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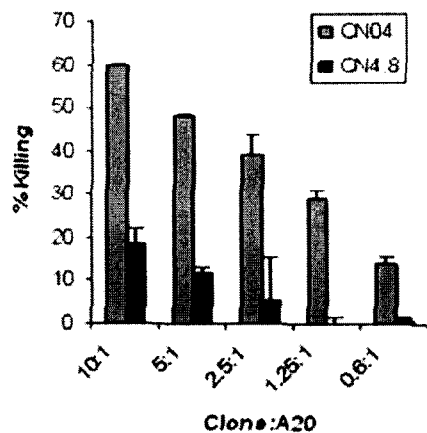
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(54) Title: MFAP4 AS A MARKER FOR REGULATORY CELLS AND ANTI-CANCER CELLS

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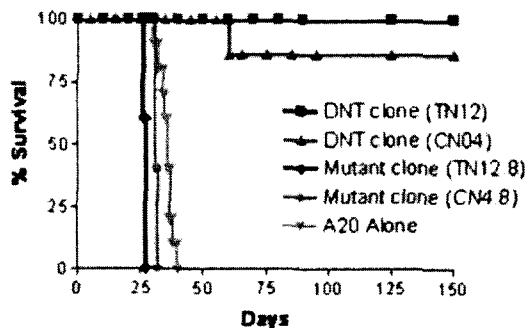


Figure 1

(57) Abstract: The present application provides a novel antibody that is specific for MFAP4. The application also provides methods and uses of MFAP4 as a marker for regulatory cells and/or anti-cancer cells. Further, the application provides methods and uses of MFAP4 binding agents for selection and activation of regulatory cells and/or anti-cancer cells.



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TITLE: MFAP4 as a Marker for Regulatory Cells and Anti-Cancer Cells**FIELD**

[001] The present application relates to a novel antibody, and
5 methods of use thereof. Further, the application relates to methods and uses
of MFAP4 as a marker for regulatory cells and anti-cancer cells. The
application also relates to MFAP4 binding agents and methods and uses for
selecting and activating regulatory cells and anti-cancer cells.

BACKGROUND

10 [002] Over the past 20 years, short-term allograft survival has been
improved significantly with the advent of immunosuppressive drugs. However,
the deterioration of drug efficacy over a prolonged period of time renders them
ineffective in preventing chronic graft rejection. Moreover, immunosuppressive
drugs render patients susceptible to infections and malignancy. A major goal
15 of transplantation is to induce long-term transplant tolerance without impairing
the recipient immune system. Many studies have established the critical role
of regulatory T cells in immune tolerance.

[003] The inventors have previously identified and cloned a novel
regulatory subset of $\alpha\beta\text{TCR}^+\text{CD4}^-\text{CD8}^-$ T (DNT) cells and demonstrated that
20 these cells can kill activated antigen-specific CD4^+ and CD8^+ effector T cells
in vitro and *in vivo* (1-5). Adoptive transfer of DNT regulatory cells activated by
donor-lymphocyte infusion can potently suppress anti-donor T cell responses
in vivo, leading to long-lasting tolerance to transplants (1-5). The inventors
have also demonstrated the suppression of autoreactive T cells by DNT
25 regulatory cells in an animal model of autoimmune diabetes, in which peptides
activated DNT regulatory cells provided protection against auto-reactive T
cells specific for an antigen expressed in pancreatic islets (6). In addition, the
inventors have demonstrated that DNT cells have potent anti-cancer effect
(7).

30 [004] DNT cells comprise only ~1% of PBMC, and no unique surface
markers have been identified to positively select DNT cells. Current *ex vivo*
expansion methods for DNT cells rely on depleting multiple subsets from the

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starting population. Moreover, there are no known functional markers that can distinguish potent suppressive DNT cells within a heterogeneous population during their activation and expansion. The lack of a reliable cell surface marker extends to other regulatory T cell subsets such as CD4⁺CD25⁺Foxp3⁺

5 T regulatory cells, CD4⁺CD25⁺ T regulatory cells, CD8⁺CD28⁻ T regulatory cells, T regulatory-1 cells, T helper-3 cells, and regulatory NKT cells (reviewed in 8). This makes it difficult to phenotypically distinguish T regulatory cells from other activated effector or memory T cells. For instance, it is still not clear whether Foxp3 is a specific marker for T regulatory cells in humans

10 because activated T cells can transiently express Foxp3 albeit at lower levels than natural CD25^{hi}CD4⁺ T regulatory cells (8). Moreover, Foxp3 is an intracellular molecule whose detection renders cells non-functional for applications subsequent to cell sorting. Other molecules such as CD25, CTLA-4, GITR, and CD127 can be used as cell surface markers for natural

15 CD4⁺CD25⁺ T regulatory cells, however they are unable to fully distinguish natural T regulatory cells from activated T cells because all T cells express these molecules upon activation (8).

[005] Thus, there is a need for methods of detecting and selecting or isolating regulatory cells.

20 **SUMMARY**

[006] The present inventors have identified and developed a novel antibody. Specifically, the inventors have identified and developed a novel antibody that binds MFAP4. Further, the inventors have identified that MFAP4 is expressed on the surface of a subset of regulatory cells and anti-cancer

25 cells. Thus, MFAP4 can be used as a marker to detect, enrich and/or select regulatory cells and/or anti-cancer cells. In addition, the inventors have shown that the regulatory cells and anti-cancer cells can be activated by binding of MFAP4. The activated regulatory cells can be used to treat or prevent the progression of many types of immune-related diseases, such as graft

30 rejection, graft-versus-host disease, autoimmune disease, allergic diseases and infectious disease. The activated anti-cancer cells can be used to treat or prevent the progression of cancer.

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[007] The inventors have cloned and sequenced a novel antibody that specifically binds MFAP4 and determined the sequence of the light and heavy chain variable regions and complementarity determining regions 1, 2 and 3 of the antibody.

5 **[008]** Accordingly, the application discloses isolated light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence QSLSSGNQKNY (SEQ ID NO:44); isolated light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence YSS (SEQ ID NO:45); and isolated light chain complementarity
10 determining region 3 (CDR3) comprising the amino acid sequence LQHYSSPFT (SEQ ID NO:46); and isolated heavy chain CDR1 comprising the amino acid sequence GFPFSNYG (SEQ ID NO:21); isolated heavy chain CDR2 comprising the amino acid sequence ISYDGRST (SEQ ID NO:22) and isolated heavy chain CDR3 comprising the amino acid sequence
15 VRHELPEDH (SEQ ID NO:23).

[009] The application also discloses isolated nucleic acid sequences encoding the light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence QSLSSGNQKNY (SEQ ID NO:44); the light chain complementarity determining region 2 (CDR2) comprising the
20 amino acid sequence YSS (SEQ ID NO:45); and the light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence LQHYSSPFT (SEQ ID NO:46); and the heavy chain CDR1 comprising the amino acid sequence GFPFSNYG (SEQ ID NO:21); the heavy chain CDR2 comprising the amino acid sequence ISYDGRST (SEQ ID
25 NO:22); and the heavy chain CDR3 comprising the amino acid sequence VRHELPEDH (SEQ ID NO:23).

[0010] Additional aspects disclosed in the present application are isolated light chain variable regions comprising light chain CDR1, CDR2 and/or CDR3 disclosed herein (SEQ ID NOS:44, 45 and/or 46), and isolated
30 heavy chain variable regions comprising heavy chain CDR1, CDR2 and/or CDR3 disclosed herein (SEQ ID NOS:21, 22 and/or 23). In one embodiment, the light chain variable region comprises the amino acid sequence shown in

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Figure 16 (SEQ ID NO:43). In another embodiment, the heavy chain variable region comprises the amino acid sequence shown in Figure 15 (SEQ ID NO:20).

[0011] Another aspect of the present application is a novel binding agent that specifically binds MFAP4, that comprises at least one light chain complementarity determining region disclosed herein (i.e. one or more of SEQ ID NOs:44-46) and/or at least one heavy chain complementarity determining region disclosed herein (i.e. one or more of SEQ ID NOs:21-23). In one embodiment, the binding agent comprises the light chain variable region of SEQ ID NO:43 and the heavy chain variable region of SEQ ID NO:20. In yet a further embodiment, the binding agent is an antibody. In another embodiment, the binding agent is a monoclonal antibody. In yet a further embodiment, the monoclonal antibody is a monoclonal antibody termed G1.

[0012] Further, the application provides a method of detecting regulatory and/or anti-cancer cells comprising the steps of:

- (1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex;
- (2) detecting the amount of binding agent-MFAP4 complex in the test sample; and
- (3) comparing the amount of binding agent-MFAP4 complex in the test sample to a control.

[0013] In addition, the application provides a method of selecting or enriching regulatory and/or anti-cancer cells comprising the steps:

- (1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex; and
- (2) selecting or enriching regulatory and/or anti-cancer cells by selecting the binding agent-MFAP4 complex from the test sample.

[0014] The application also provides a method of activating regulatory and/or anti-cancer cells comprising:

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contacting regulatory and/or anti-cancer cells with a binding agent that binds specifically to MFAP4 on the cell to crosslink MFAP4.

[0015] In one embodiment, the cells are contacted *in vitro*.

5 **[0016]** In one specific embodiment, the novel binding agent disclosed herein is the binding agent for the methods disclosed herein.

[0017] In another aspect, the application provides use of an MFAP4-selected or -activated regulatory cell obtained by the method of the application for treating or preventing an immune-related disease. The application also
10 provides a method of treating or preventing an immune-related disease comprising administering an MFAP4-selected or -activated regulatory cell obtained by the method of the application to an animal in need thereof. The application further provides use of an MFAP4-selected or -activated regulatory cell in the preparation of a medicament for treating or preventing an immune-
15 related disease. The application also provides an MFAP4-selected or -activated regulatory cell obtained by the method of the application for use in the treatment or prevention of an immune-related disease.

[0018] In yet another aspect, the application provides use of an MFAP4-selected or -activated anti-cancer cell obtained by the method of the
20 application for treating or preventing a cancer. The application also provides a method of treating or preventing a cancer comprising administering an MFAP4-selected or -activated anti-cancer cell obtained by the method of the application to an animal in need thereof. The application further provides use of an MFAP4-selected or -activated anti-cancer cell in the preparation of a
25 medicament for treating or preventing a cancer. The application also provides an MFAP4-selected or -activated anti-cancer cell obtained by the method of the application for use in the treatment or prevention of a cancer.

[0019] In a further aspect, the application provides a method of modulating an immune response in an animal comprising administering an
30 effective amount of an MFAP4 binding agent to an animal in need thereof. The application also provides a use of an effective amount of an MFAP4 binding agent for modulating an immune response in an animal in need

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thereof. The application further provides a use of an effective amount of an MFAP4 binding agent in the preparation of a medicament for modulating an immune response. The application yet further provides an MFAP4 binding agent for use in modulation of an immune response.

5 [0020] In another aspect, the present application provides a method of modulating cancer in an animal comprising administering an effective amount of an MFAP4 binding agent to an animal in need thereof. The application also provides a use of an effective amount of an MFAP4 binding agent for modulating cancer in an animal in need thereof. The application further
10 provides a use of an effective amount of an MFAP4 binding agent in the preparation of a medicament for modulating cancer. The application yet further provides an MFAP4 binding agent for use in modulation of cancer.

[0021] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be
15 understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

20 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0022] The disclosure will now be described in relation to the drawings in which:

[0023] Figure 1 shows that DNT cell clones but not their mutants can kill A20 B cell lymphoma *in vitro* and protect against lethal dose of A20 after adoptive transfer *in vivo*. (A) DNT cell clone CN04 and its natural mutant
25 clone CN48 were used as effector cells at indicated ratios. A20 tumour cells were used as targets in a standard ⁵¹Cr release assay. (B) Mice were injected with a lethal dose of A20 tumor cells either alone or together with 5x10⁵ DNT cell clones or their mutants as indicated. Percent tumor-free survival in each
30 group is shown. Each group contains 5-12 mice.

[0024] Figure 2 shows that G1 mAb selectively stains tumor-reactive and regulatory DNT cell clones, but not their non-functional mutants. (A) DNT

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cell clone TN12 (unfilled red line, labeled TN12) and its natural mutant TN12.8 (filled black histogram, labeled TN12.8), which has lost cytotoxicity against tumor cells and allo-reactive T cells, were immunostained with G1 mAb and analyzed by flow cytometry. (B) Immunostaining of another functional DNT cell clone, CNO4 (filled histogram labeled CN04), and its natural mutant CN4.8 (filled histogram labeled CN4.8), which has lost cytotoxicity against tumor cells and allo-reactive T cells, with G1 mAb.

[0025] Figure 3 shows *ex vivo* expansion of DNT cells from PBMC of healthy donors and their anti-tumor function in a xenograft tumor model. (A) Number of DNT cells after 10 days of *ex vivo* expansion from 5-10 ml of whole blood (n=10). (B) *Ex vivo*-expanded human DNT cells can inhibit human lung cancer progression in NOD-SCID mice. NOD-SCID mice were subcutaneously injected with 10^6 H460 tumor cells either alone or together with 5×10^6 *ex vivo*-expanded $CD8^+$ or DNT cells. Percent tumor free survival of the recipients is shown. $P=0.0436$ between DNT versus $CD8$ T cell-treated groups.

[0026] Figure 4 shows that G1 mAb selectively immunostains a subset of *ex vivo*-expanded human DNT cells. Ten days after expansion *ex vivo*, human DNT cells were immunostained with G1 mAb and analyzed by flow cytometry.

[0027] Figure 5 shows the validation of MFAP4 as the molecule recognized by the functional marker-specific G1 mAb. MFAP4 was identified by sequencing the protein band derived from immunoprecipitating $G1^+$ DNT cell clone lysates with G1 mAb. The protein recognized by G1 mAb and anti-MFAP4 mAb are of the same molecular sizes as determined by western blot. Recombinant mouse and human MFAP4 proteins were expressed in CHO cells and whole cell lysates were prepared as positive controls for Western blot analysis using anti-MFAP4 mAb. MFAP4 is expressed by functional $G1^+$ DNT cell clones but not $G1^-$ DNT cell mutant clones that have lost cytotoxicity against tumor cells and allo-reactive T cells.

[0028] Figure 6 shows that the molecule recognized by the functional marker-specific G1 mAb is specifically detected on the same activated DNT

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cell that expresses MFAP4, recognized by anti-MFAP4 mAb. DNT cells were purified from B6 mice, activated *in vitro* for 5 days, and subsequently co-immunostained with G1 mAb and anti-MFAP4 mAb and analyzed by flow cytometry.

- 5 **[0029]** Figure 7 shows that MFAP4 is expressed by a subset of activated DNT cells. Lymph node and spleen cells from B6.lpr mice (in which the majority of CD4 and CD8 T cells were depleted *ex vivo*) were activated *in vitro* for 5 days using alloantigen stimulation (irradiated [B6xBalb/c]F1 splenocytes), subsequently immunostained with anti-MFAP4, anti-TCR β , anti-
10 CD4, anti-CD8, and anti-NK1.1 antibodies, and then analyzed by flow cytometry. Dot plot (top) is gated on β TCR $^{+}$ cells; top highlighted box in the dot plot corresponds to CD4 $^{+}$, CD8 $^{+}$, NK1.1 $^{+}$ β TCR $^{+}$ cells, within which the smaller top box corresponds to MFAP4 $^{+}$ cells; bottom highlighted box corresponds to CD4 $^{-}$, CD8 $^{-}$, NK1.1 $^{-}$ TCR β $^{+}$ cells (DNT cells), within which the
15 smaller bottom box corresponds to MFAP4 $^{+}$ cells. Histogram plot (bottom) is gated on β TCR $^{+}$ cells and shows MFAP4 expression on CD4/CD8/NK1.1 $^{+}$ cells (filled light gray histogram), DNT cells (bold black line), and unstained control (dark gray histogram).

- [0030]** Figure 8 shows MFAP4 is expressed by a subset of regulatory
20 DNT cells converted from allo-activated CD4 $^{+}$ T cells. Purified CD4 $^{+}$ T cells from B6.Thy1.1 mice were labeled with the proliferation-tracking dye CFSE and activated *in vitro* for 6 days by alloantigen stimulation (mature bone marrow-derived dendritic cells from [B6xBalb/c]_{F1} mice), subsequently immunostained with anti-MFAP4, anti- β TCR, anti-CD4, anti-CD8, and anti-
25 NK1.1, and then analyzed by flow cytometry. Contour plot (left) is gated on $\alpha\beta$ TCR $^{+}$ cells; highlighted box within the contour plot corresponds to CD4 $^{-}$, CD8 $^{-}$, NK1.1 $^{-}$, $\alpha\beta$ TCR $^{+}$ cells (DNT cells). Histogram plot (right) shows MFAP4 expression gated on activated DNT cells based on the contour plot (left).

- [0031]** Figure 9 shows that cross-linking MFAP4 on the cell surface of
30 activated DNT cells using G1 mAb or anti-MFAP4 mAb induces rapid expression of IFN γ . DNT cells were purified and activated from B6 mice and subsequently seeded in flat-bottom wells coated with the indicated antibodies

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(10 µg/ml) to induce cross-linking of each molecule, and in the presence of a golgi inhibitor to permit intracellular measurement of IFN γ . After 5 hours of cross-linking, cells were harvested and analyzed for intracellular levels of IFN γ by flow cytometry. G1 mAb treatment induces rapid IFN γ expression in
5 activated DNT cells.

[0032] Figure 10 shows that plate-bound G1 mAb treatment of regulatory DNT cell clones induces rapid adherence and morphological differentiation. DNT regulatory clones were seeded on flat-bottom wells coated with the indicated antibodies (10 µg/ml) for 5 hours and subsequently
10 analyzed by microscopy.

[0033] Figure 11 shows that MFAP4⁺ DNT cells are potent suppressors of syngeneic allo-reactive CD4 T cells. CFSE-labeled B6.Thy1.1⁺ CD4 T cells were used as responders to alloantigen stimulation (irradiated [B6xBalb/c]_{F1} splenocytes) for 5 days, and their proliferation was analyzed by flow
15 cytometry. Indicated doses of alloantigen-activated DNT cells (purified from B6.lpr mice and activated by irradiated [B6xBalb/c]_{F1} splenocytes for 5 days prior) were added as suppressor cells. Bottom two rows (highlighted black box): Activated DNT cells from B6 mice were sorted based on MFAP4 expression into MFAP4⁻ and MFAP4⁺ cells and used respectively as
20 suppressors against syngeneic allo-reactive CD4 T cells. CD4⁺ T cells in the far left column of dot plots are highlighted by a black box (upper right quadrant), and the next two columns (middle and right) correspond to CFSE (cell division tracking dye) fluorescence gated on CD4⁺ T cells.

[0034] Figure 12 shows that enrichment of MFAP4⁺ DNT cells using G1
25 mAb selects for potent anti-tumor cells. (A) DNT cells were activated *in vitro* for 6 days by alloantigen stimulation (irradiated [B6xBAlb/c]_{F1} splenocytes) and immunostained with anti- β TCR, anti-CD4, anti-CD8, anti-NK1.1, anti-MFAP4, and G1 mAb and analyzed by flow cytometry. Left contour plot: mAb isotype controls; right contour plot: anti-MFAP4 and G1 mAb marks a subset
30 of DNT cells. G1⁺ and G1⁻ DNT cell populations were sorted and used respectively as effectors against A20 B cell lymphoma cells in a standard ⁵¹Cr-release cytotoxicity assay at the indicated effector:target ratios (B).

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[0035] Figure 13 shows the human MFAP4 sequences.

[0036] Figure 14 shows the murine MFAP4 sequences.

[0037] Figure 15 shows the V_H alignment and consensus sequences, variable regions and CDRs.

5 [0038] Figure 16 shows the V_L alignment and consensus sequences, variable regions and CDRs.

Detailed description of the disclosure

(A) Definitions

10 [0039] The term "a cell" includes a single cell as well as a plurality or population of cells.

[0040] The term "amino acid" includes all of the naturally occurring amino acids as well as modified amino acids.

[0041] The term "antibody" as used herein is intended to include, without limitation, monoclonal antibodies, polyclonal antibodies, chimeric and
15 humanized antibodies. The antibody may be from recombinant sources and/or produced in transgenic animals. The term "antibody fragment" as used herein is intended to include without limitations Fab, Fab', F(ab')₂, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, and multimers thereof, multispecific antibody fragments and Domain Antibodies. Antibodies can be fragmented
20 using conventional techniques. For example, F(ab')₂ fragments can be generated by treating the antibody with pepsin. The resulting F(ab')₂ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')₂, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, bispecific antibody
25 fragments and other fragments can also be synthesized by recombinant techniques. In one embodiment, the monoclonal antibody is a humanized monoclonal antibody.

[0042] The term "humanized antibody" as used herein means that the antibody or fragment comprises human conserved framework regions
30 (alternatively referred to as constant regions) and the hypervariable regions (alternatively referred to as the antigen binding domain) are of non-human

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origin. For example, the hypervariable region may be from a mouse, rat or other species. The humanization of antibodies from non-human species has been well described in the literature. See for example EP-B1 0 239400 and Carter & Merchant 1997 (Curr Opin Biotechnol 8, 449-454, 1997 incorporated
5 by reference in their entirety herein). Humanized antibodies are also readily obtained commercially (eg. Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.)

[0043] Humanized forms of rodent antibodies are readily generated by CDR grafting (Riechmann et al. Nature, 332:323-327, 1988). In this approach
10 the six CDR loops comprising the antigen binding site of the rodent monoclonal antibody are linked to corresponding human framework regions. CDR grafting often yields antibodies with reduced affinity as the amino acids of the framework regions may influence antigen recognition (Foote & Winter. J Mol Biol, 224: 487-499, 1992). To maintain the affinity of the antibody, it is
15 often necessary to replace certain framework residues by site directed mutagenesis or other recombinant techniques and may be aided by computer modeling of the antigen binding site (Co et al. J Immunol, 152: 2968-2976, 1994).

[0044] Humanized forms of antibodies are optionally obtained by resurfacing (Pedersen et al. J Mol Biol, 235: 959-973, 1994). In this approach
20 only the surface residues of a rodent antibody are humanized.

[0045] The term "anti-cancer cell" as used herein refers to a cell that can kill cancer cells. In one embodiment, the anti-cancer cell is cytotoxic against cancer cells. In a specific embodiment, the anti-cancer cell is a T cell.
25 In a more specific embodiment, the T cell is a double negative (DNT) T cell. The term "DNT cells" include cells that are $CD3^+TCR^+CD4^-CD8^-$. In one embodiment, DNT cells are $CD3^+\alpha\beta TCR^+CD4^-CD8^-$. In a specific embodiment, DNT cells are $CD3^+\alpha\beta TCR^+CD4^-CD8^-NK1.1^-$.

[0046] The term "binding agent" as used herein refers to agents that
30 specifically bind to another substance, such as an MFAP4 protein.

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[0047] The term "cancer cell" includes cancer or tumor-forming cells, transformed cells or a cell, such as a pre-cancerous cell, that is susceptible to becoming a cancer or tumor-forming cell.

[0048] The term "control" as used herein refers to a sample that is
5 known as having a particular trait or not having a particular trait. The term also includes a pre-determined standard.

[0049] The phrases "detecting regulatory cells" or "detecting anti-cancer cells" refer to a method or process of determining if a sample has or does not have regulatory cells or anti-cancer cells, and includes determining
10 the quantity and/or type of regulatory cells or anti-cancer cells.

[0050] The phrases "enrich and/or select regulatory cells" or "enrich and/or select anti-cancer cells" refer to a method or process of increasing the concentration of regulatory cells or anti-cancer cells, or selecting regulatory cells or anti-cancer cells from a sample.

[0051] As used herein, the phrase "effective amount" means an amount
15 effective, at dosages and for periods of time necessary to achieve the desired result. Effective amounts of therapeutic may vary according to factors such as the disease state, age, sex, or weight of the animal. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several
20 divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[0052] The term "isolated nucleic acid sequences" as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or
25 other chemicals when chemically synthesized. An isolated nucleic acid is also substantially free of sequences which naturally flank the nucleic acid (i.e. sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded, and represents
30 the sense or antisense strand. Further, the term "nucleic acid" includes the complementary nucleic acid sequences.

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[0053] The term "isolated polypeptides" refers to a polypeptide substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized.

5 **[0054]** The term "label" is preferably capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque or a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , ^{123}I , ^{125}I , ^{131}I ; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as
10 alkaline phosphatase, beta-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion.

[0055] The term "MFAP4" or "microfibrillar-associated protein-4" refers to a 255-amino acid glycoprotein of the fibrinogen-related domain family of proteins. MFAP4 contains a C-terminal fibrinogen-like domain (which has high
15 similarity to the C-terminal halves of fibrinogen β and γ chains) and the N-terminal Arg-Gly-Asp (RGD) motif sequence that serves as an integrin-binding domain, suggesting that it is an extracellular matrix protein involved in cell adhesion or intercellular interactions. In one embodiment, the MFAP4 is of murine origin. For example, the amino acid sequence of SEQ ID NO:4. In
20 another embodiment, the MFAP4 is of human origin. For example, the amino acid sequence of SEQ ID NO:1. The term also includes variants of SEQ ID NOS:1 and 4. In one embodiment, the variant amino acid sequence has at least 70%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90% and even most preferably at least 95% sequence
25 identity to SEQ ID NO:1 or 4.

[0056] The term "heavy chain variable region" or " V_H region" as used herein refers to the variable region of a heavy chain of an antibody molecule. The heavy chain variable region has three complementarity determining regions (CDRs). The term "heavy chain complementarity determining region"
30 as used herein refers to regions of hypervariability within the heavy chain variable region of an antibody molecule. The heavy chain variable region has

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three complementarity determining regions termed heavy chain complementarity determining region 1, heavy chain complementarity determining region 2 and heavy chain complementarity determining region 3 from the amino terminus to carboxy terminus.

5 **[0057]** The term "light chain variable region" or "V_L region" as used herein refers to the variable region of a light chain of an antibody molecule. Light chain variable regions have three complementarity determining regions. The term "light chain complementarity determining region" as used herein refers to regions of hypervariability within the light chain variable region of an
10 antibody molecule. Light chain variable regions have three complementarity determining regions termed light chain complementarity determining region 1, light chain complementarity determining region 2 and light chain complementarity determining region 3 from the amino terminus to the carboxy terminus.

15 **[0058]** The term "nucleic acid sequence" as used herein refers to a sequence of nucleoside or nucleotide monomers consisting of naturally occurring bases, sugars and inter-sugar (backbone) linkages. The term also includes modified or substituted sequences comprising non-naturally occurring monomers or portions thereof. The nucleic acid sequences of the
20 present application may be deoxyribonucleic acid sequences (DNA) or ribonucleic acid sequences (RNA) and may include naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The sequences may also contain modified bases. Examples of such modified bases include aza and deaza adenine, guanine, cytosine, thymidine and uracil; and xanthine
25 and hypoxanthine.

[0059] The term "regulatory cells" as used herein refers to cells that have immune regulatory function. In one embodiment, the regulatory cells are regulatory T cells, which include, without limitation, CD4⁺CD25⁺ T regulatory cells, CD4⁺CD25⁺Foxp3⁺ T regulatory cells, T regulatory-1 (Tr1) cells, T
30 helper-3 (Th3) cells, CD4⁺CD25⁻ T regulatory cells, CD8⁺CD28⁻ T regulatory cells, CD8α⁺ T regulatory cells, γδ regulatory T cells, regulatory natural killer

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(NK) T cells and double negative T (DNT) cells. In a specific embodiment, the regulatory T cells are DNT cells. The term "DNT cells" include cells that are $CD3^+TCR^+CD4^-CD8^-$. In one embodiment, DNT cells are $CD3^+\alpha\beta TCR^+CD4^-CD8^-$. In a specific embodiment, DNT cells are $CD3^+\alpha\beta TCR^+CD4^-CD8^-NK1.1^-$.

- 5 **[0060]** The term "sample" or "test sample" as used herein refers to any fluid, cell or tissue sample from a subject that contain regulatory and/or anti-cancer cells. For example, the sample can be blood, serum, plasma, lymphatic fluid, or any tissue or cell type. The term also includes cell lines.

- 10 **[0061]** The term "sequence identity" as used herein refers to the percentage of sequence identity between two polypeptide sequences or two nucleic acid sequences. To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a
15 second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The
20 percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=number of identical overlapping positions/total number of positions.times.100%). In one embodiment, the two sequences are the same length. The determination of percent identity between two sequences can also be accomplished using a
25 mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST and
30 XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide

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sequences homologous to a nucleic acid molecules of the present application. BLAST protein searches can be performed with the XBLAST program parameters set, e.g., to score=50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present disclosure. To
5 obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of
10 the respective programs (e.g., of XBLAST and NBLAST) can be used (see, e.g., the NCBI website). Another preferred non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG
15 sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity,
20 typically only exact matches are counted.

[0062] The term "subject" as used herein refers to any member of the animal kingdom, preferably a mammal, more preferably a human being.

[0063] The term "treating or preventing" includes, but is not limited to, alleviation or amelioration of one or more symptoms or conditions of a disease
25 or condition (such as cancer, graft rejection, autoimmune disease, graft versus host disease, allergy, infection etc.), diminishment of extent of disease, stabilized state of disease, preventing spread of disease, delaying or slowing of disease progression, and amelioration or palliation of the disease state, remission whether detectable or undetectable and/or prolonged survival as
30 compared to expected survival if not receiving treatment.

[0064] As used herein, the phrase "treating or preventing immune related diseases" refers to inhibiting the disease, preventing the disease,

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decreasing the severity of the disease or improving disease symptoms. The term "immune related diseases" as used herein includes, without limitation, graft rejection, graft versus host disease, autoimmune disease (e.g. diabetes, lupus, arthritis, multiple sclerosis, lymphoproliferative diseases, etc.), allergic diseases and infectious diseases. The term "treating or preventing cancer" refers to inhibiting cancer cell replication, preventing transformation of a cell to a cancer-forming cell, inhibiting cancer spread (metastasis), inhibiting tumor growth, reducing cancer cell number or tumor growth, decreasing the malignant grade of a cancer (e.g., increased differentiation), or improving cancer-related symptoms. In one embodiment, the cancer is breast cancer. In another embodiment, the cancer is blood cancer or epithelial cancer. In yet another embodiment, the blood cancer is leukemia or lymphoma. In a further embodiment, the epithelial cancer is melanoma, esophageal, or lung cancer. In yet a further embodiment, the immune related disease involves graft rejection. Thus, the term "treating or preventing graft rejection" refers to inhibiting graft rejection, preventing graft rejection, reducing the severity of graft rejection, or improving graft rejection-related symptoms.

[0065] The term "variant" as used herein includes modifications or chemical equivalents of the amino acid and nucleic acid sequences disclosed herein that perform substantially the same function as the polypeptides or nucleic acid molecules disclosed herein in substantially the same way. In one embodiment, variants of polypeptides disclosed herein include, without limitation, conservative amino acid substitutions. Variants of polypeptides also include additions and deletions to the polypeptide sequences disclosed herein. In addition, variant nucleotide sequences and polypeptide sequences include analogs and derivatives thereof.

(B) Complementarity Determining Regions and Variable Regions and Nucleic Acids Thereof

[0066] The application provides isolated light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence QSLSSGNQKNY (SEQ ID NO:44); isolated light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence YSS (SEQ

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ID NO:45); and isolated light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence LQHYSSPFT (SEQ ID NO:46); and isolated heavy chain CDR1 comprising the amino acid sequence GFPFSNYG (SEQ ID NO:21); isolated heavy chain CDR2 comprising the amino acid sequence ISYDGRST (SEQ ID NO:22); and isolated heavy chain CDR3 comprising the amino acid sequence VRHELPEDH (SEQ ID NO:23). In one embodiment, the V_L region comprises the sequence shown in SEQ ID NO:43. In another embodiment, the V_H region comprises the sequence shown in SEQ ID NO:20.

10 **[0067]** The application provides isolated light chain complementarity determining region 1 (CDR1) consisting of the amino acid sequence QSLSSGNQKNY (SEQ ID NO:44); isolated light chain complementarity determining region 2 (CDR2) consisting of the amino acid sequence YSS (SEQ ID NO:45); and isolated light chain complementarity determining region
15 3 (CDR3) consisting of the amino acid sequence LQHYSSPFT (SEQ ID NO:46); and isolated heavy chain CDR1 consisting of the amino acid sequence GFPFSNYG (SEQ ID NO:21); isolated heavy chain CDR2 consisting of the amino acid sequence ISYDGRST (SEQ ID NO:22); and isolated heavy chain CDR3 consisting of the amino acid sequence
20 VRHELPEDH (SEQ ID NO:23). In one embodiment, the V_L region consists of the sequence shown in SEQ ID NO:43. In another embodiment, the V_H region consists of the sequence shown in SEQ ID NO:20.

[0068] The application also discloses variants of the CDR sequences disclosed above. For example, the variants include polypeptides that can bind
25 to the same epitope or antigen recognized by the CDR sequences disclosed above.

[0069] Additional aspects disclosed in the present application are isolated light chain variable regions comprising light chain CDR1, CDR2 and/or CDR3 disclosed herein (SEQ ID NOS:44, 45 and/or 46), and isolated
30 heavy chain variable regions comprising heavy chain CDR1, CDR2 and/or CDR3 disclosed herein (SEQ ID NOS:21, 22 and/or 23). In one embodiment,

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the light chain variable region comprises the amino acid sequence shown in Figure 16 (SEQ ID NO:43). In another embodiment, the heavy chain variable region comprises the amino acid sequence shown in Figure 15 (SEQ ID NO:20). In one embodiment, the light chain variable region consists of the amino acid sequence shown in Figure 16 (SEQ ID NO:43). In another embodiment, the heavy chain variable region consists of the amino acid sequence shown in Figure 15 (SEQ ID NO:20).

[0070] The application also discloses variants of the isolated light chain variable regions and heavy chain variable regions disclosed above. For example, the variants include polypeptides that can bind to the same epitope or antigen recognized by the isolated light chain variable regions and isolated heavy chain variable regions disclosed above.

[0071] In one embodiment, the variant amino acid sequences of the light chain CDR1, CDR2 and CDR3, and the heavy chain CDR1, CDR2 and CDR3 have at least 50%, preferably at least 60%, more preferably at least 70%, most preferably at least 80%, even more preferably at least 90%, and even most preferably 95% sequence identity to SEQ ID NOs:44-46 or 21-23.

[0072] In another embodiment, the variant amino acid sequences of the light chain variable region and the heavy chain variable region have at least 50%, preferably at least 60%, more preferably at least 70%, most preferably at least 80%, even more preferably at least 90% and even most preferably 95% sequence identity to SEQ ID NO:20 or 43.

[0073] The application also discloses isolated nucleic acid sequences encoding the light chain complementarity determining region 1 (CDR1) comprising the amino acid sequence QSLSSGNQKNY (SEQ ID NO:44); the light chain complementarity determining region 2 (CDR2) comprising the amino acid sequence YSS (SEQ ID NO:45); and the light chain complementarity determining region 3 (CDR3) comprising the amino acid sequence LQHYSSPFT (SEQ ID NO:46); and the heavy chain CDR1 comprising the amino acid sequence GFPFSNYG (SEQ ID NO:21); the heavy chain CDR2 comprising the amino acid sequence ISYDGRST (SEQ ID

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NO:22); and the heavy chain CDR3 comprising the amino acid sequence VRHELPEDH (SEQ ID NO:23).

[0074] The application also provides isolated nucleic acid sequences encoding variants of the CDR sequences and variable region sequences
5 discussed above.

[0075] Variant nucleic acid sequences include nucleic acid sequences that hybridize to the nucleic acid sequences encoding the amino acid sequences of SEQ ID NOS:44-46 or 21-23 under at least moderately stringent hybridization conditions, or have at least 50%, 60%, 70%, 80%, 90% or 95%
10 sequence identity to the nucleic acid sequences that encode the amino acid sequence of SEQ ID NOS:44-46 and 21-23.

[0076] The application also discloses an isolated nucleic acid sequence encoding the light chain variable region disclosed herein and an isolated nucleic acid sequence encoding the heavy chain variable region disclosed
15 herein. In one embodiment, the isolated nucleic acid sequence encodes the light chain variable region comprising the amino acid sequence shown in Figure 16 (SEQ ID NO:43). In another embodiment, isolated nucleic acid sequence encodes the heavy chain variable region comprising the amino acid sequence shown in Figure 15 (SEQ ID NO:20).

[0077] The application also discloses variants of the nucleic acid sequences that encode for the light chain variable region and heavy chain variable region disclosed herein. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding the light chain variable region and heavy chain variable region disclosed herein under
20 at least moderately stringent hybridization conditions. In another embodiment, the variant nucleic acid sequences have at least 50%, preferably at least 70%, most preferably at least 80%, even more preferably at least 90% and even most preferably at least 95% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO:20.

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[0078] A person skilled in the art will appreciate that the novel nucleic acid sequences of the present application can be used in a number of recombinant methods.

[0079] Accordingly, the nucleic acid sequences of the present application may be incorporated in a known manner into an appropriate expression vector which ensures good expression of the proteins encoded thereof. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule of the present application and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. Operatively linked is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

[0080] The present application therefore contemplates a recombinant expression vector of the present application containing a nucleic acid molecule of the present application, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence.

[0081] Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes (For example, see the regulatory sequences described in (Goeddel, 1990), Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). Selection of appropriate regulatory sequences is dependent on the host cell chosen as discussed below, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen

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and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector.

[0082] The recombinant expression vectors of the present application
5 may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the present application. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β -galactosidase, chloramphenicol acetyltransferase, firefly
10 luciferase, or an immunoglobulin or portion thereof such as the Fc portion of an immunoglobulin preferably IgG. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring
15 antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive, while the other cells die. This makes it possible to visualize and assay for expression of recombinant expression vectors of the present application and in particular to determine the effect of a mutation on
20 expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

[0083] The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the recombinant protein; increased solubility of the recombinant protein; and aid
25 in the purification of the target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia),
30 pMal (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

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[0084] Recombinant expression vectors can be introduced into host cells to produce a transformed host cell. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a vector) into a cell by one of many possible techniques known in the art. The term "transformed host cell" as used herein is intended to also include cells capable of glycosylation that have been transformed with a recombinant expression vector of the present application. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. For example, nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in (Sambrook et al., 2001) (Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, 2001), and other laboratory textbooks.

[0085] Suitable host cells include a wide variety of eukaryotic host cells and prokaryotic cells. For example, the proteins of the present application may be expressed in yeast cells or mammalian cells. Other suitable host cells can be found in (Goeddel, 1990), Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991). In addition, the proteins of the present application may be expressed in prokaryotic cells, such as *Escherichia coli* (Zhang et al., 2004), Science 303(5656): 371-3). In addition, a *Pseudomonas* based expression system such as *Pseudomonas fluorescens* can be used (US Patent Application Publication No. US 2005/0186666, (Schneider et al., 2005)).

[0086] Yeast and fungi host cells suitable for carrying out the present application include, but are not limited to *Saccharomyces cerevisiae*, the genera *Pichia* or *Kluyveromyces* and various species of the genus *Aspergillus*. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 ((Baldari et al., 1987), Embo J. 6:229-234), pMFa ((Kurjan and Herskowitz, 1982), Cell 30:933-943 (1982)), pJRY88 ((Schultz et al., 1987),

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Gene 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art (see (Hinnen et al., 1978) Proc. Natl. Acad. Sci. USA 75:1929); ((Ito et al., 1983), J. Bacteriology 153:163) and ((Cullen et al., 1987) 5 BiolTechnology 5:369).

[0087] Mammalian cells suitable for carrying out the present application include, among others: COS (e.g., ATCC No. CRL 1650 or 1651), BHK (e.g. ATCC No. CRL 6281), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573) and NS-1 cells. Suitable expression vectors for 10 directing expression in mammalian cells generally include a promoter (e.g., derived from viral material such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples of mammalian expression vectors include pCDM8 ((Seed, 1987)., Nature 329:840) and pMT2PC ((Kaufman et al., 1987), EMBO 15 J. 6:187-195).

[0088] Given the teachings provided herein, promoters, terminators, and methods for introducing expression vectors of an appropriate type into plant, avian, and insect cells may also be readily accomplished. For example, within one embodiment, the proteins of the present application may be 20 expressed from plant cells (see (Sinkar et al., 1987), J. Biosci (Bangalore) 11:47-58), which reviews the use of Agrobacterium rhizogenes vectors; see also((Zambryski et al., 1984), Genetic Engineering, Principles and Methods, Hollaender and Setlow (eds.), Vol. VI, pp. 253-278, Plenum Press, New York), which describes the use of expression vectors for plant cells, including, 25 among others, PAPS2022, PAPS2023, and PAPS2034).

[0089] Insect cells suitable for carrying out the present application include cells and cell lines from Bombyx, Trichoplusia or Spodotera species. Baculovirus vectors available for expression of proteins in cultured insect cells (SF 9 cells) include the pAc series ((Smith et al., 1983), Mol. Cell Biol. 3:2156- 30 2165) and the pVL series ((Luckow and Summers, 1989), Virology 170:31-39). Some baculovirus-insect cell expression systems suitable for expression

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of the recombinant proteins of the present application are described in PCT/US/02442.

[0090] Alternatively, the proteins of the present application may also be expressed in non-human transgenic animals such as rats, rabbits, sheep and
5 pigs ((Hammer et al., 1985). Nature 315:680-683); (Brinster et al., 1985; Palmiter and Brinster, 1985; Palmiter et al., 1983) Science 222:809-814); and ((Leder and Stewart, 1988) U.S. Patent No. 4,736,866).

[0091] Accordingly, the present application provides a recombinant expression vector comprising one or more of the novel nucleic acid
10 sequences disclosed herein as well as methods and uses of the expression vectors in the preparation of recombinant proteins. Further, the application provides a host cell comprising one or more of the novel nucleic acid sequences or expression vectors comprising one or more of the novel nucleic acid sequences.

15 **(C) Novel Binding Proteins for MFAP4**

[0092] As mentioned above, the present inventors have identified a novel antibody that binds MFAP4. Further, the inventors have identified that MFAP4 is expressed on the surface of a subset of regulatory cells and/or anti-cancer cells. Thus, MFAP4 can be used as a marker to detect, enrich and/or
20 select regulatory cells and/or anti-cancer cells. In addition, the inventors have shown that the regulatory cells and anti-cancer cells can be activated by binding of MFAP4. The activated regulatory cells can be used to treat or prevent the progression of many types of immune related diseases, such as cancer, graft rejection, graft versus host disease, autoimmune disease,
25 allergic diseases and infectious diseases. The activated anti-cancer cells can be used to treat or prevent cancer.

[0093] Accordingly, the application provides a binding agent that specifically binds MFAP4. In one embodiment, the binding agent binds MFAP4 protein having the amino acid sequence of SEQ ID NO:1 or 4. In
30 another embodiment, the binding agent comprises at least one light chain complementarity determining region disclosed herein (i.e. one or more of SEQ

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ID NOs:44-46) and/or at least one heavy chain complementarity determining region disclosed herein (i.e. one or more of SEQ ID NOs:21-23).

[0094] In one embodiment, the binding protein comprises the light chain CDR sequences of SEQ ID NOS:44, 45 and 46 and/or the heavy chain CDR sequences of SEQ ID NOS:21, 22 and 23. In another embodiment, the binding protein comprises the amino acid of SEQ ID NO: 43 (light chain variable region) and/or the amino acid of SEQ ID NO:20 (heavy chain variable region).

[0095] A person skilled in the art will appreciate that the application includes variants to the specific binding proteins disclosed above, including chemical equivalents to the sequences disclosed above that perform substantially the same function as the binding proteins disclosed above in substantially the same way. For example, the functional variant of a binding protein will be able to bind to the same antigens or epitopes as the binding proteins disclosed above.

[0096] In another embodiment, the binding agent is an antibody or fragment thereof. In yet another embodiment, the binding agent is a monoclonal antibody. In yet a further embodiment, the binding agent is the novel monoclonal antibody, termed G1. In yet another embodiment, the antibody is a humanized monoclonal antibody.

[0097] A person skilled in the art will appreciate that other antibodies can be generated that bind to MFAP4. For example, to produce human monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from a human having cancer and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g. the hybridoma technique originally developed by Kohler and Milstein (*Nature* 256:495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., *Immunol. Today* 4:72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., *Methods Enzymol*, 121:140-67 (1986)), and screening of combinatorial antibody libraries (Huse et al., *Science* 246:1275 (1989)). Hybridoma cells

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can be screened immunochemically for production of antibodies specifically reactive with cancer cells and the monoclonal antibodies can be isolated. Further, specific antibodies, or antibody fragments, reactive against particular antigens or molecules, such as MFAP4, may also be generated by screening
5 expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with cell surface components. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (See for example Ward et al., *Nature* 341:544-546 (1989); Huse et al., *Science* 246:1275-1281 (1989); and
10 McCafferty et al., *Nature* 348:552-554 (1990)).

[0098] The present application also includes the use of the novel nucleic acid sequences for the preparation of binding proteins and methods thereof.

(D) Methods of Selection, Enrichment and Activation

15 **[0099]** The application provides a method of detecting regulatory and/or anti-cancer cells comprising the steps of:

- (1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex;
- 20 (2) detecting the amount of binding agent-MFAP4 complex in the test sample; and
- (3) comparing the amount of binding agent-MFAP4 complex in the test sample to a control.

[00100] The term "detecting the amount" as used herein includes both
25 qualitative and quantitative measurements. For example, the term includes both detecting whether the complex is present and the quantity of complex present.

[00101] The complex can be measured or detected directly or indirectly. For example, the binding agent can be labeled. In another embodiment, a
30 secondary detection agent (e.g. a secondary antibody that is specific for the binding agent) is used and contains a detectable label that can be used to detect the complex.

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[00102] A person skilled in the art will appreciate that the control can be a sample known to include or not to include regulatory cells or anti-cancer cells. In addition, the control can include binding agents that are known to or not to bind MFAP4.

5 **[00103]** A further aspect of the application is a method of selecting or enriching regulatory cells and/or anti-cancer cells comprising the steps:

(1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex; and

10 (2) selecting or enriching regulatory cells and/or anti-cancer cells by selecting the binding agent-MFAP4 complex from the test sample.

[00104] A person skilled in the art will appreciate that the regulatory cells and/or anti-cancer cells are indirectly selected by binding of the binding agent to MFAP4 on the surface of the regulatory cells and/or anti-cancer cells. In one embodiment, the binding agent is labeled. In a further embodiment, the binding agent is immobilized on a solid surface. A number of methods can be used to select or enrich the regulatory and/or anti-cancer cells from the test sample, including, without limitation flow cytometry, immunoprecipitation, magnetic sorting and/or panning.

20 **[00105]** The application also provides a method of activating regulatory and/or anti-cancer cells comprising:

contacting regulatory and/or anti-cancer cells with a binding agent that binds specifically to MFAP4 on the cell to crosslink MFAP4.

25 **[00106]** In one embodiment, the cells are contacted *in vitro*. In another embodiment, the binding agent is administered to a subject and the cells are contacted *in vivo*.

[00107] A person skilled in the art will appreciate that the activation of the regulatory cells and/or anti-cancer cells can be assessed. For example, activated regulatory cells and activated anti-cancer cells have increased expression of IFN γ , changes in morphological/cytoskeletal appearance, such

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as increased adherence and cell-spreading, and/or increased regulatory activity (e.g. ability to suppress allo-reactive T cells) and/or increased anti-cancer activity (e.g. ability to kill cancer cells).

[00108] In one embodiment, the binding agent is immobilized on a solid support to induce crosslinking of MFAP4. A person skilled in the art will appreciate that other methods can be used to induce crosslinking of MFAP4, and hence activation of the regulatory cells and/or anti-cancer cells. For example, a secondary antibody specific for the binding agent can be used to induce crosslinking.

5
10 **(E) Uses of MFAP4-Selected and/or -Activated Cells and/or MFAP4 binding agents**

[00109] The MFAP4-selected and/or -activated regulatory cells derived from the methods disclosed herein can be used to treat or prevent immune-related diseases. The MFAP4-selected and/or -activated anti-cancer cells can be used to treat or prevent cancer. For example, see U.S. patent no. 6,953,576 and WO 2007/056854, incorporated herein by reference.

[00110] Accordingly, in an embodiment, the application provides use of an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the application for treating or preventing an immune-related disease. The application also provides a method of treating or preventing an immune-related disease comprising administering an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the application for treating or preventing an immune-related disease. The application further provides use of an MFAP4-selected and/or -activated regulatory cell obtained according to the method of the application in the preparation of a medicament for treating or preventing an immune-related disease. The application even further provides an MFAP4-selected and/or -activated regulatory cell obtained by the method of the application for use in the treatment or prevention of an immune-related disease.

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30 **[00111]** The application also provides use of an effective amount of an MFAP4-selected and/or -activated anti-cancer cell obtained by the method of the application for treating or preventing cancer. The application also provides

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a method of treating or preventing cancer comprising administering an effective amount of an MFAP4-selected and/or -activated anti-cancer cell obtained by the method of the application for treating or preventing cancer. The application further provides use of an MFAP4-selected and/or -activated anti-cancer cell obtained according to the method of the application in the preparation of a medicament for treating or preventing cancer. The application even further provides an MFAP4-selected and/or -activated anti-cancer cell obtained by the method of the application for use in the treatment or prevention of cancer.

- 10 **[00112]** In the treatment or prevention of cancer, the cancer that can be treated can be any cancer that is amenable to treatment with DNT cells either alone or in combination with other treatment such as surgery, radiation therapy or chemotherapy, etc. Examples of cancers that may be treated or prevented according to the present application include, but are not limited to,
- 15 leukemias including chronic myelogenous leukemia, acute myelogenous leukemia, acute lymphoblastic leukemia, and T cell and B cell leukemias, lymphomas (Hodgkins and non-Hodgkins), lymphoproliferative disorders, plasmacytomas, histiocytomas, melanomas, adenomas, sarcomas, carcinomas of solid tissues, hypoxic tumours, squamous cell carcinomas,
- 20 genitourinary cancers such as cervical, ovarian, and bladder cancers, breast and lung cancers, colon cancer, esophageal cancer, hematopoietic cancers, head and neck cancers, and nervous system cancers.

- [00113]** The application also includes other therapeutic uses of the MFAP4-selected and/or -activated regulatory cells disclosed herein such as
- 25 the treatment of infectious diseases and for modulating an immune response for example in the treatment of autoimmune diseases, allergies, graft rejection and graft-versus-host disease. In such cases, the method of preparing the MFAP4-selected and/or -activated cells can include adding the appropriate infectious agent, allergen cells or tissues as the antigen.

- 30 **[00114]** Accordingly, in one embodiment, the present application provides a method of treating or preventing an infectious disease comprising administering an effective amount of an MFAP4-selected and/or -activated

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regulatory cell obtained by the method of the present application to an animal in need thereof. The present application also includes a use of an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the present application for treating or preventing an infectious disease. The present application further includes a use of an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the present application in the manufacture of a medicament for treating or preventing an infectious disease. The present application yet further includes an MFAP4-selected and/or -activated regulatory cell for use in the treatment or prevention of an infectious disease.

[00115] In a further embodiment, the present application provides a method of modulating an immune response comprising administering an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the present application to an animal in need thereof. The present application also includes a use of an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the present application for modulating an immune response. The present application further includes a use of an effective amount of an MFAP4-selected and/or -activated regulatory cell obtained by the method of the present application in the manufacture of a medicament for modulating an immune response. The present application yet further includes an MFAP4-selected and/or -activated regulatory cell for use in modulation of an immune response.

[00116] In one embodiment, the MFAP4-selected and/or -activated cells are used to treat an autoimmune disease. Autoimmune diseases that may be treated according to the present application include, but are not limited to, diabetes, arthritis, multiple sclerosis, lupus erythematosus, inflammatory bowel disease, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatic fever, Reiter's syndrome, progressive systemic sclerosis, primary biliary cirrhosis, necrotizing vasculitis, myasthenia gravis, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory

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disorder, antigen-antibody complex mediated diseases, autoimmune haemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Raynaud's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, tissue specific
5 autoimmunity, degenerative autoimmunity delayed hypersensitivities, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

[00117] In another embodiment, the MFAP4-selected and/or -activated regulatory cells can be used to treat graft-versus-host disease wherein the
10 immune cells in the transplant mount an immune attack on the recipient's immune system. This can occur when the tissue to be transplanted contains immune cells such as when bone marrow or lymphoid tissue is transplanted when treating leukemias, aplastic anemias and enzyme or immune deficiencies, for example.

[00118] In a further embodiment, the MFAP4-selected and/or -activated regulatory cells can be used to treat an allergic reaction. In an allergic reaction, the immune system mounts an attack against a generally harmless, innocuous antigen or allergen. Allergies that may be prevented or treated using the methods of the application include, but are not limited to, hay fever,
15 asthma, atopic eczema as well as allergies to poison oak and ivy, house dust mites, bee pollen, nuts, shellfish, penicillin and numerous others.

[00119] The MFAP4 binding agents described herein could also be used to modulate regulatory and/or anti-cancer cells *in vivo*. Accordingly, in another aspect, the present application provides a method of modulating an immune
25 response in an animal comprising administering an effective amount of an MFAP4 binding agent to an animal in need thereof. The application also provides a use of an effective amount of an MFAP4 binding agent for modulating an immune response in an animal in need thereof. The application further provides a use of an effective amount of an MFAP4 binding agent in
30 the preparation of a medicament for modulating an immune response. The application yet further provides an MFAP4 binding agent for use in modulation of an immune response.

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[00120] In a further aspect, the present application provides a method of modulating cancer in an animal comprising administering an effective amount of an MFAP4 binding agent to an animal in need thereof. The application also provides a use of an effective amount of an MFAP4 binding agent for
5 modulating cancer in an animal in need thereof. The application further provides a use of an effective amount of an MFAP4 binding agent in the preparation of a medicament for modulating cancer. The application yet further provides an MFAP4 binding agent for use in modulation of cancer.

[00121] For example, the MFAP4 binding agents may activate or target
10 regulatory cells and/or anti-cancer cells when administered to an area of interest, such as lymphoid tissues or non-lymphoid tissues for regulating allo- or auto-reactive T cell responses, or in tumors for activating tumor-reactive cells.

[00122] The term "modulating an immune response" as used herein
15 refers to activating or inhibiting immune cell activity and includes, without limitation, modulating an immune response in the treatment of an immune-related disease.

[00123] In one embodiment, modulating an immune response comprises activating an immune response. In another embodiment, modulating an
20 immune response comprises inhibiting an immune response.

[00124] The term "modulating a cancer" as used herein refers to activating or inhibiting cancer cells.

[00125] The MFAP4-selected or -activated regulatory and/or anti-cancer cells and/or MFAP4 binding agents described herein may be formulated into
25 pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. The substances may be administered to living organisms including
30 humans, and animals. The compositions may be administered in a convenient manner preferably by injection such as intravenous, subcutaneous, intramuscular, etc.

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[00126] The compositions described herein can be prepared by *per se* known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the cells is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, 20th ed., Mack Publishing Company, Easton, Pa., USA 2000). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

[00127] An effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the cells to elicit a desired response in the individual. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[00128] The pharmaceutical compositions of the application may include other active agents that are useful in treating the disease or condition to be treated. For example, in the treatment of a cancer, other anti-cancer agents may be administered either in the same composition or in a separate composition.

[00129] In one specific embodiment, the novel antibody, G1, disclosed herein is the binding agent for the methods disclosed herein. The G1 monoclonal antibody comprises the light chain variable region of SEQ ID NO:43 and the heavy chain variable region of SEQ ID NO:20.

[00130] The above disclosure generally describes the present disclosure. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the disclosure. Changes in form and substitution of equivalents are contemplated as

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circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

5 **[00131]** The following non-limiting examples are illustrative of the present disclosure:

EXAMPLES

10 **[00132]** DNT cells share similarity to CD4⁺CD25⁺ T regulatory cells in their potent immune regulatory function. In tumor immunity, however, DNT cells appear to play a different role from that of CD4⁺CD25⁺ T cells. The present inventors have demonstrated that DNT cells in mice possess potent cytotoxicity against syngeneic and allogeneic tumor cell lines *in vitro*, and that a single infusion of DNT cells can protect recipient mice from a lethal dose of a B cell lymphoma cell line *in vivo* (Figure 1; ref. 7). In addition, the present inventors have developed a protocol by which human DNT cells can be
15 purified from peripheral blood and expanded *ex vivo* (Figure 3A). Such *ex vivo* expanded DNT cells are cytotoxic against human leukemia, lymphoma, melanoma, esophagus and lung cancer cell lines, and adoptive transfer of these cells can significantly improve survival and inhibit cancer progression in a xeno-SCID mouse model (Figure 3B). These data provide compelling
20 evidence of the unique potential for utilizing DNT cells in anti-tumor adoptive cellular immunotherapy. Indeed, new immune cell-mediated therapy may be an effective alternative means of improving survival by eliminating residual cancer cells after chemotherapy-induced remission.

25 **[00133]** As mentioned above, DNT cells comprise only ~1% of PBMC, and no unique surface markers have been identified to positively select DNT cells. Thus, the identification of functional markers that can distinguish conventional activated T cells from activated regulatory T cells would facilitate positive selection and enrichment of *ex vivo*-expanded DNT regulatory cells with potent efficacy and thereby, as one example, potentially increase the *in*
30 *vivo* efficacy of DNT cell adoptive therapy.

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[00134] To facilitate identification of functional markers, a panel of regulatory DNT cell clones has been generated (7). During long-term culture, several natural DNT mutant clones arose that have lost suppressive function against antigen-specific T cells (7). While a single infusion of DNT cell clones prevented the rejection of skin and cardiac allografts in a donor-specific manner, adoptive transfer of the same number of mutant clones had no protective effect (1, 7, 9). Moreover, a single infusion of DNT cell clones prevented the development of B cell lymphoma from a lethal injection of B cell tumor cells, adoptive transfer of the same number of mutant clones had no protective effect (14; Figure 1). DNT cell clones and their spontaneously-derived mutants therefore provided an excellent system for the identification of specific markers that are important for DNT cell function. A large panel of monoclonal antibodies (mAbs) was thus generated against cell surface molecules on tumor-reactive DNT cells. One mAb (clone: G1) was selected for further characterization, which exhibits strong selective cell surface expression on tumor-reactive and regulatory DNT cell clones, as determined by flow cytometry (Figure 2).

1. Identification and validation of MFAP4 as novel functional cell marker recognized by G1 mAb (Figures 4-8)

[00135] To determine the identity of the DNT cell marker recognized by G1 mAb, membrane fractions of cell lysates from both DNT and mutant cell clones were immunoprecipitated with G1 mAb, resolved by 1-dimensional SDS-PAGE, and protein bands that were specifically immunoprecipitated by the G1 mAb from DNT cell clone lysates, but not mutant clone lysates, were excised and the partial amino acid sequence was obtained using LC-mass spectrometry. Genome database analyses using the derived sequence information revealed microfibrillar-associated protein-4 (MFAP4) as the cell surface molecule on regulatory DNT cells specifically recognized by the G1 mAb. MFAP4 was validated as the DNT cell marker recognized by G1 mAb: (1) determination of the molecular size of the MFAP4 protein expressed by DNT cells; (2) specific recognition of rMFAP4 retrovirally expressed in CHO cell line by G1 mAb and conversely specific recognition of MFAP4 in G1⁺ DNT

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cell clones but not G1⁻ mutant DNT cell clones by anti-MFAP4 mAb; and (3) simultaneous recognition of the cell surface marker on the same activated DNT cell by G1 mAb and anti-MFAP4 Ab. The MFAP4 protein exhibits identical molecular size as the protein recognized by G1 mAb determined by western blot (Figure 5); MFAP4 is specifically recognized by anti-MFAP4 mAb in G1⁺ DN T cell clones but not G1⁻ mutant clones; and anti-MFAP4 mAb and G1 mAb recognize a cell surface marker expressed on the surface of the same activated DNT cell (Figure 6). The G1 mAb also recognizes a cell surface protein expressed on a subset of *ex vivo*-expanded human DNT cells (Figure 4). Thus, MFAP4 is expressed on the cell surface of murine and human DNT cells, and G1 mAb immunostains this functional marker.

[00136] To determine whether MFAP4 is a specific functional marker on the cell surface of activated DNT cells, lymphocytes from B6.lpr mice were activated by alloantigen stimulation ([B6xBalb/c]_{F1} splenocytes) and cell surface expression of MFAP4 was determined on lymphocyte subsets by flow cytometry. MFAP4 is expressed on the surface of a subset of activated DNT cells (Figure 7). Thus, G1 mAb can be used to mark and positively purify activated DNT cells. It was also found that a minor subset of CD4⁺, CD8⁺, and/or NK1.1⁺ T cells stained positive for MFAP4, suggesting that the G1 mAb could also be used as a novel functional marker for other regulatory T cell subsets. Another derivation of regulatory DNT cells is via conversion from CD4⁺ T cells that have undergone extensive cell proliferation stimulated by alloantigen stimulation ([B6xBalb/c]_{F1} mature bone marrow-derived dendritic cells) (10). It was found that a subset of DNT cells with suppressor function converted from CD4⁺ T cells expresses MFAP4 on their cell surface (Figure 8).

[00137] MFAP4 is a 255-amino acid glycoprotein of the fibrinogen-related domain (FReD) family of proteins (11). It was first identified as a novel gene mapped to the chromosomal region associated with Smith-Magenis syndrome (SMS), a multiple congenital anomaly/mental retardation syndrome associated with deletion of chromosome 17p11.2 (12). MFAP4 contains a C-terminal fibrinogen-like domain (which has high similarity to the C-terminal

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halves of fibrinogen β and γ chains) and the N-terminal Arg-Gly-Asp (RGD) motif sequence that serves as an integrin-binding domain, suggesting that it is an extracellular matrix protein involved in cell adhesion or intercellular interactions. More recently, MFAP4 was shown to interact with pulmonary surfactant protein A (SP-A), a collectin involved in innate immunity against a broad range of micro-organisms by mediating microbial lysis and clearance, as well as modulating cytokine production and chemotaxis for phagocytes (13). Moreover, MFAP4 and SP-A co-localize both on the elastic fibres and lamina of pulmonary arteries of chronically inflamed lung tissue, consistent with a proposed role for MFAP4 in fixing collectins in the extracellular compartment during inflammation (13). Other members of the FReD family include fibrinogen-like protein 2/fibroleukin (FGL2), angiopoietins, ficolins, tachylectins and tenascins, and these molecules have been shown to play roles in innate and adaptive immune responses, including cell adhesion, cytokine production, and inflammation (13). With respect to an immunoregulatory role for proteins containing a fibrinogen-like domain, it has recently been shown that mice with targeted deletion of FGL2 spontaneously develop autoimmune glomerulonephritis, which correlated with increased T cell proliferation to alloantigens and increased numbers of Ab-producing B cells following immunization with T-independent Ags (14). Interestingly, FGL2 is produced by CD4⁺CD25⁺ regulatory T cells and their suppressive activity was significantly impaired by FGL2-deficiency (14). Moreover, anti-FGL2 Ab treatment completely inhibited Treg cell activity *in vitro* (14). Thus, MFAP4 as a FReD-containing protein may be important for DNT cell regulatory function.

2. Treatment of DNT cells with G1 mAb induces rapid expression of effector cytokine IFN γ , adherence and morphological activation (Figures 9-10)

[00138] It was next determined whether treatment of activated DNT cells with G1 mAb has important consequences for their function. G1 mAb-mediated crosslinking of MFAP4 on the cell surface of activated DN T cells by coating flat-bottom wells with G1 mAb induced rapid IFN γ expression (Figure 9), an effector cytokine that has been reported to have a role in promoting

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tolerance in a murine allograft model. In addition, DNT cells treated with G1 mAb gain adherence and cell-spreading indicative of morphological/cytoskeletal activation (Figure 10). Thus, G1 mAb can be used to induce rapid effector function by activated DNT cells.

5 **3. Enrichment of MFAP4-positive DNT cells using G1 mAb selects for potent suppressor cells of syngeneic allo-reactive T cells (Figure 11)**

[00139] The utility of G1 mAb to select for regulatory DNT cells expressing the cell surface marker MFAP4 was determined by sorting MFAP4-negative and MFAP4-positive activated cells and examining their
10 respective suppressor function against syngeneic allo-reactive CD4 T cells. Activated cells expressing MFAP4 were more potent at suppressing allo-reactive CD4 T cells in a mixed lymphocyte reaction compared with MFAP4-negative cells (Figure 11): allo-reactive CD4 T cells were almost completely eliminated by MFAP4+ cells. Thus, MFAP4 is a cell surface marker
15 recognized by G1 mAb and can be used to selectively enrich for potent DNT regulatory cells against allo-reactive T cells. As a minor subset of activated CD4+, CD8+, and/or NK1.1+ T cells have been found to express MFAP4 (Figure 7), G1 mAb may also recognize this cell surface marker on other regulatory T cell subsets with potent suppressive efficacy.

20 **4. Enrichment of MFAP4-positive DNT cells using G1 mAb selects for potent anti-tumor cells (Figure 12)**

[00140] The utility of G1 mAb to select anti-tumor DNT cells expressing the cell surface marker MFAP4 was determined by sorting MFAP4-negative and MFAP4-positive DNT cells (Figure 12A) and using them respectively as
25 effectors against A20 B cell lymphoma cells. Activated cells expressing MFAP4 and selected based on positive binding with G1 mAb were more potent at killing tumor cells compared with MFAP4-negative, G1-negative cells (Figure 12B). Thus, MFAP4 is a cell surface marker recognized by G1 mAb that can be used to selectively enrich for potent anti-tumor DNT cells.

30 **5. Sequencing of Novel G1 Monoclonal Antibody**

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[00141] mRNA was extracted from hybridoma cell pellets. Total RNA was extracted from the pellets. cDNA was created from the RNA by reverse-transcription with an oligo(dT) primer. PCR reactions using variable domain primers were used to amplify both the VH and VL regions of the monoclonal antibody DNA. Positive VH and VL products were cloned into the Invitrogen sequencing vector pCR2.1 (Invitrogen: K2020-20) and transformed into TOP10 E. coli. Selected colonies were analyzed by colony PCR using vector specific primers M13 forward and M13 reverse. The colony PCR products were analysed as before by 1.5% agarose gel and ethidium bromide staining.

5 Clones with positive bands around 500 bp were expanded to 5 ml culture in LB media overnight at 37°C. The plasmids were purified from the E. coli cultures using a plasmid miniprep kit (Qiagen) and the clones were sequenced.

[00142] CDRs were identified using the IMGT unique numbering system (Lefranc M.-P. et al. "IMGT unique numbering for immunoglobulin and Tcell receptor variable domains and Ig superfamily V-like domains" Dev. Comp. Immunol., 27, 55-77 (2003); Brochet, X., Lefranc, M.-P. and Giudicelli, V. IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis

15 Nucl. Acids Res, 36, W503-508 (2008)).

[00143] The V_H nucleotide sequences for 6 clones are as shown in SEQ ID NO:7, 9, 11, 13, 15, and 17 and the V_H amino acid sequences for 6 clones are as shown in SEQ ID NO:8, 10, 12, 14 and 16 respectively.

25 **[00144]** Alignment of the V_H sequences is shown in Figure 15. The consensus sequence is:

MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQGRSLKLSCAASGFPFSNYGMAWVRQAPTK
GLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYLQMDSLRSEDATYYCVRHELPEDH
WGQGVMTVSSARTTAPPVYPLAPGSL (SEQ ID NO:19)

30

[00145] The consensus heavy chain variable region comprises the amino acid sequence of SEQ ID NO:20. CDR1 of the V_H domain is as shown

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in SEQ ID NO:21; CDR2 of the V_H domain is as shown in SEQ ID NO:22; CDR3 of the V_H domain is as shown in SEQ ID NO:23.

[00146] The V_L nucleotide sequences for 9 clones are as shown in SEQ ID NOs: 24, 26, 28, 30, 32, 34, 36, 38 and 40 and the VL amino acid
5 sequences are as shown in SEQ ID NOs:25, 27, 29, 31, 33, 35, 37, 39 and 41.

[00147] Alignment of the V_L sequences is shown in Figure 16. The consensus sequence is:

10 MVLISLLFWVYGTCGDIVMTQSPFSLAVSEGEMVTINCKSGQSLSSGNQKNYLAWYQQKPG
QSPKLLIYYSTRQSGVPDRFIGSGSGTDFTLTISDVQAEDLADYYCLQHYSSPFTFGSGTKLEI
KRADAAPTVSIFPPSSKLG (SEQ ID NO:42)

[00148] The consensus light chain variable region comprises amino acid sequence of SEQ ID NO:43. The CDR1 of V_L is shown in SEQ ID NO:44; the
15 CDR2 of V_L is shown in SEQ ID NO:45; and the CDR3 of V_L is shown in SEQ ID NO:46.

[00149] While the present disclosure has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the disclosure is not limited to the disclosed examples. To the
20 contrary, the disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00150] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each
25 individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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We claim:

1. A method of detecting regulatory and/or anti-cancer cells comprising the steps of:
 - (1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex;
5
 - (2) detecting the amount of binding agent-MFAP4 complex in the test sample; and
 - (3) comparing the amount of binding agent-MFAP4 complex in the test sample to a control.
10
2. A method of selecting or enriching regulatory and/or anti-cancer cells comprising the steps:
 - (1) contacting a test sample with a binding agent that binds specifically to MFAP4 on the cell to produce a binding agent-MFAP4 complex; and
15
 - (2) selecting or enriching regulatory and/or anti-cancer cells by selecting the binding agent-MFAP4 complex from the test sample.
3. A method of activating regulatory and/or anti-cancer cells comprising:
20 contacting regulatory and/or anti-cancer cells with a binding agent that binds specifically to MFAP4 on the cell to crosslink MFAP4.
4. The method of claim 3, wherein the cells are contacted *in vitro*.
5. The method of claim 2, further comprising the step of activating the selected or enriched regulatory and/or anti-cancer cells by allowing a
25 binding agent that binds specifically to MFAP4 on the cell to crosslink MFAP4.
6. The method of any one of claims 1 to 5, wherein the regulatory cells comprise regulatory T cells.

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7. The method of claim 6, wherein the regulatory T cells comprise CD4⁺CD25⁺ T regulatory cells, CD4⁺CD25⁺Foxp3⁺ T regulatory cells, T regulatory-1 (Tr1) cells, T helper-3 (Th3) cells, CD4⁺CD25⁻ T regulatory cells, CD8⁺CD28⁻ T regulatory cells, CD8αα⁺ T regulatory cells, γδ regulatory T cells, regulatory natural killer (NK) T cells and/or double negative T (DNT) cells.
5
8. The method of claim 7, wherein the double negative T cells comprise cells that are CD3⁺TCR⁺CD4⁻CD8⁻.
9. The method of claim 8, wherein the double negative T cells comprise cells
10 that are CD3⁺αβTCR⁺CD4⁻CD8⁻.
10. The method of any one of claims 1 to 5, wherein the anti-cancer cells comprise double negative T cells.
11. The method of claim 10, wherein the double negative T cells comprise cells that are CD3⁺TCR⁺CD4⁻CD8⁻.
- 15 12. The method of claim 11, wherein the double negative T cells comprise cells that are CD3⁺αβTCR⁺CD4⁻CD8⁻.
13. The method of any one of claims 1-12, wherein the binding agent comprises a light chain complementarity determining region comprising the amino acid sequence of SEQ ID NOs: 44, 45 and/or 46.
- 20 14. The method of any one of claims 1-12, wherein the binding agent comprises a heavy chain complementarity determining region comprising the amino acid sequence of SEQ ID NOs: 21, 22 and/or 23.
15. The method of any one of claims 1-12, wherein the binding agent comprises the light chain complementarity determining regions having the
25 amino acid sequence of SEQ ID NOS: 44, 45, and/or 46, and/or the heavy chain complementarity determining regions having the amino acid sequence of SEQ ID NOS: 21, 22 and/or 23.

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16. The method of any one of claims 1-12, wherein the binding agent comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 43.
17. The method of any one of claims 1-12, wherein the binding agent
5 comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:20.
18. The method of any one of claims 1-12, wherein the binding agent comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 20 and a heavy chain variable region comprising
10 the amino acid sequence of SEQ ID NO:43.
19. The method of any one of claims 1 to 18, wherein the binding agent is an antibody.
20. The method of claim 19, wherein the antibody is a monoclonal antibody.
21. The method of claim 19, wherein the antibody is a humanized monoclonal
15 antibody.
22. A use of an effective amount of the regulatory cells selected or activated by the method of any one of claims 1-21 for treating or preventing an immune related disease.
23. The use of claim 22, wherein the immune related disease is autoimmune
20 disease, allergy, graft rejection, graft versus host disease or infectious disease.
24. A use of an effective amount of the anti-cancer cells selected or activated by the method of any one of claims 1-21 for treating or preventing cancer.
25. The use of claim 24, wherein the cancer is a blood cancer, breast cancer,
25 or epithelial cancer.
26. The use of claim 25, wherein the blood cancer is leukemia or lymphoma.

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27. The use of claim 25, wherein the epithelial cancer is lung cancer, esophageal or melanoma.

28. An isolated complementarity determining region (CDR) selected from the group consisting of:

- 5 an isolated light chain CDR 1 comprising the amino acid sequence of SEQ ID NO:44 or a variant thereof;
- an isolated light chain CDR 2 comprising the amino acid sequence SEQ ID NO:45 or a variant thereof;
- an isolated light chain CDR 3 comprising the amino acid sequence
- 10 SEQ ID NO:46 or a variant thereof;
- an isolated heavy chain CDR 1 comprising the amino acid sequence SEQ ID NO:21 or a variant thereof;
- an isolated heavy chain CDR 2 comprising the amino acid sequence SEQ ID NO:22 or a variant thereof; and
- 15 an isolated heavy chain CDR 3 comprising the amino acid sequence SEQ ID NO:23 or a variant thereof.

29. An isolated nucleic acid sequence encoding a complementarity determining region of claim 28, or a variant thereof.

30. An isolated variable region selected from the group consisting of:

- 20 an isolated light chain variable region comprising the light chain complementarity determining regions defined by SEQ ID NOS:44, 45 and/or 46, or a variant thereof; and
- an isolated heavy chain variable region comprising the heavy chain complementarity determining regions defined by SEQ ID NOS:21, 22 and/or
- 25 23, or a variant thereof.

31. The variable region of claim 30 wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO:43, or a variant thereof.

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32. The variable region of claim 30 wherein the isolated heavy chain variable region comprises the amino acid sequence of SEQ ID NO:20, or a variant thereof.
33. An isolated nucleic acid sequence encoding the variable region of any one
5 of claims 30-32, or a variant thereof.
34. A recombinant expression vector comprising the nucleic acid of claim 28 or 33.
35. A host cell comprising the recombinant expression vector of claim 34.
36. A binding agent comprising the light chain complementarity determining
10 regions defined by SEQ ID NOS: 44, 45 and/or 46, or a variant thereof.
37. A binding agent comprising the heavy chain complementarity determining regions defined by SEQ ID NOS: 21, 22 and/or 23, or a variant thereof.
38. A binding agent comprising the light chain complementarity determining regions comprising the amino acid sequences defined by SEQ ID NOS:
15 44, 45 and/or 46 and/or the heavy chain complementarity determining regions comprising the amino acid sequence defined by SEQ ID NOS: 21, 22 and/or 23, or a variant thereof.
39. A binding agent comprising the light chain variable region comprising the amino acid sequence of SEQ ID NO:43 and/or the heavy chain variable
20 region comprising the amino acid sequence of SEQ ID NO:20, or a variant thereof.
40. The binding agent of any one of claims 36-39, wherein the binding agent binds a protein comprising the amino acid sequence of SEQ ID NO:1 and/or SEQ ID NO:4.
- 25 41. The binding agent of any one of claims 36-40, wherein the binding agent is an antibody.

- 50 -

42. The binding agent of claim 41, wherein the antibody is a monoclonal antibody.
43. The binding agent of claim 41, wherein the antibody is a humanized monoclonal antibody.
- 5 44. A use of an effective amount of the binding agent of any one of claims 36-43 for modulating an immune response.
45. A use of an effective amount of the binding agent of any one of claims 36-43 for modulating cancer.

Figure 1

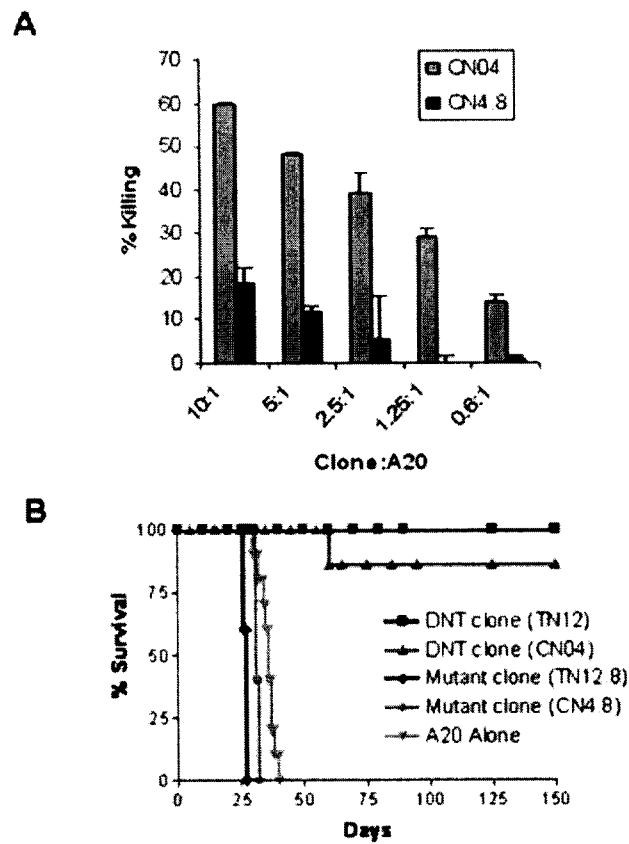


Figure 2

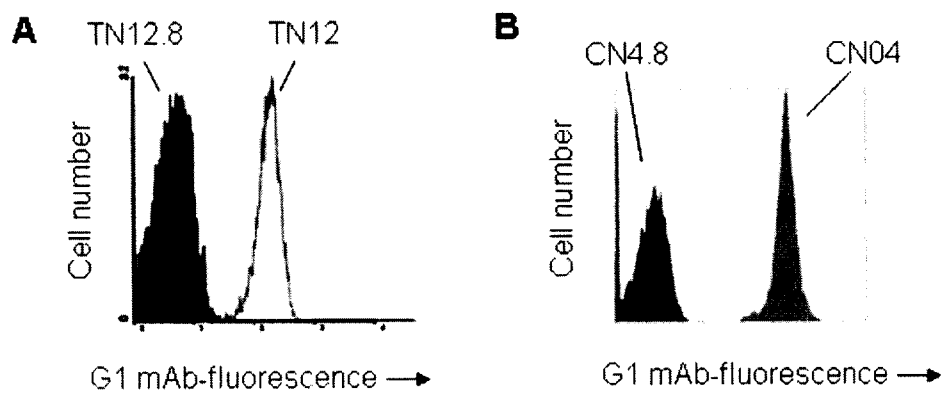


Figure 3

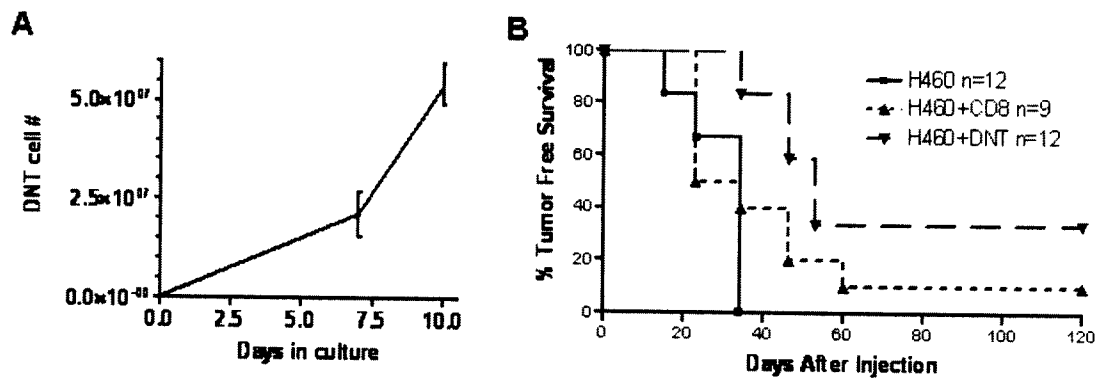


Figure 4

Human DNT cells

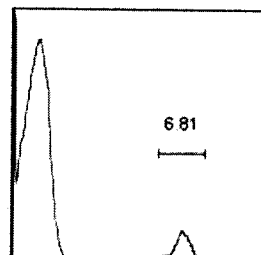


Figure 5

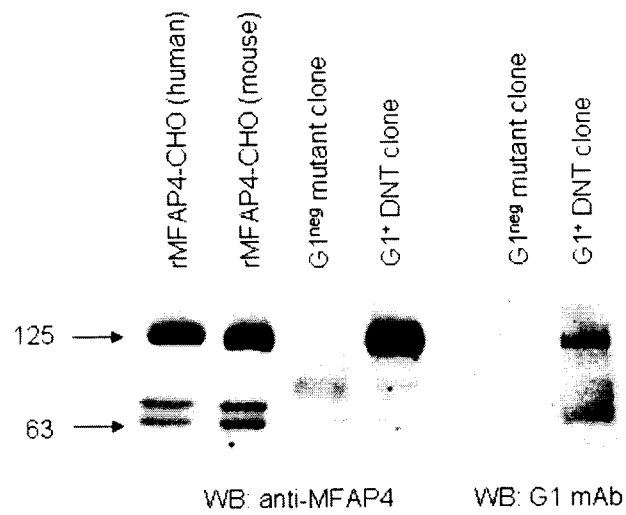


Figure 6

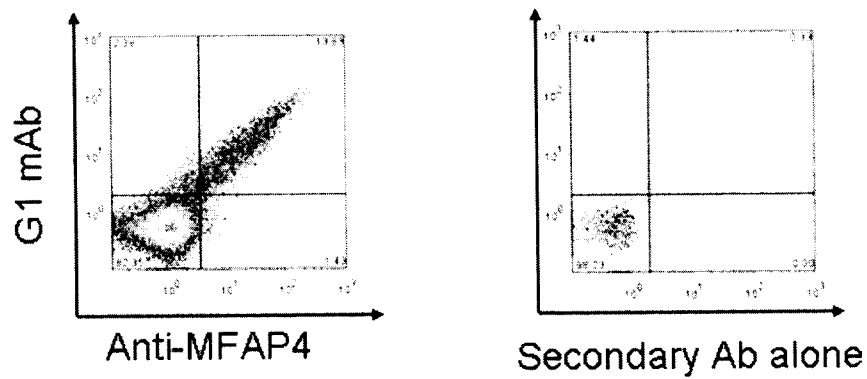


Figure 7

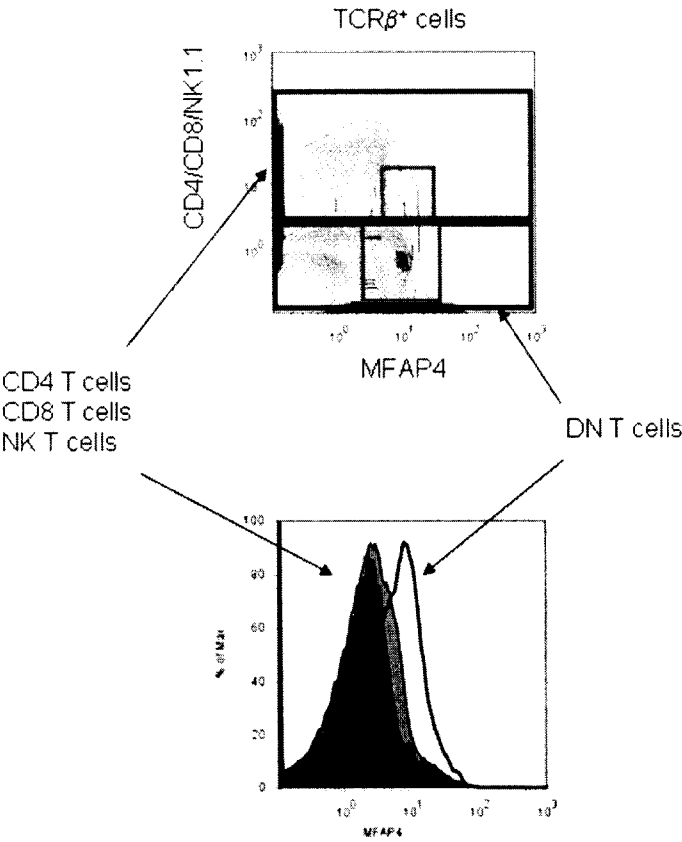


Figure 8

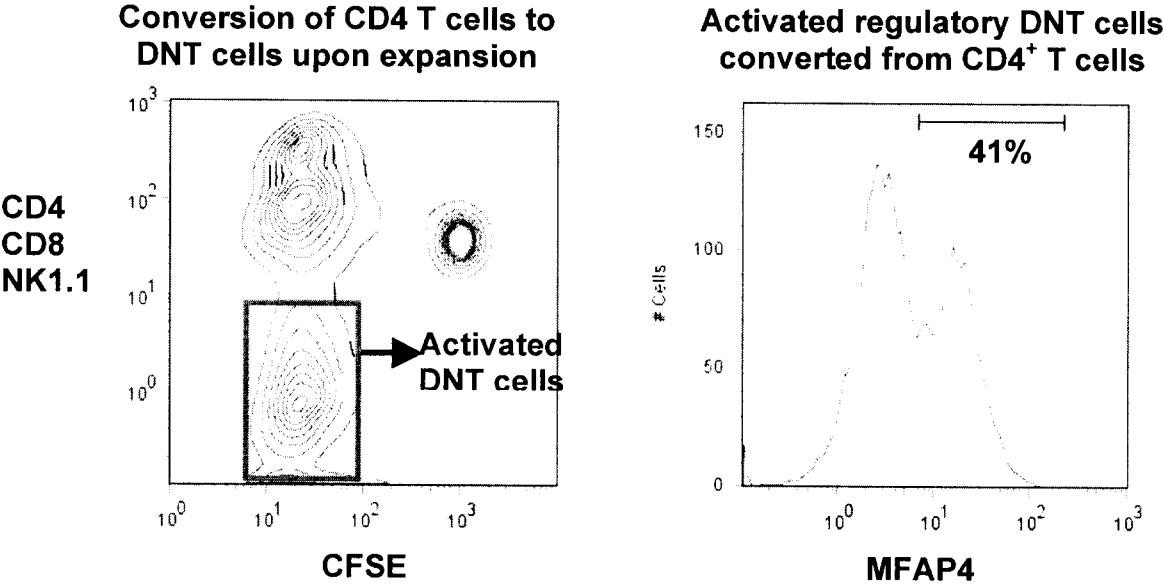
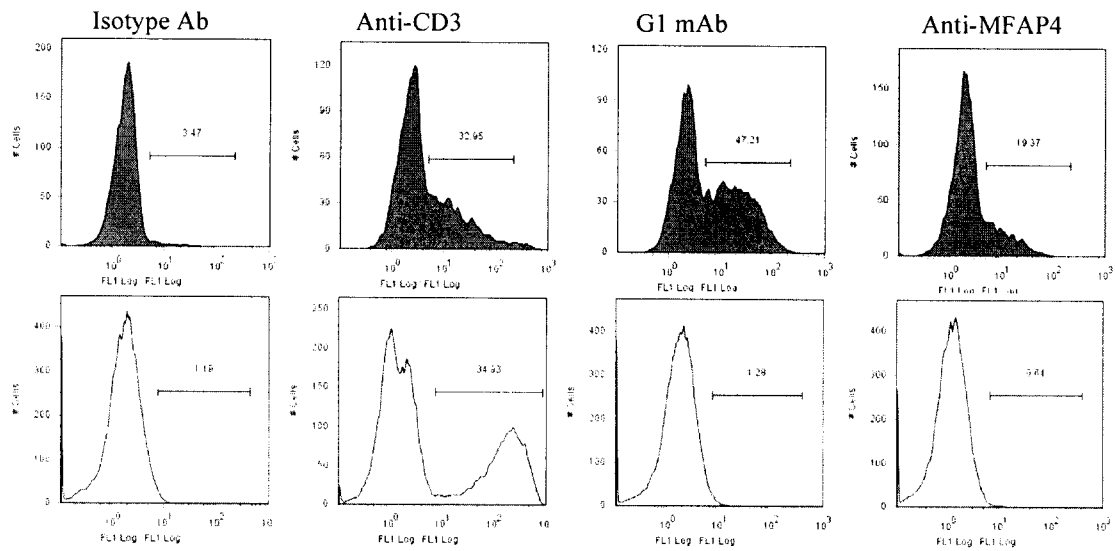


Figure 9**Interferon- γ** 

Top row: DN T cells
Bottom row: CD8 T cells

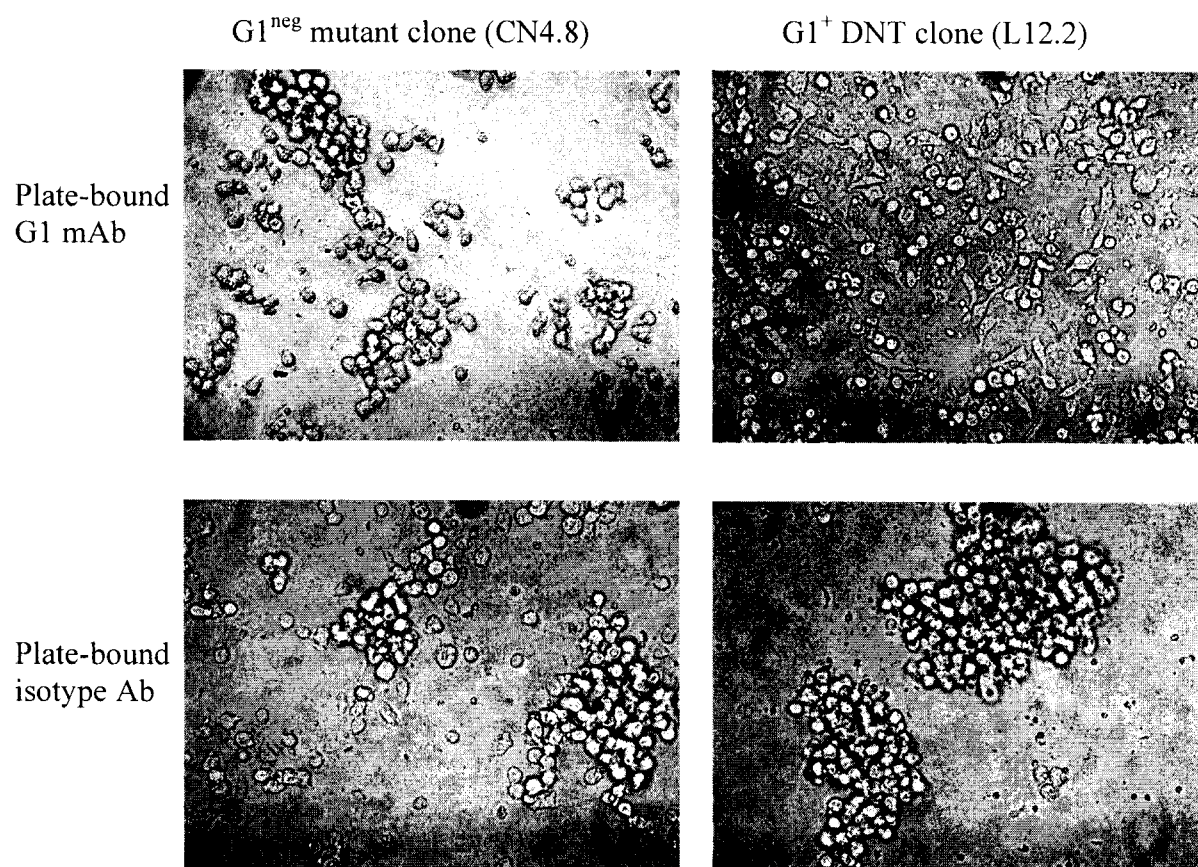
Figure 10

Figure 11

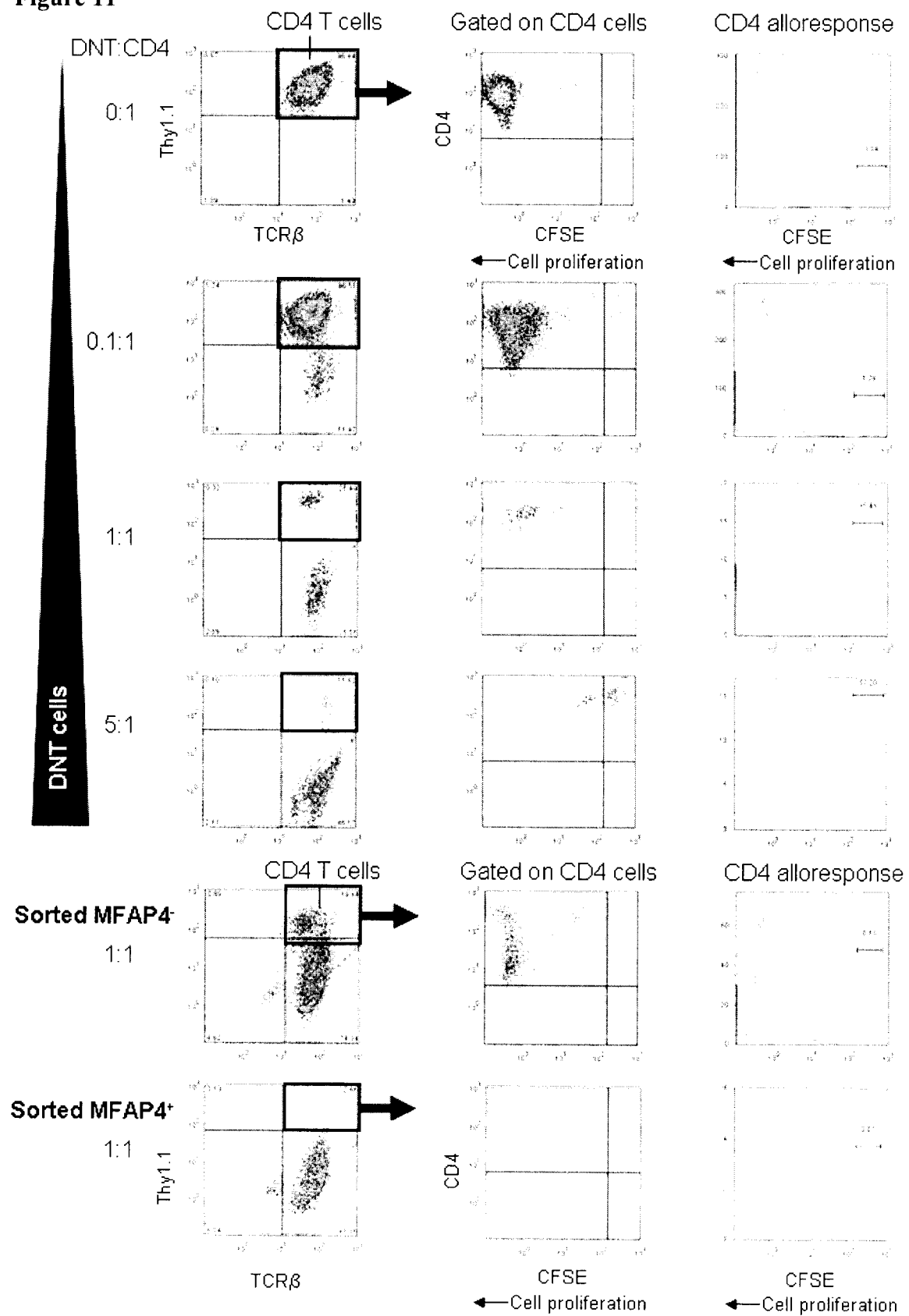
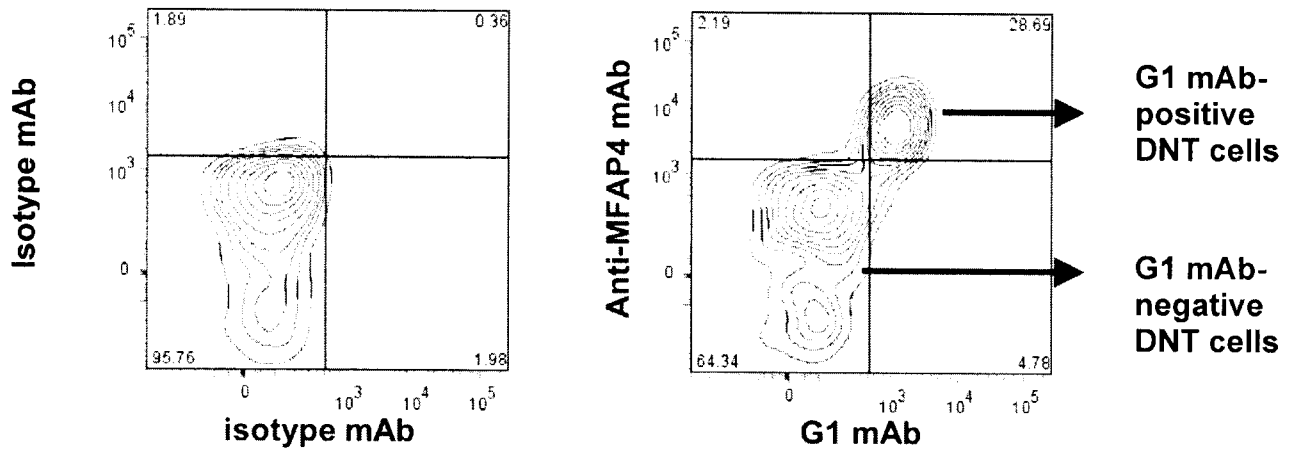


Figure 12



B Killing activity against A20 B cell lymphoma line by DNT cells sorted based on G1 mAb marker

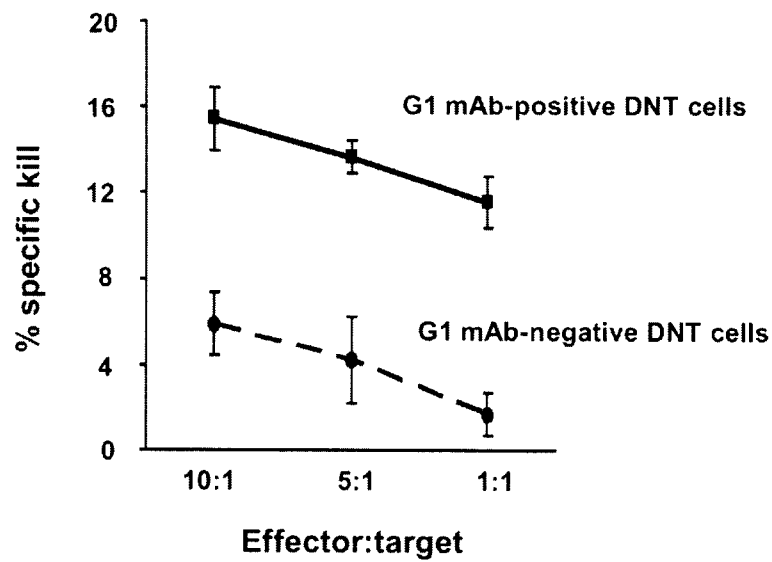


Figure 13**Human MFAP4*****Protein (SEQ ID NO:1)***

1 mkallalpll llstppcap qvsgirgdal erfclqqpld cddiyaaggyq sdgvyliyps
61 gpsvpvpvfc dmtteggkwt vfqkrfngsv sffrgwndyk lgfgradgey wlglnmhl
121 tlkqkyelrv dledfennta yakyadfsis pnavsaeedg ytlfvagfed ggagdslyh
181 sgqkfstfdr dqdlfvqnca alssgafwfr schfanlngf ylggshlsya nginwaqwk
241 fyyslkrtem kirra

mRNA (SEQ ID NO:2)

1 agccactctg agcagaactg acagcatgaa ggcactectg gccctgccgc tgetgtget
61 tctctccacg cccccgtgtg cccccaggt ctcggggtac cgaggagatg ctctggagag
121 gttttgccct cagcaacccc tggactgtga cgacatctat gccagggct accagtcaga
181 cgcgctgtac ctcatctacc ctcggggccc cagtgtgcct gtgcccgtct tctgtgacat
241 gaccaccgag ggcgggaagt ggacgggttt ccagaagaga ttcaatggct cagtaagttt
301 ctccgcggcg tggaatgact acaagctggg ctccggccgt gctgatggag agtactggct
361 ggggctgcag aacatgcacc tctgacact gaagcagaag tatgagctgc gactggactt
421 ggaggacttt gagaacaaca cggcctatgc caagtacgt gacttctcca tctccccgaa
481 cgcggtcagc gcagaggagg atggctacac cctctttgtg gcaggctttg aggatggcgg
541 ggcaggtgac tccctgtcct accacagtgg ccagaagttc tctaccteg accgggacca
601 ggacctcttt gtgcagaact ggcagctct ctctcagga gccttctggt tccgcagctg
661 ccactttgcc aacctcaatg gcttctacct aggtggetcc cactctctt atgccaatgg
721 catcaactgg gccagtgga agggcttcta ctactccctc aaacgcactg agatgaaaat
781 ccgccgggccc tgaagggtg gccccctcag gcaccttcc tccctggac acccatggc
841 tccatgagt ctcctctgc tgccccgat gcatgttct getgattccc gacaccaac
901 tcttacaag ggggccttgt ggctctcagc catgccacat cctgtcaca caccagggc
961 atccattcct aagccagacc cggtccct acacctgaag ttactgcc agcagttccc
1021 caggcctctt ccgagaggca catggttcta gcctggacct ggctgggctc catgagaatg
1081 agttgctcc accctgtccc aacagctgac agccaggagc cactctccca gctgcaggcc
1141 tttgtgtcc atctgtcct gcttctcacc tgtggacccc tgtctgggccc accctagtgt
1201 gctaagctga gcagtgcagt gtgaacaggg cccatggtgt attctaggcc acagcccagc
1261 actcctctgg gctgcttca aaccatgtcc catctcagc atccctccca ccaacttact
1321 cccctgtggt gactaccgtg gaaccccagc ccacctcact atcatactca gttccccctg
1381 atggcccat ccagcccctg aagctctatg ccaagaacac agctaccgca caccaccctg
1441 aaacagccac agccaaggta ggcattcata tgaggtcttc cccataacct ctgggtgttg
1501 agaggtttag ccacatgagg gagcagagga caatctctgc agggctggga gtgggtaggg
1561 actgaaggte tcaataaacc ttcagaacct gaatgaactg gttcataca cacaacata
1621 ttgtttatc ccccaaatgt aggcacctgg ctctccttg ctccctgct gatggtgtcc
1681 taccgccaac tccaaaatt acactggag tcaggtgcag aagggaacct tgtatttacc
1741 aggcctcatt ttgatggcaa aaagacagt taataataac ataataataa taaaaatata
1801 atactgaaaa ggaaaaaaaa aaaaaaaaaa

Figure 13 – Continued**Gene (SEQ ID NO:3)**

1 agccactctg agcagaactg acagcatgaa ggtacggggc ccagggtcgg gggactcata
61 gcatggggga actgagccca ctccagaggc cctggccac agagggcact atgaaggcac
121 aaggagtctt ctgaaactc gtaagctatg agggtagaca gcgtcccaga gagtgggggt
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241 gcaagggcag tatgtcatg catggtgggg gcaaatgcaa agagaacagg ggatctctgg
301 agctgggaag gggctgtgct gacctgtct cccatcgcc aggcactct ggccctgccg
361 ctgctgctgc ttctctccac gccccgtgt gccccccagg tctccgggat ccgaggagat
421 ggtaagcagc cccacactca cagacgcggc ctggcccaca ggttgtgcaa gaagaggctg
481 gactgtcaga acagactgtc agggccgagc cccagctctg caggactcag gtctctctt
541 taaaaatgg ggcagatgga tgacataagc attgtctatc tccctgaag aaattctcca
601 tgactccttg tgtcaaatg cccagcccc agggactgag gcaaaccttg ggggtgctgag
661 gtgtcagtga tgccatagtc ccactctct cactgtccc atgtccctac cccagctct
721 ggagagggtt tgccttcagc aaccctgga ctgtgacgac atctatgcc agggctacca
781 gtcagacggc gtgtacctca tctacccctc gggccccagt gtgctgtgc ccgtctctg
841 tgacatgacc accgagggcg ggaagtggac ggtgagtggg agggagggaac agaggccaga
901 gctggttcta atcccagccc tgtgacctg ggcttcacat atgcgaacct cagtttctt
961 atcttataaa tggggataat gatacccaca gcacagcttc tgagcagctg ccaacgctta
1021 attctaagt cctgaggtag gtgtttgtt gttgtttt aggcggagcc tctctgtc
1081 atccaggctt gagtgcagt gctccatctc agtctactgc tggaaactcc acctcccagg
1141 ttcaaaagat tctctgcct caggctccc agtagctggg attacaggca tgtgccaca
1201 tgcccagcta attttttat ttttagtaga gatgagggtt caccatgtt gccaaactgg
1261 ccttgaactc ctgacctcag gtgatccgc cgcctcagcc tccaaagtg ctgagataac
1321 aggcgtgagc caccgcgcc agctgaggtg ggttaatgca cataagcctc acaataatcc
1381 tgggaagttag tgaggaacta aagcccagag aggtgaagta acttggccag atcccacagc
1441 taggcactcg aactctagaa cgcctccctc ttagcctctt tctgcctct atacgaaact
1501 gggtatgaag agcccagggt ggtgctggca actgctccac agggggcctg gtgccaatgt
1561 ggacgttgtt ttagcaacc cactcccacc acctcccca ccccaacc agtctttaa
1621 caaactggcc acagcatcac aggtccatg ctgaggttg gcagggcagg accagaacct
1681 tgccattgg accttgatg atccagtctc atctctctg ctccagggtt tccagaagag
1741 attcaatggc tcagtaagt tctccgcgg ctggaatgac tacaagctgg gcttcggccg
1801 tgtgatgga gactactggc tgggtaaggc agggaccagg tgggtgagg ggctgcccc
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2041 gctacacct cttgtggca ggtttgagg atggcggggc aggtataacc cgtgttagc
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2341 tcagggaggg tgccccatc ccgccaggct gccacgtgat atctctaca gtctcattg
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2461 tctctgcca ggtgactccc tgtctacca cagtggccag aagtctcta ccttcgaccg
2521 ggaccaggac ctcttgtgc agaactgcgc agctctctc tcaggagct tctggttccg

Figure 13 – Continued

2581 cagctgccac ttgccaacc tcaatggctt ctacctaggt ggetcccacc tctttatgc
2641 caatggcatc aactgggccc agtgggaaggg ettctactac tccctcaaac gcactgagat
2701 gaaaatccgc cgggcctgaa gggctggccc cctcaggcac ctttctccc ctggacaccc
2761 atggtetcca tgagtgtcc ctctgtgcc cctgatgat gcttctgtg attcccagc
2821 accaactcct tacaaggggg cttgtggtt ctacgcatg ccacatccct gtcacacacc
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3061 caggcctttg tggccaatc tgcctgctt cctactgtg gacctgtg tgggccaccc
3121 tagtgtgcta agctgagcag tgcagtgtga acagggccca tgggtgtatc taggccacag
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3301 cccctgatgg cccatcccag cccctgaagc tctatgcaa gaacacagct accgcacacc
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3481 gtagggactg aaggtctcaa taaacctca gaacctgaat gaactggctt catacacaca
3541 aacatatttg ttatcccc aaatgtaggc acctggctc tcttctcc cctgctgatg
3601 gtgtctacc ccgaactcca aaaattacac ctggagtcag gtgcagaagg gaaccttgta
3661 ttcacaggc ctcatcttga tggcaaaaag acagtgtaat aataacataa taataataaa
3721 aatataatac tgaaaagga

Figure 14**Mouse MFAP4****Protein (SEQ ID NO:4)**

1 mkalpalplm lmllsmpppc apqasgird aleksclqqp ldcdiyaag yqedgvyliy
61 pygpsvpvpv fcdmtteggk wtvfqkrfng svsfirgwsd yklgfggradg eywlglnlh
121 ltlkqkyel rvdledfenn tayakyidfs ispnaisae dgytlyvagf edggagdsls
181 yhsgqkfstf drdqdlfvqn caalssgafw frschfanln gfylggshls yanginwaqw
241 kgfyyslkrt emkirra

mRNA (SEQ ID NO:5)

1 ccagacagc cagcctctc caactgagct gacacatga aggcctccc agcctgcca
61 ctgatgctga tctgctctc catgcctccc cctgcgccc cgcaagcctc tgggatccgg
121 ggagatgctc tggagaagtc ctgtctcag caaccctgg actgtgatga tatctacgcc
181 cagggtatc aggaagacgg cgtgtatctc atctaccct atggccccag tgtgccggtg
241 cccgtctct ggcacatgac aactgagggc ggcaagtga cggttttcca gaaaagattc
301 aacggctcag tgagttctt ccggggctgg agcgactaca agctgggctt tggccgtgct
361 gacggggagt actggctggg gctgcagaac ctgcacctc tgacactgaa gcagaagtat
421 gagctgcgcg tggacttga agactttgag aacaacacag cctatgcaa gtacattgac
481 ttctccatc ccccaacgc catcagtgt gaggaggatg gctataccct ctacgtggct
541 ggcttcgagg atggcggggc aggtgactca ctgtctacc acagtggcca gaagttctc
601 accttgatc gggaccagga cctctcgtg cagaactgtg cagcctctc ctacaggacc
661 ttctggttc gaagtgcga ttctgccaat ctcaacggtt tctacctggg tggttccat
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1141 ttctgttgg ggtacctc gacagagtc tgcagacct gtggcattgc caatcagtc
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1261 ctcatcatt tactccctac gtgagcatc tggagcccaa tccatctgc ctctactat
1321 cctcaaaaat taccaccaa acaatactc accacggcta ctgtcaact ctgaagtcgt
1381 catggcaaag ataggttgt tgacttggc cctacttgc ctagcgatc gtcagagag
1441 gcagcaggga tcaatatgt gggctggaag tgggtggga gcagaggtct caataaact
1501 caggatctga tgg

Gene (SEQ ID NO:6)

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61 aagaccattt gtggagacaa gccacttgc agataggga cagcgaagct cagtggagt
121 ctctgaaac ttaagttctg gggatattg gccttctct gaaagttgag agggggtaaa
181 aaagacagag ggcagagttc aggcaggag agccttggga ggtgggaggc gtctggccca
241 tgttggggc tacactacc ccatttcca tcccaggec ctcccagccc tgcactgat
301 gctgatgtg ctctccatg ctccccctg cgccccgaa gcctctggga tccggggaga

Figure 14 – Continued

361 tggtaagcag cccccattca cagacacggc ctggtcagca ggctggatga ggccattcta
421 agtgaggctc tgcaggactg ctctctctc tacaaagtgg caggatccc cctagagaaa
481 tctccagatt tctctgata gagtccctta ggctggagc aaaactggga tatggagggg
541 ggtgtcctta tgccttagtc ttctctca cggccccac actgccactc gcagctctgg
601 agaagtcctg tctcagcaa cccctggact gtgatgatat ctacgccag ggctatcagg
661 aagacggcgt gtatctcatc taccctatg gccccagtgt gccgggtgcc gtctctgcg
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781 gggttcaggc agctgcgca tcccggctgt catctctgtg aagtggattt tcatctca
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1081 cttatagac caggtggcc tgaattcag aaatctgcct gcctctgcct cccgattgt
1141 gggattaaag gcgtgggcca ccacggcgg cctctctgt ggtttatca tgcagtctg
1201 ggatggaacc caggccctc aagctaagt gggtcttgc cactaagcta tgtctacagg
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1621 gccaccctc cctagggctg cagaacctgc acctctgac actgaagcag aagtatgagc
1681 tgcgctgga ctggaagac ttgagaaca acacagccta tgccaagtac attgactct
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2521 cctcaagcg cacggagatg aaaatctgc gggcctgagg ggctggcca agcaggcccc
2581 atcttcccc tgaagtcca aggtccatg ttctctcc acgtttacc cacaattct
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2761 tggatctggc tgaaatccat taaaactcc aagtgtctc taccctca cgacagctga
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2881 gcagagcctt ctgttggg ctacctga cagagctatg cagcactgt ggcattgcca
2941 atcagctct gcactgcc acaccagc actcacatag ctctctcag aatactct

Figure 14 – Continued

3001 accttgccct catcacttta ctcctacgt gagcatcatg gagcccaatc ccactgcct
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3181 catgagaggc agcagggatc aatatgtggg gctggaagtg ggtgggtagc agaggtctca
3241 ataaacttca ggatctgatg g

Figure 15

		1	50
VH4-27	(1)	MEFGFSLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
VH4-26	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
VH4-23	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
VH4-12	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
VH4-5	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
VH4-4	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
Consensus	(1)	MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSN	
		51	100
VH4-27	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
VH4-26	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
VH4-23	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
VH4-12	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
VH4-5	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
VH4-4	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
Consensus	(51)	YGMWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYL	
		101	150
VH4-27	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLAPGS	
VH4-26	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLAPWK	
VH4-23	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLVPWK	
VH4-12	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPL----	
VH4-5	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLVPGS	
VH4-4	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLAPGS	
Consensus	(101)	QMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLAPGS	
		151	
VH4-27	(151)	L- (SEQ ID NO:18)	
VH4-26	(151)	LG (SEQ ID NO:16)	
VH4-23	(151)	LG (SEQ ID NO:14)	
VH4-12	(147)	- (SEQ ID NO:12)	
VH4-5	(151)	L- (SEQ ID NO:10)	
VH4-4	(151)	L- (SEQ ID NO:8)	
Consensus	(151)	L (SEQ ID NO:19)	

P3CZG1 VH domain consensus amino acid sequence:

MDSRLNLVFLVLFIKGVQCEVKLVESGGGLVQPGRSLKLSCAASGFPPFSNYGMAWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYLQMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSSARTTAPPVYPLAPGS (SEQ ID NO:19)

Signal peptide is in *italics*

Variable domain

EVKLVESGGGLVQPGRSLKLSCAASGFPPFSNYGMAWVRQAPT KGLAWVATISYDGRSTYYRDSVKGRFTISRNNAKSTLYLQMDSLRS EDTATYYCVRHEL PEDHWGQGVMVT VSS (SEQ ID NO:20)

Complementarity Determining Regions

CDR1: GFPFSNYG (SEQ ID NO:21)

CDR2: ISYDGRST (SEQ ID NO:22)

CDR3: VRHEL PEDH (SEQ ID NO:23)

Figure 16

		1		50
VL3-17	(1)	MVLMSLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-20	(1)	MVLMSLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-3	(1)	MVLMSLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-5	(1)	-GSYALLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-2	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-21	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-22	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-31	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
VL3-4	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
Consensus	(1)	MVLISLLFWVYGT	CGDIVMTQSPFSLAVSE	GEMVTINCKSGQSLLSSGNQ
		51		100
VL3-17	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-20	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-3	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-5	(50)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-2	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-21	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-22	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-31	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
VL3-4	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
Consensus	(51)	KNYLAWYQQKPGQSP	PKLLIYYSSSTRQSGVP	DRFIGSGSGTDFTLTISDVQ
		101		146
VL3-17	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:25
VL3-20	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:27
VL3-3	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:29
VL3-5	(100)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:31
VL3-2	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:33
VL3-21	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:35
VL3-22	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:37
VL3-31	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG SEQ ID NO:39
VL3-4	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSK-- SEQ ID NO:41
Consensus	(101)	AEDLADYYCLQHYSS	PFTFGSGTKLEIKRADA	AAPTVSIFPPSSKLG (SEQ ID NO:42)

P3CZG1 VL domain consensus amino acid sequence:

*MVLISLLFWVYGT*CGDIVMTQSPFSLAVSEGEMVTINCKSGQSLLSSGNQKNYL
AWYQQKPGQSPKLLIYYSSSTRQSGVPDRFIGSGSGTDFTLTISDVQAEDLADY
YCLQHYSSPFTFGSGTKLEIKRADAAPTVSIFPPSSKLG (SEQ ID NO:42)

Signal peptide is in *italics*

Variable domain:

DIVMTQSPFSLAVSEGEMVTINCKSGQSLLSSGNQKNYLAWYQQKPGQSPKL
LIYYSSSTRQSGVPDRFIGSGSGTDFTLTISDVQAEDLADYYCLQHYSSPFTFGS
GTKLEIK (SEQ ID NO:43)

Complementarity Determining Regions

CDR1: QSLLSSGNQKNY (SEQ ID NO:44)

CDR2: YSS (SEQ ID NO:45)

CDR3: LQHYSSPFT (SEQ ID NO:46)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2009/000197

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C07K 16/28, A61K 35/14, A61K 39/395, A61P 35/00, A61P 35/02, A61P 37/06, C07K 16/18, C12N 15/13, C12N 5/00, G01N 33/567, G01N 33/574, C07K 14/705* (all, 2006.01)

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)

IPC: *C07K 16/28, A61K 35/14, A61K 39/395, A61P 35/00, A61P 35/02, A61P 37/06, C07K 16/18, C12N 15/13, C12N 5/00, G01N 33/567, G01N 33/574, C07K 14/705* (all, 2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent Database, Delphion, Pubmed, GQPAT, Swiss-Prot, TrEMBL, RefSeq Protein, GenPept, IPI, ENSEMBL, Drug Bank. **Terms:** MFAP4, microfibril-associated, antibody, anti-cancer, regulatory, cell, double negative T cell, disease, therapy, humanized.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THOMSON, C. W. ET AL. Double-Negative Regulatory T Cells. Immunol., Res. 2006, Vol. 35, No. 1-2, pages 163-77. whole document	22-27
X	WO 2007/056854 A1 ZHANG, L. ET AL. 24 May 2007 whole document	22-27
X	US 7214493 B2 KUNKEL, M. J. ET AL. 8 May 2007 whole document	28, 29, 36, 38, 39 and 41 to 45
X	EP 1033406 A1 SAKAGUCHI, N. 6 Sept. 2000	28, 29, 37 to 39, 41, 42, 44
Y	whole document	43

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search

8 May 2009 (08-05-2009)

Date of mailing of the international search report

23 June 2009 (23-06-2009)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
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Facsimile No.: 001-819-953-2476

Authorized officer

Mimi Yurack 819- 994-5077

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2009/000197**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. ☒ Claim Nos. : 3 and 6 to 21, as depends on claim 3
because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 3 and 6 to 21, as depends on claim 3, encompass a method of treatment of the human or animal body by surgery or therapy which this Authority is not obliged to search under Rule 39.1(iv) of the PCT, the search has been carried out based on the alleged effects or purposes/uses of the agent defined therein.
2. ☐ Claim 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. ☐ Claim 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 :

This Authority found that unity of invention is not complied with but chose not to invite the applicant to pay additional fees.

Continued in Supplemental box.

1. ☐ As all required additional 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 additional fees, this Authority did not invite payment of 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 claim 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 claim 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

International application No.
PCT/CA2009/000197

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RIECHMANN, L. ET AL. Reshaping Human Antibodies For Therapy. Nature, 24 March 1988, Vol. 332, pages 323-327. whole document	43
A	ZHAO, Z. ET AL. The gene for a human microfibril-associated glycoprotein is commonly deleted in Smith-Magen's syndrome patients. Hum. Mol. Genetics, 1995, Vol. 4, No. 4, pages 589-597. whole document	1 to 45
A	MEZA-ZEPEDA, L. A. Array Comparative Genomic Hybridization Reveals Distinct DNA Copy Number Differences between Gastrointestinal Stromal Tumors and Licomysarcomas. Cancer Res., 15 Sept. 2006, vol. 66, No. 18, pages 8984-8993. whole document	1 to 45
A	LAUSEN, M. ET AL. Microfibril-associated Protein 4 Is Present in Lung Washings and Binds to the Collagen Region of Lung Surfactant Protein D. J. Biol. Chem., 5 Nov. 1999, Vol. 274, No. 45, pages 32234-40. whole document	1 to 45
A	SCHLOSSER, A. ET AL. Microfibril-associated Protein 4 Binds to Surfactant Protein A (SP-A) and Colocalizes with SP-A in the Extracellular Matrix of the Lung. Scand. J. Immunol., 2006, Vol. 64, pages 104-116.	1 to 45

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2009/000197

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO 2007056854A1	24-05-2007	CA 2629532A1	24-05-2007
		CN 101313061A	26-11-2008
		EP 1948788A1	30-07-2008
		US 2009098095A1	16-04-2009

US 7214493B2	08-05-2007	AU 2003259294A1	16-02-2004
		AU 2003259294A8	16-02-2004
		EP 1539237A2	15-06-2005
		EP 1539237A4	24-05-2006
		IS 7668A	27-01-2005
		JP 2006500921T	12-01-2006
		NO 20050416A	02-03-2005
		PL 375144A1	28-11-2005
		US 6887673B2	03-05-2005
		US 2004105855A1	03-06-2004
		US 2005202022A1	15-09-2005
		WO 2004010947A2	05-02-2004
		WO 2004010947A3	23-09-2004

EP 1033406A1	06-09-2000	EP 1033406A4	09-03-2005
		WO 9922008A1	06-05-1999

Supplemental Box.

The International Searching Authority found multiple inventions in the present international application, as follows:

1. Claims 1 to 27 (in whole): A method of detecting, selecting or enriching, or activating regulatory and/or anti-cancer cells and use of said regulatory and/or anti-cancer cells for treating or preventing disease.
2. Claims 28 to 30, 33 to 36, 38 and 40 to 45 (all in part): A light chain (LC) complementarity determining region (CDR)1 comprising the sequence SEQ ID NO:44 and related nucleic acid; an isolated variable region comprising said LC CDR1 and related nucleic acid, vector and host cell; and a binding agent comprising said LC CDR1 and use of said binding agent for modulating immune response or for modulating cancer.
3. Claims 28 to 30, 33 to 36, 38 and 40 to 45 (all in part): A LC CDR2 comprising the sequence SEQ ID NO:45 and related nucleic acid; an isolated variable region comprising said LC CDR2 and related nucleic acid, vector and host cell; and a binding agent comprising said LC CDR2 and use of said binding agent for modulating immune response or for modulating cancer.
4. Claims 28 to 30, 33 to 36, 38 and 40 to 45 (all in part): A LC CDR3 comprising the sequence SEQ ID NO:46 and related nucleic acid; an isolated variable region comprising said LC CDR3 and related nucleic acid, vector and host cell; and a binding agent comprising said LC CDR3 and use of said binding agent for modulating immune response or for modulating cancer.
5. Claims 28 to 30, 33 to 35, 37, 38 and 40 to 45 (all in part): A heavy chain (HC) CDR1 comprising the sequence SEQ ID NO:21 and related nucleic acid; an isolated variable region comprising said HC CDR1 and related nucleic acid, vector and host cell; and a binding agent comprising said HC CDR1 and use of said binding agent for modulating immune response or for modulating cancer.
6. Claims 28 to 30, 33 to 35, 37, 38 and 40 to 45 (all in part): A HC CDR2 comprising the sequence SEQ ID NO:22 and related nucleic acid; an isolated variable region comprising said HC CDR2 and related nucleic acid, vector and host cell; and a binding agent comprising said HC CDR2 and use of said binding agent for modulating immune response or for modulating cancer.
7. Claims 28 to 30, 33 to 35, 37, 38 and 40 to 45 (all in part): A HC CDR3 comprising the sequence SEQ ID NO:23 and related nucleic acid; an isolated variable region comprising said HC CDR1 and related nucleic acid, vector and host cell; and a binding agent comprising said HC CDR3 and use of said binding agent for modulating immune response or for modulating cancer.
8. Claim 31 (in whole) and claims 33 to 35 and 39 to 45 (all in part): A light chain variable region (VL) which comprises the sequence SEQ ID NO:43 and related nucleic acid, vector and host cell; and a binding agent comprising said VL and use of said binding agent for modulating immune response or for modulating cancer.
9. Claim 32 (in whole) and claims 33 to 35 and 39 to 45 (all in part): A heavy chain variable region (VH) which comprises the sequence SEQ ID NO:20 and related nucleic acid, vector and host cell; and a binding agent comprising said VH and use of said binding agent for modulating immune response or for modulating cancer.

According to Article 3(4)(III)PCT and Rule 13 PCT, a group of inventions may be claimed in one international application provided there is a technical relationship among those inventions involving one or more special technical features. However, the inventions listed above fail to share a common special technical feature, and therefore, they are viewed as encompassing multiple inventions