 2.

[144] [Table 1]

	Light chain						Heavy chain					
	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3	SEQ ID NO	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3	SEQ ID NO
1-19	SSNIGNNY	1	ADSKRP	9	GAWDLSLNGYV	17	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
1-23	SSNIGNNY	1	ADSKRP	9	GAWDVSHNGYV	18	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
3-07	SSNIGNNY	1	ADSKRP	9	GAWDQSLNGYV	19	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
3-27	SSNIGNNY	1	ADSKRP	9	GAWDSMGNGYV	20	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
4R9	SSNIGNNY	1	ADSRRP	10	GAWDLSLNGYV	17	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
4R20	SSNIGNNY	1	ADSRRP	10	GAWDASYNGYV	21	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
4R26	SSNIGNNY	1	ADSKRL	11	GAWDGRLNGYV	22	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
4R9_H2-2	SSNIGNNY	1	ADSRRP	10	GAWDLSLNGYV	17	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPLFDY	48
4R9_H4-n20	SSNIGNNY	1	ADSRRP	10	GAWDLSLNGYV	17	GFNFSNYA	31	ISHGGGSI	39	ARDPTKGYAPTFDY	47
C25	SSNIGNNY	1	ADSKRP	9	GAWDASLNGYV	23	GFTFSNYA	30	ISHGGGSI	39	ARDPTKGYAPTFDY	47
C15	SSSNIGNNY	2	ANSNRP	12	GTWDASLSAYV	24	GFTFSSYS	32	ISPNGGNK	40	AKDPYNIYQPLFDY	49
C16	SSNIGSNT	3	ADNNRP	13	GTWDYSLSGYV	25	GFTFSNYS	33	ISSDGGSK	41	ARDPRKHVDRYFDY	50
C17	SSNIGNNA	4	ANSHRP	14	GAWDASLNGYV	23	GFTFSDYS	34	IYPDDGNT	42	ARGSIWWLSLIPSSYNAMDV	51
C18	SSNIGSNA	5	ADSHRP	15	GSWDDSLNAYV	26	GFTFSNYD	35	ISHSSGSK	43	ARDPLPCLIPKCSYYYAMDV	52
C19	SSNIGSNY	6	SNSHRP	16	AAWDSSLNGYV	27	GFTFSGYA	36	IYHDGGST	44	ARVTVLCTTYGCSSYDGMDV	53
C22	SSNIGSNN	7	ANSHRP	14	GSWDSSLNAYV	28	GFTFSDYD	37	IYSGSSSK	45	AKAPLPFYFRPKSYYYAMDV	54
C26	SSNIGNN	8	ADSHRP	15	GAWDYSLSGYV	29	GFTFSGYD	38	ISYGGGSI	46	AKDRLPQKAVRHSYANGMDV	55

[145] [Table 2]

	Light chain variable region	Heavy chain variable region	Light chain	Heavy chain
	SEQ ID NO	SEQ ID NO	SEQ ID NO	SEQ ID NO
1-19	SEQ ID NO: 56, 57	SEQ ID NO: 86, 87	SEQ ID NO: 106	SEQ ID NO: 121
1-23	SEQ ID NO: 58, 59	SEQ ID NO: 86, 87	SEQ ID NO: 107	SEQ ID NO: 121
3-07	SEQ ID NO: 60, 61	SEQ ID NO: 86, 87	SEQ ID NO: 108	SEQ ID NO: 121
3-27	SEQ ID NO: 62, 63	SEQ ID NO: 86, 87	SEQ ID NO: 109	SEQ ID NO: 121
4R9	SEQ ID NO: 64, 65	SEQ ID NO: 86, 87	SEQ ID NO: 110	SEQ ID NO: 121
4R20	SEQ ID NO: 66, 67	SEQ ID NO: 86, 87	SEQ ID NO: 111	SEQ ID NO: 121
4R26	SEQ ID NO: 68, 69	SEQ ID NO: 86, 87	SEQ ID NO: 112	SEQ ID NO: 121
4R9_H2-2	SEQ ID NO: 64, 65	SEQ ID NO: 88, 89	SEQ ID NO: 110	SEQ ID NO: 122
4R9_H4-n20	SEQ ID NO: 64, 65	SEQ ID NO: 90, 91	SEQ ID NO: 110	SEQ ID NO: 123
C25	SEQ ID NO: 70, 71	SEQ ID NO: 86, 87	SEQ ID NO: 113	SEQ ID NO: 121
C15	SEQ ID NO: 72, 73	SEQ ID NO: 92, 93	SEQ ID NO: 114	SEQ ID NO: 124
C16	SEQ ID NO: 74, 75	SEQ ID NO: 94, 95	SEQ ID NO: 115	SEQ ID NO: 125
C17	SEQ ID NO: 76, 77	SEQ ID NO: 96, 97	SEQ ID NO: 116	SEQ ID NO: 126
C18	SEQ ID NO: 78, 79	SEQ ID NO: 98, 99	SEQ ID NO: 117	SEQ ID NO: 127
C19	SEQ ID NO: 80, 81	SEQ ID NO: 100, 101	SEQ ID NO: 118	SEQ ID NO: 128
C22	SEQ ID NO: 82, 83	SEQ ID NO: 102, 103	SEQ ID NO: 119	SEQ ID NO: 129
C26	SEQ ID NO: 84, 85	SEQ ID NO: 104, 105	SEQ ID NO: 120	SEQ ID NO: 130

[146] Thirty milliliters of ExpiHEK293F cells (ThermoFisher scientific; Cat. No. A14527) at a concentration of 2.5×10^6 cells/ml were treated and transfected with 30 μg of the extracted antibody DNA. On the next day following the transfection, an enhancer (ThermoFisher; Cat. No. A14524) was added to the transfected ExpiHEK293F cells and cultured in a shaking incubator for 7 days under the condition of 37℃, 8% CO_2 and 125 rpm, to produce anti-CEACAM1 antibody.

[147] After the culture, the supernatant separated from the culture medium by centrifugation was incubated with 100 $\mu\ell$ of protein A beads (Repligen; Cat. No. CA-PRI-0100) for 2 hours. The beads were then washed with 10 ml of a binding buffer (ThermoFisher Scientific; Cat. No. 21019). Thereafter, 200 $\mu\ell$of an elution buffer (ThermoFisher Scientific; Cat. No. 21004) was added to the beads, to separate the antibodies conjugated to the beads. The separated antibodies were neutralized by the addition of 10 $\mu\ell$ of 1.5 M Tris-HCl pH 8.8 solution (Bio-Rad; Cat. No. 210005897).

[148] **Experimental Example 1. Evaluation of binding ability of anti-**CEACAM1 antibody

[149] **Experimental Example 1.1. Evaluation of binding ability of anti-**CEACAM1 antibody according to CEACAM1 domain

[150] Thirty milliliters of ExpiHEK293F cells (ThermoFisher scientific; Cat. No. A14527) at a concentration of 2.5×10^6 cells/ml were treated and transfected with 30 μg of the DNA of CEACAM1 mutant proteins conjugated with a human immunoglobulin C

kappa domain. Also, the enhancer (ThermoFisher; Cat. No. A14524) was added to the transfected ExpiHEK293F cells and cultured in a shaking incubator for 7 days under the condition of 37℃, 8% CO_2 and 125 rpm.

[151] Then, the supernatant was separated from the culture medium and reacted with a kappa selection bead (GE Healthcare; Cat. No. 17-5458-01) for 2 hours. Thereafter, the beads were washed with 10 ml of a binding buffer and added with 200 ㎕ of an elution buffer, to separate and purify the CEACAM1 mutant protein from the beads (Fig. 1).

[152] Each of the purified CEACAM1 mutant proteins (2.5 ㎍) was dissolved in 1,000 ㎕ of PBS and dispensed into each well at 20 ㎕/well, and then reacted at 4℃ for 16 hours. Also, 1 ㎕ of C25 was diluted in 1,000 ㎕ of PBS and dispensed into each well at 25 ㎕/well, and then reacted at 37℃ for 1 hour. Then, each well was washed 3 times with a washing buffer prepared by diluting 10 ㎕ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 ㎕ of PBS. Thereafter, human IgG conjugated with 1 ㎕ of horse radish peroxidase (HRP) (HRP-conjugated anti-human IgG: Sigma; Cat. No. A0170) was diluted in 5000 ㎕ of PBS, which was then dispensed into each well at 25㎕/well and incubated at 37℃ for 1 hour.

[153] After completion of the reaction, the wells were washed three times with the washing buffer, and 25 ㎕ of TMB solution (KPL; Cat. No. 52-00-03) was added to each well to induce color development. Then, 25 ㎕ of 2 M H_2SO_4 was added to each well to stop the reaction, and the absorbance was measured at a wavelength of 450 nm.

[154] As a result, in the case of C25, the N-domain and the B-domain were found to be essential sites for binding with full affinity. It was found that A1 and A2 are not necessary for direct binding. Thus, C25 mainly binds to the N-domain of CEACAM1, and for the binding with full affinity, B-domain is additionally required. In contrast, some of the C25-derived mutant clones bind to the N-domain of CEACAM1 with minimal or residual dependence on the B-domain for the binding with their full affinities as compared to C25 (Fig. 2).

[155] **Experimental Example 1.2. Evaluation of binding ability of anti-**CEACAM1 antibody to CEACAM1 protein

[156] Two micrograms of the recombinant CEACAM1 protein were dissolved in 1000 ㎕ of PBS, which was dispensed into 96-well plate (Nunc; Cat. No. 467679) at 50 ㎕/well and incubated at 4℃ for 16 hours. Thereafter, 300 ㎕ of 3% (v/v) bovine serum albumin was added to each well and incubated at 37℃ for 1 hour. C25 antibody (0.75 ㎍) was dissolved in 1000 ㎕ of PBS. The diluted C25 solution was subjected to serial dilutions in PBS at a 1:1 volume ratio for 14 times. Each of the 15 different diluted C25 solutions was dispensed into each well at 50 ㎕/well, and incubated at 37℃ for 1 hour. Then, each well was washed 3 times with a washing buffer prepared by diluting 10 ㎕ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 ㎕ of PBS. Thereafter,

human IgG conjugated with 1 μℓ of HRP was diluted in 5000 μℓ of PBS, which was then dispensed into each well at 50 μℓ/well and incubated for 1 hour. The wells were washed three times with the washing buffer, and 50μℓ of TMB solution was added to each well to induce color development, and then, 50 μℓ of 2 M H_2SO_4 was added to each well to stop the reaction. The absorbance was measured at a wavelength of 450 nm.

[157] As a result, the EC_{50} (Half maximal effective concentration) value was measured to be 0.35 nM (Fig. 3).

[158] Also, 2.5 μg of the recombinant CEACAM-1/CD66a protein (R&D systems; Cat. No. 2244-CM) was dissolved in 10 ml of PBS, and dispensed into 96-well plate (Nunc; Cat. No. 467679) at 100 μℓ/well and incubated overnight at 4°C. Thereafter, 300 μℓ of 1% (v/v) bovine serum albumin was added to each well, which was incubated at 37°C for 1 hour.

[159] Each of C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibodies (3μg) was dissolved in 1000 μℓ of PBS. Each of the diluted antibodies was subjected to serial dilutions in PBS at a 1:1 volume ratio for 6 times. Each of the antibodies diluted to 7 concentrations was dispensed into each well at 100 μℓ/well, which was incubated at 37°C for 1 hour. Then, each well was washed 3 times with a washing buffer prepared by diluting 10 μℓ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 μℓ of PBS. Thereafter, 2 μℓ of human IgG conjugated with HRP was diluted in 10 ml of PBS, which was then dispensed into each well at 100 μℓ/well and incubated for 1 hour.

[160] After completion of the reaction, the wells were washed three times with the washing buffer, and 100 μℓ of TMB solution was added to each well to induce color development. Then, 100 μℓ of 2 M H_2SO_4 was added to each well to stop the reaction, and the absorbance was measured at a wavelength of 450 nm.

[161] As a result, it was found that C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibody bind to CEACAM1 protein (Fig. 4)

[162] **Experimental Example 1.3. Evaluation of binding ability of anti-**CEACAM1 antibodies to CEACAM1 protein expressed on cell surface

[163] Jurkat T cells (Jurkat E6.1 (ATCC; TIB-152TM)) were transfected with CEACAM1 cDNA, and treated with 700 μg/ml of G418 antibiotic for selection. The selected CEACAM1-Jurkat T cell lines were resuspended in the DPBS supplemented with 2% (v/v) FBS (hereinafter, referred to as FACS buffer), centrifuged at 1,500 rpm, and then resuspended in a FACS buffer such that the number of cells was $3x10^6$/ml. The cells were dispensed into each well of a U-bottom 96-well plate at 100 μℓ/well. Then, the cells were centrifuged at 1,500 rpm, and the supernatant was discarded. After resuspending the recovered cells in 50 μℓ of the FACS buffer to which 0.5 μℓ of human Fc block (BD Pharmingen; Cat. No. 564220) solution was added, the cells were

incubated at 4°C for 15 minutes (Fig. 5).

[164] Anti-CEACAM1 antibody or human IgG4 (Sigma; Cat. No. I4639) was diluted in 50 μℓ of the FACS buffer to obtain the concentrations of 20 μg/ml, 10 μg/ml, 5 μg/ml, 2.5 μg/ml, 1.25 μg/ml, 0.625 μg/ml, 0.3125 μg/ml and 0.15625 μg/ml. Fifty microliters of anti-CEACAM1 antibody or human IgG4 diluted above was added to the cells and incubated at 4°C for 1.5 hour.

[165] The cells incubated with antibodies were repeatedly subjected to the washing procedure of resuspending the cells in a FACS buffer and centrifuging the solution at 1,500 rpm. Goat anti-human $F(ab)_2$ labeled with phycoerythrin (hereinafter, referred to as PE) (Phycoerythrin-conjugated goat anti-human $F(ab)_2$; (Sigma; Cat. No. P8047)) was diluted in the FACS buffer at a volume ratio of 1:200, and then 100 μℓ of each solution was added to each well, which was incubated at 4°C for 30 minutes in a dark condition.

[166] The cells were repeatedly subjected to the washing procedure of resuspending the cells in a FACS buffer, centrifuging the solution at 1,500 rpm, and discarding the supernatant. The cells were then resuspended in 100 μℓ of a fixation buffer (BD Cytofix ™; Cat. No. 554655) and incubatedat 4°C for 30 minutes in a dark place.

[167] The cells incubated with the fixation buffer were repeatedly subjected to the washing procedure of resuspending the cells in a FACS buffer, centrifuging the solution at 1,500 rpm, and discarding the supernatant. The washed cells were resuspended in 200 μℓ of a FACS buffer, and the mean fluorescence intensities (MFIs) of PE-labeled cells were compared in a FACS LSR-Fortessa. All FACS analyses were conducted using the FlowJo software.

[168] It was observed that the maximal level of C25 binding to the target (MFI 1800 or more) was reached at 5 μg/ml of concentration, but its binding ability decreased rapidly below the concentration of 5 μg/ml (Fig. 5a).

[169] The CEACAM1-Jurkat T cells prepared above were treated with Fc block solution and incubated for 15 minutes. Then, C25 and C25-derived clones including 4R9, 4R9_H2-2, 4R9_H4-n20 and 4R26 along with human IgG4 were diluted respectively in 50 μℓ of a FACS buffer to the concentrations of 25 μg/ml, 5 μg/ml, 1 μg/ml, 0.2 μg/ml, 0.04 μg/ml, 0.008 μg/ml, 0.0016 μg/ml and 0.00032 μg, and then dispensed into the cells, which were incubated at 4°C for 1.5 hour. Herein, it is noted that the cell numbers were adjusted to $1x10^5$. The cells incubated with the antibodies were repeatedly subjected to the washing procedure of resuspending the cells in a FACS buffer and centrifuging the solution at 1,500 rpm.

[170] PE-conjugated goat anti-human $F(ab)_2$; (Sigma; Cat. No. P8047)) was diluted in the FACS buffer at a volume ratio of 1:200, and then added to the cells at 100 μℓ/well. Cells were resuspended well and incubated at 4°C for 30 minutes in a dark condition.

[171] The cells incubated with the fixation buffer were repeatedly subjected to the washing procedure of resuspending the cells in a FACS buffer, centrifuging the solution at 1,500 rpm, and discarding the supernatant. The washed cells were resuspended in 200 ㎕ of a FACS buffer, and the mean fluorescence intensities (MFIs) of the PE-labeled cells were monitored by a FACS LSR-Fortessa. All FACS analyses were conducted using the FlowJo software.

[172] Consistent with the results in Figure 5a, C25 showed the maximal levels of its target-binding (MFI 8008 or more) at the concentration of 5 ㎍/ml, but the binding ability rapidly decreased below the concentration of 5 ㎍/ ml., On the other hand, the target-binding abilities of C25-derived clones 4R9, 4R26, and 4R9_H2-2 were maintained up to 80% or higher of their maximal levels even at the concentration of 0.2 ㎍/ml. In addition, the MFI value of 4R9_H4-n20 clone reached up to 12,000 or more, showing 1.5 times higher in MFI values than those of other clones (12,000 vs 8,000) , but, the binding ability decreased rapidly below the concentration of 5 ㎍/ml similar to that of C25 (Fig. 5b).

[173] **Experimental Example 1.4. Measurement of target-binding affinity of each anti-**CEACAM1 antibody

[174] The quantitative binding abilities of C25, 4R9, 4R26, 4R9_H2-2, 4R9_H4-n20 and 4R9_H4-n20HC+4R26LC to CEACAM1 were measured using Octet QK^{e} (Pall ForteBio). Antibodies isolated and purified in Example 1 at the concentration of 400 nM were serially 1:1 diluted for 6 times, and the resulting antibody solutions at the concentrations of 400 nM, 200 nM, 100 nM, 50 nM, 25 nM, 12.5 nM and 6.25 nM were dispensed into Greiner 96-well plate (Greiner; Cat. No. 655209) in a row. The last well in each row was with a sample at the antibody concentration of 0 nM. Re-combinant human CEACAM1 protein (R&D Systems; Cat. No. 2244-CM) was diluted to obtain a concentration of 6.25 ㎍/㎕ and dispensed into each well of another column at 200 ㎕/well.

[175] As for the washing buffer, neutralization buffer and baseline buffer, Reagent/Kinetics buffer (10X) (Fortebio; Cat. No. 18-1092) were 1:10 diluted and dispensed into each well of a column in a row at 200 ㎕/well, and the regeneration buffer was dispensed into each well at 200 ㎕/well. Greiner 96-well plate was separately prepared, Reagent/ Kinetics buffer (1X) was dispensed into each well of the number of biosensors to be used in a row at 200 ㎕/well, and Biosensors/Anti-His (His1K) (Fortebio; Cat. No. 18-5120) cassette was installed. Association and dissociation periods were set to 300 seconds and 600 seconds respectively, and K_D values were measured.

[176] As a result, their affinities to CEACAM1 were determined as affinity (K_D) values of 1.82 nM to 39.0 nM (Fig. 6).

[177] **Experimental Example 2. Evaluation of physical properties of anti-CEACAM1**

antibody

[178] **Experimental Example 2.1. Identification of** isoelectric point of anti-CEACAM1 antibody

[179] Twenty milliliters of IEF anode buffer (50x) were mixed with 980 ml of deionized water (hereinafter, referred to as DW) to make a 1X IEF anode buffer, and 20 ml of IEF cathode buffer pH 3-10 (10X) was mixed with 180 ml of DW to make a 1X IEF cathode buffer. 1X IEF anode buffer and 1xIEF cathode buffer were cooled to 4°C and used.

[180] Using an IEF gel (Invitrogen/pH 3-10 IEF gel; 1.0 mm x 10 well/EC6655BOX), the upper chamber was filled with 200 ml of 1X IEF cathode buffer and the lower chamber was filled with 600 ml of 1X IEF anode buffer. Fifteen microliters $\mu\ell$ out of 20 $\mu\ell$ of the solution containing 10 $\mu\ell$ of C25 at the concentration of 1.12 mg/ml mixed with 10 $\mu\ell$ of IEF sample buffer (pH 3-10, 2X) were loaded, and 5 $\mu\ell$ of IEF Markers 3-10 (SERVA/10 mg/mL/SERVA Liquid Mix; 39212.01) was used for a marker.

[181] Electrophoresis was carried out with the voltage changed in three steps of 100V: 1 hour, 200V: 1 hour and 500V: 30 minutes, and fixation was carried out using 12% TCA solution for 30 minutes. After fixation, the isoelectric point was evaluated using Coomassie Blue R-250 Intron Biotechnology (IBS-BC006).

[182] As a result, the actual isoelectric point value of C25 turned out 8.0, which was slightly higher than its theoretical value of 7.76 (Fig. 7).

[183] **Experimental Example 3. Measurement of anti-**CEACAM1 antibody effect on T cell activation

[184] **Experimental Example 3.1. Evaluation of T cell activation by anti-**CEACAM1 antibody

[185] Jurkat E6.1 (ATCC; TIB-152TM) cells were resuspended at 1×10^5/200 $\mu\ell$ in complete Iscove's Modified Dulbecco's Medium (IMDM; Invitrogen; Cat. No. 12440053) supplemented with 10% (v/v) Fetal Bovine Serum (Gibco; Cat. No. 16000044) and 1X Penicillin/Streptomycin (100X; Gibco; Cat. No. 15140122), and incubated with plate-coated anti-CD3 (OKT3; 0.1 μg/ml; eBioscience; Cat. No. 16-0037), in the presence of 10 μg/ml or 25 μg/ml, at 37°C for 96 hours with 5% CO_2. HuIgG4 was used as a control.

[186] After 96 hours, the cells and culture medium were recovered and centrifuged at 1,500 rpm. While the supernatant was set aside for IL-2 measurement, the remaining cells were recovered, replaced with a FACS buffer, and subjected to a blocking step with Fc receptor at 4°C for 15 minutes.

[187] The cells were incubated with anti-CD25-PE-Cy7 antibody or anti-CD69-APC antibody (eBioscience; anti-CD25-PE-Cy7: Cat. No. 25-0259; anti-CD69-APC: Cat. No. 17-0699) at 4°C for 15 minutes.

[188] Cells were filled with a FACS buffer up to 200 ㎕, and centrifuged at 1,200 rpm for 5 minutes, with the supernatant removed. After resuspending the cells in a fresh FACS buffer, this procedure was repeated three times for complete removal of unbound antibodies. The cells were resuspended in DPBS supplemented with 1% (v/v) paraformaldehyde and fixed at 4℃ for 30 minutes.

[189] Cells were resuspended in lX FoxP3 staining buffer (eBioscience; Cat. No. 00-5523-00) and centrifuged. After repeating this procedure twice, anti-Ki67 antibody (eBioscience; Cat. No. 350520) labeled with a different dye was diluted at 1: 100 volume ratio in 1X FoxP3 staining buffer, which was added to the cells to obtain the total volume of 50 ㎕, and then the mixture was incubated at 4℃ for 30 minutes. Cells were filled with 1X FoxP3 staining buffer and centrifuged. After repeated washing three times, cells were applied to flow cytometry for counting activated $CD25^+$ $CD69^+$ cells and further $Ki67^+$ proliferating populations in the FACS LSR-Fortessa device and analyzed using the FlowJo software (Figs. 8 and 10).

[190] As a result, when Jurkat T cells were treated with C25 at the antibody concentration of 10 ㎍/ml and 25 ㎍/ml in the CCM1 induction condition (OKT3: 0.1 ㎍/ml, culture in cIMDM for 4 days) for Jurkat E6.1 T cells, high levels of activation were observed. More specifically, the percentage and the number of activated $CD25^+$ $CD69^+$ populations of Jurkat T cells were increased two-fold or higher, and the percentage and the number of proliferating $Ki67^+$ cells were also increased two-fold or higher as compared to the control which was treated with huIgG4 (Figs. 9b and 11b).

[191] **Experimental Example 3.2. Evaluation of IL-2 secretion of T cells by anti-CEACAM1 antibody**

[192] To evaluate the changes in IL-2 secretion from T cells by anti-CEACAM1 antibody, anti-IL-2 antibodies (capture Ab: eBioscience; Cat. No. 14-7029-85) were diluted in a coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6) first. Then, the diluted anti-IL-2 antibody solution was dispensed into each well of a 96-well plate at 200 ㎕/well, and incubated at 4℃ for 16 to 18 hours. Then, the 96-well plate was washed with DPBS and 200 ㎕ of a blocking buffer (SuperBlock™ Blocking Buffer: ThermoFisher Scientific; Cat. No. 37515) was added to each well, which was then incubated at room temperature for 30 minutes.

[193] To obtain a standard curve, IL-2 recombinant protein (R&D Systems; Cat. No. P60568) was diluted in a blocking buffer to obtain a concentration of 20 ㎍/ml. The diluted IL-2 recombinant protein solution was serially diluted at a volume ratio of 1:1 for 11 times. Twelve IL-2 recombinant protein samples and the supernatants from the Jurkat E6.1 cell culture stored at -80℃ in Example 3.1 were dispensed into each well of a 96-well plate coated with anti-IL-2 antibody at 100 ㎕/well, and incubated at room temperature for 2 hours.

[194] After washing the 96-well plate 4 times with a washing buffer prepared by diluting 10 μℓ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 μℓ of PBS, a solution prepared by diluting biotin-conjugated anti-IL-2 antibodies (Detection antibody: eBioscience; Cat. No. 13-7028) in a blocking buffer at a volume ratio of 1:1,000 was dispensed into each well at 100 μℓ/well, which was then incubated at room temperature for 2 hours.

[195] After washing the 96-well plate with a washing buffer 4 times, a solution prepared by diluting a peroxidase-labeled streptavidin (Sigma; Cat. No. S5512) in a blocking buffer at a volume ratio of 1: 1,000 was dispensed into each well at 100 μℓ/well, which was incubated at room temperature for 2 hours.

[196] After washing the 96-well plate 4 times again with a washing buffer, TMB peroxidase substrate solution (KPL; Cat. No. 52-00-02) was dispensed into each well at 100 μℓ/well, which was incubated for 10 minutes. Then, a stop solution (KPL; Cat. No. 50-85-05) was added to the wells at 100 μℓ/well, to stop the reaction. The O.D values were measured at a wavelength of 450 nm using a molecular dynamics reader device. The measured values were analyzed using SoftMax Pro 5.4.1.

[197] As a result, when the cells were treated with C25 at the antibody concentration of 10 μg/ml and 25 μg/ml in the CEACAM1 induction condition (OKT3 at 0.1 μg/ml, culture for 4 days) for Jurkat E6.1, the secreted IL-2 levels measured using the supernatants were about three times higher than those of the control (Figs. 9c and 11c).

[198] **Experimental Example 3.3. Evaluation of activation of T cells overexpressing** CEACAM1 by anti-CEACAM-1 antibody

[199] EF1 promoter-CEACAM1-GFP vector was transfected into Jurkat E6.1 T cells, and then treated with G418 disulfate salt solution (Sigma; G8168) at a concentration of 700 μg/ml, for selection. GFP^+ $CEACAM1^+$ cells were separated using Fluorescence-Activated Cell Sorter, to construct Jurkat cells overexpressing CEACAM1 (CEACAM1-Jurkat T cells).

[200] CEACAM1-Jurkat T cells derived from Jurkat E6.1 (ATCC; TIB-152TM) cells were resuspended in 200 μℓ of IMDM supplemented with 10% FBS and 1X Penicillin/ Streptomycin to obtain a cell number of 1×10^5, and then stimulated with plate-coated OKT3 (0.1 or 1 μg/ml; eBioscience; 16-0037), in the presence of 10 μg/ml or 25 μg/ml of C25, for 48 hours. HuIgG4 was used as a control.

[201] After 48 hours, the cells and culture medium were recovered and centrifuged at 1,500 rpm. The supernatant was stored at -80°C for IL-2 ELISA, and the remaining cells were recovered, replaced with a FACS buffer, and subjected to blocking of the Fc receptor at 4°C for 15 minutes. Cells were treated with CD25-PE-Cy7 antibody or anti-CD69-APC antibody, and incubated at 4°C for 15 minutes.

[202] Cells were recovered, and filled with a FACS buffer up to 200 μℓ, and centrifuged at

1,200 rpm for 5 minutes. After resuspending the cells in a fresh FACS buffer, this procedure was repeated three times, for complete removal of unbound antibodies. The cells were resuspended in DPBS supplemented with 1% (v/v) paraformaldehyde and fixed at 4℃ for 30 minutes.

[203] Cells were filled in lX FoxP3 staining buffer up to 200 ㎕, and centrifuged at 1,200 rpm for 5 minutes, to remove the supernatant. After repeating this procedure twice, anti-Ki67 antibody labeled with a different dye was diluted at 1: 100 volume ratio, which was added to the cells to obtain the total volume of 50 ㎕, and then the mixture was incubated at 4℃ for 30 minutes.

[204] Cells were filled in lX FoxP3 staining buffer up to 200 ㎕, and centrifuged at 1,200 rpm for 5 minutes, to remove the supernatant. After repeating this procedure three times, and replacing with FACS buffer finally, activated $CD25^+$ $CD69^+$ cells and proliferating $Ki67^+$ cells among them were identified in the FACS LSR-Fortessa device and analyzed using the FlowJo software (Figs. 12 and 15).

[205] As a result, CEACAM1-Jurkat T cells by a low concentration of OKT3 (0.1 ㎍/ml) in the presence of anti-CEACAM1 showed high levels of activation. More specifically, $CD25^+$ $CD69^+$ populations of CEACAM1-Jurkat T cells were increased by 1.5-fold (C25 at 25 ㎍/ml) or 4-fold or higher (C25 at 10 ㎍/ml) as compared to those of the huIgG4-treated group (Figs. 13b and 14b).

[206] Also, CEACAM1-Jurkat T cells by a high concentration of OKT3 (1 ㎍/ml) in the presence of anti-CEACAM1 showed high levels of activation. More specifically, $CD25^+$ $CD69^+$ populations of CEACAM1-Jurkat T cells were increased by 2-fold (C25 at 25 ㎍/ml) or by 6-fold or higher (C25 at 10 ㎍/ml) as compared to those of the huIgG4-treated group (Figs. 16b and 17b).

[207] **Experimental Example 3.4. Evaluation of IL-2 secretion of** CEACAM1 -overexpressing T cells by anti-CEACAM1 antibody

[208] To evaluate the anti-CEACAM1 antibody-induced changes in secreted IL-2 levels of CEACAM1-overexpressing T cells, the supernatant separated from CEACAM1-Jurkat E6.1 T cells stored at -80℃ in Experimental Example 3.3 was subjected to ELISA by the same method as in Experimental Example 3.2.

[209] As a result, when the CEACAM1-Jurkat T cells overexpressinf CEACAM1, were treated with C25 at the antibody concentrations of 10 ㎍/ml and 25 ㎍/ ml in a culture condition (OKT3 at the concentration of 0.1 ㎍/ml, culture for 2 days), the IL-2 secretion levels were measured using the supernatants were 20 times or more higher than the control (Figs. 13c and 14c).

[210] Further, when the CEACAM1-Jurkat T cells overexpressing CEACAM1 were treated with C25 at the antibody concentrations of 10 ㎍/ml and 25 ㎍/ ml in another culture condition (OKT3 at the concentration of 1 ㎍/ml, culture for 2 days), the IL-2

secretion levels were measured using the supernatants were 1.5 times or more higher than the control (Figs. 16c and 17c).

[211] **Experimental Example 3.5. Evaluation of T cell activation by anti-CEACAM1 antibody using** NFAT-luciferase assay

[212] Jurkat-GFP/NFAT-luc cells (cells not expressing CEACAM1, NFAT-luciferase reporter-overexpressing cells) or Jurkat-CCM1/NFAT-luc cells (CEACAM1-overexpressing cells, NFAT-luciferase reporter-overexpressing cells) were resuspended in each well at a cell number of 6 x 10^5, and dispensed into 96-well plate coated with 0.05 or 0.1 ㎍/ml of OKT3, and added with each antibody at a concentration of 10 ㎍/ml prepared in Example 1 and anti-CD28 (28.2; eBioscience; Cat. No. 16-0289-85; at 1 ㎍/ml). Human IgG4 was used as a negative control.

[213] After incubation for 6 hours, the cells were harvested, washed once with PBS, and lysed with 80 ㎕ of a passive lysis buffer. Twenty microliters of the cell lysate were dispensed into each well of a white 96-well-assay plate, which was then placed in a luminometer device. A luciferase buffer was loaded in a luminometer injector, and 80 ㎕ of the luciferase buffer was injected into each well, to measure the activity of luciferase.

[214] As a result, the inhibitory effect by C25 on CCM1-dependent T cell inhibition was not observed in Jurkat-GFP/NFAT-luc which did not express CEACAM1 in a 6-hour culture condition, whereas enhancement levels of TCR-induced NFAT activation by C25 during 6-hour duration was 2 times or more higher in Jurkat-CCM1/NFAT-luc cells with the OKT3 at both the concentrations of 0.05 and 0.1 ㎍/ml (Figs. 18 and 19).

[215] Also, the experimental group treated with C25-derived anti-CEACAM1 antibodies showed 1.5 to 2 times higher NFAT-luc luciferase activities than that of the IgG4-treated group at 0.1 ㎍/ml of OKT3 concentration, which were statistically significant (Fig. 20).

[216] **Experimental Example 4. Evaluation of expression of** CEACAM1 in human and monkey tissues

[217] A 10mM solution of EZ-Link™ Sulo-NHS-LC-LC-Biotin (ThermoFisher Scientific; Cat. No. 21338) was prepared and 13.3 ㎕ of the solution per 1 mg of C25 was added and biotinylated at 4℃ for 2 hours. Human and monkey TMA slides were deparaffinized in an oven at 60℃ for 1 hour. Hydration was progressively carried out in the order of xylene (100%), alcohol (90%), alcohol (80%) and alcohol (alcohol-DW).

[218] For antigen retrieval, the TMA slides were treated in a constant temperature water bath containing 1.5 L of Tris-EDTA pH 9.0 buffer, which were incubated at 100℃ for 20 minutes. To prevent intracellular peroxidase activity, 100 ㎕ of 3% hydrogen peroxide was added thereto and incubated for 6 minutes, and then 100 ㎕ of 5% (v/v) normal horse serum was added thereto and incubated for 30 minutes.

[219] The primary antibodies (biotin-conjugated hIgG4 or C25; 1 mg/ml) were diluted in 100 ㎕ of a staining buffer at a 1: 1 or 1: 5 volume ratio, and incubated at room temperature for 2 hours. One hundred microliters of ABC reagent were added and incubated for 30 minutes. DAB substrate kit (VECTOR LABORATORIES; Cat. No. SK-4100) was added, followed by color development for 2 minutes and comparative staining with hematoxylin for analysis. Since CEACAM1 expression levels in normal cells were very low, the antibodies were used at relatively high concentrations.

[220] The staining intensities of C25 on normal tissues of Cynomolgus monkeys were similar to those on human normal tissues, and the levels of CEACAM1 expression in normal tissues were very low. Therefore, CEACAM1 expression was detected only when the tissues were treated with high concentrations of C25 (Fig. 21). Human IgG4 was used at the same concentration, as a control for C25 (Fig. 22).

[221] **Experimental Example 5. Evaluation of cross-reactivity of anti-**CEACAM1 antibody

[222] **Experimental Example 5.1. Evaluation of cross-reactivity of anti-**CEACAM1 antibody to CEACAM family proteins using human IgG Fab antibody

[223] All of the recombinant human CEACAM-1/CD66a protein (R&D systems; Cat. No. 2244-CM, HCCM1), recombinant human CEACAM-5/CD66e protein (R&D systems; Cat. No. 4128-CM, HCCM5), recombinant human CEACAM-6/CD66c protein (R&D systems; Cat. No. 3934-CM, HCCM6), recombinant human CEACAM-3/CD66d protein (Sino Biological; Cat. No. 11933-H08H, HCCM3), recombinant mouse PD-1 Fc chimeric protein (R&D systems; Cat. No. 1021-PD, mPD-1-Fc), recombinant CEACAM1-Fc (IgG4) protein (Mogam, in-house production, HCCM1-Fc), recombinant human CEACAM-1/CD66a protein Bulk (R&D systems; Cat. No. 2244-CM, HCCM1(bulk)) and mouse CEACAM1/CD66a protein (Sino Biological; Cat. No. 50571-M08H, mouse CCM1) (2.5 ㎍ each) were dissolved in 10 ml of PBS, respectively and dispensed into a 96-well plate (Nunc; Cat. No. 467679) at 100 ㎕/well, which was incubated overnight at 4℃. Thereafter, each well was treated with 300 ㎕ of 1% (v/v) bovine serum albumin, which was incubated at 37℃ for 1 hour.

[224] Three micrograms of C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibodies were dissolved in 1000 ㎕ of PBS, respectively. The diluted antibodies were dispensed into each well coated with each protein at 100 ㎕/well, which was incubated at 37℃ for 1 hour. Then, each well was washed 3 times with a washing buffer prepared by diluting 10 ㎕ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 ㎕ of PBS. Thereafter, 2 ㎕ of HRP-conjugated human anti-IgG-Fab antibody (anti-human IgG Fab; Sigma; Cat. No. A0293) was diluted in 10 ml of PBS, added to the wells at 100 ㎕/well, and incubated for 1 hour.

[225] After completion of the reaction, the wells were washed three times with the washing

buffer, and 100 ㎕ of TMB solution was added to each well to induce color development. Then, 100 ㎕ of 2 M H_2SO_4 was added to each well to stop the reaction, and the absorbance was measured at a wavelength of 450 nm.

[226] As a result, all of the C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibodies showed high levels of binding ability to HCCM1, HCCM1 (bulk) and HCCM1-Fc. The CEACAM1 antibodies excluding C17, C19, C22 showed basal levels of binding ability to HCCM3, HCCM5 and HCCM6 (Fig. 23). Thus, it was concluded that the anti-CEACAM antibodies of the present invention bind specifically to CEACAM1 only.

[227] **Experimental Example 5.2. Cross-reactivity of** CEACAM family protein with anti-CEACAM1 antibody using human IgG Fc antibody

[228] All of the recombinant human CEACAM-1/CD66a protein (R&D systems; Cat No. 2244-CM, HCCM1), recombinant human CEACAM-5/CD66e protein (R&D systems; Cat. No. 4128-CM, HCCM5), recombinant human CEACAM-6/CD66c protein (R&D systems; Cat. No. 3934-CM, HCCM6), recombinant human CEACAM-3/CD66d protein (Sino Biological; Cat. No. 11933-H08H, HCCM3), human B7-H5/Gi24/VISTA protein (Sino Biological; Cat. No. 13482-H08H, HVISTA), recombinant human CEACAM-1/CD66a protein Bulk (R&D systems; Cat. No. 2244-CM, HCCM1 (bulk)), recombinant human CEACAM1-N domain-Kappa protein (Mogam, in-house production, HCCM1-N domain-kappa), and BSA protein (2.5 ㎍ each) were dissolved in 10 ml of PBS, respectively, and dispensed into a 96-well plate at 100 ㎕/well, which was incubated at 4℃ overnight. Thereafter, each well was treated with 300 ㎕ of 1% (v/v) bovine serum albumin, and incubated at 37℃ for 1 hour.

[229] Each of C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibodies (3 ㎍) was dissolved in 1000 ㎕ of PBS. The diluted antibodies were dispensed into each well coated with each protein at 100 ㎕/well, and incubated at 37℃ for 1 hour. Then, each well was washed 3 times with a washing buffer prepared by diluting 10 ㎕ of Tween 20 (Sigma-Aldrich; Cat. No. P9146) in 990 ㎕ of PBS. Thereafter, 2 ㎕ of HRP-conjugated human anti-IgG-Fc antibody (anti-human IgG Fc, Sigma Cat. No. A0170) was diluted in 10 ml of PBS, added to the wells at 100 ㎕/well, and incubated for 1 hour.

[230] After completion of the reaction, the wells were washed three times with the washing buffer, and 100 ㎕ of TMB solution was added to each well to induce color development. Then, 100 ㎕ of 2 M H_2SO_4 was added to each well to stop the reaction, and the absorbance was measured at a wavelength of 450 nm.

[231] As a result, all of the C15, C16, C17, C18, C19, C20, C21, C22, C23, C25 and C26 antibodies showed high levels of binding ability to HCCM1, HCCM1 (bulk) and

HCCM1-N-domain-kappa, and the CEACAM1 antibodies excluding C17, C19, C22 showed basal levels of binding ability to HCCM3, HCCM5 and HCCM6 (Fig. 24). Thus, it was found that the anti-CEACAM1 antibodies of the present invention bind specifically to CEACAM1 only.

[232] **Experimental Example 5.3. Evaluation of cross-reactivity of** CEACAM family protein expressed on cell surface with anti-CEACAM1 antibody

[233] HEK293 cells were added with 10 ㎍ of each pEF1α-AcGFP-N1 vector (Clontech, Cat. No. 631973) as a control vector, CEACAM1, CEACAM3, CEACAM5, CEACAM6 and CEACAM8 plasmid vectors, followed by transfection under the condition of pulse voltage 1,100 V, pulse width 20 ms and pulse number 2. After 48 hours of transfection, GFP expression was confirmed by fluorescence microscopy. The cells were detached by treatment with 1 ml of TrypLE Express Solution (ThermoFisher Scientific; Cat. No. 12605010), resuspended in 9 ml of DMEM (Gibco, Cat. No. 11995-065) supplemented with 10% (v/v) FBS, and centrifuged at 1,200 rpm for 5 minutes, to remove the resulting supernatant.

[234] After washing once with PBS, the cells were resuspended in a FACS buffer to obtain a concentration of 5 x 10^5 cells/100 ㎕. Human Fc Block solution was added to the samples at 1 ㎕/sample, which were incubated at 4℃ for 10 minutes. The cells were treated with hulgG4 or one of the anti-CEACAM1 clones listed at 1 ㎍/5x10^5 cells, which were incubated at 4℃ for 10 minutes. After washing with FACS buffer, the PE-labeled goat-anti-huIgG4 antibodies were diluted at a ratio of 1: 200 in a FACS buffer, and added to the samples at 100 ㎕/sample, which were incubated at 4℃ for 30 minutes. The samples were filled with a FACS buffer up to 200 ㎕, and then centrifuged at 1,200 rpm for 5 minutes, to remove the resulting supernatant. The fixation buffer (300 ㎕) was added to resuspend the cells. The cells expressing GFP were selected in the FACS LSR-Fortessa device, and the expression of CEACAM members were evaluated respectively using the antibody specific to each CEACAM member (Table 3). Also, the binding of anti-CEACAM1 antibodies was evaluated by PE fluorescence channel and analyzed using the FlowJo software.

[235] [Table 3]

Type	Form	Substance name	Purchased from:
CEACAM1	cDNA clone	CEACAM1	R&D Systems, RDC0951
	Isotype	MouseIgG1 Isotype Control PE	R&D Systems, IC002P
	Anti-CEACAM1 antibody	Human CEACAM1/CD66a PEconjugated Antibody	R&D Systems,FAB2244P
CEACAM3	cDNA clone	CEACAM3-GFP	Origene, RG217469
	Isotype	Sheep IgG	R&D systems, 5-001-A
	Anti-CEACAM3 antibody	HUMAN CEACAM-3/CD66d Antibody	R&D systems, AF4166P
	Secondary antibody	Donkey Anti-Sheep IgG (H+L) Phycoerythrin	R&D Systems,F0126
CEACAM5	cDNA clone	CEACAM5-GFP	Origene, RG206434
	Isotype	MouseIgG2a Isotype Control PE	R&D Systems, IC003P
	Anti-CEACAM5 antibody	HUMAN CEACAM-5/CD66e PE-conjugated Antibody	R&D systems, FAB41281P
CEACAM6	cDNA clone	CEACAM6-GFP	Origene, RG202454
	Isotype	Mouse IgG1 K Isotype Control PE	eBioscience,12-4714-82
	Anti-CEACAM6 antibody	Human CD66c-PE	eBioscience,12-0667-42
CEACAM8	cDNA clone	CEACAM8	Origene/RG204740
	Isotype	MouseIgG1 Isotype Control PE	R&D Systems, IC002P
	Anti-CEACAM8 antibody	Human CEACAM8 PE-conjugated Antibody	R&D systems/ FAB4246P

[236] As a result, it was found that C25 and C25-derived clones specifically bound to CEACAM1 only among the CEACAM members to which CEACAM1 belonged (Fig.

25).

[237] Further, HEK293 cells were mixed with 10 μg of each control vector, Cynomolgus CEACAM1 plasmid vector and human CEACAM1 plasmid vector, followed by transfection using a Neon transfection system under the condition of pulse voltage 1,100 V, pulse width 20 ms and pulse number 2. After 48 hours of transfection, GFP expression was confirmed by a fluorescence microscope, and the cells were detached by treatment with TrypLE Express Solution, and transferred to the FACS buffer, to stop the reaction.

[238] After washing once with PBS, the cells were resuspended in a FACS buffer to obtain a concentration of 5 x 10^5 cells/100 $\mu\ell$. Human Fc Block solution was added to the samples at 1 $\mu\ell$/sample, which were incubated at 4°C for 10 minutes. The samples were treated with human IgG4 or C25 at a concentration of 1 μg/5x10^5 cells, which were incubated at 4°C for 1 hour. After washing with FACS buffer, the PE-labeled goat-anti-huIgG4 antibodies were diluted at a ratio of 1: 200 in a FACS buffer, and added to the samples at 100 $\mu\ell$/sample, which were incubated at 4°C for 30 minutes. The samples were filled with a FACS buffer up to 200 $\mu\ell$, and then centrifuged at 1,200 rpm for 5 minutes, to remove the resulting supernatant. the fixation buffer (300 $\mu\ell$) was added to resuspend the cells. The cells expressing GFP were selected in the FACS LSR-Fortessa device, and the expression of each of CEACAM members was evaluated using the antibody specific to each member of CEA family. Also, the binding with C25 was evaluated by PE fluorescence channel and analyzed using the FlowJo software.

[239] As a result, it was found that C25 recognized not only human CEACAM1 but also monkey (Cynomolgus) CEACAM1 (Fig. 26a). C25-derived clones also recognized the CEACAM1 of cynomolgus as well as the human protein (Fig. 26b).

[240] **Experimental Example 6. Evaluation of anti-cancer effect of anti-CEACAM1 antibody**

[241] Cancer cells (MKN45: JCRB Cell Bank; Cat. No. JCRB0254; MKN1: JCRB Cell Bank; Cat. No. JCRB0252) were resuspended in RPMI 1640 medium (ThermoFisher Scientific; Cat. No. 11875093) at a concentration of 1x10^4/200 $\mu\ell$, and dispensed into each well of the 96-well plate at 200 $\mu\ell$/well, and cultured overnight under the condition of 37°C and 5% CO_2. Unconjugated cells were removed along with the medium, and TALL-104 (ATCC; Cat. No. CRL-11386TM) or NK92MI (ATCC; Cat. No. CRL-2408TM) cells were added thereto at various ratios relative to cancer cells (0:1, 0.1:1: 1:1, 10:1). Herein, C25 was also added to the wells at a concentration of 10 μg/ml. As a control, huIgG4 was treated in the same manner.

[242] After co-culture for 6 hours, unconjugated soluble cells were removed along with the medium, and Cell Titer 96 Aqueous One Solution (Promega; Cat. No. G3582), an

MTS assay reagent, was diluted at a ratio of 1:4 in RPMI 1640 medium. The diluted solution was added to the wells at 200 $\mu\ell$/well, and further cultured in a dark place for 3 hours. Thereafter, O.D value of each sample was measured with a spectrophotometer at a wavelength of 490 nm, and the measured values were analyzed using SoftMax Pro 5.4.1 program.

[243] As a result, when the anti-cancer effects of CTL (Fig. 27: TALL-104) or NK (Fig. 28: NK-92MI) cells were evaluated by the method of evaluating the *in vitro* efficacy of C25, it was found that C25 increased the anti-cancer immune reactions of CTLs and natural killer cells in the case of MKN45 gastric cancer cell line in which CEACAM1 was expressed at a high level, whereas no anti-cancer effect of C25 was observed in the case of MKN1, a gastric cancer cell line, in which CEACAM1 expression was not detected (Figs. 27 and 28).

[244] In addition, when the *in vitro* effect of C25-derived anti-CEACAM1 antibody clones were tested in the CEACAM1$^+$ MKN45 cancer cell line in the same manner as above, cancer cell death by anti-CEACAM1 antibodies reached 40 to 50% levels as compared to those of the control (Fig. 29).

[245]

Claims

[Claim 1] An anti-CEACAM1 antibody or a fragment thereof comprising:
light chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 1 to 8;
light chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 9 to 16;
light chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 17 to 29;
heavy chain CDR1 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 30 to 38;
heavy chain CDR2 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 39 to 46; and
heavy chain CDR3 comprising one of the amino acid sequences selected from the group consisting of SEQ ID NOS: 47 to 55.

[Claim 2] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 3] The anti-CEACAM1 antibody or the fragment thereof of claim 2, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 56, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 4] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 18, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39,

and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 5] The anti-CEACAM1 antibody or the fragment thereof of claim 4, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 58, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 6] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 19, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 7] The anti-CEACAM1 antibody or the fragment thereof of claim 6, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 60, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 8] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 20, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 9] The anti-CEACAM1 antibody or the fragment thereof of claim 8, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 62, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 10] The anti-CEACAM1 antibody or the fragment thereof of claim 1,

wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 11] The anti-CEACAM1 antibody or the fragment thereof of claim 10, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 12] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 21, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 13] The anti-CEACAM1 antibody or the fragment thereof of claim 12, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 66, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 14] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 11, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 22, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence rep-

resented by SEQ ID NO: 47.

[Claim 15] The anti-CEACAM1 antibody or the fragment thereof of claim 14, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 68, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 16] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 48.

[Claim 17] The anti-CEACAM1 antibody or the fragment thereof of claim 16, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 88.

[Claim 18] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 10, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 17, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 31, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 19] The anti-CEACAM1 antibody or the fragment thereof of claim 18, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 64, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 90.

[Claim 20] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the

amino acid sequence represented by SEQ ID NO: 2, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 12, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 24, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 32, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 40, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 49.

[Claim 21] The anti-CEACAM1 antibody or the fragment thereof of claim 20, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 72, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 92.

[Claim 22] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 3, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 13, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 25, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 33, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 41, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 50.

[Claim 23] The anti-CEACAM1 antibody or the fragment thereof of claim 2, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 74, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 94.

[Claim 24] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 4, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 14, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 34, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 42, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 51.

[Claim 25] The anti-CEACAM1 antibody or the fragment thereof of claim 24, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 76, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 96.

[Claim 26] The anti-CEACAM1 antibody or the fragment thereof ofclaim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 5, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 15, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 26, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 35, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 43, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 52.

[Claim 27] The anti-CEACAM1 antibody or the fragment thereof of claim 26, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 78, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 98.

[Claim 28] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 6, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 16, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 27, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 36, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 44, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 53.

[Claim 29] The anti-CEACAM1 antibody or the fragment thereof of claim 28, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 80, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 100.

[Claim 30] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 7, light chain CDR2

comprising the amino acid sequence represented by SEQ ID NO: 14, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 28, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 37, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 45, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 54.

[Claim 31] The anti-CEACAM1 antibody or the fragment thereof of claim 30, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 82, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 102.

[Claim 32] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 8, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 15, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 29, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 38, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 46, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 55.

[Claim 33] The anti-CEACAM1 antibody or the fragment thereof of claim 32, wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 84, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 104.

[Claim 34] The anti-CEACAM1 antibody or the fragment thereof of claim 1, wherein the antibody comprises light chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 1, light chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 9, light chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 23, heavy chain CDR1 comprising the amino acid sequence represented by SEQ ID NO: 30, heavy chain CDR2 comprising the amino acid sequence represented by SEQ ID NO: 39, and heavy chain CDR3 comprising the amino acid sequence represented by SEQ ID NO: 47.

[Claim 35] The anti-CEACAM1 antibody or the fragment thereof of claim 34,

wherein the antibody comprises a light chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 70, and a heavy chain variable domain comprising the amino acid sequence represented by SEQ ID NO: 86.

[Claim 36] The anti-CEACAM1 antibody or the fragment thereof of any one of claims 1 to 35, wherein the antibody fragment is one selected from the group consisting of Fab, scFv, $F(ab)^2$ and Fv.

[Claim 37] An anti-cancer agent comprising the anti-CEACAM1 antibody or the fragment thereof of any one of claims 1 to 35 as an active ingredient.

[Claim 38] The anti-cancer agent of claim 37, wherein the cancer is one selected from the group consisting of gastric cancer, pancreatic cancer, melanoma, lung cancer, thyroid cancer and myeloma.

[Claim 39] An anti-cancer adjuvant comprising the anti-CEACAM1 antibody or the fragment thereof of any one of claims 1 to 35 as an active ingredient.

[Claim 40] A composition for treating cancer comprising the anti-cancer adjuvant of claim 39 and a cell therapeutic agent.

[Claim 41] The composition for treating cancer of claim 40, wherein the cell therapeutic agent is cytotoxic T cells or natural killer cells.

[Claim 42] A method for treating cancer comprising administering to a subject lymphocytes contacted with the anti-CEACAM1 antibody or the fragment thereof of any one of claims 1 to 35.

[Claim 43] The method for treating cancer of claim 42, wherein the lymphocytes comprise at least one of cytotoxic T cells and natural killer cells.

[Claim 44] The method for treating cancer of claim 42, wherein the lymphocytes are obtained from a subject.

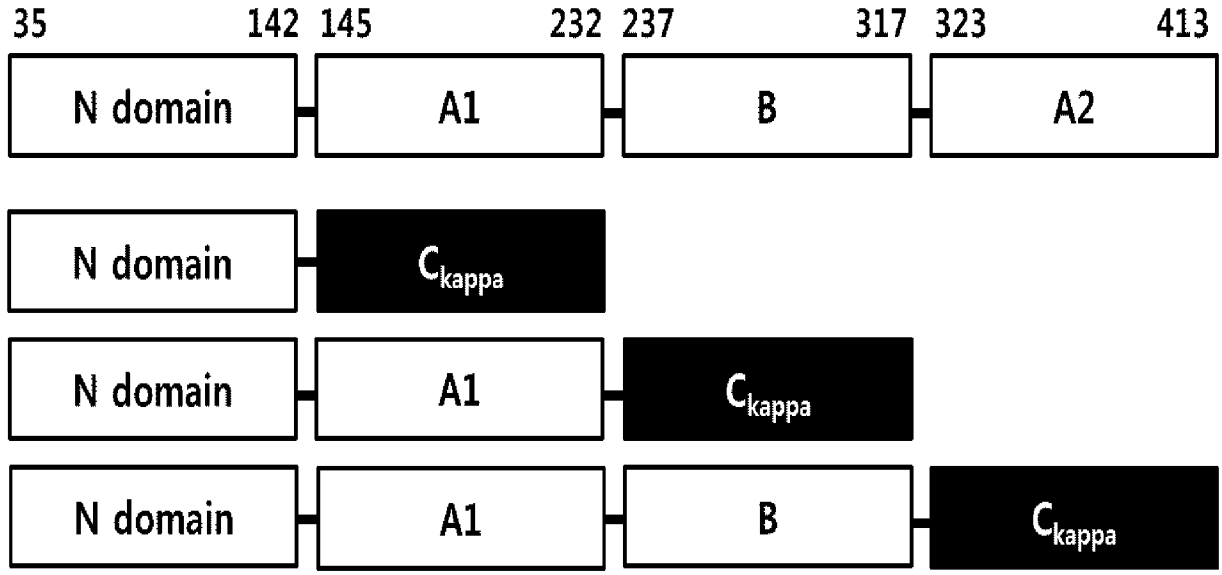
[Claim 45] The method for treating cancer of claim 42, wherein the cancer is one selected from the group consisting of gastric cancer, pancreatic cancer, melanoma, lung cancer, thyroid cancer and myeloma.

[Claim 46] A method for inhibiting proliferation of CEACAM1-expressing tumor cells, comprising contacting the CEACAM1-expressing tumor cells with the anti-CEACAM1 antibody or the fragment thereof of claim 1.

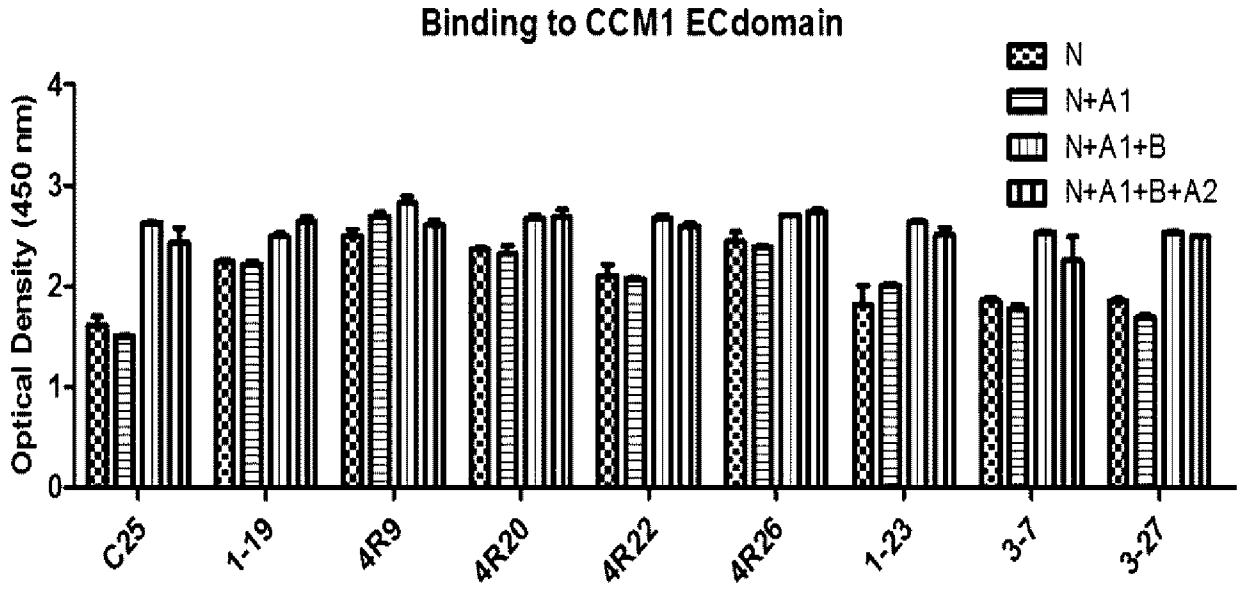
[Claim 47] A use of the antibody or the fragment thereof of claim 1 for the prevention or treatment of cancer.

[Claim 48] A use of the antibody or the fragment thereof of claim 1 for preparing a drug for the prevention or treatment of cancer.

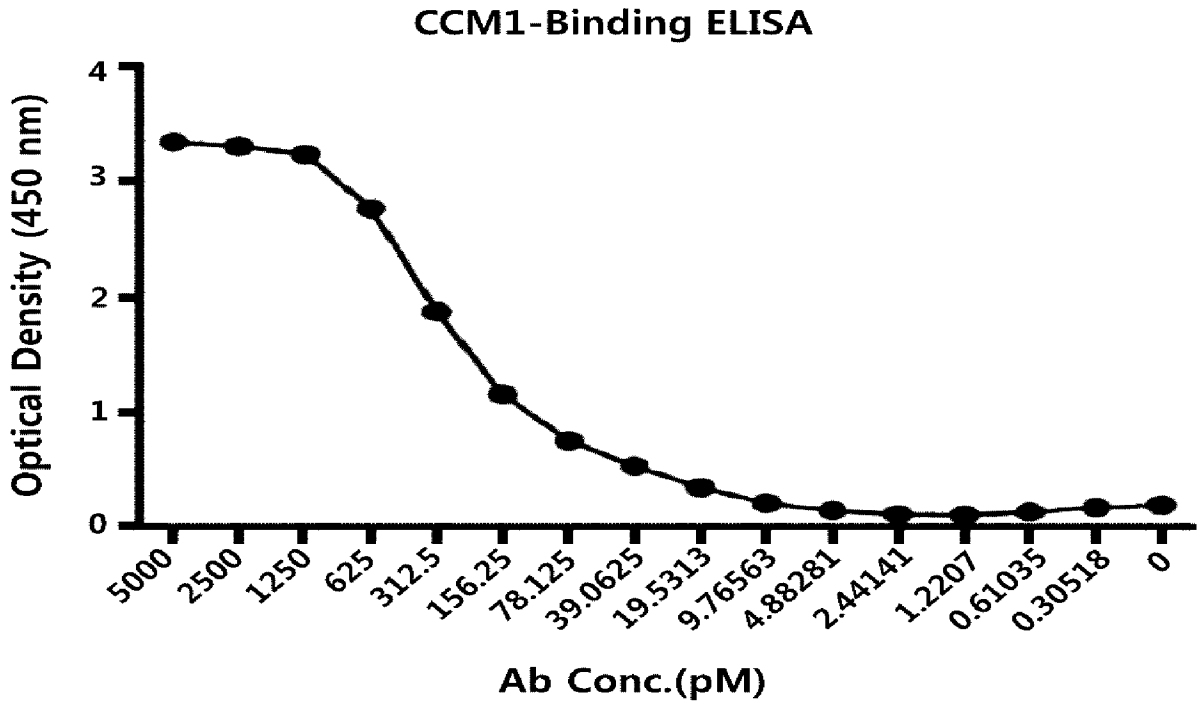
[Fig. 1]


35
142
145
232
237
317
323
413
N domain
A1
B
A2
N domain
Ckappa
N domain
A1
Ckappa
N domain
A1
B
Ckappa

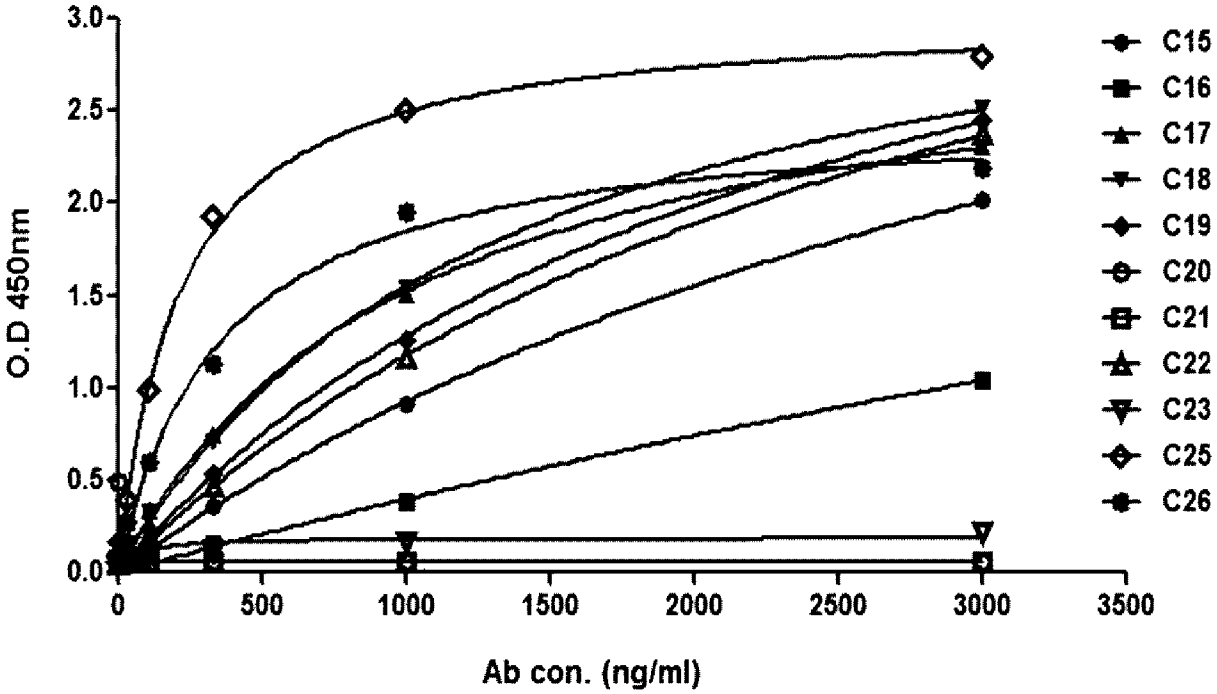
[Fig. 2]


Binding to CCM1 ECdomain
N
N+A1
N+A1+B
N+A1+B+A2
Optical Density (450 nm)
0
1
2
3
4
C25
1-19
4R9
4R20
4R22
4R26
1-23
3-7
3-27

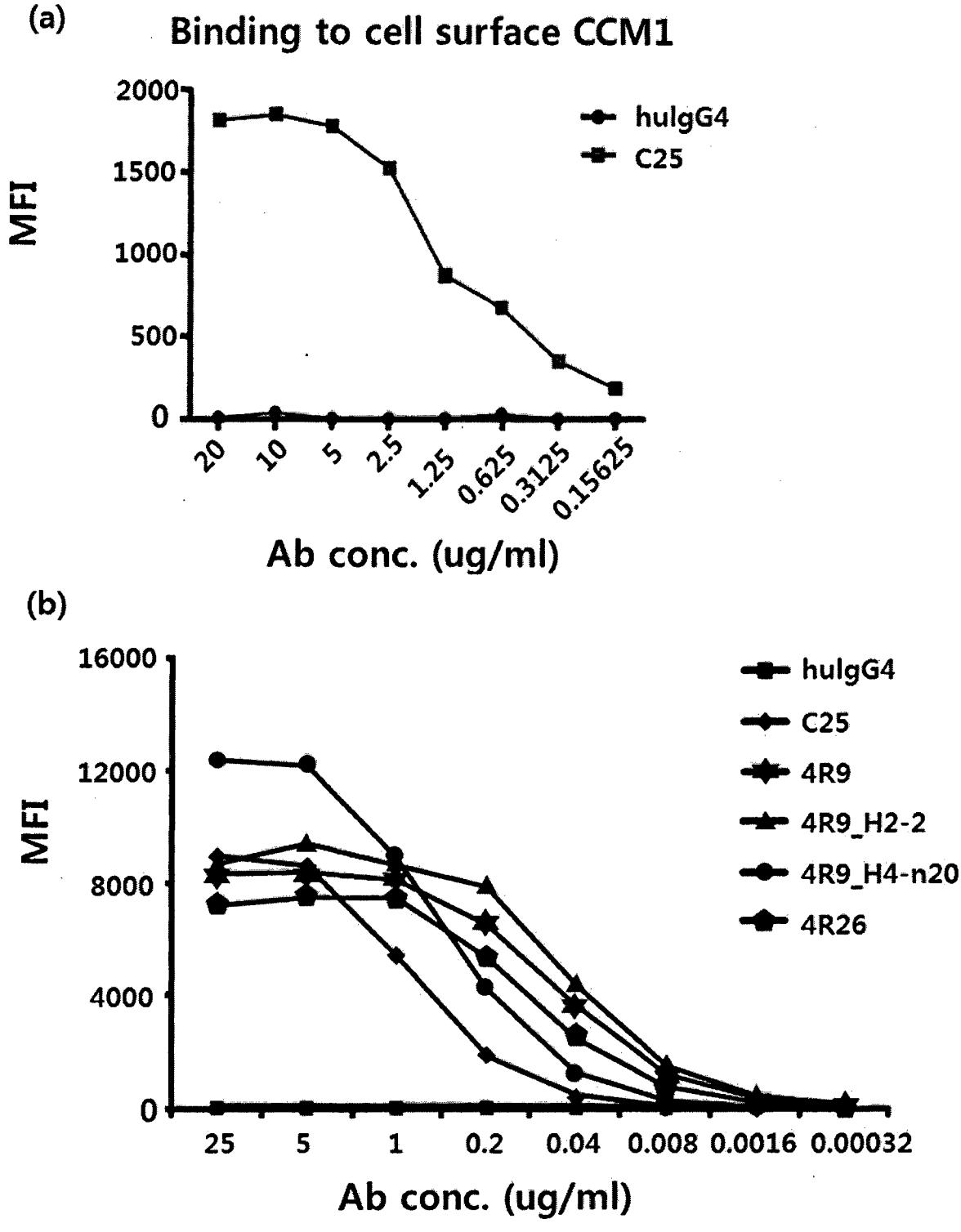
[Fig. 3]


CCM1-Binding ELISA
Optical Density (450 nm)
4
3
2
1
0
5000
2500
1250
625
312.5
156.25
78.125
39.0625
19.5313
9.76563
4.88281
2.44141
1.2207
0.61035
0.30518
0
Ab Conc.(pM)

[Fig. 4]


O.D 450nm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
3000
3500
Ab con. (ng/ml)
C15
C16
C17
C18
C19
C20
C21
C22
C23
C25
C26

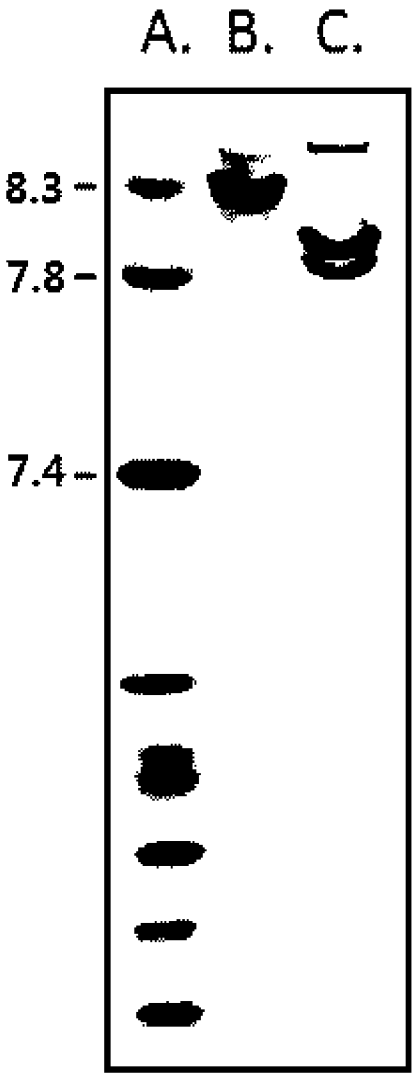
【Fig. 5】


(a)
Binding to cell surface CCM1
MFI
2000
1500
1000
500
0
20
10
5
2.5
1.25
0.625
0.3125
0.15625
Ab conc. (ug/ml)
hulgG4
C25
(b)
MFI
16000
12000
8000
4000
0
25
5
1
0.2
0.04
0.008
0.0016
0.00032
Ab conc. (ug/ml)
hulgG4
C25
4R9
4R9_H2-2
4R9_H4-n20
4R26

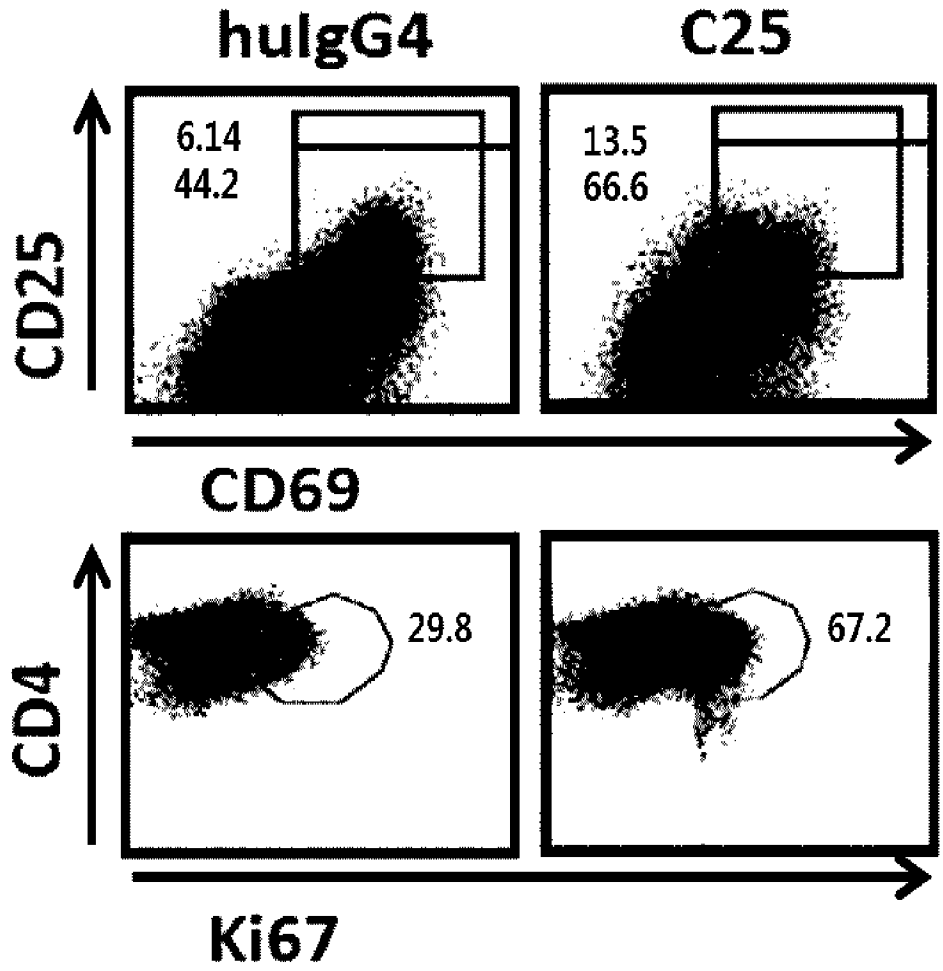
[Fig. 6]

Clone	K_D (M)	k_{on} (1/Ms)	K_{off}(1/s)
C25	3.49×10^{-8}	1.82×10^{4}	5.05×10^{-4}
4R9	3.39×10^{-9}	1.63×10^{5}	5.67×10^{-4}
4R26	1.82×10^{-9}	1.33×10^{5}	2.66×10^{-4}
4R9_H2-2	3.54×10^{-9}	1.69×10^{5}	6.27×10^{-4}
4R9_H4-n20	3.90×10^{-8}	2.61×10^{4}	3.33×10^{-4}
4R9_H4-n20HC +4R26LC	2.57×10^{-8}	1.90×10^{4}	3.21×10^{-4}

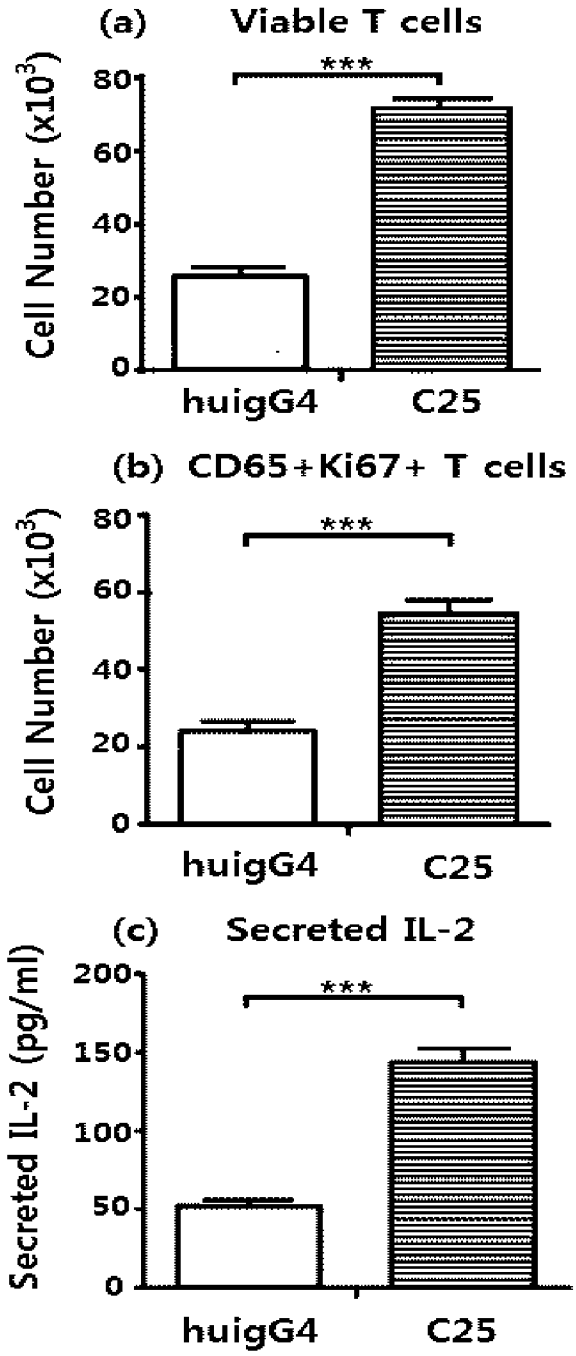
[Fig. 7]



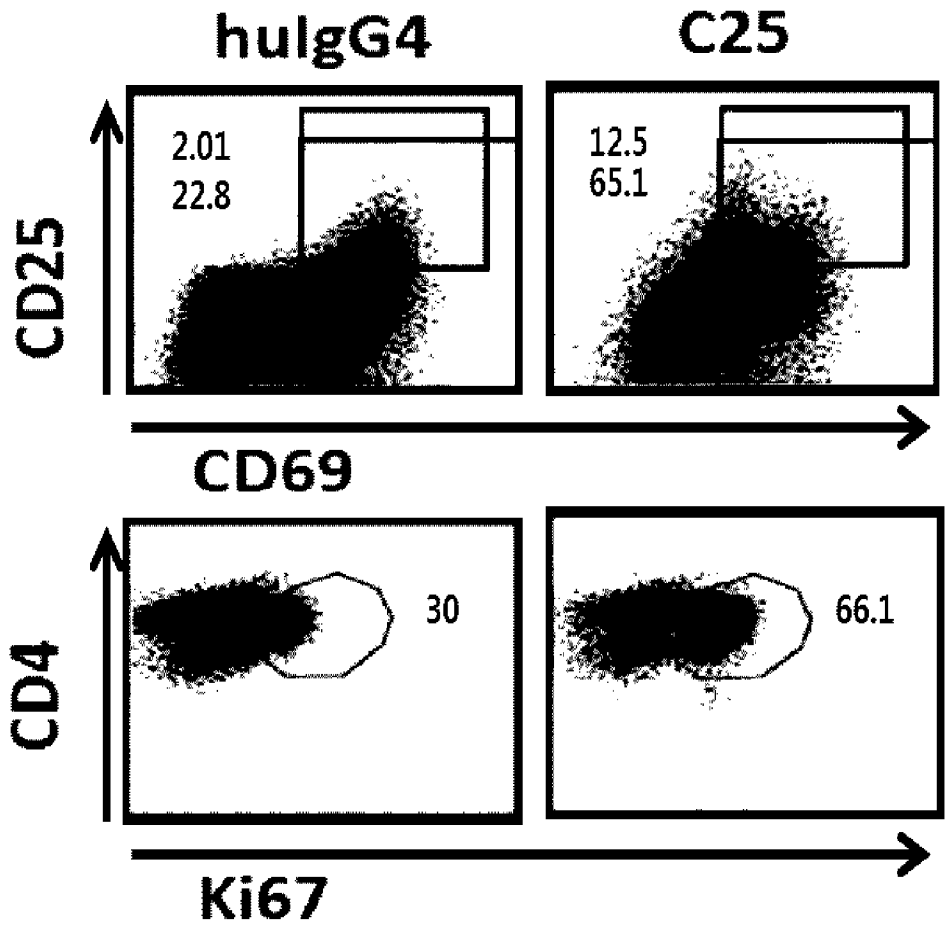
[Fig. 8]



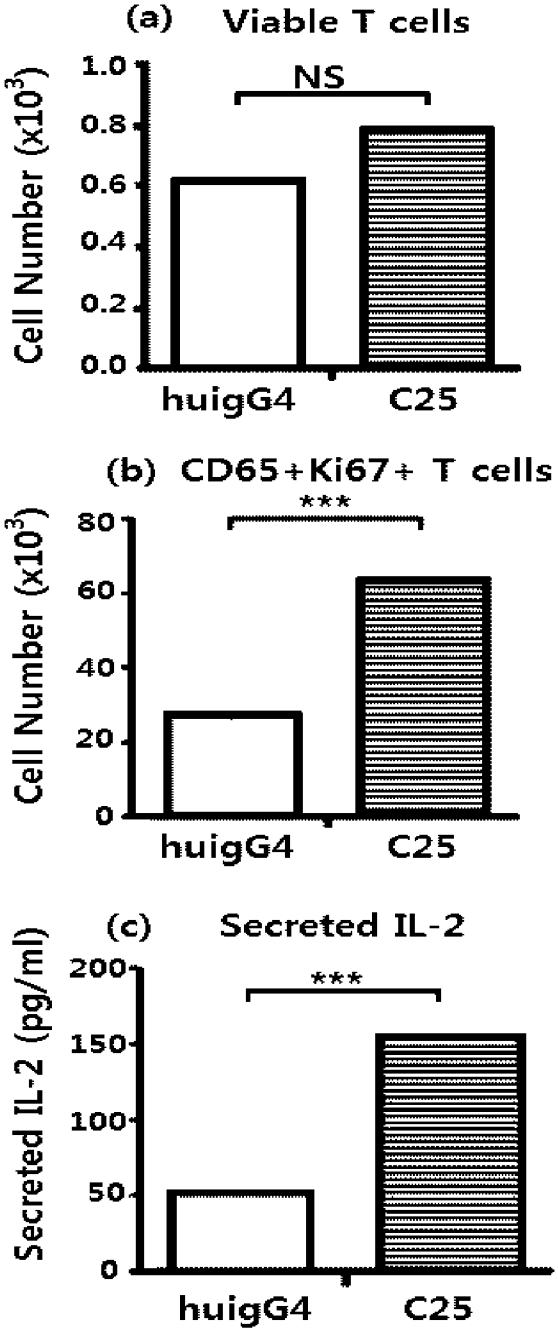
[Fig. 9]


(a) Viable T cells
Cell Number (x10^3)
***
80
60
40
20
0
huigG4
C25
(b) CD65+Ki67+ T cells
Cell Number (x10^3)
***
80
60
40
20
0
huigG4
C25
(c) Secreted IL-2
Secreted IL-2 (pg/ml)
***
200
150
100
50
0
huigG4
C25

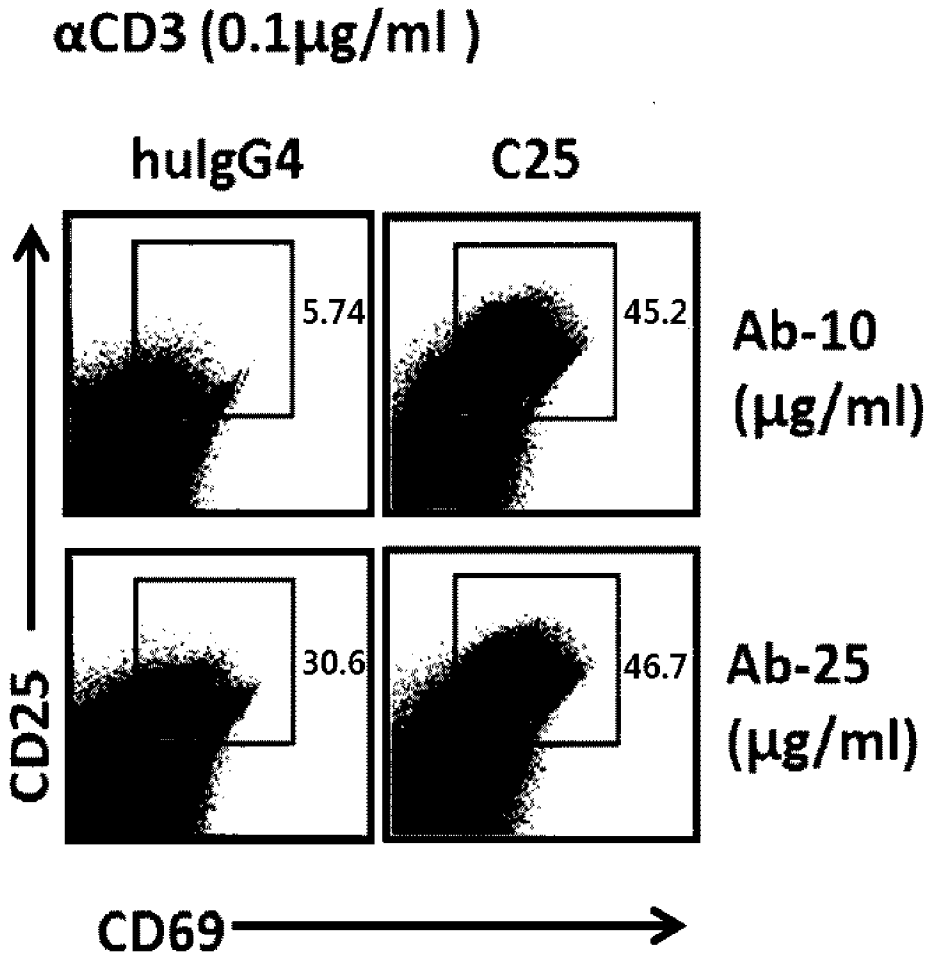
[Fig. 10]



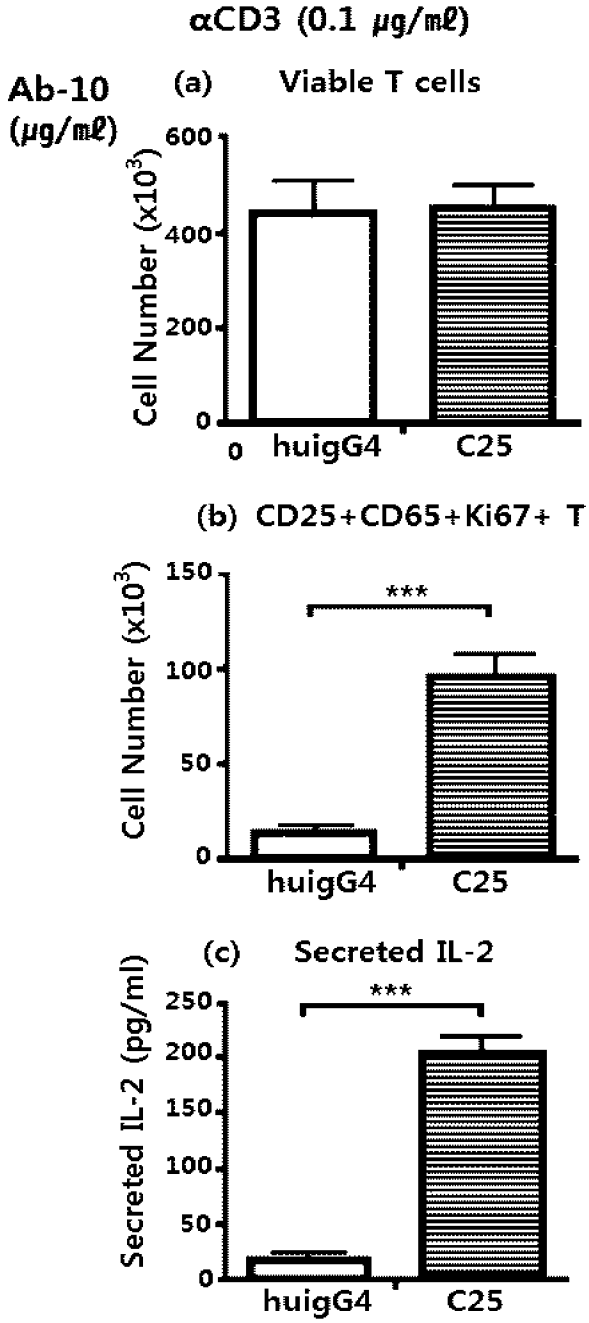
[Fig. 11]


(a) Viable T cells
NS
Cell Number (x10^3)
1.0
0.8
0.6
0.4
0.2
0.0
huigG4
C25
(b) CD65+Ki67+ T cells
***
Cell Number (x10^3)
80
60
40
20
0
huigG4
C25
(c) Secreted IL-2
***
Secreted IL-2 (pg/ml)
200
150
100
50
0
huigG4
C25

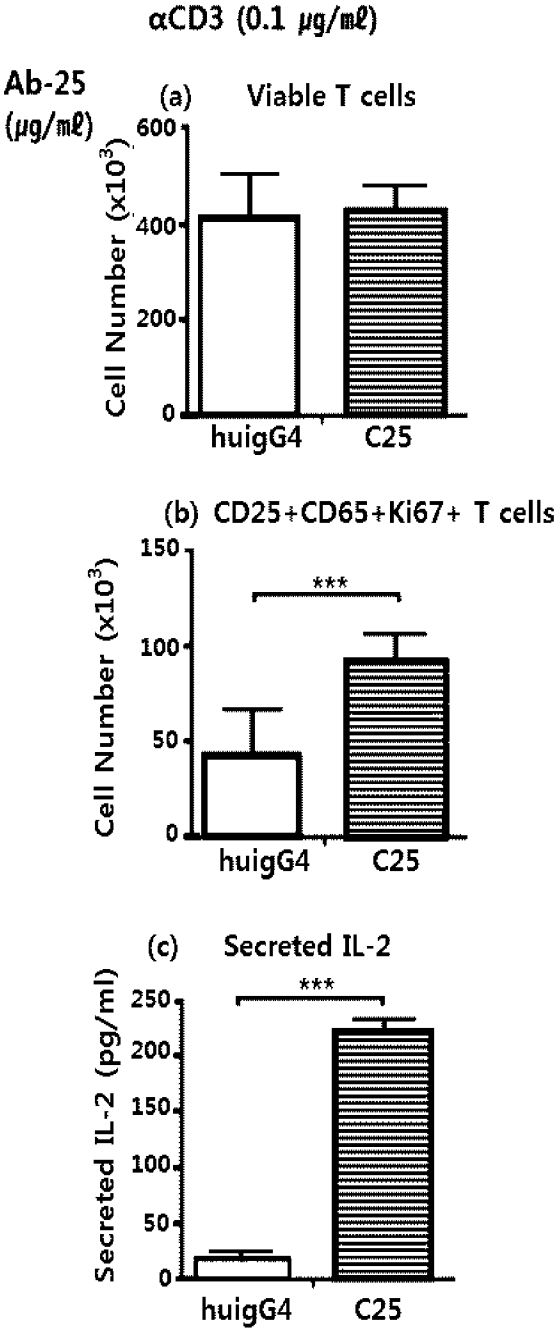
[Fig. 12]


αCD3 (0.1μg/ml)
hulgG4
C25
5.74
45.2
Ab-10
(μg/ml)
30.6
46.7
Ab-25
(μg/ml)
CD25
CD69

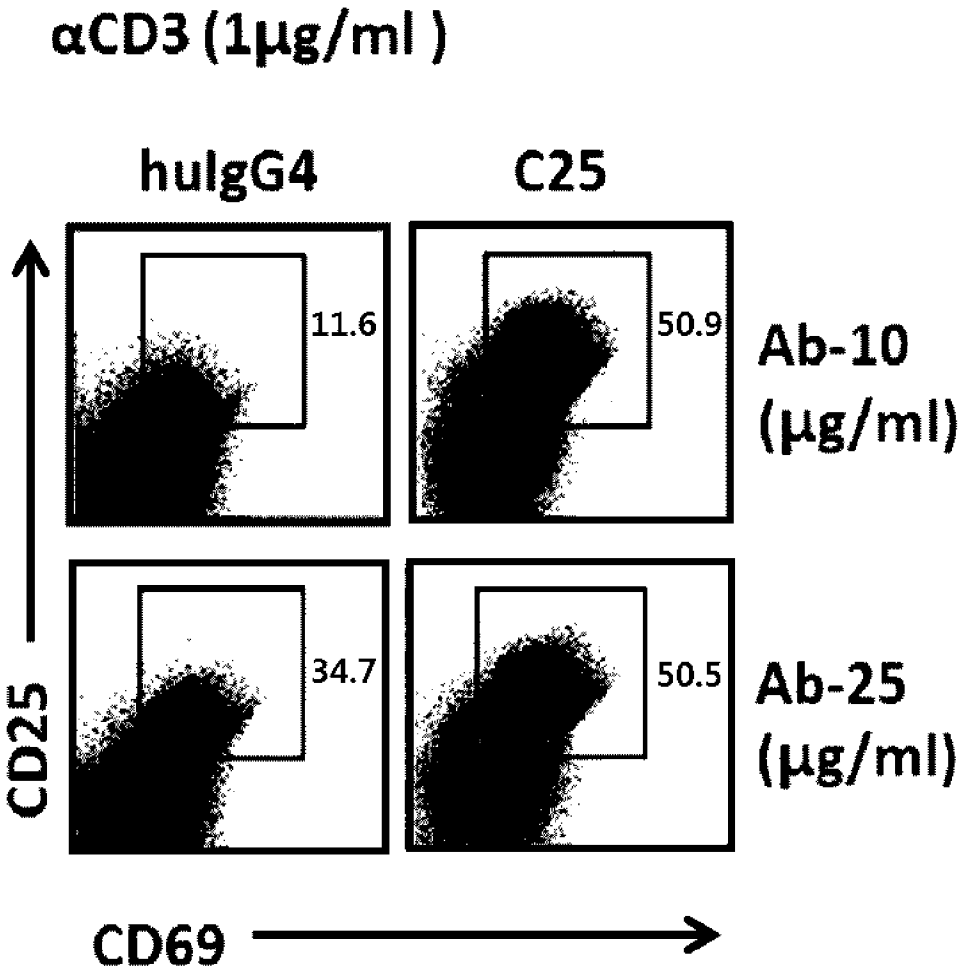
[Fig. 13]


αCD3 (0.1 μg/mℓ)
Ab-10 (μg/mℓ)
(a) Viable T cells
Cell Number (x10³)
600
400
200
0
0
huigG4
C25
(b) CD25+CD65+Ki67+ T
Cell Number (x10³)
150
100
50
0
***
huigG4
C25
(c) Secreted IL-2
Secreted IL-2 (pg/ml)
250
200
150
100
50
0
***
huigG4
C25

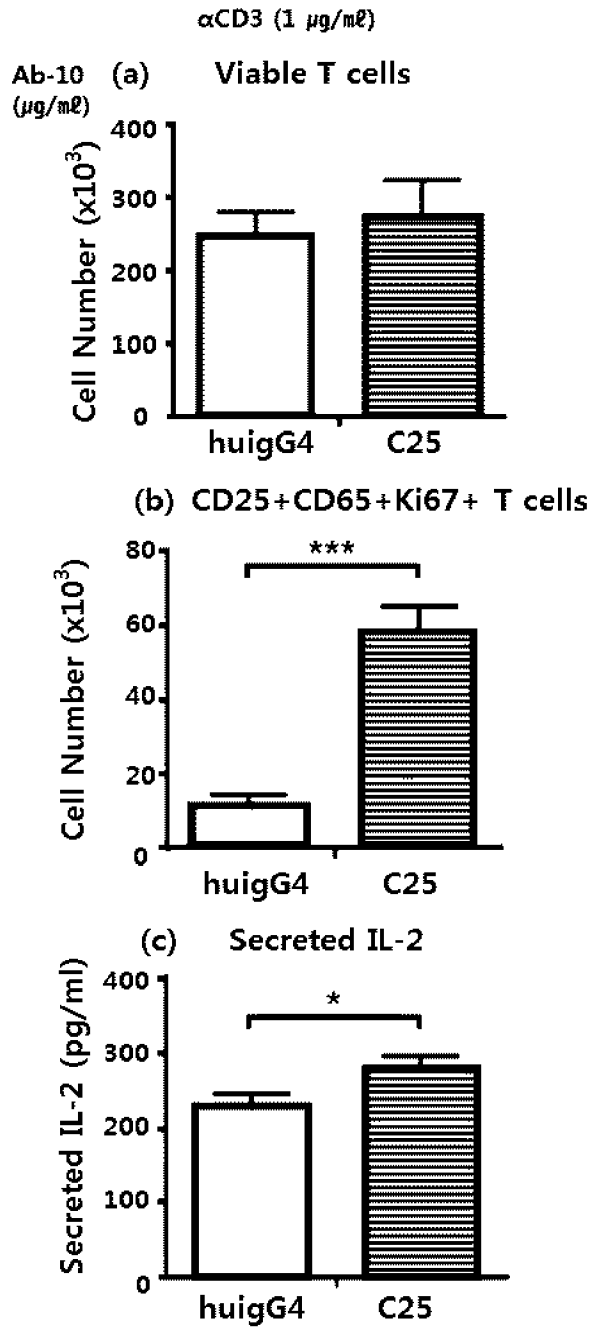
[Fig. 14]


αCD3 (0.1 μg/mℓ)
Ab-25 (μg/mℓ)
(a) Viable T cells
Cell Number (x10³)
600
400
200
0
huigG4
C25
(b) CD25+CD65+Ki67+ T cells
Cell Number (x10³)
150
100
50
0
***
huigG4
C25
(c) Secreted IL-2
Secreted IL-2 (pg/ml)
250
200
150
100
50
0
***
huigG4
C25

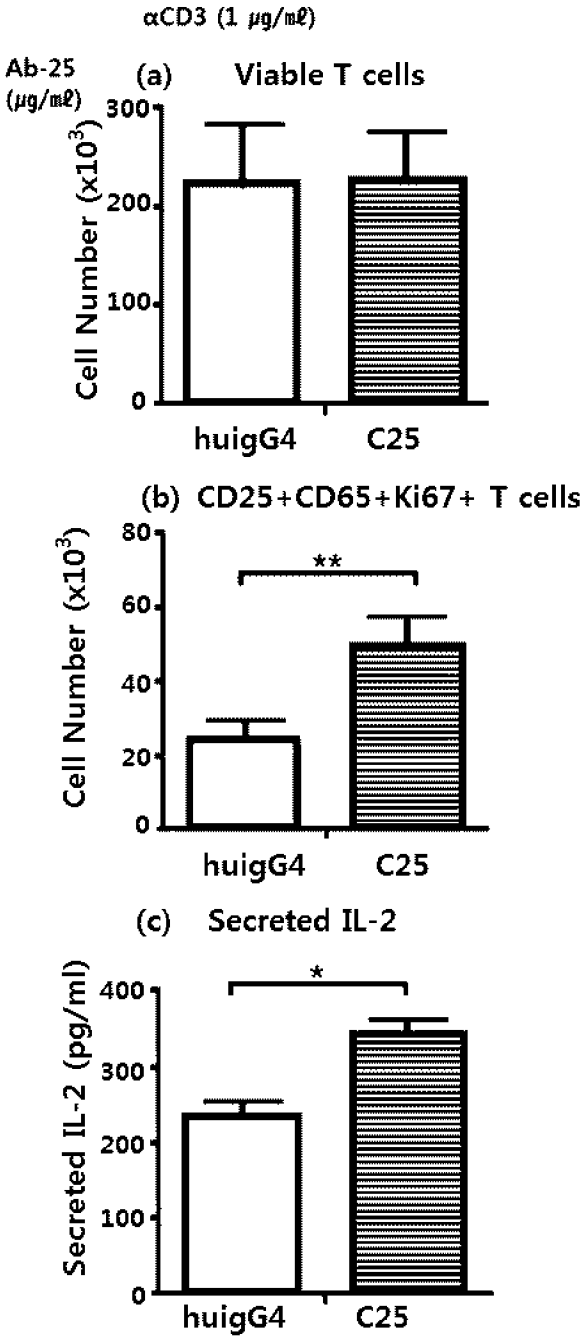
[Fig. 15]


αCD3 (1μg/ml)
huIgG4
C25
11.6
50.9
Ab-10 (μg/ml)
34.7
50.5
Ab-25 (μg/ml)
CD25
CD69

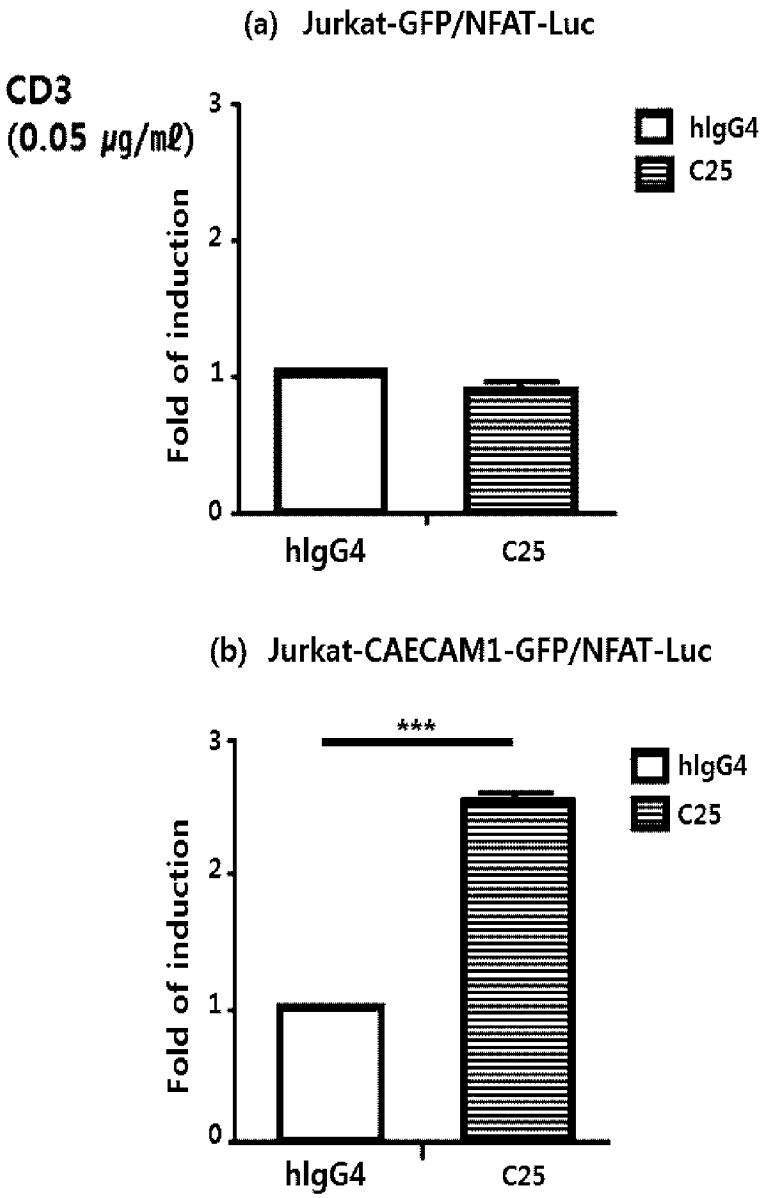
[Fig. 16]


αCD3 (1 μg/mℓ)
Ab-10 (μg/mℓ)
(a) Viable T cells
Cell Number (x10³)
400
300
200
100
0
huigG4
C25
(b) CD25+CD65+Ki67+ T cells
Cell Number (x10³)
80
60
40
20
0
***
huigG4
C25
(c) Secreted IL-2
Secreted IL-2 (pg/ml)
400
300
200
100
0
*
huigG4
C25

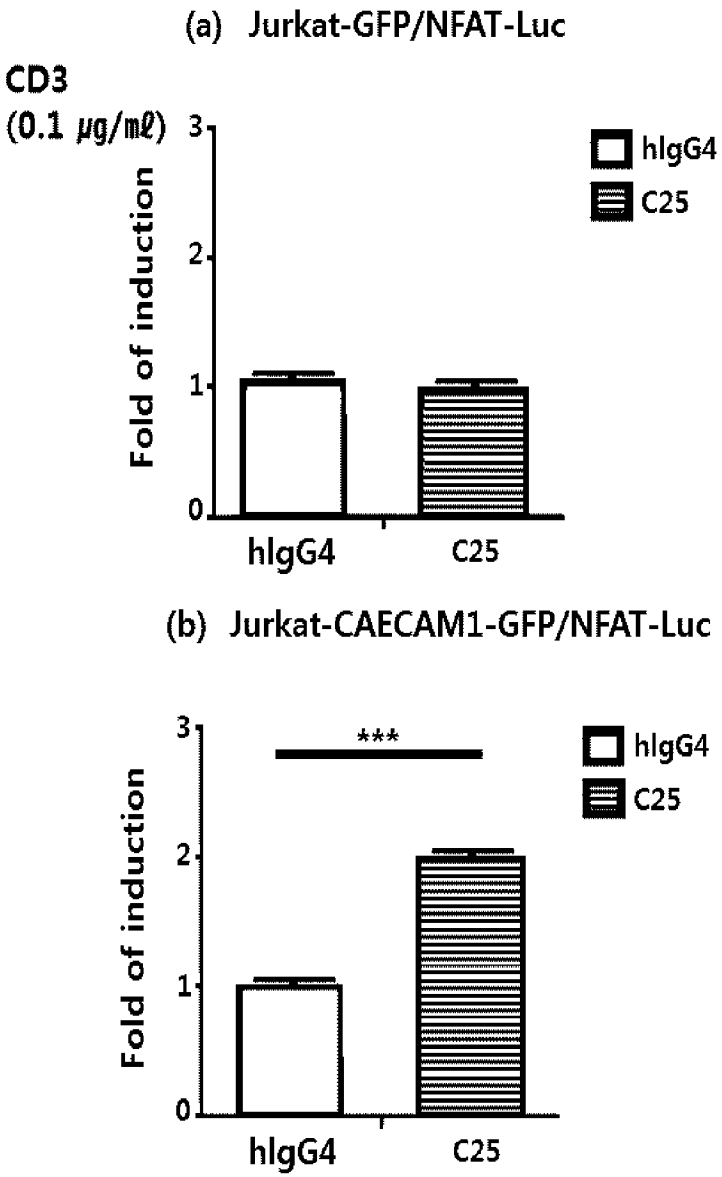
[Fig. 17]


αCD3 (1 μg/mℓ)
Ab-25 (μg/mℓ)
(a) Viable T cells
Cell Number (x10³)
300
200
100
0
huigG4
C25
(b) CD25+CD65+Ki67+ T cells
Cell Number (x10³)
80
60
40
20
0
**
huigG4
C25
(c) Secreted IL-2
Secreted IL-2 (pg/ml)
400
300
200
100
0
*
huigG4
C25

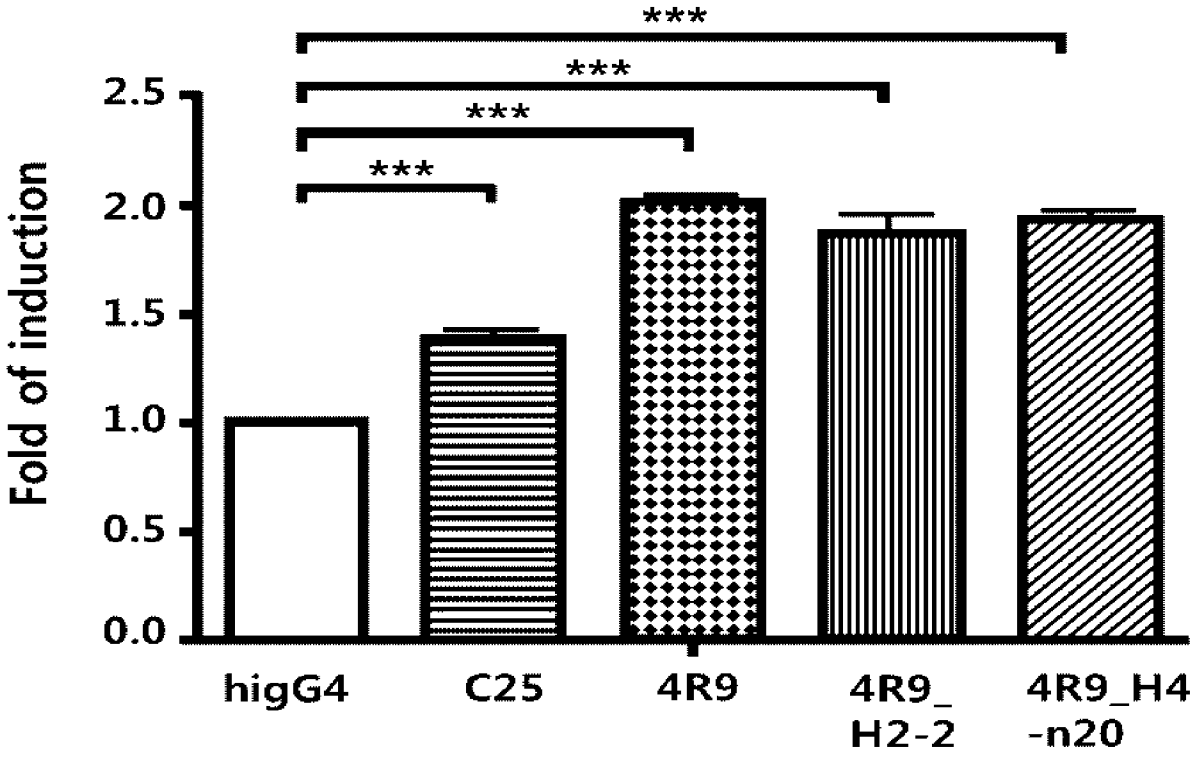
[Fig. 18]


(a) Jurkat-GFP/NFAT-Luc
CD3
(0.05 μg/mℓ)
Fold of induction
0
1
2
3
hIgG4
C25
hIgG4
C25
(b) Jurkat-CAECAM1-GFP/NFAT-Luc
***
Fold of induction
0
1
2
3
hIgG4
C25
hIgG4
C25

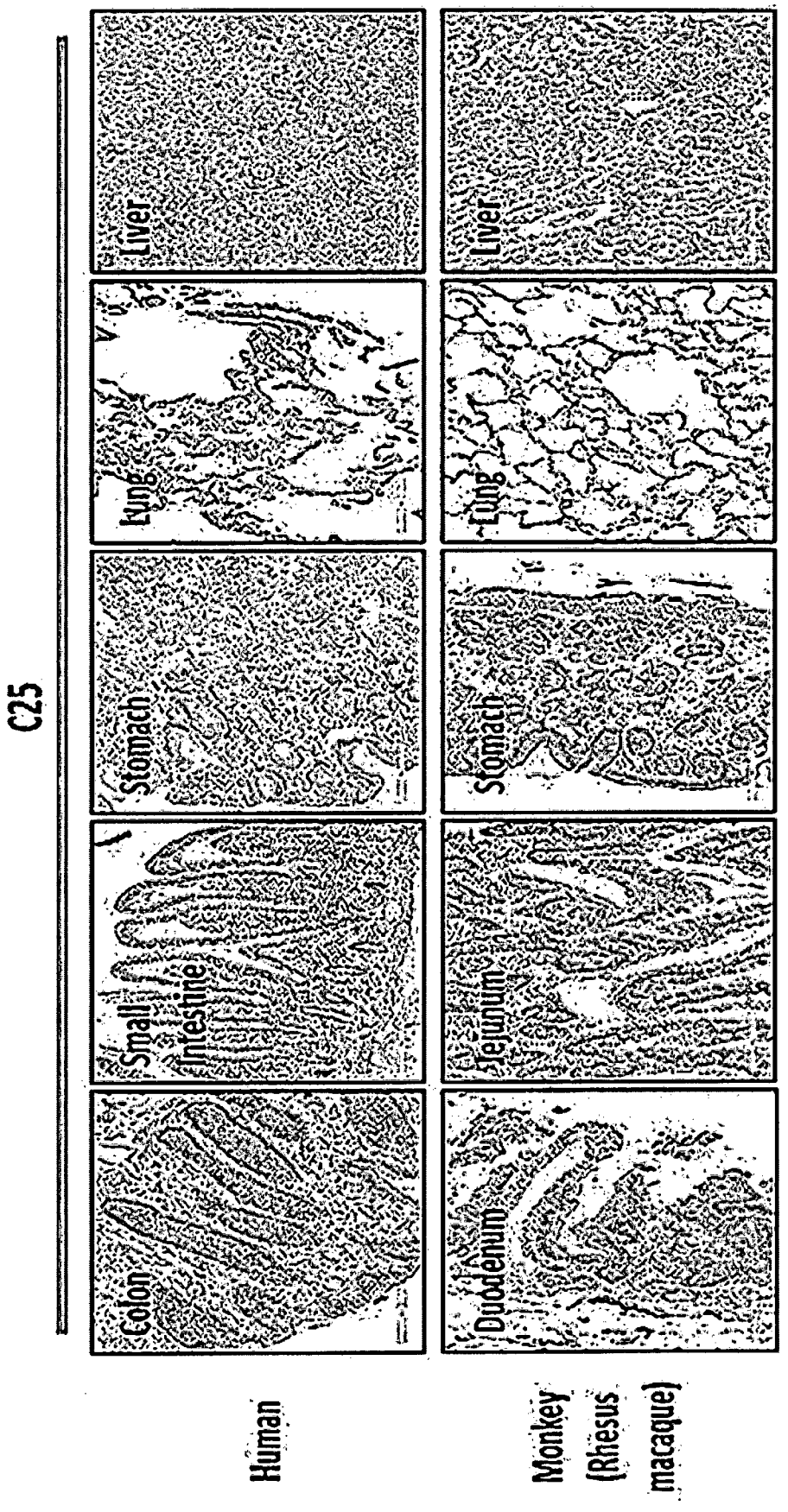
[Fig. 19]


(a) Jurkat-GFP/NFAT-Luc
CD3
(0.1 μg/mℓ)
Fold of induction
0
1
2
3
hIgG4
C25
hIgG4
C25
(b) Jurkat-CAECAM1-GFP/NFAT-Luc
***
Fold of induction
0
1
2
3
hIgG4
C25
hIgG4
C25

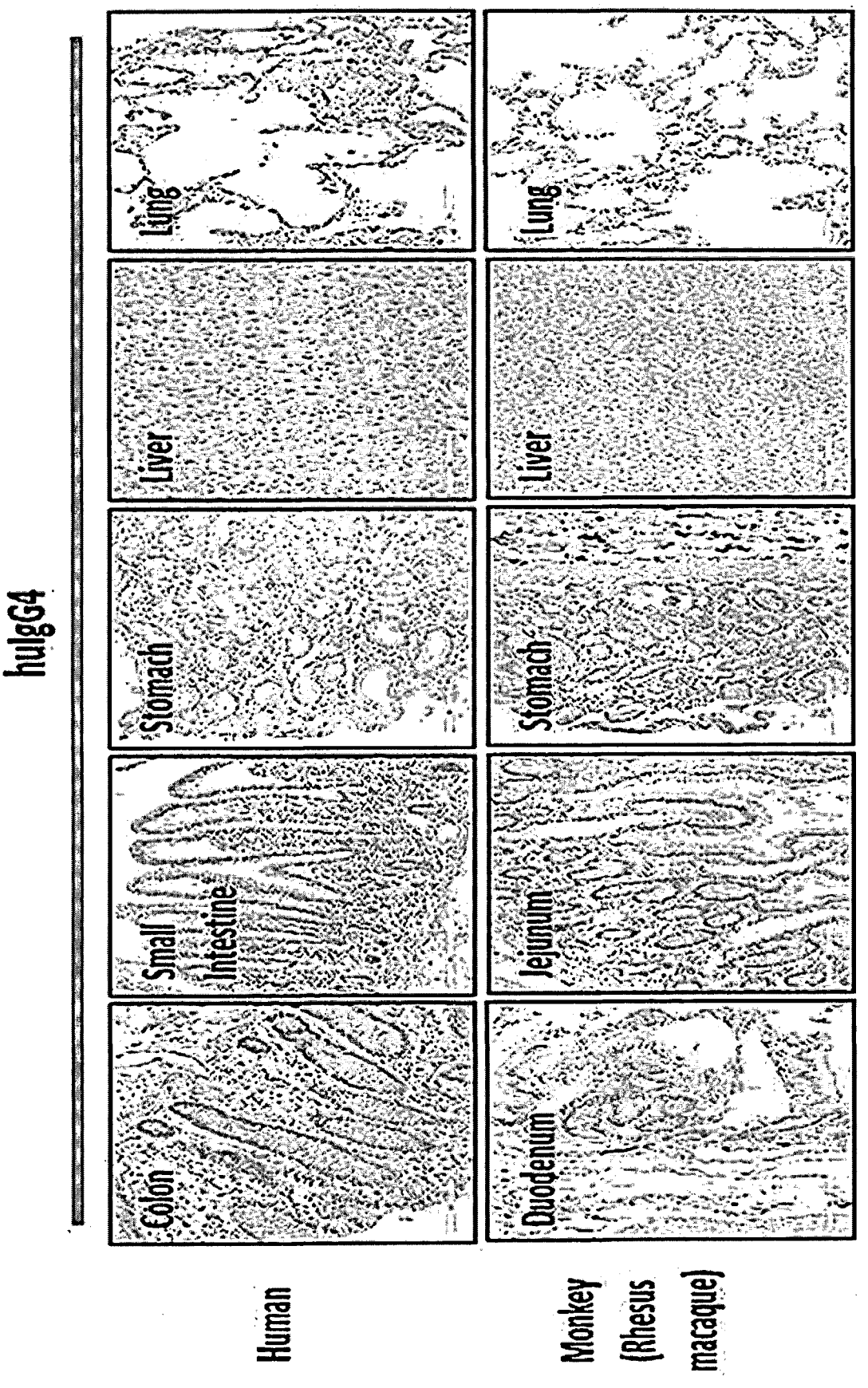
[Fig. 20]


***
***
***
***
2.5
2.0
1.5
1.0
0.5
0.0
Fold of induction
higG4
C25
4R9
4R9_
H2-2
4R9_H4
-n20

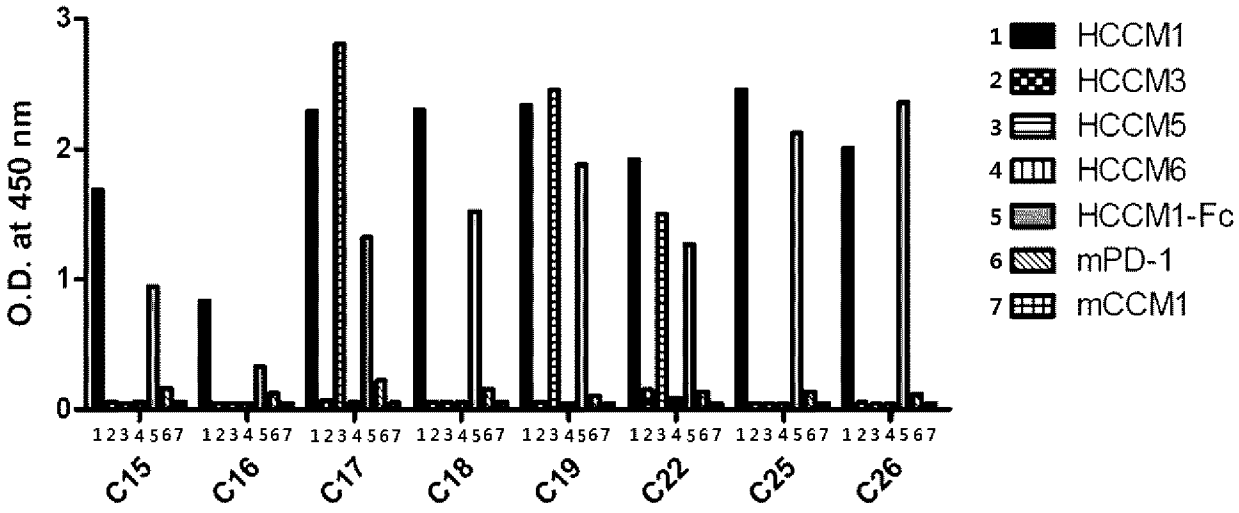
【Fig. 21】


C25
Human
Monkey (Rhesus macaque)
Colon
Small intestine
Stomach
Lung
Liver
Duodenum
Jejunum
Stomach
Lung
Liver

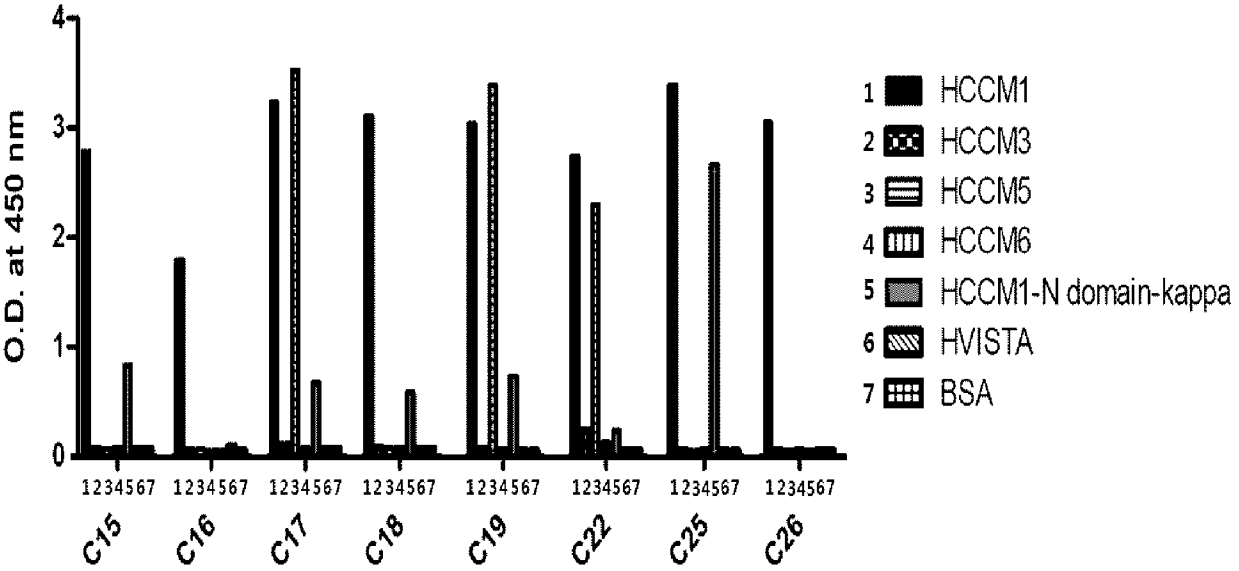
【Fig. 22】


hulgG4
Colon
Small Intestine
Stomach
Liver
Lung
Duodenum
Jejunum
Stomach
Liver
Lung
Human
Monkey (Rhesus maraque)

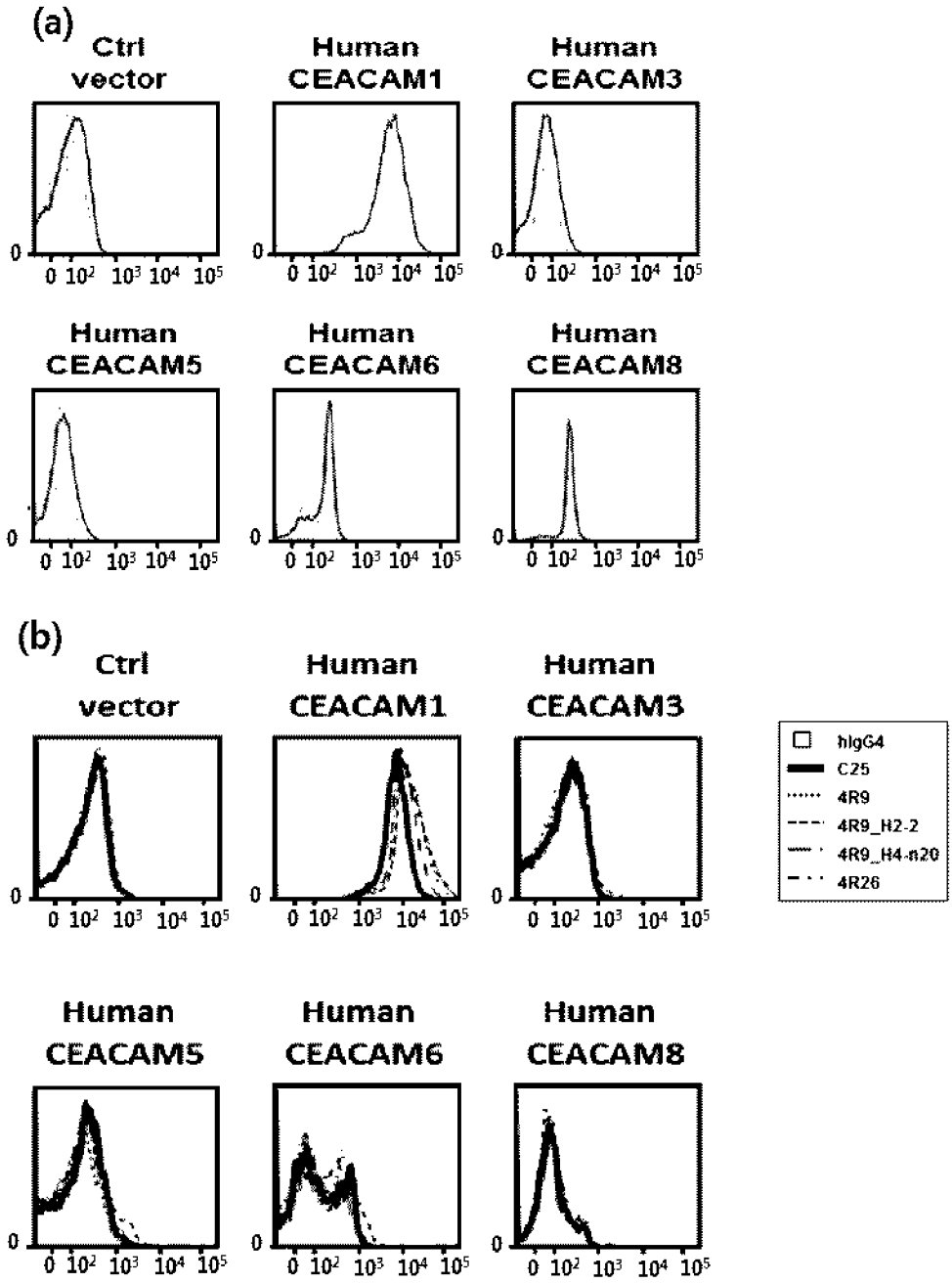
[Fig. 23]


O.D. at 450 nm
0
1
2
3
1234567
C15
C16
C17
C18
C19
C22
C25
C26
1 HCCM1
2 HCCM3
3 HCCM5
4 HCCM6
5 HCCM1-Fc
6 mPD-1
7 mCCM1

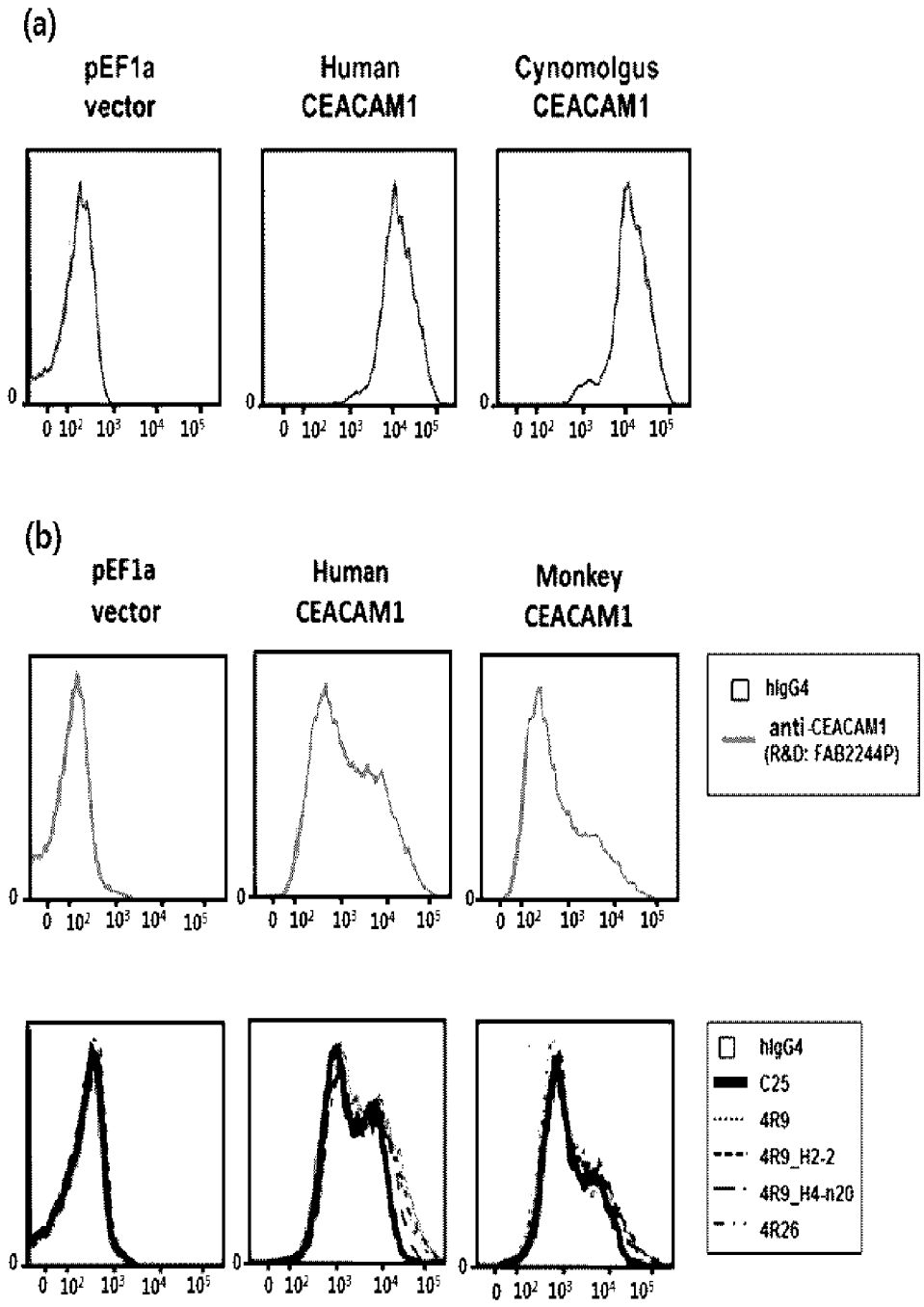
[Fig. 24]


O.D. at 450 nm
0
1
2
3
4
1234567
C15
C16
C17
C18
C19
C22
C25
C26
1 HCCM1
2 HCCM3
3 HCCM5
4 HCCM6
5 HCCM1-N domain-kappa
6 HVISTA
7 BSA

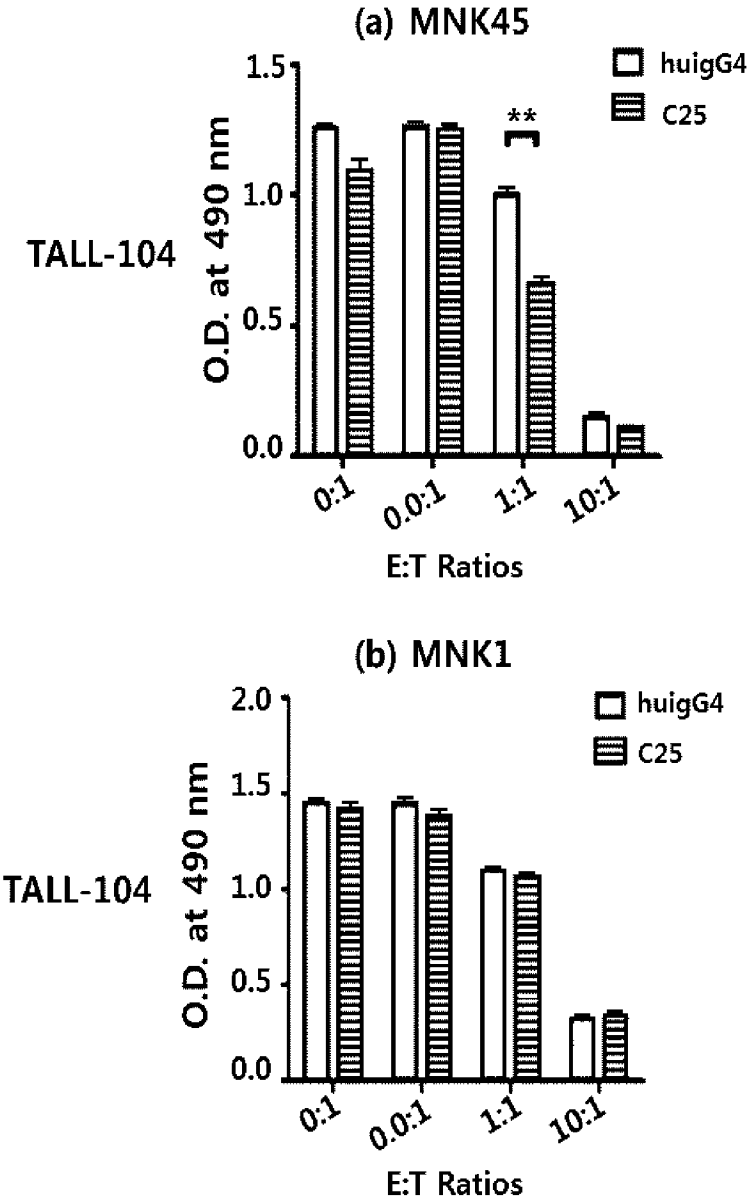
[Fig. 25]


(a)
Ctrl vector
Human CEACAM1
Human CEACAM3
Human CEACAM5
Human CEACAM6
Human CEACAM8
(b)
Ctrl vector
Human CEACAM1
Human CEACAM3
Human CEACAM5
Human CEACAM6
Human CEACAM8
hIgG4
C25
4R9
4R9_H2-2
4R9_H4-n20
4R26
0
0 10² 10³ 10⁴ 10⁵

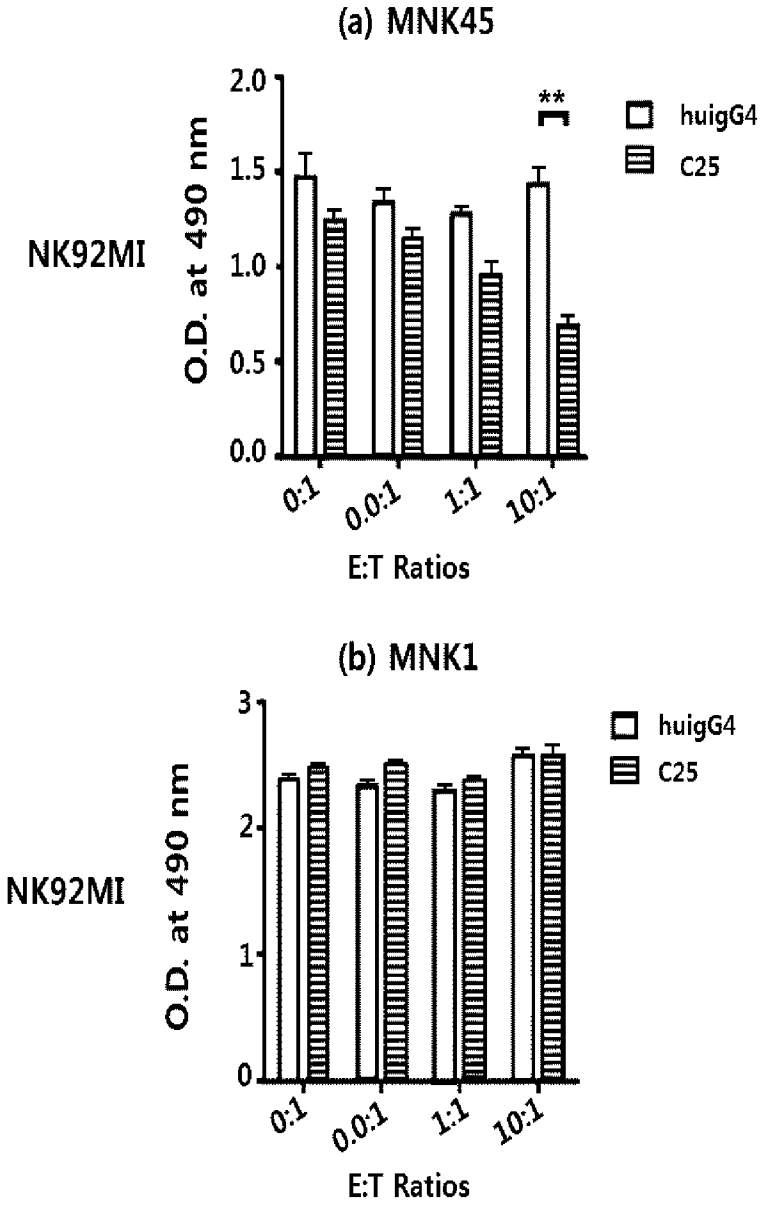
[Fig. 26]


(a)
pEF1a vector
Human CEACAM1
Cynomolgus CEACAM1
0
0 10² 10³ 10⁴ 10⁵
(b)
pEF1a vector
Human CEACAM1
Monkey CEACAM1
hIgG4
anti-CEACAM1 (R&D: FAB2244P)
hIgG4
C25
4R9
4R9_H2-2
4R9_H4-n20
4R26

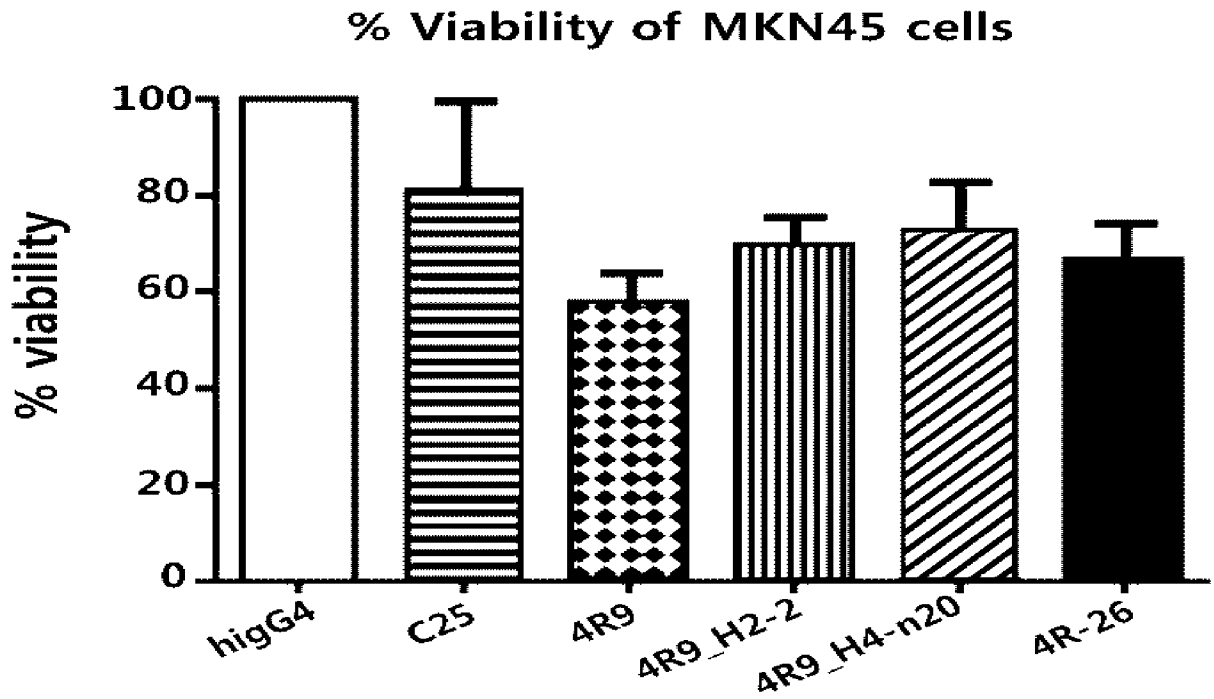
[Fig. 27]


(a) MNK45
huigG4
C25
**
TALL-104
O.D. at 490 nm
1.5
1.0
0.5
0.0
0:1
0.0:1
1:1
10:1
E:T Ratios
(b) MNK1
huigG4
C25
TALL-104
O.D. at 490 nm
2.0
1.5
1.0
0.5
0.0
0:1
0.0:1
1:1
10:1
E:T Ratios

[Fig. 28]


(a) MNK45
NK92MI
O.D. at 490 nm
2.0
1.5
1.0
0.5
0.0
**
huigG4
C25
0:1
0.0:1
1:1
10:1
E:T Ratios
(b) MNK1
NK92MI
O.D. at 490 nm
3
2
1
0
huigG4
C25
0:1
0.0:1
1:1
10:1
E:T Ratios

[Fig. 29]


% Viability of MKN45 cells
% viability
100
80
60
40
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4R9_H4-n20
4R-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/003419

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i, A61K 39/395(2006.01)i, A61K 35/17(2014.01)i, A61K 39/39(2006.01)i, A61K 39/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 16/28; A61K 39/395; C12N 5/0783; C07K 16/00; A61K 35/17; A61K 39/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: anti-CEACAM1 antibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013-054320 A1 (TEL HASHOMER MEDICAL RESEARCH INFRASTRUCTURE AND SERVICES LTD.) 18 April 2013 See claim 1.	1-41,47-48
A	US 2014-0328841 A1 (THE BRIGHAM AND WOMEN`S HOSPITAL, INC.) 06 November 2014 See claim 1.	1-41,47-48
A	US 2017-0051058 A1 (LANG, KARL SEBASTIAN et al.) 23 February 2017 See claims 16, 24 and 28.	1-41,47-48
A	US 2016-0176966 A1 (TEL HASHOMER MEDICAL RESEARCH INFRASTRUCTURE AND SERVICES LTD. et al.) 23 June 2016 See claim 1.	1-41,47-48
A	US 9512220 B2 (LEE, EUNKYUNG et al.) 06 December 2016 See claim 1; SEQ ID NOs: 8 and 52.	1-41,47-48

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 July 2018 (30.07.2018)	**30 July 2018 (30.07.2018)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
KAM, Yoo Lim
Telephone No. +82-42-481-3516

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International application No.
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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 42-46
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 42-46 pertain to a method for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2018/003419

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013-054320 A1	18/04/2013	AU 2012-322272 A1	10/04/2014
		AU 2012-322272 B2	17/08/2017
		AU 2012-322272 C1	02/11/2017
		BR 112014008893 A2	13/06/2017
		CA 2851762 A1	18/04/2013
		CN 103987729 A	13/08/2014
		CN 103987729 B	31/05/2017
		EP 2744829 A1	25/06/2014
		EP 2744829 B1	13/12/2017
		ES 2661488 T3	02/04/2018
		HK 1199646 A1	10/07/2015
		IL 231931 A	28/05/2014
		IN 873MUN2014 A	03/07/2015
		JP 2015-502138 A	22/01/2015
		JP 6170926 B2	26/07/2017
		KR 10-2014-0069206 A	09/06/2014
		LT 2744829 T	26/03/2018
		RU 2014118773 A	20/11/2015
		RU 2650869 C2	17/04/2018
		US 2014-0271618 A1	18/09/2014
		US 2017-0355781 A1	14/12/2017
		US 9771431 B2	26/09/2017
		WO 2013-054331 A1	18/04/2013
		WO 2013-054331 A8	10/04/2014
US 2014-0328841 A1	06/11/2014	CA 2892371 A1	06/06/2013
		EP 2785743 A1	08/10/2014
		JP 2015-502355 A	22/01/2015
		JP 2018-0064569 A	26/04/2018
		US 2017-0327578 A1	16/11/2017
		US 9556271 B2	31/01/2017
		WO 2013-082366 A1	06/06/2013
US 2017-0051058 A1	23/02/2017	WO 2016-120331 A1	04/08/2016
US 2016-0176966 A1	23/06/2016	AU 2010-243211 A1	24/11/2011
		AU 2010-243211 B2	30/01/2014
		BR PI1015350 A2	10/05/2016
		CA 2760385 A1	04/11/2010
		CN 102482354 A	30/05/2012
		CN 102482354 B	25/02/2015
		CY 1117502 T1	26/04/2017
		DK 2424896 T3	14/12/2015
		EP 2424896 A1	07/03/2012
		EP 2424896 B1	09/09/2015
		EP 2990421 A1	02/03/2016
		EP 2990421 B1	21/02/2018
		ES 2563527 T3	15/03/2016
		ES 2668874 T3	22/05/2018

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2018/003419

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		HK 1167264 A1	29/01/2016
		HK 1222862 A1	14/07/2017
		HR P20151099 T1	15/01/2016
		HR P20151099 T8	29/01/2016
		HU E028294 T2	28/12/2016
		IL 215677 A	31/01/2012
		IL 215677 B	31/12/2014
		JP 2012-525142 A	22/10/2012
		JP 5726170 B2	27/05/2015
		KR 10-2012-0028301 A	22/03/2012
		LT 2990421 T	25/04/2018
		PT 2424896 E	30/11/2015
		RU 2011148598 A	10/06/2013
		RU 2598710 C2	27/09/2016
		SI 2424896 T1	31/12/2015
		US 2012-0100158 A1	26/04/2012
		US 2014-0120554 A1	01/05/2014
		US 2018-0016338 A1	18/01/2018
		US 8598322 B2	03/12/2013
		US 9261507 B2	16/02/2016
		WO 2010-125571 A1	04/11/2010
US 9512220 B2	06/12/2016	US 2014-0112933 A1	24/04/2014
		WO 2012-137993 A1	11/10/2012