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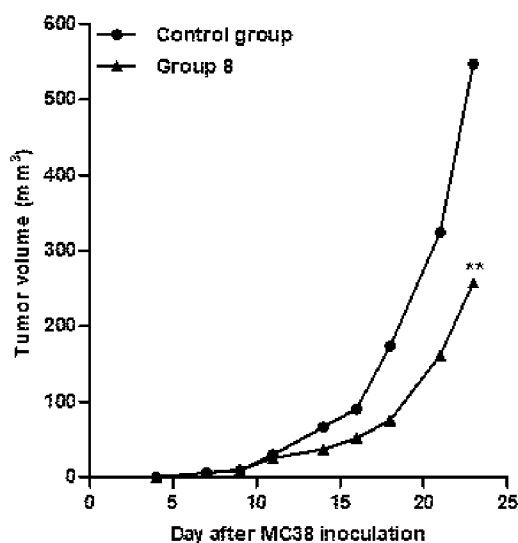
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(54) Title: NOVEL TARGET FOR ANTI-CANCER AND IMMUNE-ENHANCING



(57) Abstract: The present disclosure provides a pharmaceutical composition for treating or preventing cancer, comprising inhibitors of KIRREL3, CNTN4 and/or CD351. In addition, the present disclosure provides a pharmaceutical composition for immune-enhancing, comprising inhibitors of KIRREL3, CNTN4 and/or CD351. Furthermore, the present disclosure provides a method of screening of anti-cancer agent using KIRREL3, CNTN4 and/or CD351, and a method of providing information necessary for analysis of cancer prognosis using KIRREL3, CNTN4 and/or CD351.

Description

Title of Invention: NOVEL TARGET FOR ANTI-CANCER AND IMMUNE-ENHANCING

Technical Field

- [1] This application claims priority from U.S. Application No.: 62/652,948 filed on April 5, 2018, the disclosure of which is incorporated herein in its entirety by reference.
- [2] The present disclosure provides a pharmaceutical composition for treating or preventing cancer, comprising one or more inhibitors of KIRREL3, CNTN4 and CD351, and a method of treating or preventing cancer by administering one or more inhibitors of KIRREL3, CNTN4 and CD351 to a subject in need thereof. In addition, the present disclosure provides a pharmaceutical composition for immune-enhancing, comprising one or more inhibitors of KIRREL3, CNTN4 and CD351, and a method of immune-enhancing by administering one or more inhibitors of KIRREL3, CNTN4 and CD351 to a subject in need thereof. Furthermore, the present disclosure provides a method of screening of anti-cancer agent using one or more of KIRREL3, CNTN4 and CD351, and a method of providing information necessary for analysis of cancer prognosis using one or more of KIRREL3, CNTN4 and CD351.

Background Art

- [3] Despite advances in understanding the etiology of cancer and the methods for treating cancer over the past several years, it is still the leading cause of death worldwide. Although anti-cancer treatments exist for many malignancies, such treatments often do not fully control such malignancies or are not effective in all patients. Most of the methods currently being used to treat cancer are relatively non-selective. The affected tissue is removed through surgery, the size of solid tumors is reduced through radiation therapy, or chemotherapy is used to kill cancer cells rapidly. In particular, the chemotherapy can cause the drug resistance, and sometimes restricts the administrable dose. It causes severe side effects so that they may rule out the use of potentially effective agents. Accordingly, there is a need to develop more target-specific and effective cancer therapies.
- [4] The adaptive immune system of the human is a very precise system which is able to specifically remove cancer cells. In particular, T cells determine cell mediated adaptive immunity, and recognize and remove non-self antigens or abnormal antigens that a cell is exposed to. T cells express about 20,000 to 40,000 TCR molecules per cell, and recognize several antigens (determined by their peptide sequences) among the 100,000 pMHC molecules of APC to begin signal transfer. Such TCR molecules should function as highly sensitive sensors which need to recognize very minute changes in

the antigen and transfer signals. This cell-mediated adaptive immunity operates in a very precise manner to effectively remove cancer cells. If an antigen-specific adaptive immune system does not operate normally, serious problems are caused in the ability to remove cancer cells. For example, if the protein PD-L1 or PD-L2 on the surface of a cancer cell binds to the protein PD-1 on the surface of a T cell, the T cell is not able to attack cancer cells. Therefore, for effective cancer treatment, it is necessary to remove the factors that hinder T cell's ability to remove cancer cells.

- [5] Accordingly, the inventors have conducted research to develop a method of cancer treatment using the human immune system, and identified that inhibition of the activity and expression of one or more of KIRREL3, CNTN4 and CD351 leads to substantial suppression of development, growth, invasion and metastasis of cancer.

Disclosure of Invention

Technical Problem

- [6] One purpose of the present disclosure is to provide a pharmaceutical composition for treating or preventing cancer, and a method of treating or preventing cancer.
- [7] Another purpose of the present disclosure is to provide a pharmaceutical composition for immune-enhancing, and a method of immune-enhancing.
- [8] Another purpose of the present disclosure is to provide a method of screening of anti-cancer agent.
- [9] Another purpose of the present disclosure is to provide a method of providing information necessary for analysis of cancer prognosis.

Solution to Problem

- [10] To achieve the purposes of the present disclosure, one aspect of the present disclosure provides a pharmaceutical composition for treating or preventing cancer comprising one or more inhibitors of KIRREL3, CNTN4 and CD351 as an active ingredient, and a method of treating or preventing cancer by administering one or more inhibitors of KIRREL3, CNTN4 and CD351 to a subject in need thereof.
- [11] The term "KIRREL3 (Kin of IRRE-like protein 3)" refers to a protein that is encoded by *KIRREL3* gene, which belongs to the member of the nephrin-like protein family, and is also known as 'NEPH2'. It is expressed in fetal and adult brain, and in podocytes of kidney glomeruli, and is reported to be involved in blood filtration function of the kidney and synapse formation.
- [12] The term "CNTN4 (Contactin-4)" refers to a protein that is encoded by *CNTN4* gene, which belongs to the immunoglobulin superfamily. It is reported to be a glycosylphosphatidylinositol (GPI)-anchored neuronal membrane protein that functions as a cell adhesion molecule, and to be involved in formation of axon connections in developing nervous system.

- [13] The term "CD351 (Cluster of Differentiation 351)" refers to a Fc receptor that binds to IgA and IgM with high affinity, and is also known as 'Fc α / μ R'. It is reported that in mice, the receptor is expressed on macrophages, follicular dendritic cells, marginal zone and follicular B cells, and kidney tubular epithelial cells. It is reported that in human, it is expressed on intestinal lamina propria cells, Paneth cells, follicular dendritic cells in tonsils, activated macrophages, and some types of pre-germinal centre IgD+/CD38+ B cells.
- [14] The KIRREL3, CNTN4 and CD351 may be human-derived KIRREL3, CNTN4 and CD351. More specifically, the amino acid sequence of KIRREL3 may be or comprise the sequence of NCBI Reference Sequence: NP_115920.1 disclosed in NCBI. The amino acid sequence of CNTN4 may comprise the sequence of NCBI Reference Sequence: NP_783200.1 disclosed in NCBI. The amino acid sequence of CD351 may be or comprise the sequence of NCBI Reference Sequence: AAL51154.1 disclosed in NCBI. In addition, each of the amino acid sequence of KIRREL3, CNTN4 and CD351 may be or comprise, but is not limited to, amino acid sequences having at least 80%, 85%, 90% or 95% identity with each sequence of NCBI Reference Sequence: NP_115920.1, NP_783200.1, AAL51154.1, as well as amino acid sequences having the property or function of KIRREL3, CNTN4 and CD351.
- [15] The gene of KIRREL3 may be or comprise a nucleic acid sequence encoding the amino acid sequence of human-derived KIRREL3, or the nucleic acid sequence of NCBI Reference Sequence: NM_032531.4 disclosed in NCBI. The gene of CNTN4 may be or comprise a nucleic acid sequence encoding the amino acid sequence of human-derived CNTN4, or the nucleic acid sequence of NCBI Reference Sequence: NM_175607.3 disclosed in NCBI. The gene of CD351 may be or comprise a nucleic acid sequence encoding the amino acid sequence of human-derived CD351, or the nucleic acid sequence of NCBI Reference Sequence: AY063125.1 disclosed in NCBI. In addition, each of the nucleic acid sequence of KIRREL3, CNTN4 and CD351 may be or comprise, but are not limited to, nucleic acid sequences having at least 80%, 85%, 90% or 95% identity with each sequence of NCBI Reference Sequence: NM_032531.4, NM_175607.3, AY063125.1, as well as nucleic acid sequences that can produce amino acids having the property or function of KIRREL3, CNTN4 and CD351.
- [16] The term "one or more inhibitors of KIRREL3, CNTN4 and CD351" refers to substances that inhibit the activity or expression of at least one selected among KIRREL3, CNTN4 and CD351. "One or more inhibitors of KIRREL3, CNTN4 and CD351" and "inhibitors of KIRREL3, CNTN4 and/or CD351" are used interchangeably. For example, the inhibitors may be a substance that inhibits the activity or expression of all of KIRREL3, CNTN4, and CD351. As another example, the in-

hibitors may be a substance that inhibits the activity or expression of KIRREL3 and CNTN4, or of KIRREL3 and CD351, or of CNTN4 and CD351. As another example, the inhibitors may be a substance that inhibits the activity or expression of KIRREL3, or of CNTN4, or of CD351. As another example, the inhibitors may be a combination of KIRREL3 inhibitor, CNTN4 inhibitor and CD351 inhibitor. As another example, the inhibitors may be a combination of KIRREL3 inhibitor and CNTN4 inhibitor, or a combination of KIRREL3 inhibitor and CD351 inhibitor, or a combination of CNTN4 inhibitor and CD351 inhibitor.

- [17] The inhibitors of KIRREL3, CNTN4 and/or CD351 can preferably suppress the function of cancer cells evading T cells. The inhibitors of KIRREL3, CNTN4 and/or CD351 block the activity of KIRREL3, CNTN4 and/or CD351 existing in a cancer cell, thereby suppressing the mechanism that T cells are rendered unable to attack cancer cells by KIRREL3, CNTN4 and/or CD351 and maintaining the immune activity of T cells against cancer cells. Alternatively, the inhibitors of KIRREL3, CNTN4 and/or CD351 specifically bind to KIRREL3, CNTN4 and/or CD351 protein, and interfere with binding of KIRREL3, CNTN4 and/or CD351 to T cells. Alternatively, the inhibitors of KIRREL3, CNTN4 and/or CD351 suppress a particular metabolic pathway of KIRREL3, CNTN4 and/or CD351 to reduce the expression of protein, or cause KIRREL3, CNTN4 and/or CD351 to denature so that the protein loses its activity. Therefore, the inhibitors of KIRREL3, CNTN4 and/or CD351 according to the present disclosure are very effective in treating or preventing cancer.
- [18] The inhibitors of KIRREL3, CNTN4 and/or CD351 may include, but are not limited to, any compounds, proteins, fusion proteins, antibodies, amino acids, peptides, viruses, carbohydrates, lipids, nucleic acids, extracts or fractions so long as it inhibits the activity or expression of KIRREL3, CNTN4 and/or CD351. The KIRREL3 inhibitor, CNTN4 inhibitor and CD351 inhibitor may be the same or different type independently of each other. For example, all inhibitors may be antibodies. As another example, two inhibitors may be antibodies, and one inhibitor may be a compound.
- [19] In one embodiment, the inhibitors of KIRREL3, CNTN4 and/or CD351 are ones that reduce the expression of KIRREL3, CNTN4 and/or CD351 in a cancer cell compared to a cancer cell not treated with inhibitors of KIRREL3, CNTN4 and CD351. Reduction in expression of KIRREL3, CNTN4 and/or CD351 may refer to lowered or no level of mRNA and/or protein produced from KIRREL3, CNTN4 and/or CD351 gene. The inhibitors of KIRREL3, CNTN4 and/or CD351 may include, but are not limited to, antisense nucleic acid, siRNA, shRNA, miRNA, ribozyme, etc. which binds in a complementary manner to DNA or mRNA of KIRREL3, CNTN4 and/or CD351 gene. The KIRREL3 inhibitor, CNTN4 inhibitor and CD351 inhibitor may be the same or different type independently of each other. For example, all inhibitors may be

siRNAs. As another example, two inhibitors may be siRNAs, and one inhibitor may be an antisense nucleic acid.

[20] The term "antisense nucleic acid" refers to DNAs or RNAs comprising nucleic acid sequences complementary to the sequence of certain mRNA, or fragments or derivatives thereof, which bind to or hybridize with the complementary sequences in mRNA and inhibit the translation of mRNA into protein.

[21] The term "siRNA (small interfering RNA)" refers to a short double chain RNA which is able to induce the RNAi (RNA interference) through cleavage of certain mRNA. The siRNA comprises a sense RNA strand having a sequence homologous to the mRNA of the target gene, and an antisense RNA strand having a sequence complementary thereto. The siRNA can inhibit the expression of the target gene, and thus can be used in gene knockdown, genetic therapy, etc.

[22] The term "shRNA (short hairpin RNA)" is a single strand RNA, which comprises a stem portion forming a double strand portion through hydrogen bonds, and a loop portion. It is processed by a protein such as Dicer to be converted into siRNA, and performs the same function as siRNA.

[23] The term "miRNA (micro RNA)" refers to 21 to 23 non-coding RNAs which modulate gene expression after transcription by promoting the degradation of target RNA or by suppressing its translation.

[24] The term "ribozyme" refers to an RNA molecule that has an enzyme-like function, recognizing a particular base sequence and cutting the same. The ribozyme comprises an area that specifically binds to a complementary base sequence of a target messenger RNA strand, and an area that cleaves the target RNA.

[25] The antisense nucleic acid, siRNA, shRNA, miRNA, ribozyme, etc. that binds complementarily to the DNA or mRNA of KIRREL3, CNTN4 and/or CD351 gene can inhibit the translation of mRNA of KIRREL3, CNTN4 and/or CD351, its translocation into the cytoplasm, its maturation, or any other activities crucial for the biological functions of KIRREL3, CNTN4 and/or CD351.

[26] In one embodiment, the inhibitors of KIRREL3, CNTN4 and/or CD351 are ones that deactivate the function of KIRREL3, CNTN4 and/or CD351 or reduce the activity thereof in a cancer cell compared to a cancer cell not treated with inhibitors of KIRREL3, CNTN4 and CD351. The inhibitors of KIRREL3, CNTN4 and/or CD351 may include, but are not limited to, compounds, peptides, peptide mimetics, fusion proteins, antibodies, aptamers, etc. that bind specifically to KIRREL3, CNTN4 and/or CD351 protein. The KIRREL3 inhibitor, CNTN4 inhibitor and CD351 inhibitor may be the same or different type independently of each other.

[27] The term "specific" or "specifically" refers to the ability to bind to only a target protein without affecting other proteins in the cell.

- [28] The term "antibody" may include monoclonal antibodies, polyclonal antibodies, bispecific antibodies, multispecific antibodies, chimera antibodies, humanized antibodies and human antibodies, and may also include new antibodies as well as antibodies known to the art or commercialized in the art. The antibody may include not only the forms having a full length comprising two heavy chains and two light chains but also the functional fragments of antibody molecules, so long as they specifically bind to one or more of KIRREL3, CNTN4 and CD351. The functional fragment of antibody molecule refers to a fragment having at least its antigen-binding function, and may include, but are not limited to, Fab, F(ab'), F(ab')₂, Fv, etc.
- [29] The term "peptide mimetics" refers to a peptide or non-peptide which inhibits the binding domain of one or more of protein of KIRREL3, CNTN4 and CD351 that induces KIRREL3, CNTN4 and/or CD351 activities.
- [30] The term "aptamer" refers to a single strand nucleic acid (DNA, RNA or modified nucleic acid) having in itself a stable tertiary structure and being able to bind to a target molecule with high affinity and specificity.
- [31] The substance inhibiting the activity or expression of KIRREL3, CNTN4 and/or CD351 which is comprised in the pharmaceutical composition of the present disclosure can inhibit suppression of T cell function by KIRREL3, CNTN4 and/or CD351, and accordingly can increase or maintain the ability of T cells to attack and kill cancer cells. Here, the ability of T cells to attack and kill cancer cells in a group treated with inhibitors of KIRREL3, CNTN4 and/or CD351 may be increased by 5% to 200% as compared to a group not treated with inhibitors of KIRREL3, CNTN4 and/or CD351. Thus, the pharmaceutical composition of the present disclosure can be useful in the preventing or treating cancer.
- [32] The cancer that can be treated or prevented by the pharmaceutical composition of the present disclosure may include, but are not limited to, stomach cancer, lung cancer, liver cancer, colorectal cancer, colon cancer, small intestinal cancer, pancreatic cancer, brain cancer, bone cancer, melanoma, breast cancer, sclerosing adenosis, uterine cancer, cervical cancer, head and neck cancer, esophageal cancer, thyroid cancer, parathyroid cancer, renal cancer, sarcoma, prostate cancer, urethral cancer, bladder cancer, blood cancer, leukemia, lymphoma, fibroadenoma, etc.
- [33] The pharmaceutical composition according to the present disclosure may comprise the active ingredient alone, or may additionally comprise one or more pharmaceutically acceptable carriers, excipients, diluents, stabilizing agents, preserving agent, etc.
- [34] The pharmaceutically acceptable carriers may include, for example, carriers for oral administration or non-oral administration. The carriers for oral administration may include, for example, lactose, starch, cellulose derivatives, magnesium stearate, stearic

acid, etc. The carriers for non-oral administration may include, for example, water, suitable oils, saline, aqueous glucose, glycols, etc. The pharmaceutically acceptable stabilizing agents may include, for example, antioxidants such as sodium bisulfate, sodium sulfite or ascorbic acid. The pharmaceutically acceptable preserving agents may include, for example, benzalkonium chloride, methyl- or propyl-paraben, chlorobutanol, etc. Other pharmaceutically acceptable carriers may be those disclosed in the literature "Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Company, Easton, PA, 1995".

- [35] The pharmaceutical composition of the present disclosure may be administered to animals including human using various methods. For example, it may be administered orally or parenterally. The parenteral administration may include, but are not limited to, intravenous, intramuscular, intraarterial, intramarrow, intradural, percutaneous, subcutaneous, intraperitoneal, intranasal, intrainestinal, topical, sublingual, rectal administration, etc.
- [36] The pharmaceutical composition of the present disclosure may be prepared into formulations for oral or parenteral administration, depending on the administration route as described in the above.
- [37] The formulation for oral administration may be prepared in the form of powders, granules, tablets, pills, sugar-coated pills, capsules, liquids, gels, syrups, slurries, suspensions, etc., using methods known in the art. For example, the active ingredient of the present disclosure may be mixed with suitable excipient(s) and/or adjuvant(s), and then processed into a granule mixture to obtain a tablet or a sugar-coated tablet for oral administration. Examples of suitable excipients may include, but are not limited to, sugars including lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, etc., starches including corn starch, wheat starch, rice starch, potato starch, etc., celluloses including cellulose, methyl cellulose, sodium carboxymethyl cellulose, hydroxypropylmethyl cellulose, etc., and fillers such as gelatin, polyvinyl pyrrolidone, etc. Optionally, disintegrating agents such as crosslinked polyvinyl pyrrolidone, agar, alginic acid or sodium alginate may be added. Further, the pharmaceutical composition of the present disclosure may further comprise anticoagulants, lubricants, wetting agents, aromatic agents, emulsifiers and preservatives, etc.
- [38] The formulation for parenteral administration may be prepared in the form of injections, gels, aerosols, nasal inhalers using methods known in the art.
- [39] These administration forms may refer to those disclosed in the literature known in the art "Remington's Pharmaceutical Science, 15th Edition, 1975. Mack Publishing Company, Easton, Pennsylvania 18042, Chapter 87: Blaug, Seymour".
- [40] The total effective dose of the pharmaceutical composition according to the present disclosure may be administered to a subject in a single dose, or in multiple doses

through a fractionated treatment protocol.

- [41] The appropriate dose of the pharmaceutical composition according to the present disclosure or the contents of active ingredient in the pharmaceutical composition may be determined considering various factors such as administration route, times administered, patient age, body weight, health, gender, severity of disease, diet and excretion rate, etc. by a person having ordinary skill in the art. For example, the total dose of the pharmaceutical composition according to the present disclosure may be about 0.01 μ g to 1,000mg per 1kg body weight of a patient per day, or 0.1 μ g to 100mg. There is no particular limit to the dosage form, administration route and administration method, so long as the pharmaceutical composition shows the effect of the invention.
- [42] Another aspect of the present disclosure provides a pharmaceutical composition for immune-enhancing in a subject, comprising one or more inhibitors of KIRREL3, CNTN4 and CD351 as an active ingredient.
- [43] When the pharmaceutical composition is administered to a subject in need thereof, it can fully or partially reduce the expression or activity of one or more of KIRREL3, CNTN4 and CD351 in the subject to increase the level of T cell-mediated immune response.
- [44] Accordingly, the pharmaceutical composition of the present disclosure can be used for immune-enhancing. For example, it can be used for the subject in need of prevention, treatment or improvement of diseases related to immunodeficiency, lower immune function, immune system damage, immunocompromising, etc.
- [45] Another aspect of the present disclosure is to provide a method of treating or preventing cancer in a subject, comprising administering to the subject one or more inhibitors of KIRREL3, CNTN4 and CD351. And also, another aspect of the present disclosure provides a method of immune-enhancing in a subject, comprising administering to the subject one or more inhibitors of KIRREL3, CNTN4 and CD351. In these methods, unless specifically mentioned otherwise, the terms associated have the same meaning as the terms explained for the pharmaceutical compositions in the above.
- [46] Another aspect of the present disclosure is to provide a method of screening an anti-cancer agent comprising:
- [47] (a) treating a cancer cell with a candidate of anti-cancer agent; and
- [48] (b) measuring the expression or activity of one or more of KIRREL3, CNTN4 and CD351 in the cancer cell.
- [49] Optionally, the method of screening an anti-cancer agent may further comprise a step of determining the candidate anti-cancer agent to be the anti-cancer agent if a group treated with the candidate anti-cancer agent shows a lower (or significantly lower) level of expression of one or more of KIRREL3, CNTN4 and CD351 mRNA or

protein or a lower (or significantly lower) level of suppression of T cell activity by one or more of KIRREL3, CNTN4 and CD351 compared to a group not treated with the candidate anti-cancer agent. Here, the lower (or significantly lower) level may indicate an amount decreased by 5% to 95% (e.g., 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% and 90%). The decreased amounts may also mean the sum of each decreased amount of KIRREL3, CNTN4 and CD351, or the independently decreased amounts of KIRREL3, CNTN4, and CD351. The group not treated with the candidate anti-cancer agent may be cancer cells to which no substance is added, or to which any substance such as anti-cancer agent other than one or more inhibitors of KIRREL3, CNTN4 and CD351 is treated.

[50] The term "screening" refers to finding the target materials having the particular properties such as sensitivity or activity among proteins, fusion proteins, antibodies, peptides, antibiotics, enzymes, compounds or any other substances.

[51] The term "candidate anti-cancer agent" may refer to a nucleic acid, protein, antibody, compound, extract or natural substance that is randomly selected or is thought to be able to inhibit the expression or activity of KIRREL3, CNTN4 and/or CD351 according to the usual selection method. The candidate of anti-cancer agent may preferably be a substance that inhibits the expression and/or activity of KIRREL3, CNTN4 and/or CD351.

[52] The expression or activity of KIRREL3, CNTN4 and/or CD351 may be measured by determining the level of expression of the mRNA or protein of KIRREL3, CNTN4 and/or CD351, or by determining the degree to which T cell activity is suppressed by KIRREL3, CNTN4 and/or CD351.

[53] The method of determining the level of expression of the mRNA of KIRREL3, CNTN4 and/or CD351 may include, but are not limited to, any method conventionally known to the art such as reverse transcriptase PCR, competitive reverse transcriptase PCR, real-time reverse transcriptase PCR, RNase protection assay, Northern blotting, DNA chip or RNA chip.

[54] The method of determining the level of expression of KIRREL3, CNTN4 and/or CD351 protein may include, but are not limited to, any method conventionally known to the art such as Western blot, ELISA, radioimmunoassay analysis, radial immunodiffusion, Ouchterlony immunodiffusion, rocket immunoelectrophoresis, tissue immunohistochemistry, immunoprecipitation assay, complement fixation assay, FACS or protein chip.

[55] The method of determining the degree of T cell activity inhibition by KIRREL3, CNTN4 and/or CD351 may include, but are not limited to, any method conventionally known to the art such as RT-PCR, Western Blot, ELISA, radioimmunoassay, radioimmunodiffusion, Ouchterlony immunodiffusion, rocket immunoelectrophoresis, im-

munohistochemistry, immunoprecipitation, complete fixation assay, or FACS.

- [56] In addition, in the method of screening of the present disclosure, confirmation of KIRREL3, CNTN4 and/or CD351 activity inhibition may be performed using conventional methods such as reacting KIRREL3, CNTN4 and/or CD351 protein and a candidate substance to measure an activity, yeast two-hybrid, searching for phage-displayed peptide clones binding to KIRREL3, CNTN4 and/or CD351 protein, HTS (high throughput screening) using natural material and chemical libraries, drug hit HTS, cell-based screening, or DNA array-based screening.
- [57] The method of screening an anti-cancer agent may be performed either in vitro or in vivo. For in vivo, the step of treating a cancer cell with a candidate of anti-cancer agent may be substituted by a step of administering a candidate of anti-cancer agent to a subject having cancer cells or suffering from cancer. Such a subject may be an animal such as human, mouse, etc.
- [58] The method of screening an anti-cancer agent is based on the novel disclosure in the present invention that inhibition of the activity or expression of KIRREL3, CNTN4 and/or CD351 can suppress the function of cancer cells evading T cells. The method of screening of the present disclosure is very advantageous in that it allows for easy development of new anti-cancer agents through a simple and inexpensive method.
- [59] Another aspect of the present disclosure provides a method of providing information necessary for analysis of cancer prognosis, comprising measuring expression or activity of one or more of KIRREL3, CNTN4 and CD351 in cells or tissues isolated from a subject.
- [60] In the method, the terms associated with the expression or activity of one or more of KIRREL3, CNTN4 and CD351 and its measurement, unless specifically mentioned otherwise, have the same meaning as the terms explained for the composition and the screening method.
- [61] The term "prognosis" refers to predictions as to progress of disease, improvement of disease, recurrence of disease, metastasis, and likelihood of death. For example, in the present disclosure, the prognosis refers to the possibility of curing a cancer patient or improving the condition of cancer patient.
- [62] The cell or tissue isolated from the subject may be a cancer cell or a tissue wherein cancer have occurred or cancer cells exist.
- [63] The method of providing information necessary for analysis of cancer prognosis is based on the fact that the lower activity or expression of one or more of KIRREL3, CNTN4 and CD351 in cancer cells can increase T cell activity and proliferation, thereby increasing cancer treatment effect.
- [64] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an ingredient"

means one ingredient or more than one ingredient. The term "A, B and/or C" is used herein to refer to A, or B, or C, or A and B, or A and C, or B and C, or A, B and C.

Brief Description of Drawings

- [65] FIG. 1 shows the proliferation (%) of CD4+ T cells suppressed by KIRREL3.
- [66] FIG. 2 shows the proliferation (%) of CD8+ T cells suppressed by KIRREL3.
- [67] FIG. 3 shows the proliferation (%) of CD4+ T cells suppressed by CNTN4.
- [68] FIG. 4 shows the proliferation (%) of CD8+ T cells suppressed by CNTN4.
- [69] FIG. 5 shows the proliferation (%) of CD4+ T cells suppressed by CD351.
- [70] FIG. 6 shows the proliferation (%) of CD8+ T cells suppressed by CD351.
- [71] FIGS. 7a, 7b, 7c and 7d show the cytotoxicity (%) of PBMC when lung cancer cell line A549 and PBMC were treated with KIRREL3 inhibitors.
- [72] FIGS. 8a, 8b, 8c and 8d show the cytotoxicity (%) of PBMC when colon cancer cell line HCT-116 and PBMC were treated with KIRREL3 inhibitors.
- [73] FIGS. 9a, 9b, 9c and 9d show the cytotoxicity (%) of PBMC when breast cancer cell line MDA-MB-231 and PBMC were treated with KIRREL3 inhibitors.
- [74] FIGS. 10a, 10b, 10c and 10d show the cytotoxicity (%) of PBMC when gastric cancer cell line MKN-74 and PBMC were treated with KIRREL3 inhibitors.
- [75] FIGS. 11a, 11b, 11c and 11d show the cytotoxicity (%) of PBMC when leukemia cell line U937 and PBMC were treated with KIRREL3 inhibitors.
- [76] FIGS. 12a, 12b, 12c and 12d show the cytotoxicity (%) of PBMC when lung cancer cell line A549 and PBMC were treated with CNTN4 inhibitors.
- [77] FIGS. 13a, 13b, 13c and 13d show the cytotoxicity (%) of PBMC when colon cancer cell line HCT-116 and PBMC were treated with CNTN4 inhibitors.
- [78] FIGS. 14a, 14b, 14c and 14d show the cytotoxicity (%) of PBMC when breast cancer cell line MDA-MB-231 and PBMC were treated with CNTN4 inhibitors.
- [79] FIGS. 15a, 15b, 15c and 15d show the cytotoxicity (%) of PBMC when gastric cancer cell line MKN-74 and PBMC were treated with CNTN4 inhibitors.
- [80] FIGS. 16a, 16b, 16c and 16d show the cytotoxicity (%) of PBMC when leukemia cell line U937 and PBMC were treated with CNTN4 inhibitors.
- [81] FIGS. 17a, 17b, 17c and 17d show the cytotoxicity (%) of PBMC when lung cancer cell line A549 and PBMC were treated with CD351 inhibitors.
- [82] FIGS. 18a, 18b, 18c and 18d show the cytotoxicity (%) of PBMC when colon cancer cell line HCT-116 and PBMC were treated with CD351 inhibitors.
- [83] FIGS. 19a, 19b, 19c and 19d show the cytotoxicity (%) of PBMC when breast cancer cell line MDA-MB-231 and PBMC were treated with CD351 inhibitors.
- [84] FIGS. 20a, 20b, 20c and 20d show the cytotoxicity (%) of PBMC when gastric cancer cell line MKN-74 and PBMC were treated with CD351 inhibitors.

- [85] FIGS. 21a, 21b, 21c and 21d show the cytotoxicity (%) of PBMC when leukemia cell line U937 and PBMC were treated with CD351 inhibitors.
- [86] FIG. 22 shows the tumor size in mouse treated with KIRREL3 inhibitors.
- [87] FIG. 23 shows the tumor size in mouse treated with CNTN4 inhibitors.
- [88] FIGS. 24a, 24b and 24c show the tumor size in mouse treated with CD351 inhibitors.

Mode for the Invention

- [89] In the following, exemplary embodiments of the inventive concept will be explained in further detail with reference to examples. However, the following examples are meant to exemplify the present invention, and the scope of the invention is not restricted by these examples.

[90]

[91] **Example 1. Inhibition of the proliferation and activity of T cells**

- [92] This example is to confirm whether KIRREL3, CNTN4 and CD351 suppress the proliferation and activity of the T cell, and ensures that cancer cells evade the T cell-mediated immune system.

[93]

[94] **1.1. Preparation of CD4+ cells and CD8+ T cells**

- [95] Human blood was placed in a 10 ml tube coated with EDTA (or heparin) and mixed with PBS at a ratio of 1:1. Ficoll-Paque PLUS was placed in a 50 ml tube, and then the blood sample was added. After centrifugation, human PBMCs (peripheral blood mononuclear cells) were collected. The resultant was centrifuged, and the supernatant was removed. Then, RBC lysis (1x) was added, pipetted, and stored on ice for 3 minutes. After that, 50 ml of 10% FBS RPMI1640 was added, and the mixture was centrifuged to remove the supernatant. Then, FACS buffer was added, and the supernatant was removed by centrifugation. Subsequently, 50 ml of MACS buffer (PBS containing 0.5% bovine serum albumin and 2mM EDTA) was added, the number of cells was counted, and the supernatant was completely removed after centrifugation.
- [96] CD4+ T cells and CD8+ T cells were resuspended using 40 μ l of MACS buffer based on the number of 1×10^7 cells in a 50 ml tube. 10 μ l of anti-CD4 and anti-CD8 biotin antibodies were added to the tube respectively, and then stored in the refrigerator for 5 minutes. Subsequently, 30 μ l of MACS buffer based on the number of 1×10^7 cells was added to the resultant, and 20 μ l of anti-biotin microbeads were added and mixed. Then, CD4+ T cells and CD8+ T cells were separated using LS column, and were counted.
- [97] The prepared CD4+ T cells and CD8+ T cells were mixed with 1 μ l of CFSE (carboxyfluorescein succinimidyl ester) based on the number of 2×10^6 cells, and stored at 37°C for 3 minutes. Then, FBS was added into tubes containing the CD4+ T cells

and CD8+ T cells respectively, and stored on ice 10 minutes. Thereafter, the supernatant was removed by centrifugation. The resultant was added with 30 ml of FACS buffer, pipetted, and centrifuged to remove the supernatant. Then, the resultant was mixed with 10 ml of 10% FBS RPMI1640, and the number of cells was counted.

[98]

[99] 1.2. Measurement of T cell activity

[100] 1.2.1. Inhibition of T cell activity by KIRREL3

[101] The recombinant human IgG1 Fc protein (Cat. No. 110-HG) and the recombinant human PD-L1/B7-H1 Fc chimera protein (Cat. No. 156-B7) were purchased from R&D systems. The recombinant human KIRREL3 His Tag protein (Cat. No. 4910-K3) was purchased from R&D systems.

[102] 7.5 µg/ml or 10 µg/ml of each protein was mixed with 2.5 µg/ml of anti-CD3 antibody (BioLegend, Cat. No. 317325) in PBS, respectively. The resultant mixture was coated on 96-well plates at 4°C, and the wells were washed three times with PBS.

[103] The CD4+ T cells and CD8+ T cells prepared in the Example 1.1 were added to each well of the 96-well plate at the number of 2×10^6 cells in an amount of 200 µl, and then incubated.

[104] CD4+ T cells and CD8+ T cells were activated by anti-CD3 antibody for 72 hours. The proliferation of CD4+ T cells and CD8+ T cells can be confirmed by the degree of CFSE fluorescent cell staining, and was analyzed by flow cytometry using FACSDiVa software (BD Biosciences).

[105]

[106] 1.2.2. Inhibition of T-cell activity by CNTN4

[107] The recombinant human IgG1 Fc protein (Cat. No. 110-HG) and the recombinant human PD-L1/B7-H1 Fc chimera protein (Cat. No. 156-B7) were purchased from R&D systems. The recombinant human CNTN4 His Tag protein (Cat. No. 2205-CN) was purchased from R&D systems.

[108] 7.5 µg/ml or 10 µg/ml of each protein was mixed with 2.5 µg/ml of anti-CD3 antibody (BioLegend, Cat. No. 317325) in PBS, respectively. The resultant mixture was coated on 96-well plates at 4°C, and the wells were washed three times with PBS.

[109] The CD4+ T cells and CD8+ T cells prepared in the Example 1.1 were added to each well of the 96-well plate at the number of 2×10^6 cells in an amount of 200 µl, and then incubated.

[110] CD4+ T cells and CD8+ T cells were activated by anti-CD3 antibody for 72 hours. The proliferation of CD4+ T cells and CD8+ T cells can be confirmed by the degree of CFSE fluorescent cell staining, and was analyzed by flow cytometry using FACSDiVa software (BD Biosciences).

[111]

[112] 1.2.3. Inhibition of T-cell activity by CD351

[113] The recombinant human IgG1 Fc protein (Cat. No. 110-HG) and the recombinant human PD-L1/B7-H1 Fc chimera protein (Cat. No. 156-B7) were purchased from R&D systems. The recombinant human CD351 His Tag protein (Cat. No. 9278-FC) was purchased from R&D systems.

[114] 10 µg/ml of each protein was mixed with 1.0 µg/ml, 2.0 µg/ml, 4.0 µg/ml, or 6.0 µg/ml of anti-CD3 antibody (BioLegend, Cat. No. 317325) in PBS, respectively. The resultant mixture was coated on 96-well plates at 4°C, and the wells were washed three times with PBS.

[115] The CD4+ T cells and CD8+ T cells prepared in the Example 1.1 were added to each well of the 96-well plate at the number of 2×10^6 cells in an amount of 200 µl, and then incubated.

[116] CD4+ T cells and CD8+ T cells were activated by anti-CD3 antibody for 72 hours. The proliferation of CD4+ T cells and CD8+ T cells can be confirmed by the degree of CFSE fluorescent cell staining, and was analyzed by flow cytometry using FACSDiVa software (BD Biosciences).

[117]

[118] 1.3. Results

[119] 1.3.1. Inhibition of T-cell activity by KIRREL3

[120] FIG. 1 and FIG. 2 show the percent proliferation (%) of CD4+ T cells and CD8+ T cells, respectively.

[121] The control group treated with PD-L1 inhibited the proliferation of both CD4+ T cells and CD8+ T cells compared to the control group treated with IgG1. The PD-L1 binds to PD-1, a protein on the surface of T cells, and inhibits the proliferation of T cells. Accordingly, it results in suppressing the function of T cells attacking and killing cancer cells.

[122] The group treated with KIRREL3 remarkably inhibited the proliferation of both CD4+ T cells and CD8+ T cells compared to the control group treated with IgG1. And also, the group treated with KIRREL3 inhibited the proliferation of both CD4+ T cells and CD8+ T cells similarly to the control group treated with PD-L1.

[123] It means that if KIRREL3 is neutralized by blocking or knockdown, the T cell proliferation inhibition of KIRREL3 can be suppressed. Accordingly, the cancer treatment can be effectively achieved.

[124]

[125] 1.3.2. Inhibition of T-cell activity by CNTN4

[126] FIG. 3 and FIG. 4 show the percent proliferation (%) of CD4+ T cells and CD8+ T cells, respectively.

[127] The control group treated with PD-L1 inhibited the proliferation of both CD4+ T

cells and CD8+ T cells compared to the control group treated with IgG1.

[128] The group treated with CNTN4 remarkably inhibited the proliferation of both CD4+ T cells and CD8+ T cells compared to the control group treated with IgG1. And also, the group treated with CNTN4 inhibited the proliferation of both CD4+ T cells and CD8+ T cells similarly to the control group treated with PD-L1.

[129] It means that if CNTN4 is neutralized by blocking or knockdown, the T cell proliferation inhibition of CNTN4 can be suppressed. Accordingly, the cancer treatment can be effectively achieved.

[130]

[131] 1.3.3. Inhibition of T-cell activity by CD351

[132] FIG. 5 and FIG. 6 show the percent proliferation (%) of CD4+ T cells and CD8+ T cells, respectively.

[133] The control group treated with PD-L1 significantly inhibited the proliferation of CD4+ T cells compared to the control group treated with IgG1, whereas it did not show a significant inhibition on the proliferation of CD8+ T cells compared to the control group treated with IgG1.

[134] The group treated with CD351 remarkably inhibited the proliferation of both CD4+ T cells and CD8+ T cells compared to the control group treated with IgG1 as well as the control group treated with PD-L1.

[135] It means that CD351 is neutralized by blocking or knockdown, the T cell proliferation inhibition of CD351 can be suppressed. Accordingly, the cancer treatment can be effectively achieved.

[136]

[137] **Example 2. PBMC cytotoxic function assay**

[138] This example is to confirm whether the cytotoxic ability of PBMC against cancer cells is increased when KIRREL3, CNTN4 or CD351 is neutralized using inhibitors of KIRREL3, CNTN4 or CD351.

[139]

[140] 2.1. Preparation of PBMC

[141] Human blood was placed in a 10 ml tube coated with EDTA (or heparin) and mixed with PBS at a ratio of 1:1. Ficoll-Paque PLUS was placed in a 50 ml tube, and then the blood sample was added. After centrifugation, human PBMCs were collected. The resultant was centrifuged, and the supernatant was removed. Then, RBC lysis (1x) was added, pipetted, and stored on ice for 3 minutes. After that, 50 ml of 10% FBS RPMI1640 was added, and the mixture was centrifuged to remove the supernatant. Then, FACS buffer was added, and the supernatant was removed by centrifugation. Subsequently, 50 ml of MACS buffer (PBS containing 0.5% bovine serum albumin and 2mM EDTA) was added, the number of cells was counted, and the supernatant

was completely removed after centrifugation.

- [142] 96-well plates were coated with 1.0 µg/ml of anti-CD3 antibody (BioLegend, Cat. No. 317325) in PBS at 4°C, and the wells were washed three times with PBS. The PBMC prepared in the above was mixed with 10% FBS RPMI1640, and was added to each well of the 96-well plate at the number of 6×10^5 cells in an amount of 100 µl. The PBMC was activated by anti-CD3 antibody for 72 hours.

[143]

[144] 2.2. Preparation of cancer cells

- [145] Lung cancer cell line A549, colon cancer cell line HCT-116, breast cancer cell line MDA-MB-231, gastric cancer cell line MKN-74, and leukemia cell line U937 were respectively mixed with 1 µl of CFSE (carboxyfluorescein succinimidyl ester), and then stored at 37°C for 3 minutes. Subsequently, FBS was added into tubes containing cancer cells and stored on ice for 10 minutes. Thereafter, the supernatant was removed by centrifugation. The resultant was added with 30 ml of FACS buffer, pipetted, and centrifuged to remove the supernatant. Then, 10% FBS RPMI1640 was added, pipetted, and centrifuged to remove the supernatant. Thereafter, the resultant was mixed with 10 ml of 10% FBS RPMI1640, and the number of cells was counted.

- [146] Each PBMC-containing well of the 96-well plate prepared in the Example 2.1 was added with the cancer cells at the number of 3×10^4 cells in an amount of 100 µl.

[147]

[148] 2.3. Measurement of cytotoxicity of PBMC against cancer cells

- [149] The mixtures of PBMCs and cancer cells were prepared in the Example 2.2. These mixtures were incubated for 24 hours with 10 µg/mL of anti-human KIRREL3 antibody, anti-human CNTN4 antibody or anti-human CD351 antibody, or 50 nM of KIRREL3 siRNA, CNTN4 siRNA or CD351 siRNA.

- [150] Table 1 below provides the non-treated control group and Groups 1 to 4 using four neutralizing antibodies for blocking KIRREL3, and Table 2 below provides the non-treated control group and Groups 5 to 7 using three siRNAs for knockdown of KIRREL3.

- [151] [Table 1]

	human KIRREL3 neutralizing antibody
Control group	Not treated
Group 1	anti-human KIRREL3 antibody (R&D, AF4910)
Group 2	anti-human KIRREL3 antibody (Bioss, bs-11864R)
Group 3	anti-human KIRREL3 antibody (genetex, GTX32140)
Group 4	anti-human KIRREL3 antibody (LSbio, LS-C336219)

[152] [Table 2]

	human KIRREL3 siRNA
Control group	Not treated
Group 5	Sense (5'-CUCUCAAGUUACCCACAGUtt-3') (SEQ ID NO: 1)Antisense (5'-ACUGUGGGUAACUUGAGAGtt-3') (SEQ ID NO: 2)
Group 6	Sense (5'-GGAGAGGUGUACAGGACCAtt-3') (SEQ ID NO: 3)Antisense (5'-UGGUCCUGUACACCUCUCCtt-3') (SEQ ID NO: 4)
Group 7	Sense (5'-UCUCAAGUUACCCACAGUAAtt-3') (SEQ ID NO: 5)Antisense (5'-UACUGUGGGUAACUUGAGAtt-3') (SEQ ID NO: 6)

[153] Table 3 below provides the non-treated control group and Groups 1 to 5 using five neutralizing antibodies for blocking CNTN4, and Table 4 below provides the non-treated control group and Groups 6 to 8 using three siRNAs for knockdown of CNTN4.

[154] [Table 3]

	human CNTN4 neutralizing antibody
Control group	Not treated
Group 1	anti-human CNTN4 antibody (R&D, MAB2205)
Group 2	anti-human CNTN4 antibody (abcam, ab137107)
Group 3	anti-human CNTN4 antibody (abcam, ab131285)
Group 4	anti-human CNTN4 antibody (LSbio, LS-C119876)
Group 5	anti-human CNTN4 antibody (Abnova, PAB27653)

[155] [Table 4]

	human CNTN4 siRNA
Control group	Not treated
Group 6	Sense (5'-CAGUAUCUUUGCCAGAAGUtt-3') (SEQ ID NO: 7)Antisense (5'-ACUUCUGGCAAAGAUAUCUGtt-3') (SEQ ID NO: 8)
Group 7	Sense (5'-GAUAAUGAGUCGGAAGUAAtt-3') (SEQ ID NO: 9)Antisense (5'-UUACUUCCGACUCAUUAUCtt-3') (SEQ ID NO: 10)
Group 8	Sense (5'-GUGACAAUAGACGAAAUCAtt-3') (SEQ ID NO: 11)Antisense (5'-UGAUUUCGUCUAUUGUCACtt-3') (SEQ ID NO: 12)

[156] Table 5 below provides the non-treated control group and Groups 1 to 3 using three neutralizing antibodies for blocking CD351, and Table 6 below provides the non-treated control group and Groups 4 to 6 using three siRNAs for knockdown of CD351.

[157] [Table 5]

	human CD351 neutralizing antibody
Control group	Not treated
Group 1	anti-human CD351 antibody (Biolegend, 13730)
Group 2	anti-human CD351 antibody (Creative diagnostics, CABT-BL4657)
Group 3	anti-human CD351 antibody (Biobyte, orb183662)

[158] [Table 6]

	human CD351 siRNA
Control group	Not treated
Group 4	Sense (5'-GAGAGAUGAACUGCUCAGUtt-3') (SEQ ID NO: 13)Antisense (5'-ACUGAGCAGUUCAUCUCUCtt-3') (SEQ ID NO: 14)
Group 5	Sense (5'-GAGAACUUCCAACUCAGUAtt-3') (SEQ ID NO: 15)Antisense (5'-UACUGAGUUGGAAGUUCUCtt-3') (SEQ ID NO: 16)
Group 6	Sense (5'-AGAGAACUUCCAACUCAGUtt-3') (SEQ ID NO: 17)Antisense (5'-ACUGAGUUGGAAGUUCUCUtt-3') (SEQ ID NO: 18)

- [159] After twenty-four hours from incubating the mixtures of PBMCs and cancer cells with antibody or siRNA, cells were stained with 7-aminoactinomycin D (7-AAD; BD Pharmingen, San Diego, CA, USA) to detect lysed cells. The cytotoxicity of PBMC against cancer cells was analyzed by determining FL-1 (CFSE) and FL-3 (7-AAD) staining using a FACSDiVa software (BD Biosciences).
- [160]
- [161] 2.4. Results
- [162] For the lung cancer cell line A549, FIGS. 7a, 7b, 7c and 7d show the results treated with KIRREL3 neutralizing antibody or siRNA, FIGS. 12a, 12b, 12c and 12d show the results treated with CNTN4 neutralizing antibody or siRNA, and FIGS. 17a, 17b, 17c and 17d show the results treated with CD351 neutralizing antibody or siRNA.
- [163] When the lung cancer cell line A549 and PBMC were treated with KIRREL3 neutralizing antibody, CNTN4 neutralizing antibody or CD351 neutralizing antibody, the cytotoxicity against lung cancer cell was significantly increased compared to the non-treated control group even though there is more or less degree of difference depending on the type of antibody. Further, the cytotoxicity against lung cancer cell was also significantly increased when it was treated with KIRREL3 siRNA, CNTN4 siRNA or CD351 siRNA.
- [164] Using KIRREL3 neutralizing antibody or siRNA, the results on the colon cancer cell line HCT-116 are shown in FIGS. 8a, 8b, 8c and 8d, the result on the breast cancer cell line MDA-MB-231 are shown in FIGS. 9a, 9b, 9c and 9d, the results on the gastric cancer cell line MKN-74 are shown in FIGS. 10a, 10b, 10c and 10d, and the results on the leukemia cell line U937 are shown in FIGS. 11a, 11b, 11c and 11d.
- [165] In addition, using CNTN4 neutralizing antibody or siRNA, the results on the colon cancer cell line HCT-116 are shown in FIGS. 13a, 13b, 13c and 13d, the results on the breast cancer cell line MDA-MB-231 are shown in FIGS. 14a, 14b, 14c and 14d, the results on the gastric cancer cell line MKN-74 are shown in FIGS. 15a, 15b, 15c and 15d, and the results on the leukemia cell line U937 are shown in FIGS. 16a, 16b, 16c and 16d.
- [166] In addition, using CD351 neutralizing antibody or siRNA, the results on the colon cancer cell line HCT-116 are shown in FIGS. 18a, 18b, 18c and 18d, the results on the breast cancer cell line MDA-MB-231 are shown in FIGS. 19a, 19b, 19c and 19d, the results on the gastric cancer cell line MKN-74 are shown in FIGS. 20a, 20b, 20c and 20d, and the results on the leukemia cell line U937 are shown in FIGS. 21a, 21b, 21c and 21d.
- [167] As shown in FIGS. 8a to 11d, 13a to 16d, and 18a to 21d, when one or more of KIRREL3, CNTN4 and CD351 were neutralized by antibodies or siRNAs, the results of increasing the cytotoxicity of PBMC were also observed in colon cancer, breast

cancer, gastric cancer and leukemia.

[168]

[169] **Example 3. Tumor-mouse model experiment**

[170] This example is to confirm whether the growth of tumor in mouse is suppressed when KIRREL3, CNTN4 or CD351 is neutralized using inhibitors of KIRREL3, CNTN4 or CD351.

[171]

[172] **3.1. Establishment of tumor-mouse model**

[173] MC-38 cell line derived from C57bL6 colon adenocarcinoma cells was resuspended in 50 µl PBS at the number of 2×10^5 cells, and was subcutaneously injected into the flanks of 6-week-old female C57bL6 mice.

[174] Table 7 below provides the non-treated control group and Group 8 using a siRNA for knockdown of KIRREL3.

[175] [Table 7]

	mouse KIRREL3 siRNA
Control group	Not treated
Group 8	Sense (5'-GUAAAGGAGAGGUCAUCAA-3') (SEQ ID NO: 19) Antisense (5'-UUGAUGACCUCUCCUUUAC-3') (SEQ ID NO: 20)

[176] Table 8 below provides the non-treated control group and Group 9 using a siRNA for knockdown of CNTN4.

[177] [Table 8]

	mouse CNTN4 siRNA
Control group	Not treated
Group 9	Sense (5'-GUGUAGACAAACUCUCUGU-3') (SEQ ID NO: 21) Antisense (5'-ACAGAGAGUUUGUCUACAC-3') (SEQ ID NO: 22)

[178] Table 9 below provides the non-treated control group and Groups 7, 8 and 9 using three siRNAs for knockdown of CD351.

[179] [Table 9]

	mouse CD351 siRNA
Control group	Not treated
Group 7	Sense (5'-GUCCAUCCAACACCACCUA-3') (SEQ ID NO: 23) Antisense (5'-UAGGUGGUGUUGGAUGGAC-3') (SEQ ID NO: 24)
Group 8	Sense (5'-CUGAUGAGGGGAAAGAACUU-3') (SEQ ID NO: 25) Antisense (5'-AAGUUCUUUCCCUCaucag-3') (SEQ ID NO: 26)
Group 9	Sense (5'-CAGCUAAGCCCAGUGAACA-3') (SEQ ID NO: 27) Antisense (5'-UGUUCACUGGGCUUAGCUG-3') (SEQ ID NO: 28)

[180] In all Groups, the siRNA targeting mouse KIRREL3, mouse CNTN4 or mouse CD351 was injected into the tumor of mice three times at the interval of 5 days from the 11th day after injecting MC-38 cells. Specifically, 10 µg siRNA and 7.5 µl oligofectamine (Invitrogen) in PBS were mixed according to manufacturer's instruction, and then injected into the tumor tissue induced in mice at a dose of 0.5 mg/kg.

[181]

[182] 3.2. Results

[183] FIG. 22 provides the result on the size of tumor in mice of the non-treated control group and Group 8 wherein KIRREL3 was knocked down. FIG. 23 provides the result on the size of tumor in mice of the non-treated control group and Group 9 wherein CNTN4 was knocked down. FIGS. 24a, 24b and 24c provide the results on the size of tumor in mice of the non-treated control group and Groups 7 to 9 wherein CD351 was knocked down.

[184] In the non-treated control group, the tumor continued to grow after it occurred. Compared to the non-treated control group, the growth rate of tumor in mouse was remarkably inhibited in Groups wherein KIRREL3, CNTN4 or CD351 was knocked down. It means that when one or more of KIRREL3, CNTN4 and CD351 are blocked or knocked down to inhibit its activity or expression, the development of cancer is delayed or stopped and the occurrence of cancer is inhibited. Accordingly, one or more inhibitors of KIRREL3, CNTN4 and CD351 can be efficiently used to prevent cancer.

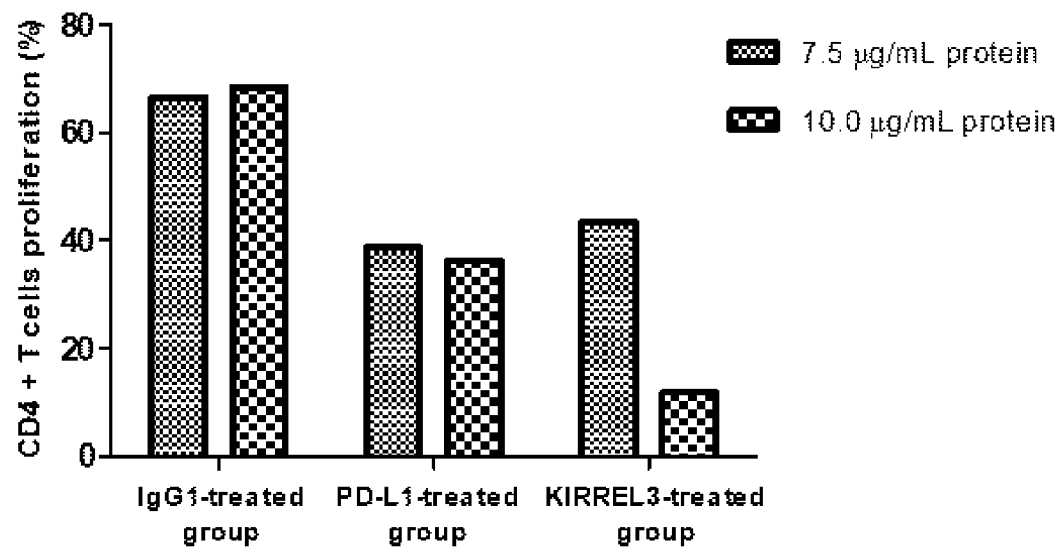
[185] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the present disclosure described herein. Such equivalents are intended to be encompassed by the following claims.

Claims

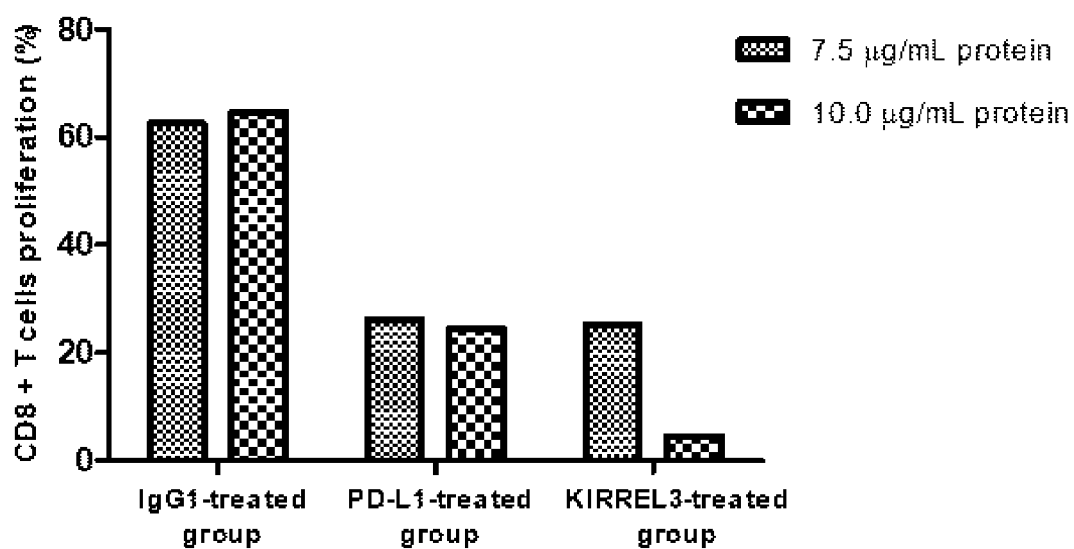
- [Claim 1] A pharmaceutical composition for treating or preventing cancer, comprising one or more inhibitors of KIRREL3, CNTN4 and CD351.
- [Claim 2] The pharmaceutical composition for treating or preventing cancer according to claim 1, wherein the inhibitor is an antisense nucleic acid, a siRNA, a shRNA, a miRNA or a ribozyme that binds in a complementary manner to a DNA or a mRNA of one or more of KIRREL3, CNTN4 and CD351 gene.
- [Claim 3] The pharmaceutical composition for treating or preventing cancer according to claim 1, wherein the inhibitor is a compound, a peptide, a peptide mimetic, a fusion protein, an antibody or an aptamer that binds specifically to one or more of KIRREL3, CNTN4 and CD351 protein.
- [Claim 4] The pharmaceutical composition for treating or preventing cancer according to claim 1, wherein the cancer is stomach cancer, lung cancer, liver cancer, colorectal cancer, colon cancer, small intestinal cancer, pancreatic cancer, brain cancer, bone cancer, melanoma, breast cancer, sclerosing adenosis, uterine cancer, cervical cancer, head and neck cancer, esophageal cancer, thyroid cancer, parathyroid cancer, renal cancer, sarcoma, prostate cancer, urethral cancer, bladder cancer, blood cancer, leukemia, lymphoma, or fibroadenoma.
- [Claim 5] The pharmaceutical composition for treating or preventing cancer according to claim 1, wherein the inhibitor suppresses a function of cancer cells evading T cells.
- [Claim 6] A pharmaceutical composition for immune-enhancing in a subject, comprising one or more inhibitors of KIRREL3, CNTN4 and CD351.
- [Claim 7] The pharmaceutical composition for immune-enhancing according to claim 6, wherein the inhibitor inhibits expression or activity of one or more of KIRREL3, CNTN4 and CD351 in the subject to increase a level of T cell-mediated immune response.
- [Claim 8] The pharmaceutical composition for immune-enhancing according to claim 6, wherein the subject is in need of prevention, treatment or improvement of diseases related to immunodeficiency, lower immune function, immune system damage, or immunocompromising.
- [Claim 9] A method of screening an anti-cancer agent comprising:
(a) treating a cancer cell with a candidate anti-cancer agent; and
(b) measuring expression or activity of one or more of KIRREL3, CNTN4 and CD351 in the cancer cell.

- [Claim 10] The method according to claim 9, wherein the step (b) is conducted by determining a level of expression of a mRNA or a protein of one or more of KIRREL3, CNTN4 and CD351 or a level of suppression of T cell activity by one or more of KIRREL3, CNTN4 and CD351.
- [Claim 11] The method according to claim 9, further comprising:
(c) determining the candidate anti-cancer agent to be the anti-cancer agent if a group treated with the candidate anti-cancer agent shows a significantly lower level of expression of mRNA of one or more of KIRREL3, CNTN4 and CD351 or protein of one or more of KIRREL3, CNTN4 and CD351; or
a significantly lower level of suppression of T cell activity by one or more of KIRREL3, CNTN4 and CD351
compared to a group not treated with the candidate anti-cancer agent.
- [Claim 12] A method of providing information necessary for analysis of cancer prognosis comprising: measuring expression or activity of one or more of KIRREL3, CNTN4 and CD351 in cells or tissues isolated from a subject.
- [Claim 13] The method according to claim 12, wherein the expression or activity of one or more of KIRREL3, CNTN4 and CD351 is measured by determining a level of expression of a mRNA or a protein of one or more of KIRREL3, CNTN4 and CD351 or a level of suppression of T cell activity by one or more of KIRREL3, CNTN4 and CD351.

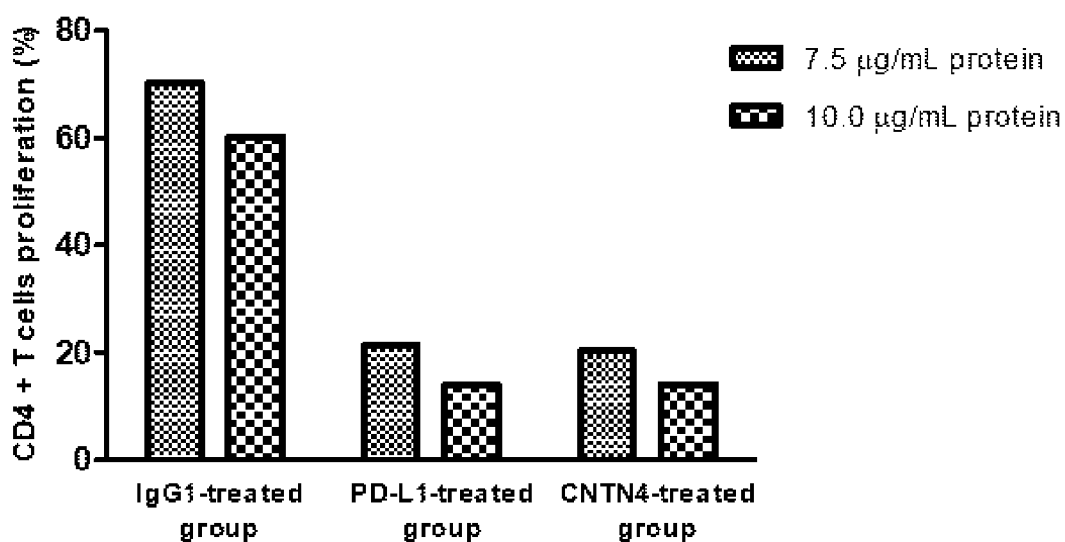
[Fig. 1]



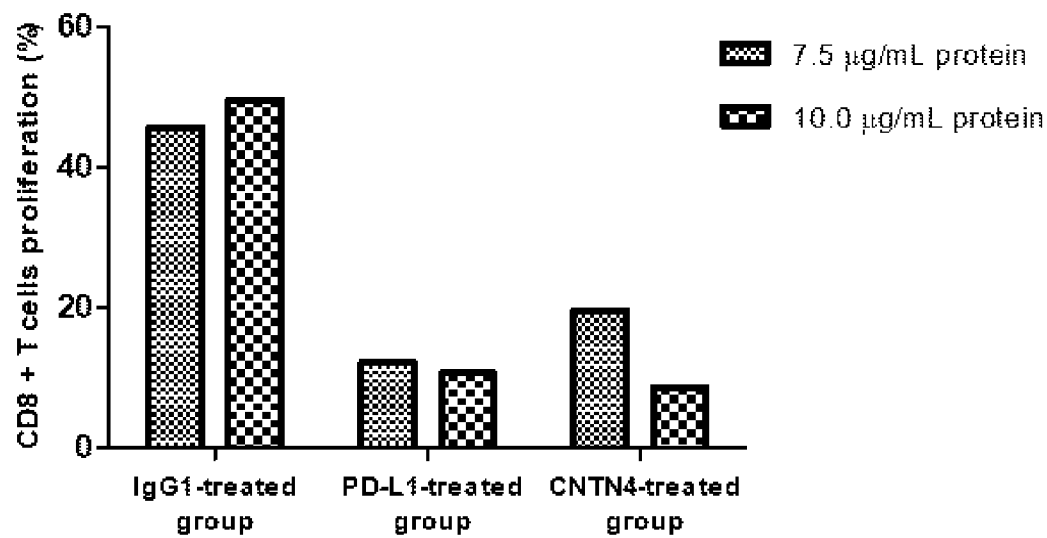
[Fig. 2]



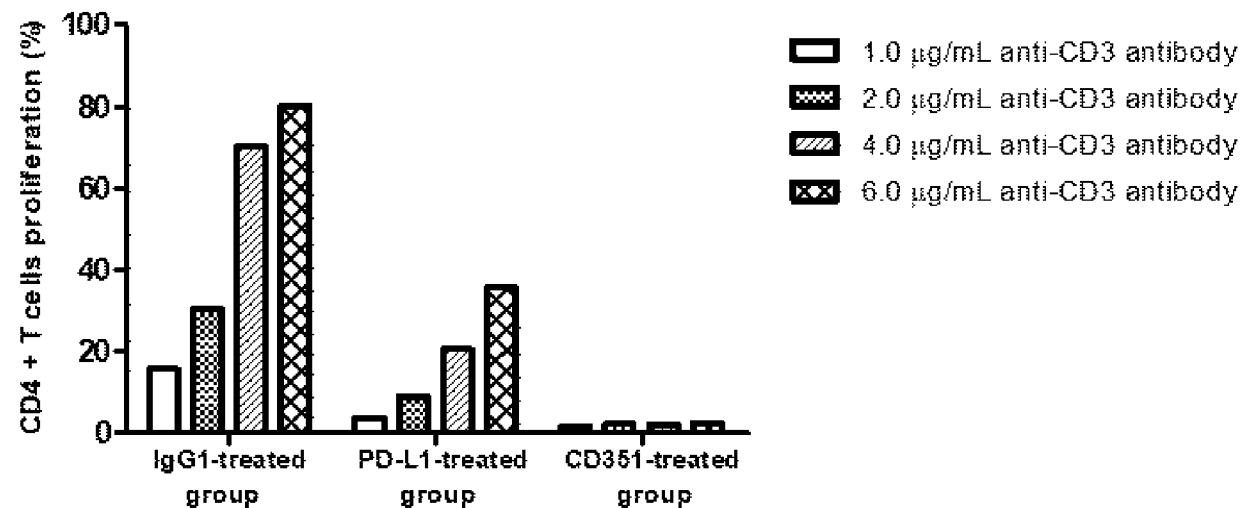
[Fig. 3]



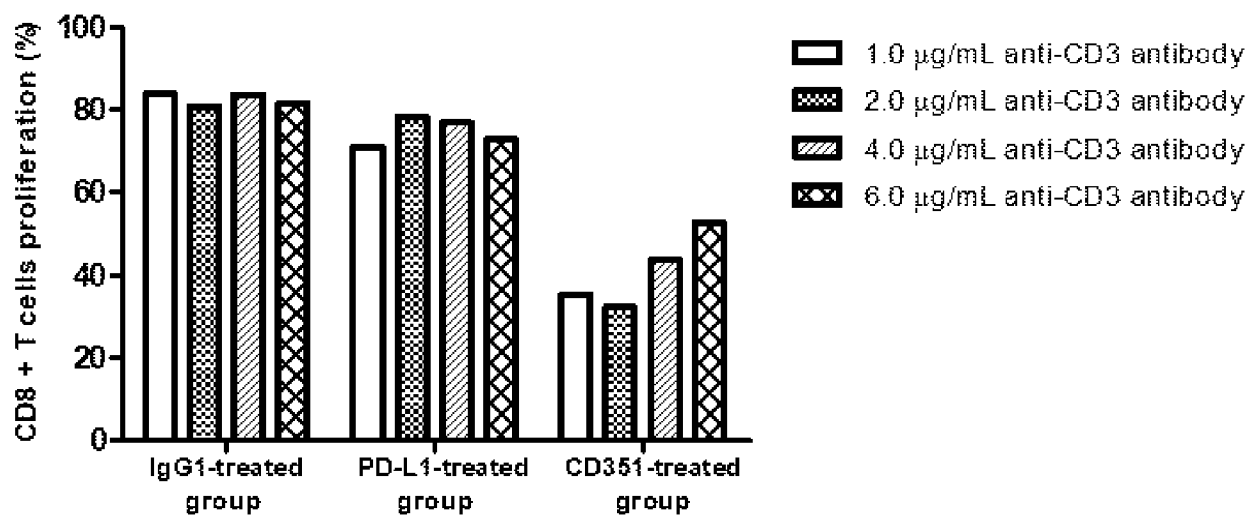
[Fig. 4]



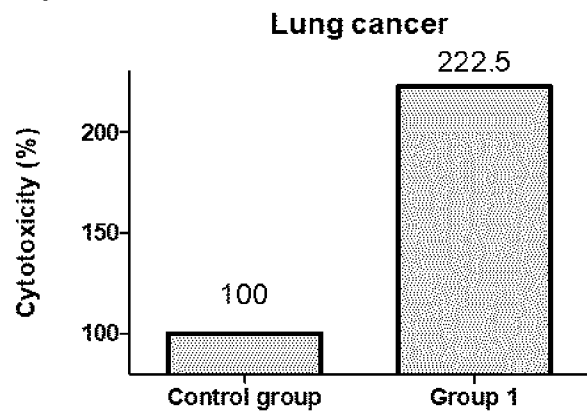
[Fig. 5]



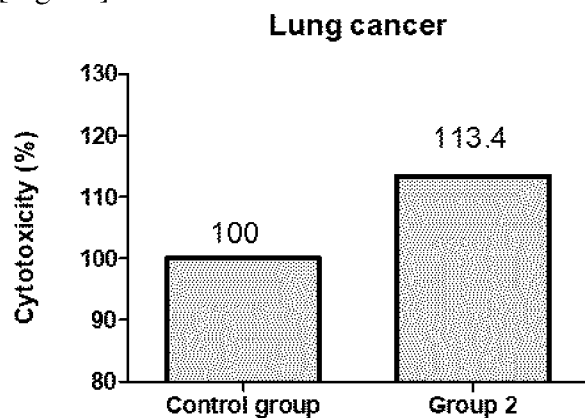
[Fig. 6]



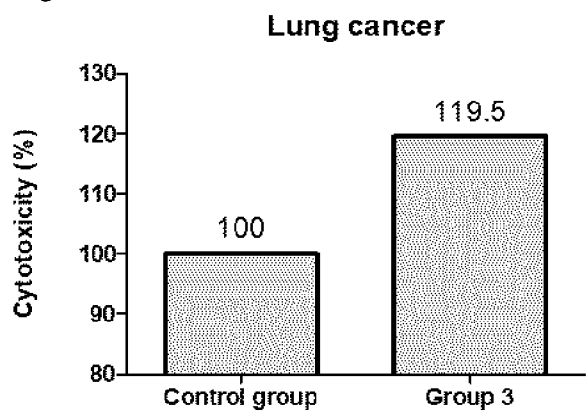
[Fig. 7a]



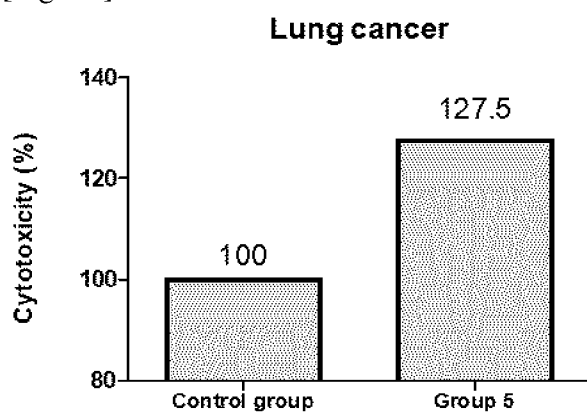
[Fig. 7b]



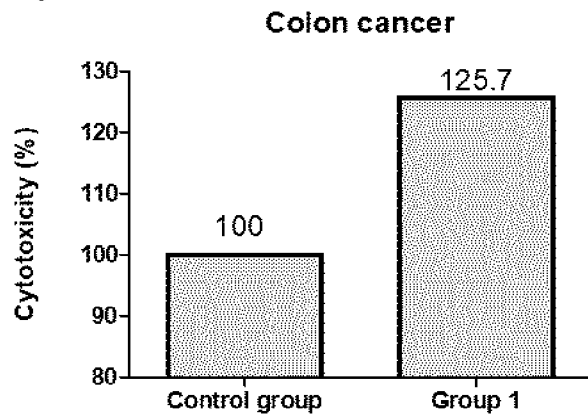
[Fig. 7c]



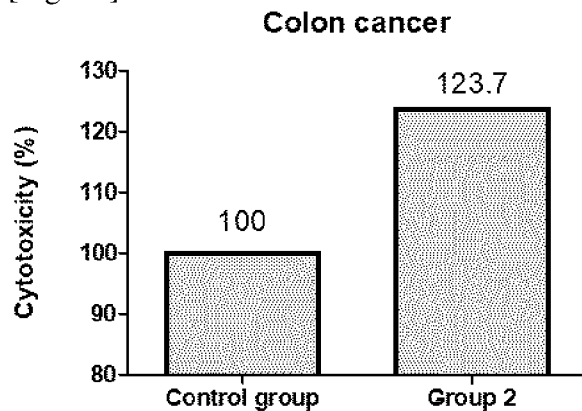
[Fig. 7d]



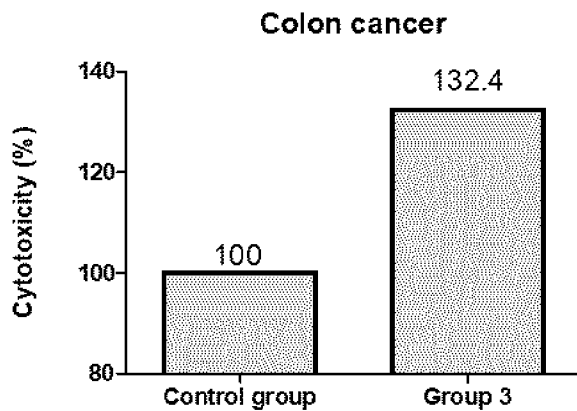
[Fig. 8a]



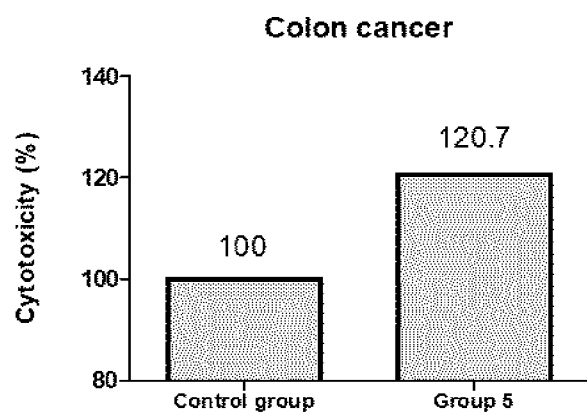
[Fig. 8b]



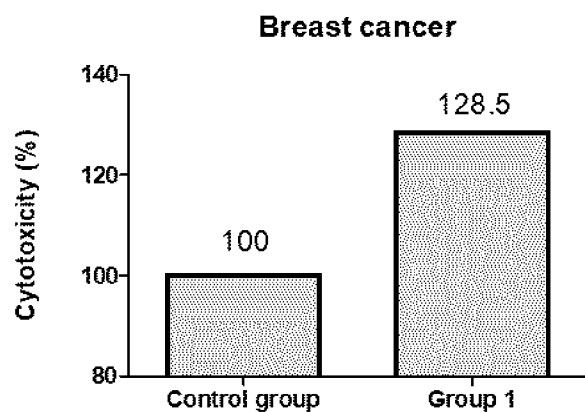
[Fig. 8c]



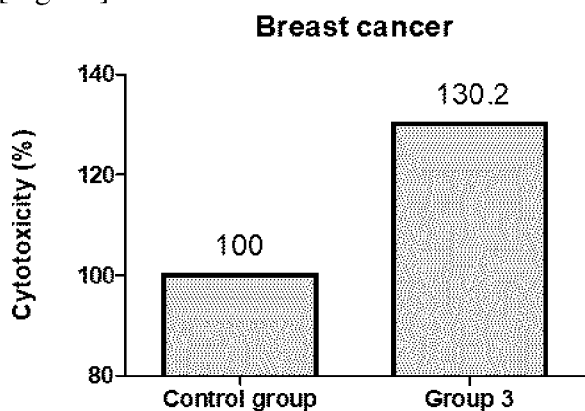
[Fig. 8d]



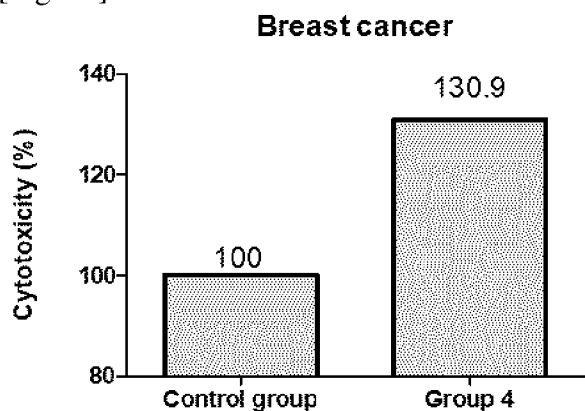
[Fig. 9a]



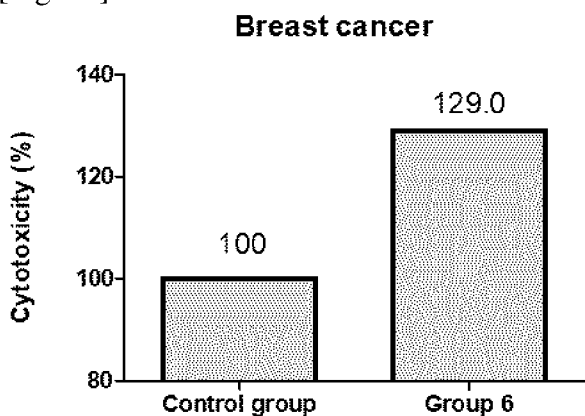
[Fig. 9b]



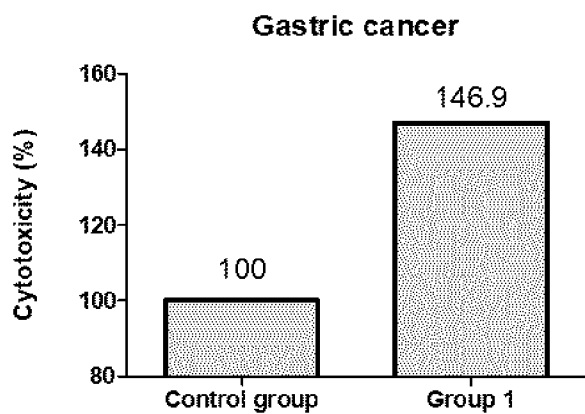
[Fig. 9c]



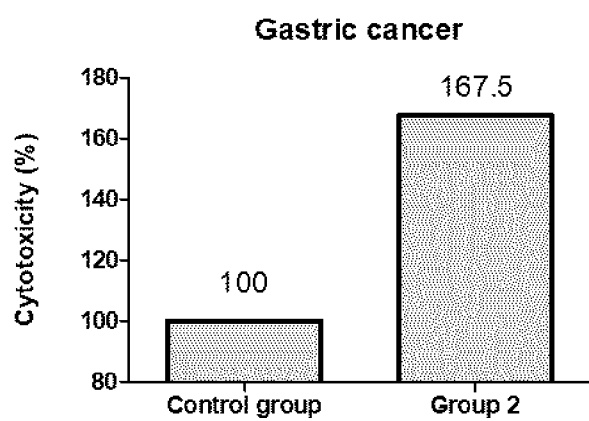
[Fig. 9d]



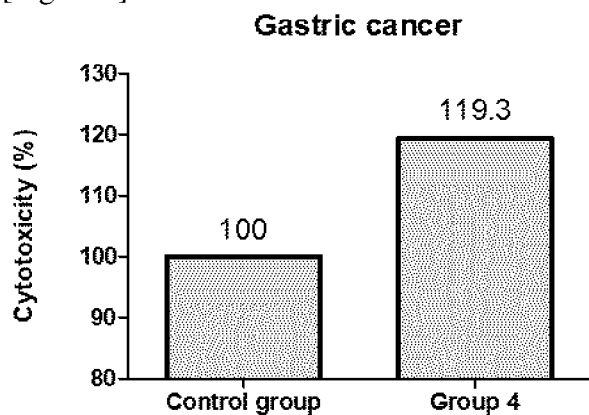
[Fig. 10a]



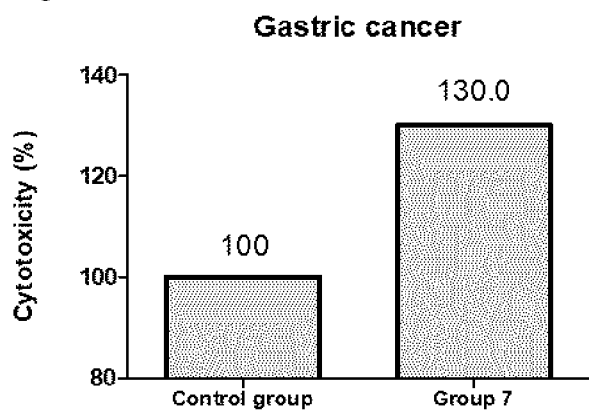
[Fig. 10b]



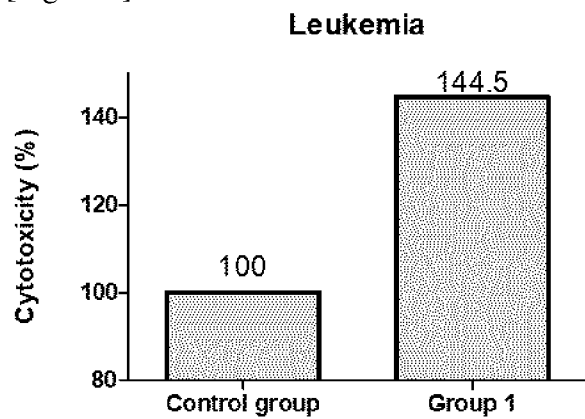
[Fig. 10c]



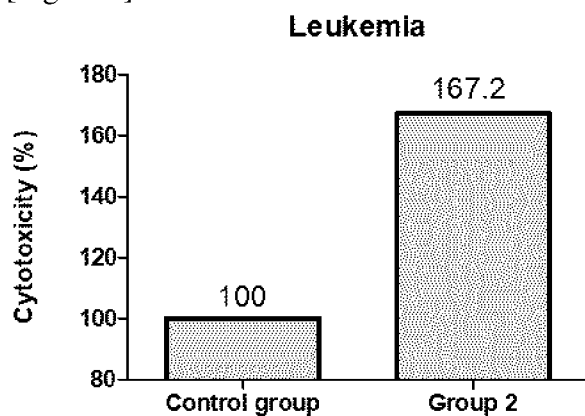
[Fig. 10d]



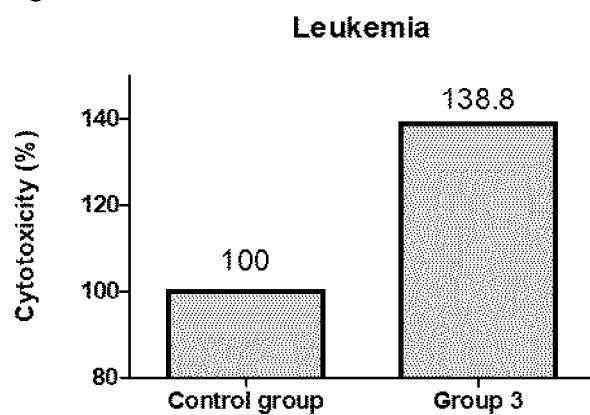
[Fig. 11a]



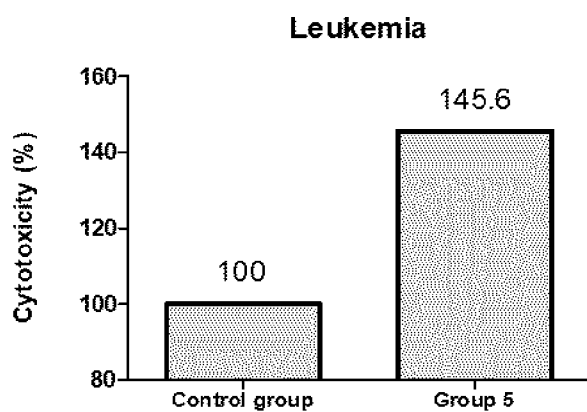
[Fig. 11b]



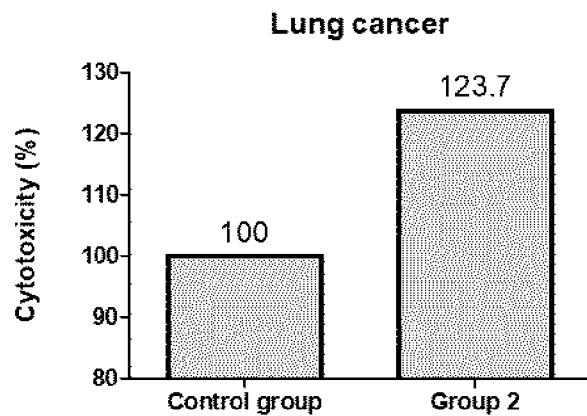
[Fig. 11c]



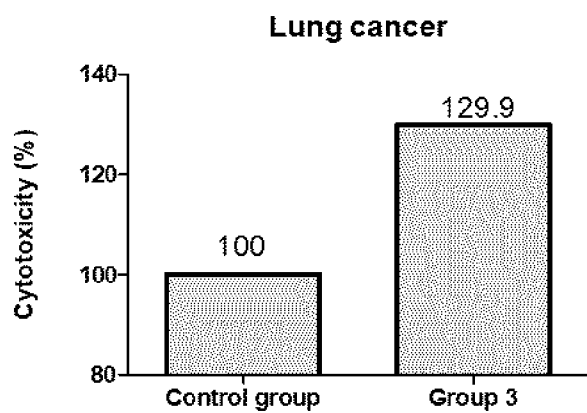
[Fig. 11d]



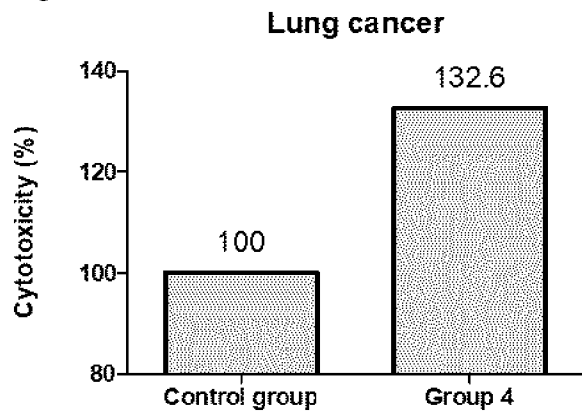
[Fig. 12a]



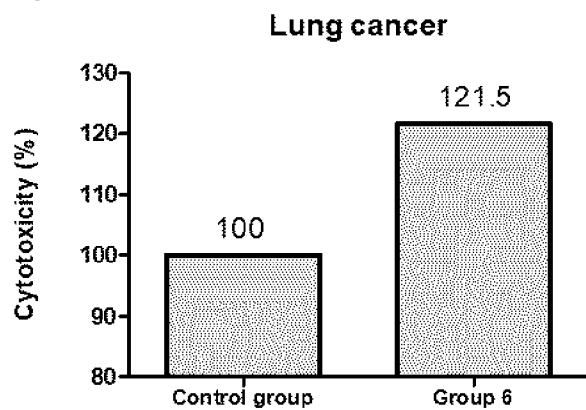
[Fig. 12b]



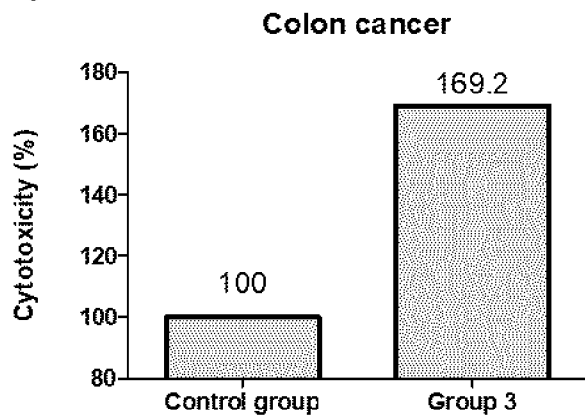
[Fig. 12c]



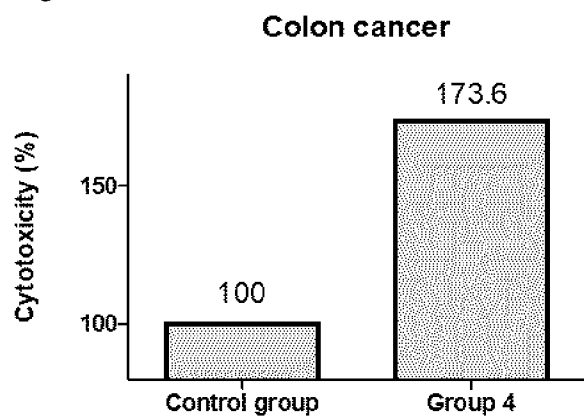
[Fig. 12d]



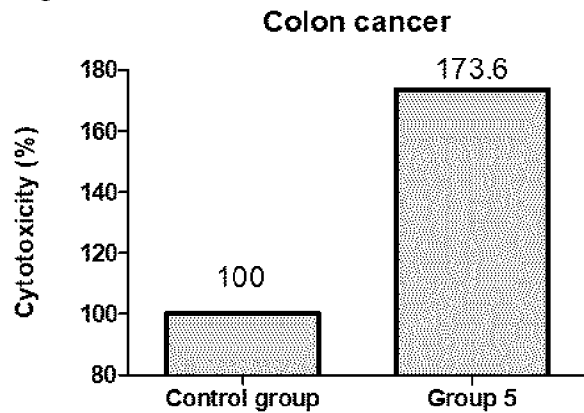
[Fig. 13a]



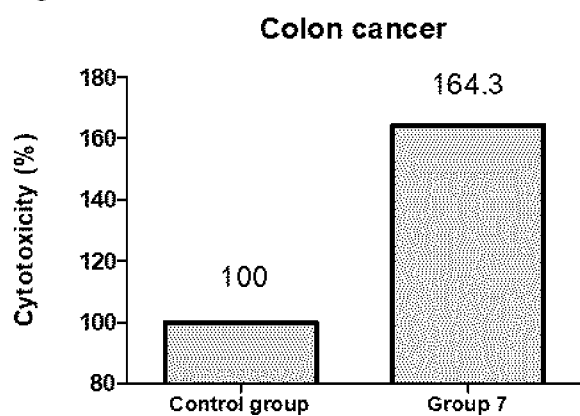
[Fig. 13b]



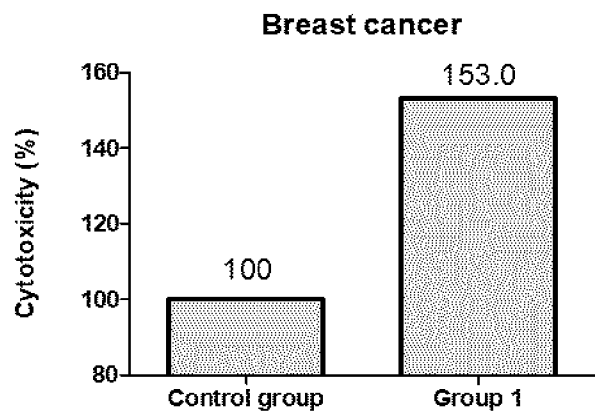
[Fig. 13c]



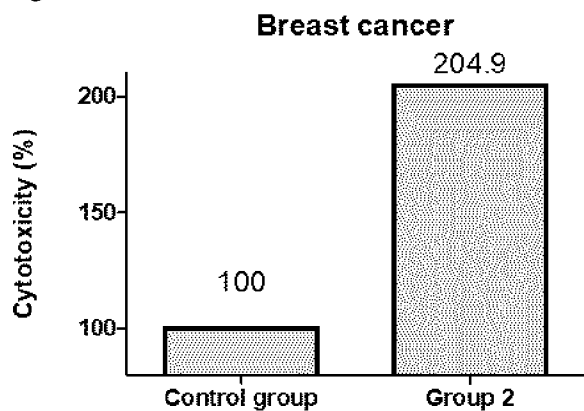
[Fig. 13d]



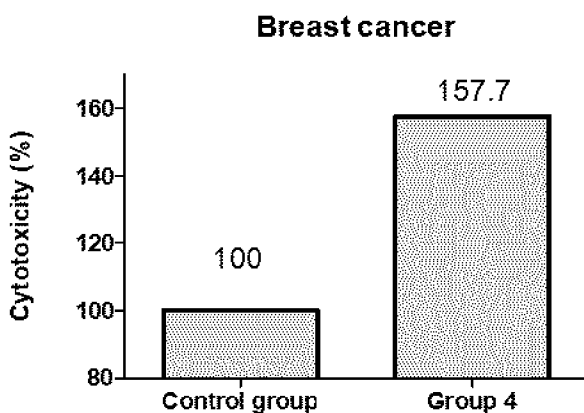
[Fig. 14a]



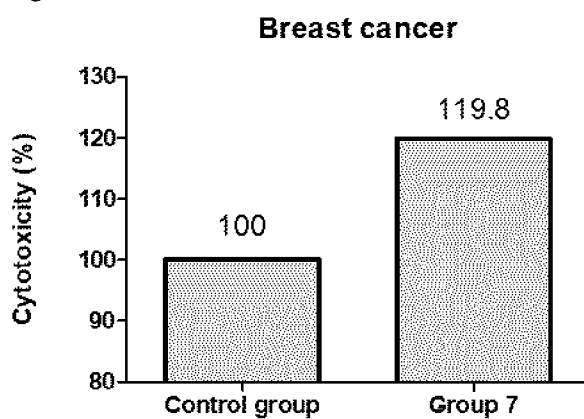
[Fig. 14b]



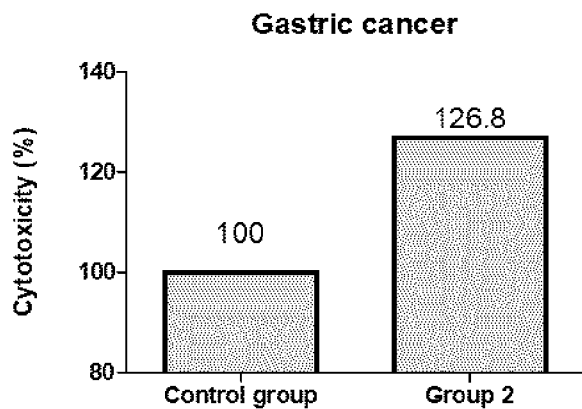
[Fig. 14c]



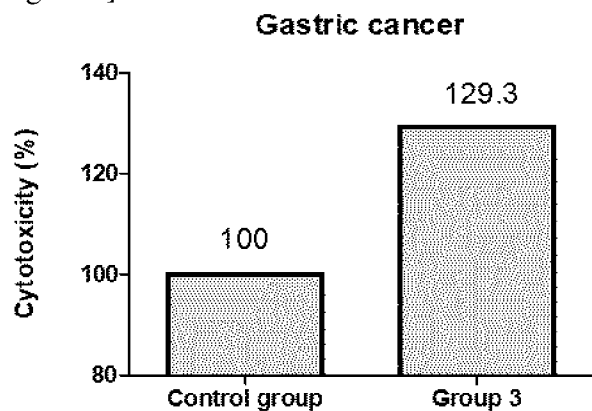
[Fig. 14d]



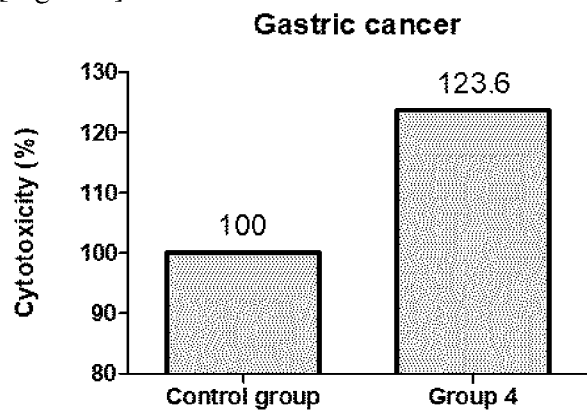
[Fig. 15a]



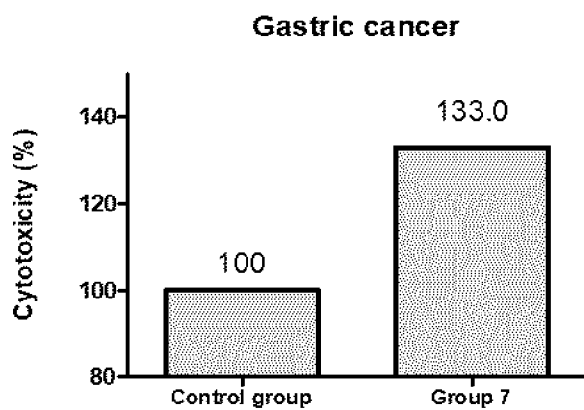
[Fig. 15b]



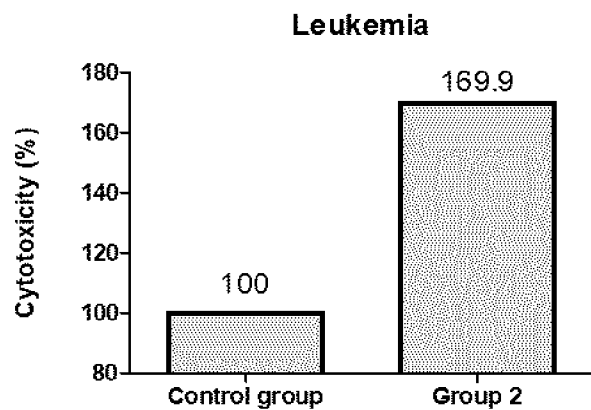
[Fig. 15c]



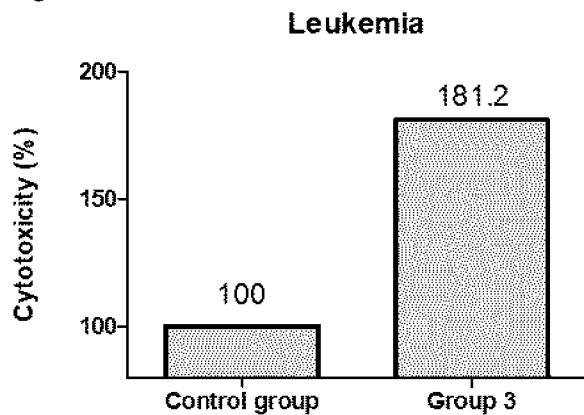
[Fig. 15d]



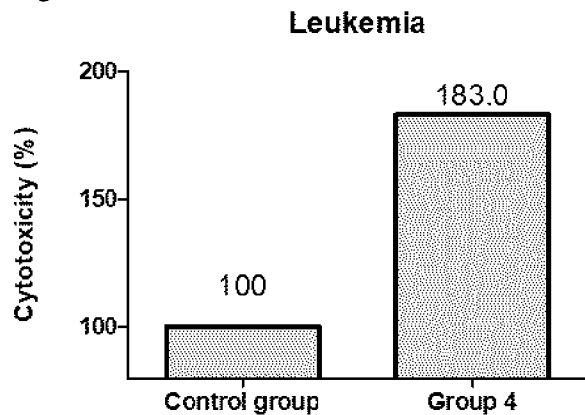
[Fig. 16a]



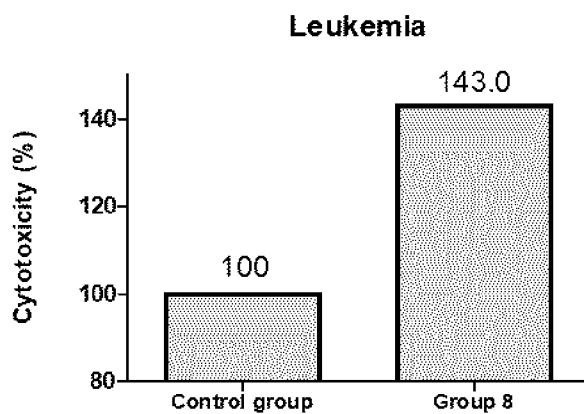
[Fig. 16b]



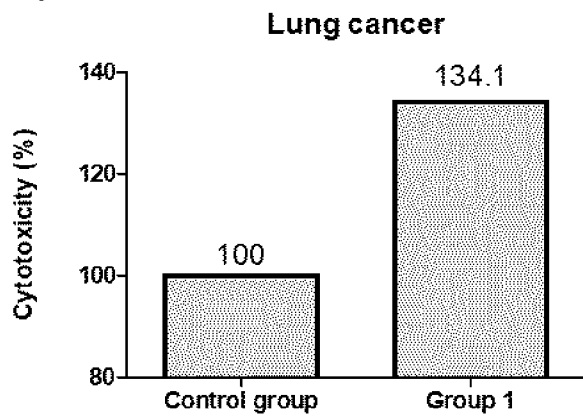
[Fig. 16c]



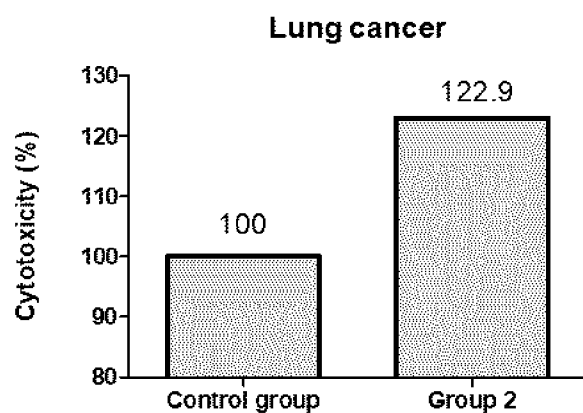
[Fig. 16d]



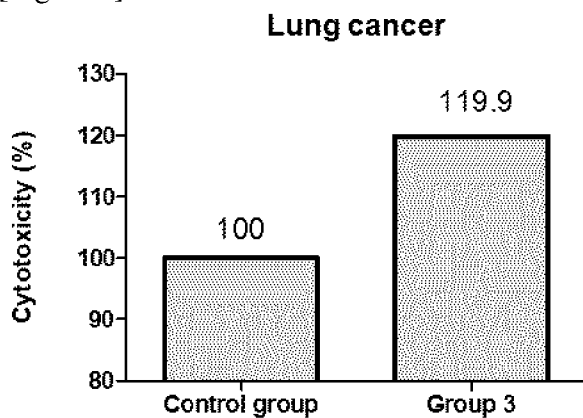
[Fig. 17a]



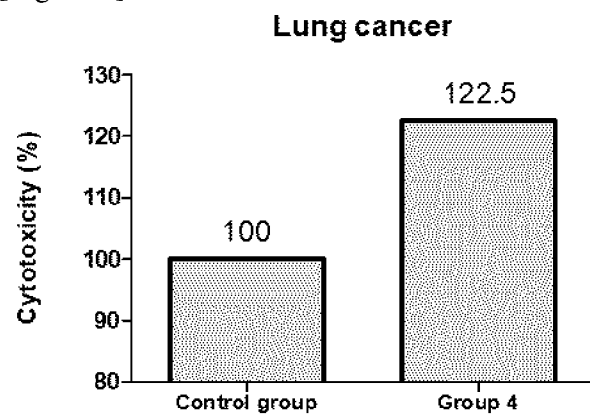
[Fig. 17b]



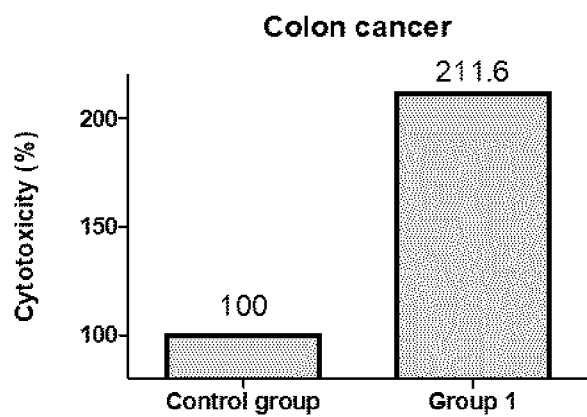
[Fig. 17c]



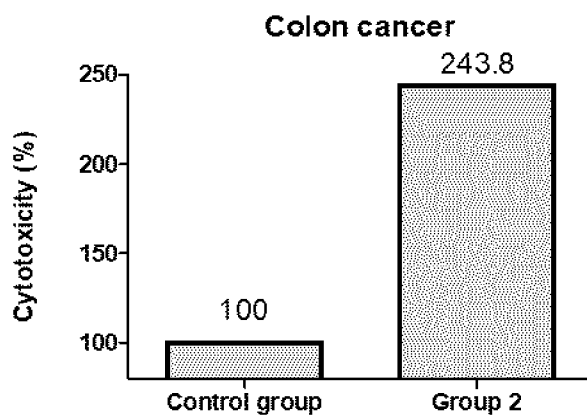
[Fig. 17d]



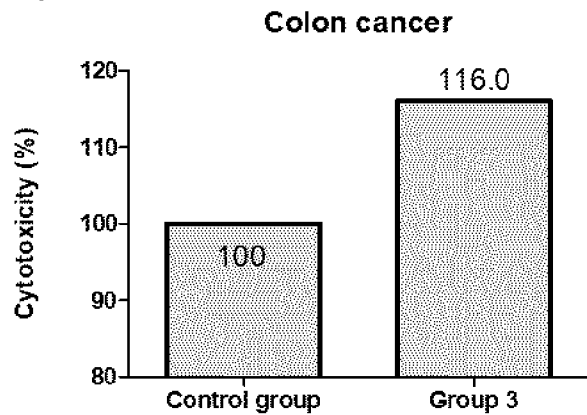
[Fig. 18a]



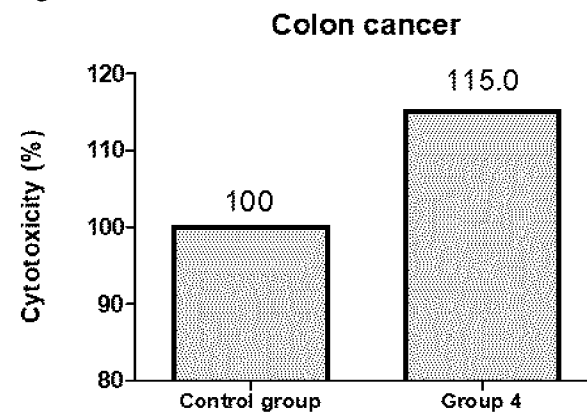
[Fig. 18b]



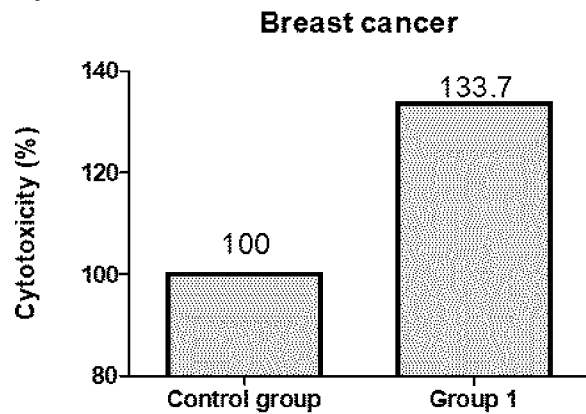
[Fig. 18c]



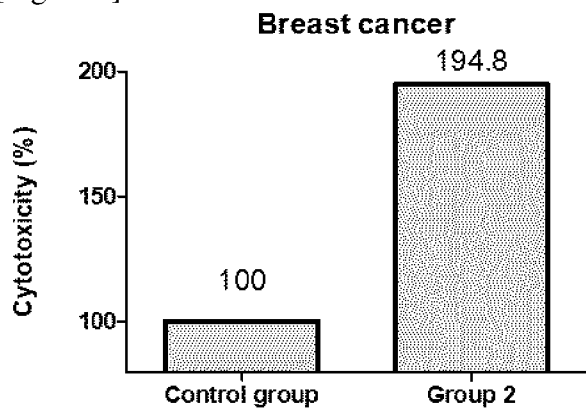
[Fig. 18d]



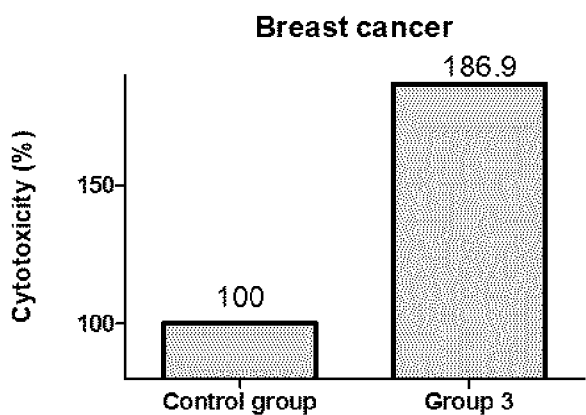
[Fig. 19a]



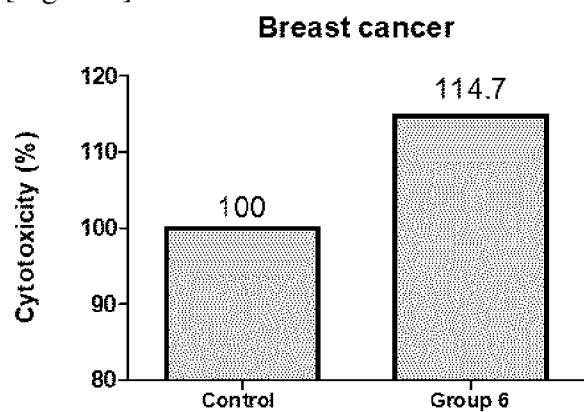
[Fig. 19b]



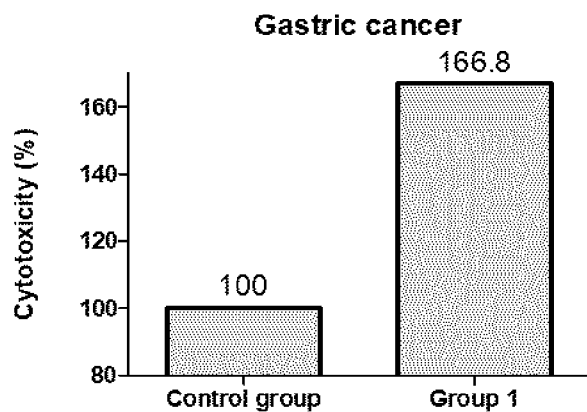
[Fig. 19c]



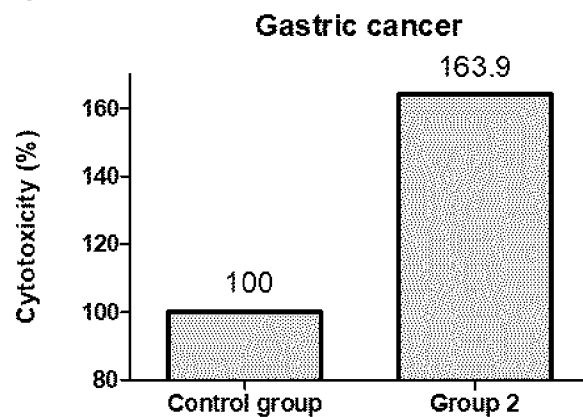
[Fig. 19d]



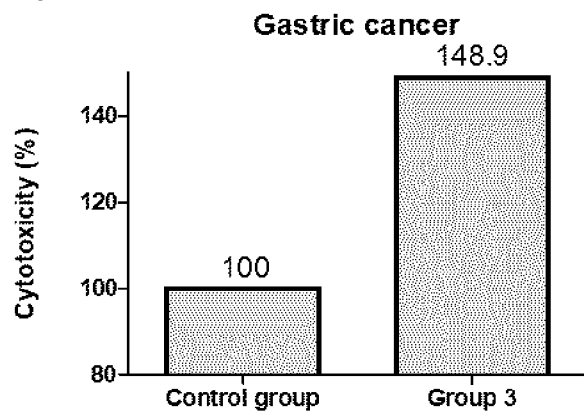
[Fig. 20a]



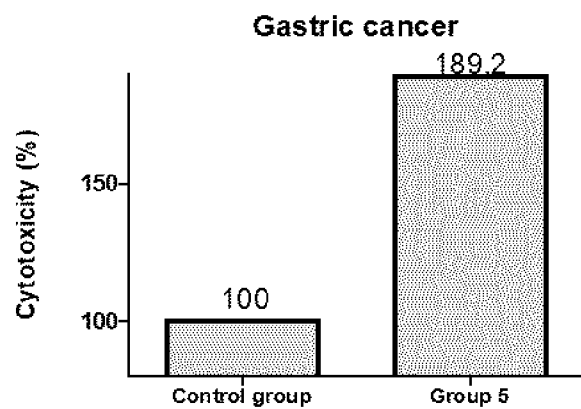
[Fig. 20b]



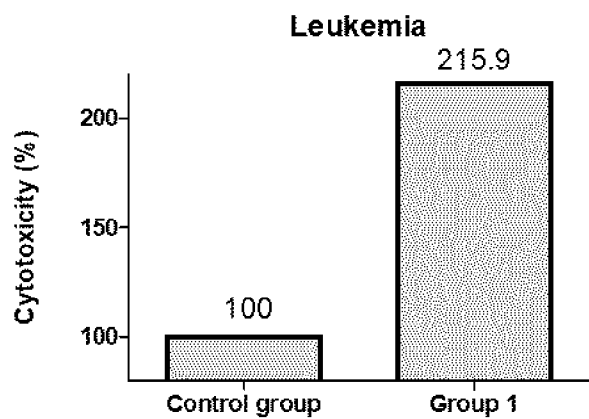
[Fig. 20c]



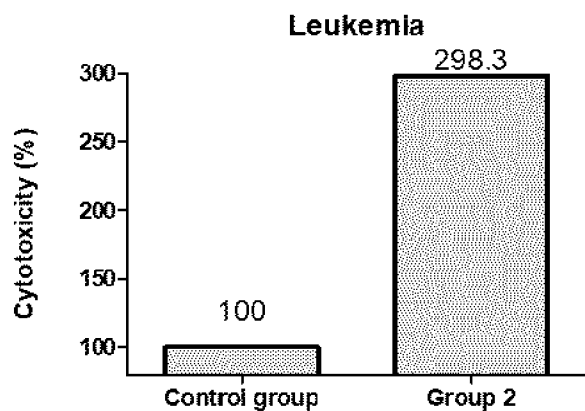
[Fig. 20d]



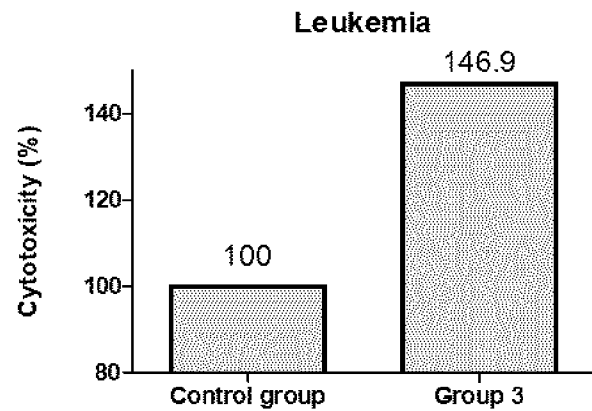
[Fig. 21a]



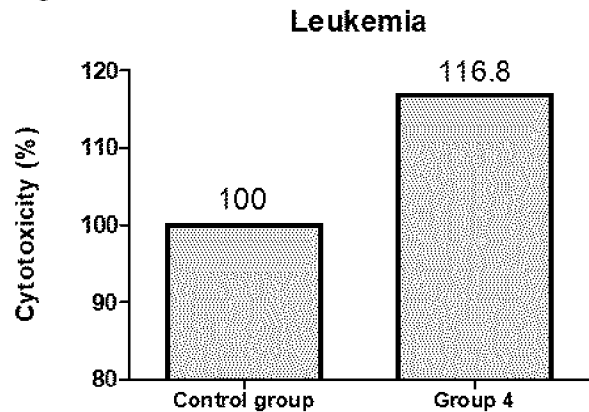
[Fig. 21b]



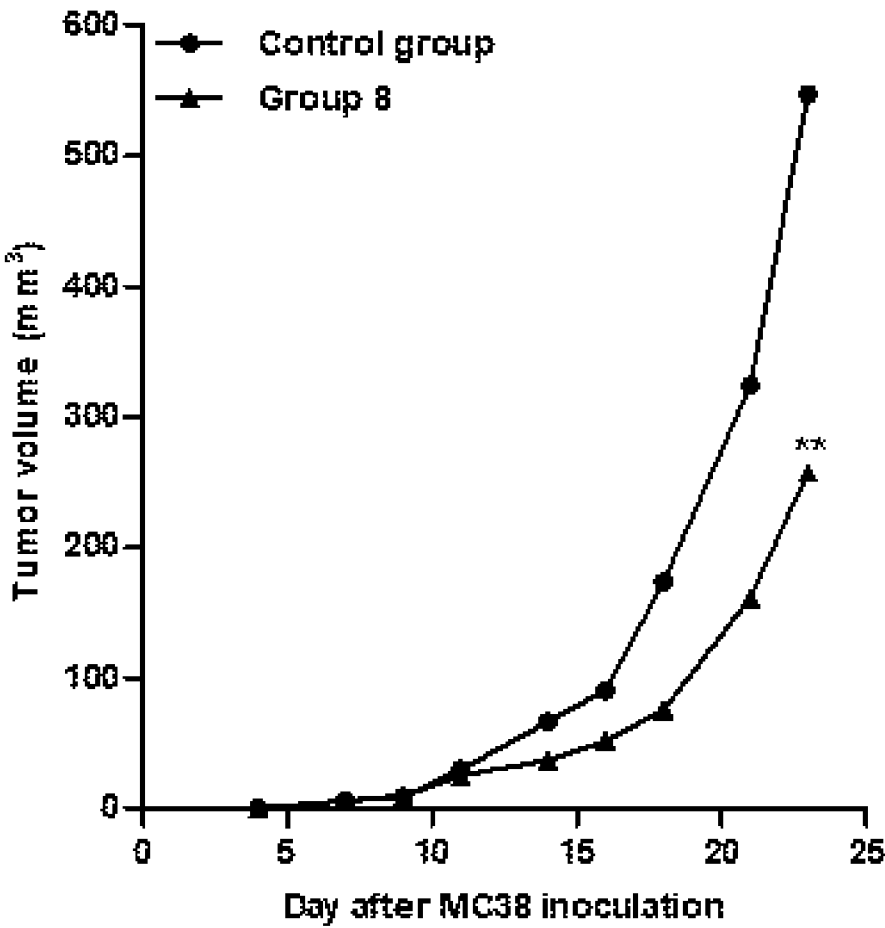
[Fig. 21c]



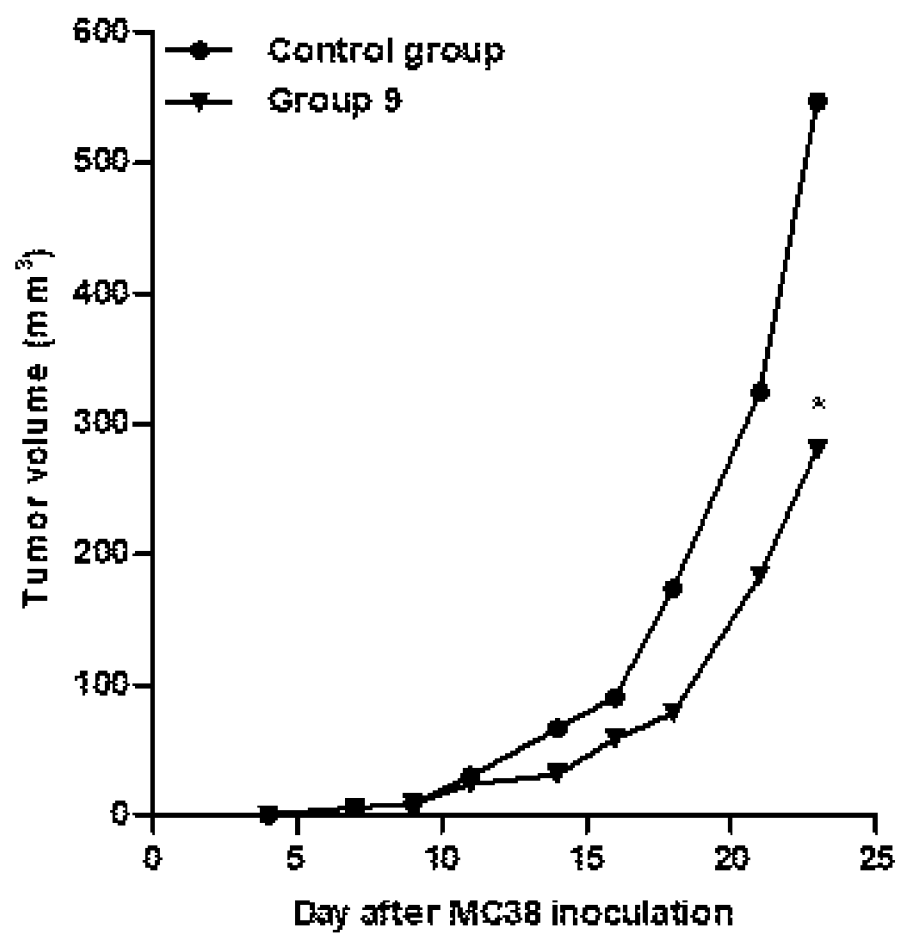
[Fig. 21d]



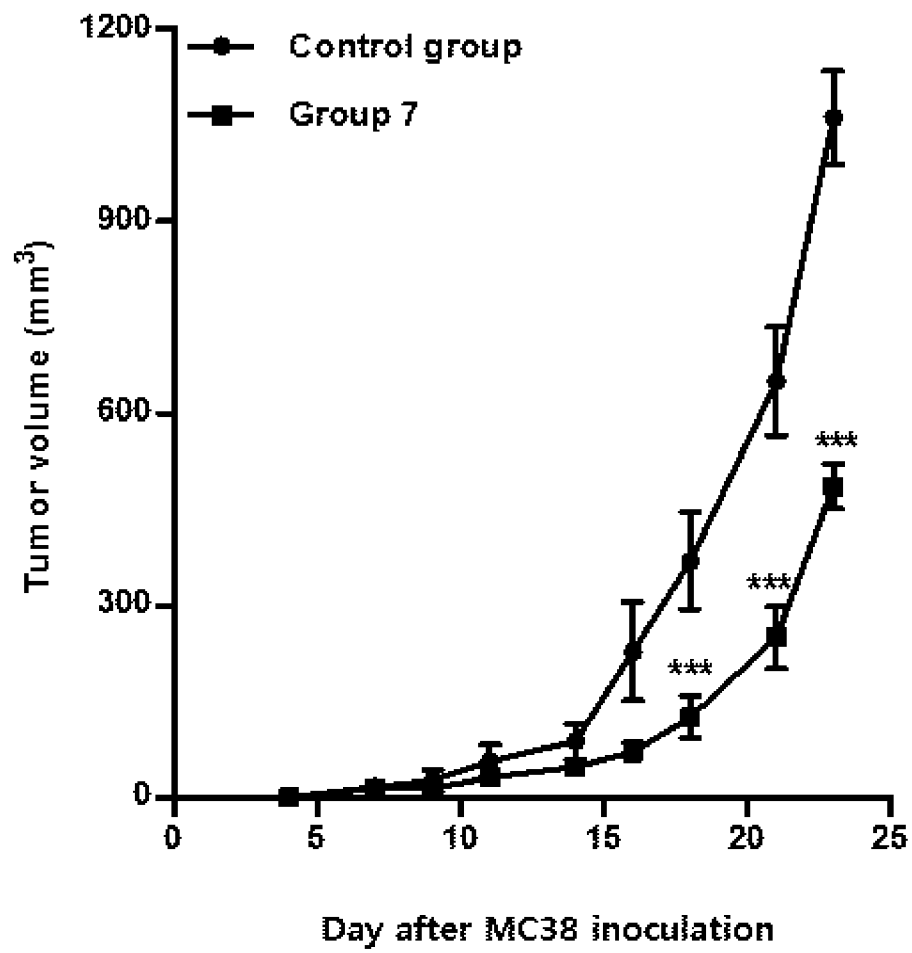
[Fig. 22]



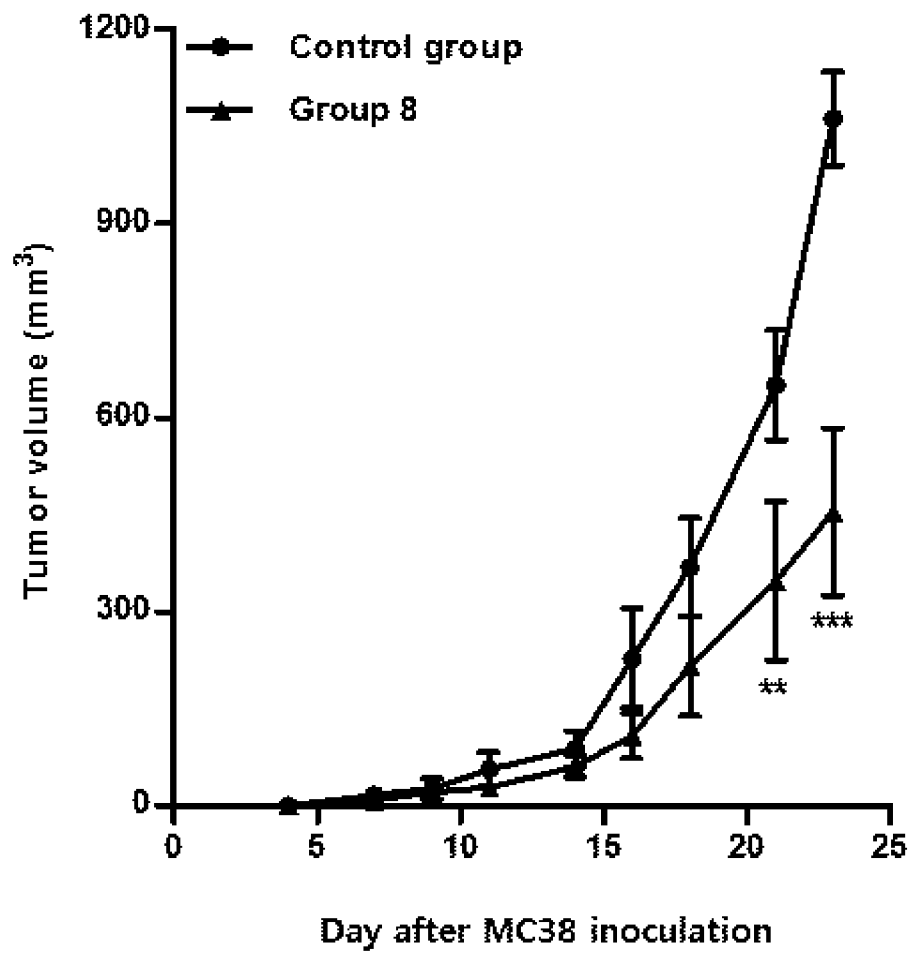
[Fig. 23]



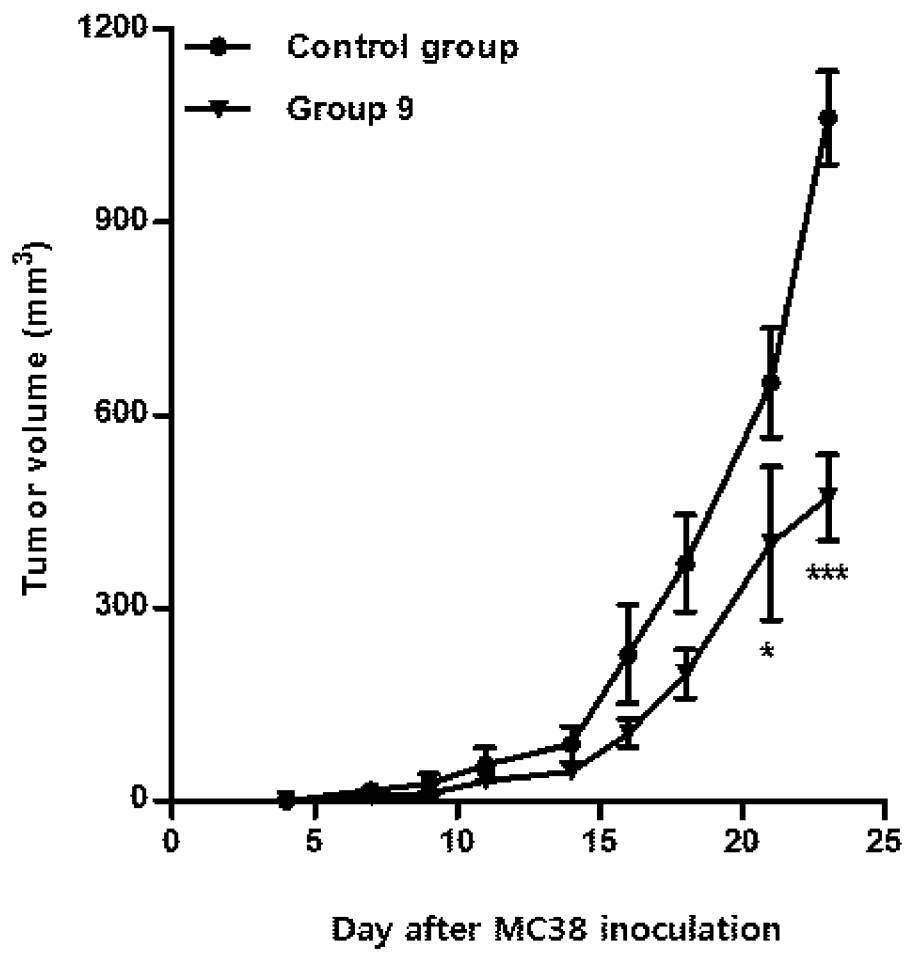
[Fig. 24a]



[Fig. 24b]



[Fig. 24c]



A. CLASSIFICATION OF SUBJECT MATTER**G01N 33/50(2006.01)i, G01N 33/68(2006.01)i, C12Q 1/6886(2018.01)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)

G01N 33/50; C12Q 1/02; G01N 33/566; G01N 33/68; C12Q 1/6886

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: KIRREL3, CNTN4, CD351, cancer, immunity, inhibitor, target

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EVENEPOEL, L. et al., 'Expression of contactin 4 is associated with malignant behavior in pheochromocytomas and paragangliomas', The Journal of Clinical Endocrinology and Metabolism, January 2018, Vol.103, No.1, pp.46-55 See abstract; and discussion.	9-13
A		1-8
Y	US 2012-0015374 A1 (JUNG, C. R. et al.) 19 January 2012 See paragraph [0018]; and claims 1-4.	9-13
A	BAIG, D. N. et al., 'Distortion of the normal function of synaptic cell adhesion molecules by genetic variants as a risk for autism spectrum disorders', Brain Research Bulletin, 2017, Vol.129, pp.82-90 See the whole document.	1-13
A	TAMIR-LIVNE, Y. et al., 'Adhesion molecule Kirrel3/Neph2 is required for the elongated shape of myocytes during skeletal muscle differentiation', The International Journal of Developmental Biology, 2017, Vol.61, pp.337-345 See the whole document.	1-13
A	SHIBUYA, A. et al., 'A pro-inflammatory role of Fcα/μR on marginal zone B cells in sepsis', International Immunology, 2017, Vol.29, No.11, pp.519-524 See the whole document.	1-13



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

12 July 2019 (12.07.2019)

Date of mailing of the international search report

12 July 2019 (12.07.2019)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

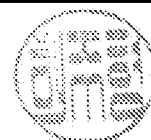
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2019/003965Patent document
cited in search reportPublication
datePatent family
member(s)Publication
date

US 2012-0015374 A1

19/01/2012

EP 2354158 A1

10/08/2011

JP 2010-222348 A

07/10/2010

JP 5377358 B2

25/12/2013

KR 10-1087617 B1

29/11/2011

KR 10-2010-0104841 A

29/09/2010

US 2010-0239566 A1

23/09/2010

US 8088750 B2

03/01/2012

US 8404435 B2

26/03/2013