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(54) Title: MYELOID-DERIVED SUPPRESSOR CELL-SPECIFIC PEPTIDES FOR DIAGNOSTIC AND THERAPEUTIC USE

(57) Abstract: Provided herein are novel diagnostic and therapeutic MDSC-specific peptide-Fc fusion (peptibody) reagents for the depletion of blood, splenic, and intratumoral MDSC.



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DESCRIPTION

MYELOID-DERIVED SUPPRESSOR CELL-SPECIFIC PEPTIDES FOR

DIAGNOSTIC AND THERAPEUTIC USE

BACKGROUND OF THE INVENTION

5 **[0001]** The present application claims the priority benefit of United States provisional application number 61/884,581, filed September 30, 2013, the entire contents of which are incorporated herein by reference.

[0002] The invention was made with government support under Grant No. P50 CA136411 awarded by the National Institutes of Health. The government has certain rights
10 in the invention.

1. Field of the Invention

[0003] The present invention relates generally to the fields of immunology and cancer biology. More particularly, it concerns peptides and peptibodies for use in depleting myeloid-derived suppressor cells in a subject.

15 2. Description of Related Art

[0004] Activating the immune system has emerged as a promising cancer treatment. Recent positive Phase III clinical trials of therapeutic cancer vaccines include FDA-approved Sipuleucel-T prostate cancer vaccine, melanoma peptide vaccines, and personalized lymphoma vaccines (Cheever and Higano, 2011; Schwartzentruber *et al.*, 2011; Schuster *et al.*, 2011). However, tumor-induced immune suppression remains an obstacle limiting their
20 *al.*, 2011). However, tumor-induced immune suppression remains an obstacle limiting their potency. Myeloid-derived suppressor cells (MDSC) are heterogeneous cells that co-express Gr-1 and CD11b myeloid lineage differentiation markers (Marigo *et al.*, 2008; Gabrilovich and Nagaraj, 2009; Peranzoni *et al.*, 2010). Functional studies showed that MDSC are potent inhibitors of T-cells in mice (Bronte and Zanoello, 2005; Rodriguez *et al.*, 2002; Ochoa *et al.*, 2007; Ugel *et al.*, 2009), but more specific surface markers that would allow isolation of
25 viable cells would facilitate additional studies to precisely understand tumor-MDSC interactions in the microenvironment. Up to now, identification of MDSC has relied on a combination of cell surface molecules, none of which is MDSC specific. Lack of MDSC-specific markers makes it difficult to deplete those immune suppressive cells that contribute
30 to the immune escape mechanism of tumor cells. Therefore, there is a need for MDSC-

specific peptides and peptibodies for identifying and depleting of MDSC. New specific markers are also needed for targeting MDSC *in vivo* to test the hypothesis that MDSC inhibition enhances antitumor immunity (Marigo *et al.*, 2008; Ugel *et al.*, 2009).

SUMMARY OF THE INVENTION

5 **[0005]** Provided herein are 12-mer linear peptides that specifically bind to myeloid-derived suppressor cells (MDSC). These MDSC-specific binding peptides may be used to develop reagents, such as peptibodies, for diagnostic and therapeutic use.

[0006] In one embodiment, a peptibody is provided that comprises a peptide that specifically binds to a myeloid-derived suppressor cell (MDSC), said peptide being fused to
10 an Fc portion of an IgG antibody. In various aspects, the Fc may be from an IgG1, IgG2, or IgG3 antibody. In some aspects, the Fc portion is derived from a human or a mouse IgG antibody. In some aspects, the peptibody may be conjugated to an imaging agent (*e.g.*, fluorochrome) or a radioligand.

[0007] In one aspect, the MDSC may be a human MDSC and the peptide may
15 specifically bind to said human MDSC.

[0008] In one aspect, the MDSC may be a mouse MDSC. The peptide that specifically binds to a mouse MDSC may have a sequence according to either SEQ ID NOs: 1 or 2. In certain aspects, this peptide may be comprised in a peptibody having a sequence that is at least 90%, 95%, 98%, or 99% identical to the sequence of either SEQ ID NOs: 3 or
20 4. In other certain aspects, the peptibody may have a sequence that is the sequence of SEQ ID NOs: 3 or 4.

[0009] In another embodiment, there is provided a nucleic acid comprising a nucleotide sequence that encodes a peptibody of the present embodiments. In one embodiment, said nucleic acid may be comprised within an expression vector. In yet another
25 embodiment, said expression vector may be comprised within a host cell (*e.g.*, bacterial cell, a fungal cell, an insect cell, or a mammalian cell).

[0010] In one embodiment, there is provided a method of producing a peptibody of the present embodiments comprising the peptibody in a host cell (*e.g.*, bacterial cell, a fungal cell, an insect cell, or a mammalian cell) and purifying the polypeptide therefrom.

[0011] In one embodiment, a pharmaceutical formulation is provided comprising a peptibody of the present embodiments in a pharmaceutically acceptable carrier.

[0012] In another embodiment, a method of depleting MDSC in a subject is provided comprising administering to the subject a formulation of the present embodiments, or an agent that specifically binds to a cell surface S100 protein on a MDSC. In one aspect, the method is a method of inhibiting tumor growth. In another aspect, the method is a method of treating cancer.

[0013] In some aspects, the cancer may be a breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, brain cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, adrenal cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer, blood cancer, or skin cancer. The cancer may be metastatic, recurrent, or multi-drug resistant.

[0014] In one aspect, the subject may be a human patient. In another aspect, the subject may be a non-human mammal.

[0015] In some aspects, the formulation may be administered systemically, intravenously, intradermally, intraperitoneally, intramuscularly, subcutaneously, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a catheter, or via a lavage.

[0016] In some aspects, the method may further comprise administering at least a second therapy to the subject, wherein the second therapy is an anticancer therapy (*e.g.*, a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy or cytokine therapy).

[0017] In one aspect, the formulation may be administered at least a second time. In one aspect, the formulation may be administered over a period of 1 week to 6 months.

[0018] In one embodiment, a method is provided for identifying a MDSC in a patient sample. The method comprises (a) obtaining a sample from a subject; (b) contacting the sample with an agent that specifically binds to a cell surface S100 protein on a MDSC; and (c) detecting binding of the agent to a cell in the sample, wherein said binding identifies the cell as a MDSC.

[0019] In one aspect, an agent that specifically binds to a cell surface S100 protein on a MDSC may be a peptide. The peptide may have a sequence according to either SEQ ID NOs: 1 or 2. In one aspect, the peptide may be conjugated to an imaging agent or radioligand. In one aspect, detecting binding may comprise detecting binding of the imaging agent or radioligand to the cell. In some aspects, the peptide may be comprised within a peptibody of the embodiments.

[0020] In some aspects of the present embodiments, an agent that specifically binds to a cell surface S100 protein on a MDSC may be an antibody, such as IgG, IgM, IgA, IgD, IgE, or a polypeptide comprising an antibody CDR domain that retains S100-binding activity. The antibody may be a chimeric antibody, an affinity matured antibody, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, or an antigen-binding antibody fragment or a natural or synthetic ligand. Examples of antibody fragments suitable for the present embodiments include, without limitation: (i) the Fab fragment, consisting of V_L , V_H , C_L , and C_{H1} domains; (ii) the “Fd” fragment consisting of the V_H and C_{H1} domains; (iii) the “Fv” fragment consisting of the V_L and V_H domains of a single antibody; (iv) the “dAb” fragment, which consists of a V_H domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments; (vii) single chain Fv molecules (“scFv”), wherein a V_H domain and a V_L domain are linked by a peptide linker that allows the two domains to associate to form a binding domain; (viii) bi-specific single chain Fv dimers; and (ix) diabodies, multivalent or multispecific fragments constructed by gene fusion. Fv, scFv, or diabody molecules may be stabilized by the incorporation of disulphide bridges linking the V_H and V_L domains. Minibodies comprising a scFv joined to a CH3 domain may also be made.

[0021] In one embodiment, a method is provided for identifying a MDSC in a patient sample. The method comprises (a) obtaining a sample from a subject; (b) contacting the sample with a peptide that specifically binds to a MDSC; and (c) detecting binding of the peptide to a cell in the sample, wherein said binding identifies the cell as a MDSC. The peptide may have a sequence according to either SEQ ID NOs: 1 or 2. In one aspect, the peptide may be conjugated to an imaging agent or radioligand. In one aspect, detecting may comprise detecting binding of the imaging agent or radioligand to the cell. In some aspects, the peptide may be comprised within a peptibody of the embodiments.

[0022] In one embodiment, a method of identifying an MDSC is provided, the method comprising (a) obtaining a sample from a subject; (b) contacting the sample with a peptibody of the embodiments; and (c) detecting binding of the peptibody to a cell in the sample, wherein said binding identifies the cell as a MDSC. In one aspect, the subject is a human patient. In some aspects, the sample is a tumor sample, a spleen sample, or a serum sample.

[0023] In one embodiment, a kit is provided comprising a peptide or peptibody of the present embodiments.

[0024] In one embodiment, a composition comprising a peptibody or a nucleic acid encoding a peptibody is provided for use in the treatment of a cancer in a subject. In another embodiment, the use of a peptibody or a nucleic acid encoding a peptibody in the manufacture of a medicament for the treatment of a tumor is provided. Said peptibody may be any peptibody of the embodiments.

[0025] In one embodiment, a composition comprising a peptide or peptibody is provided for use in identifying a MDSC in a sample from a subject. In one aspect, the peptide or peptibody may be conjugated to an imaging agent or radioligand. Said peptibody may be any peptibody of the embodiments.

[0026] Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well.

[0027] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising,” the words “a” or “an” may mean one or more than one.

[0028] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

[0029] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0030] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0032] **FIGs. 1a-d. Identification and characterization of MDSC-binding peptides.** (FIG. 1a) Identification of Gr-1⁺CD11b⁺ MDSC in spleens of *C57BL/6* mice ($N = 5$) challenged subcutaneously with EL4 mouse lymphoma cells for three weeks. Double positive cells contain two distinct populations including Gr-1^{high}CD11b⁺ granulocytic (P7) and Gr-1^{int}CD11b⁺ monocytic (P10) MDSC subsets. (FIGs. 1b-c) Biopanning with Ph.D.-12 peptide phage display library on Gr-1- and CD11b-labeled splenocytes showed enriched phage eluted from sorted MDSC subsets. Biopanning enrichment was expressed in either “Number of plaques / 10⁶ cells” or phage “Output / Input ratio” ($\times 10^{-8}$). (FIG. 1d) Binding of synthetic FITC-conjugated G3 and H6 peptides on Gr-1⁺CD11b⁺ gated MDSC from EL4-bearing *C57BL/6* mice ($N = 4$), compared with Gr-1⁻CD11b⁻ gated non-MDSC splenocytes. A non-specific peptide (irrel peptide) was used as a negative control to exclude non-specific binding. The data are representative of three identical experiments.

[0033] **FIGs. 2a-g. Generation and characterization of MDSC-specific peptibodies.** (FIG. 2a) Schematic representation of peptibody construction. (FIG. 2b) Characterization of recombinant peptibodies (Pep-H6, Pep-G3 and a control Pep-irrel) that were purified using Protein A chromatography. The identity of peptibodies was verified by

Western blot using HRP-conjugated anti-mouse IgG (left) or anti-His tag antibodies (right). (FIG. 2c) Binding of FITC-conjugated Pep-H6 or Pep-G3 on $CD11b^+Ly6G^+Ly6C^{int/low}$ gated granulocytic MDSC and $CD11b^+Ly6G^-Ly6C^{high}$ gated monocytic MDSC in splenocytes from EL4-bearing *C57BL/6* mice ($N = 5$). (FIG. 2d) Binding of the peptibodies with granulocytic (upper panel) and monocytic (lower panel) MDSC subsets in splenocytes from different species of mice (*C57BL/6* and *Balb/c*) challenged with various tumors ($N = 3$ for each tumor type). (FIG. 2e) Characterization of binding specificity of the peptibodies on $Ly6G^+CD11c^-$ gated granulocytic MDSC (gMDSC) versus $CD11c^+Ly6G^-$ gated DC in splenocytes pooled from EL4-bearing *C57BL/6* mice ($N = 5$). (FIG. 2f) Identification of peptibody binding on $CD11b^+Gr-1^+$ immature myeloid cells in the bone marrow from EL4-bearing *C57BL/6* mice ($N = 5$). (FIG. 2g) Co-staining of APC-labeled Pep-G3 and FITC-labeled Pep-H6 with $CD11b^+Gr-1^+$ MDSC in splenocytes pooled from EL4-bearing *C57BL/6* mice ($N = 5$). The data represent three independent experiments.

[0034] FIGs. 3a-h. Peptibodies specifically depleted tumor-induced MDSC in multiple tumor models and inhibited tumor growth *in vivo*. (FIGs. 3a-b) Depletion of $Gr-1^+CD11b^+$ MDSC in the blood and spleens in EL4-challenged *C57BL/6* mice ($N = 5$ per group) after treatment with 50 μ g peptibody i.v. for three consecutive days. Control mice received Gr-1-depleting mAb (positive control), irrelevant control peptibody (Pep-irrel), or PBS. Plots are shown for individual representative mice (FIG. 3a) and composite results (FIG. 3b) in a representative experiment out of five, with percentages of MDSC subsets indicated (g = granulocytic, m = monocytic) (mean $\pm$ s.e.m). (FIGs. 3c-d) Depletion of MDSC in the blood and subcutaneous tumors of EG.7-challenged *C57BL/6* mice ($N = 5$ per group) by peptibody treatment. Percentage of $Gr-1^+CD11b^+$ MDSC from ficoll blood and single cell suspensions prepared from harvested tumors are shown for individual representative mice (FIG. 3c) and composite results (mean $\pm$ s.e.m) (FIG. 3d) in a representative experiment out of two. (FIG. 3e) Peptibody treatment depleted MDSC *in vivo* from the blood and spleens of A20 lymphoma-challenged *Balb/c* mice (data pooled from 2 experiments). (FIG. 3f) Frequencies of $Gr-1^+CD11b^+$ MDSC, $Ly6G^-CD11c^+$ DC, $CD3^+$ T cells, $CD19^+$ B cells, and $CD3^-CD49b^+$ NK cells in spleens and $Gr-1^+CD11b^+$ immature myeloid cells in the bone marrow from peptibody-treated, EL4-bearing *C57BL/6* mice. Data are shown as the mean $\pm$ s.e.m of 5 mice per group. (FIGs. 3g-h) Inhibition of EL4 tumor growth in *C57BL/6* mice following every-other-day peptibody treatment. Tumor size is

shown as the mean $\pm$ s.d of five mice per group in a representative experiment out of four (FIG. 3g). Tumor mass data are pooled results from four independent experiments (FIG. 3h). * $P < 0.05$, ** $P < 0.01$ compared with tumor-challenged mice without peptibody treatment (PBS) by two-tailed Student's t -test.

5 **[0035] FIGs. 4a-f. Peptibodies recognize extracellular S100 family proteins on the surface of MDSC.** (FIG. 4a) Schematic representation of a strategy for peptibody-based isolation of candidate cell type-specific surface markers. (FIG. 4b) Proteomic analysis from sorted Gr-1⁺CD11b⁺ splenic MDSC from EL4-bearing *C57BL/6* mice revealing predominant peptides with homology to S100A9. The data are representative of two independent
10 experiments. (FIG. 4c) Identification of S100A9 protein in Protein A eluates of Pep-H6-bound, sorted MDSC lysate (without biotinylation) by Western blot. Recombinant mouse S100A9 protein served as a positive control (left panel), and lysates from unbound MDSC were negative controls (right panel). Input lysates were blotted with actin as an internal control. (FIG. 4d) Detection of both S100A9 and S100A8 proteins in Protein A eluates of
15 Pep-H6-bound, sorted MDSC lysate by Western blot. All Western blot data shown are representative of three individual experiments. (FIG. 4e) Binding of Pep-H6 and Pep-G3 peptibodies with CD11b⁺Gr-1⁺ gated splenic MDSC from EL4-bearing, S100A9-deficient *C57BL/6* mice ($N = 3$). The data are representative of two independent experiments. (FIG. 4f) Frequencies of CD11b⁺Gr-1⁺ splenic MDSC from EL4-bearing, S100A9-deficient
20 *C57BL/6* mice ($N = 3$) after peptibody treatment as in FIG. 3a.

[0036] FIGs. 5a-b. Peptibodies recognized MDSC in tumor-bearing mice. (FIG. 5a) Binding of FITC-conjugated Pep-H6 or Pep-G3 on Gr-1^{high}CD11b⁺ gated granulocytic MDSC (gMDSC) and Gr-1^{low}CD11b⁺ gated monocytic MDSC (mMDSC) in splenocytes from EL4-bearing *C57BL/6* mice ($N = 5$). A non-specific peptibody (Pep-irrel) was used as a
25 negative control. (FIG. 5b) Increase of MDSC in tumor-challenged mice. Splenocytes from naïve mice or mice challenged with various tumors ($N = 3$ for each tumor type) were stained for CD11b and Gr-1 to identify MDSC. Frequencies of splenic MDSC are shown as mean $\pm$ s.e.m. (** $P < 0.01$ compared with naïve mice by two-tailed student's t -test).

[0037] FIGs. 6a-c. Lack of peptibody binding on lymphocyte subsets.
30 Splenocytes pooled from EL4-bearing *C57BL/c* mice ($N = 5$) were gated on CD3⁺ T cells

(FIG. 6a), CD19⁺ B cells (FIG. 6b), and CD3⁻CD49b⁺ NK cells (FIG. 6c), respectively, and analyzed for peptibody binding.

[0038] FIG. 7. Depletion of intratumoral MDSC in EL4 tumor-challenged mice by peptibody treatment. Groups of five *C57BL/6* mice were challenged s.c. with EL4 tumor cells followed by peptibody treatment as in FIG. 3a. The percentage of Gr-1⁺CD11b⁺ MDSC from single cell suspensions prepared from tumors harvested on Day 20 is shown as mean $\pm$ s.e.m. (* $P < 0.01$ compared with PBS by two-tailed Student's *t*-test).

[0039] FIG. 8. Long-term administration did not diminish peptibody-induced MDSC depletion. As in FIG. 3g, EL4-bearing *C57BL/6* mice were treated with peptibodies every other day for two weeks. At the end of treatment, splenocytes were harvested and stained for Gr-1⁺CD11b⁺ MDSC, shown as the mean $\pm$ s.e.m. of five mice per group. Plots are shown with frequency of splenic MDSC for individual representative mice. Differences between groups were analyzed by two-tailed Student's *t*-test. The data represent two independent experiments.

[0040] FIG. 9. Pep-G3 immunoprecipitated S100 family proteins. Proteomic analysis of eluates of Pep-G3-bound, sorted MDSC lysates isolated from immobilized Protein A revealed predominant peptides with homology to S100A9 and A8 proteins. Control eluates of non-peptibody bound, sorted MDSC lysates from Protein A displayed peptides possessing homology only to keratin.

[0041] FIGs. 10a-b. Peptibodies did not cause bone marrow toxicity. Frequencies of Gr1⁺CD11b⁺ splenic MDSC (FIG. 10a) or bone marrow immature myeloid cells (FIG. 10b) from naive *C57BL/6* mice treated with peptibodies for three consecutive days. Untreated (No Pep) or Pep-irrel-treated mice served as negative controls. Data are shown as the mean $\pm$ s.e.m of three mice per group. ** $P < 0.01$ compared with untreated mice (No pep) by two-tailed Student's *t*-test.

[0042] FIGs. 11a-c. Identification of S100A9 on tumor-infiltrated human MDSC in a lymphoma patient. Staining of human splenic lymphoma samples identified a HLA-DR⁻CD33⁺CD11b⁺ subpopulation that contained both CD15⁺ (92%) and CD14⁺ (8%) cells, suggesting granulocytic and monocytic MDSC, respectively (FIG. 11a). These tumor-infiltrated human MDSC suppressed *in vitro* activation of HLA-matched, allogeneic antigen-

specific T cells (FIG. 11b). The FACS data revealed the presence of S100A9 on the surface of these tumor-infiltrated human MDSC (FIG. 11c).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0043] Cancer immune evasion is an emerging hallmark of disease progression.

5 Functional studies to understand the role of myeloid-derived suppressor cells (MDSC) in the tumor microenvironment however, are limited by the lack of available specific cell surface markers. A competitive peptide phage display platform was used herein to generate novel diagnostic and therapeutic MDSC-specific peptide-Fc fusion (peptibody) reagents. A signal peptide that leads to secretion of translated protein and a 6 histidine tag was engineered into
10 the peptibody construct as shown in FIG. 2A. Peptibodies successfully depleted blood and splenic MDSC in multiple syngeneic tumor models, as well as intratumoral MDSC. Superiority of the peptibody therapeutic effects over an available Gr-1 mAb was suggested by their ability to deplete both granulocytic and monocytic MDSC subsets (Gr-1 depleted primarily granulocytic MDSC) and more consistent tumor inhibition *in vivo*.

15 [0044] The S100 family of calcium binding proteins are intracellular molecules released into the extracellular milieu by myeloid cells in response to inflammation and function as proinflammatory danger signals (alarmins) (Cheng *et al.*, 2008). Though there is limited evidence showing that soluble S100A9 binds with MDSC *in vitro* (Sinha *et al.*, 2008), it is likely that MDSC secrete S100A9 in an autocrine feedback mechanism through
20 receptors. The precise nature of the peptibody target remains to be elucidated. Although the epitope(s) recognized may be S100 protein-derived, because the protocol used viable cells to screen the peptide library, combinatorial native conformational epitopes comprised of the S100-receptor complex would have also been preserved. Given that the peptibody did not bind or deplete DC, the cell surface receptor for S100A9 and S100A8 on MDSC is as yet
25 unidentified or different from those on DC, such as RAGE and TLR4.

[0045] Without being bound by theory, a MDSC-depleting peptibody could cause tumor regression by (1) depleting MDSC by complement-dependent cytotoxicity or ADCC, (2) inducing apoptosis by blocking the binding of S100 family protein with its receptor(s) on MDSC or on tumor cells (Ichikawa *et al.*, 2011; Kallberg *et al.*, 2012) and interfering with
30 S100-induced survival signals, and (3) inducing direct cytotoxicity against tumor cells

(Ichikawa *et al.*, 2011; Kallberg *et al.*, 2012). Indeed, S100A9 was recently reported on human MDSC isolated from patients with colon cancer (Zhao *et al.*, 2012).

[0046] MDSC-specific binding affinity, *in vivo* MDSC-depletion, and low toxicity make the present peptibodies an attractive adjuvant for cancer immunotherapy, as amelioration of tumor suppressive microenvironment is required for maximizing the therapeutic benefits of cancer immunotherapy (Corzo *et al.*, 2010; Marigo *et al.*, 2010; Fernandez *et al.*, 2011). In addition, its high binding affinity can also be utilized to develop therapeutic reagents, such as a cytotoxic radiolabeled peptibody. The MDSC-specific binding peptides may also serve as MDSC-targeting agents for any conjugated cargo, preferably cytotoxic cargo or a reporter. The peptides and peptibodies also provide new diagnostic markers for MDSC.

I. Myeloid Derived Suppressor Cells

[0047] The term “myeloid derived suppressor cell (MDSC)” refers to a cell with an immunosuppressive function that is of hematopoietic lineage.

[0048] Myeloid-derived suppressor cells (MDSC), also known as myeloid suppressor cells (MSCs), are a heterogeneous population of immature myeloid cells with immunoregulatory activity. The term “MDSC” is used throughout the application and is synonymous with the term “MSC.” Human MDSCs are characterized by at least the expression of the cell markers CD11b and CD33. Human MDSCs may also express the markers CD15 and/or CD14. Murine MDSCs at least express the markers CD11b and Gr-1. Murine MDSCs may also express CD115 and/or F4/80 (see Li *et al.*, 2004). Murine MDSCs may also express CD31, c-kit, vascular endothelial growth factor (VEGF)-receptor, or CD40 (Bronte *et al.*, 2000). MDSCs may further differentiate into several cell types, including macrophages, neutrophils, dendritic cells, Langerhand cells, monocytes or granulocytes. MDSCs may be found naturally in normal adult bone marrow of human and animals or in sites of normal hematopoiesis, such as the spleen in newborn mice.

[0049] Upon distress, MDSCs may be found in the adult spleen. MDSCs can suppress the immunological response of T cells, induce T regulatory cells, and produce T cell tolerance. Morphologically, MDSCs usually have large nuclei and a high nucleus-to-cytoplasm ratio. MDSCs can secrete TFG- β and IL-10 and produce nitric oxide (NO) in the presence of IFN- γ or activated T cells. MDSCs may form dendriform cells; however, MDSCs

are distinct from dendritic cells (DCs) in that DCs are smaller and express CD11c; MDSCs do not express CD11c, or express only a low level of CD11c. T cell inactivation by MDSCs *in vitro* can be mediated through several mechanisms: IFN- γ -dependent nitric oxide production (Kusmartsev *et al.*, 2000); Th2-mediated-IL-4/IL-13-dependent arginase 1
5 synthesis (Bronte *et al.*, 2003); loss of CD ξ signaling in T cells (Rodriguez *et al.*, 2003); and suppression of the T cell response through reactive oxygen species (Bronte *et al.*, 2003; Bronte *et al.*, 2003; Kusmartsev *et al.*, 2004; Schmielau and Finn, 2001).

II. Therapeutic Peptibodies

[0050] In certain embodiments, a peptide that binds to at least a portion of an MDSC-
10 specific epitope, fused either directly or indirectly to another molecule, such as an Fc domain of an antibody (*e.g.*, a peptibody), and identifies and/or depletes MDSC and its associated use in treatment and diagnosis of diseases are contemplated. Preferably, the MDSC-specific epitope-binding peptibody is humanized. By known means and as described herein, peptibodies may be created that are specific to a MDSC-specific antigen, one or more of its
15 respective epitopes, or conjugates of any of the foregoing, whether such antigens or epitopes are isolated from natural sources or are synthetic derivatives or variants of the natural antigen or epitope.

[0051] As used herein, the term “fusion protein” refers to a chimeric protein containing proteins or protein fragments operably linked in a non-native way. A peptide or
20 peptibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the accompanying claims, all such fused proteins are included in the definition of peptibodies.

[0052] The term “peptibody” refers to a molecule comprising peptide(s) fused either directly or indirectly to other molecules such as an Fc domain of an antibody, where the
25 peptide moiety specifically binds to a desired target. The peptide(s) may be fused to either an Fc region or inserted into an Fc-Loop. Fc-Loops are described in U.S. Patent Application Publication No. US2006/0140934, incorporated herein by reference in its entirety. The invention includes such molecules comprising an Fc domain modified to comprise a peptide as an internal sequence of the Fc domain. The Fc internal peptide molecules may include
30 more than one peptide sequence in tandem in a particular internal region, and they may include further peptides in other internal regions. While the putative loop regions are

exemplified, insertions in any other non-terminal domains of the Fc are also considered part of this invention. As used herein, a peptibody does not comprise an antigen-binding antibody Fab domain.

[0053] The term “Fc” refers to a molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of a whole antibody, whether in monomeric or multimeric form. Typically, a Fc comprises a CH2 and CH3 domain. The original immunoglobulin source of the Fc is in one aspect of human origin and may be any of the immunoglobulins. A Fc is a monomeric polypeptide that may be linked into dimeric or multimeric forms by covalent association (*i.e.*, disulfide bonds), non-covalent association or a combination of both. The number of intermolecular disulfide bonds between monomeric subunits of Fc molecules ranges from one to four depending on class (*e.g.*, IgG, IgA, IgE) or subclass (*e.g.*, IgG1, IgG2, IgG3, IgA1, IgGA2). One example of a Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (Ellison *et al.*, 1982). The term “Fc” as used herein is generic to the monomeric, dimeric, and multimeric forms.

[0054] The term “Fc variant” refers to a molecule or sequence that is modified from a native Fc, but still comprises a binding site for the salvage receptor, FcRn. International PCT Application Publications WO 97/34631 and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference. In one aspect, the term “Fc variant” comprises a molecule or sequence that is humanized from a non-human native Fc. In another aspect, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term “Fc variant” comprises a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC).

[0055] The term “Fc domain” encompasses native Fc and Fc variant molecules and sequences as defined above. As with Fc variants and native Fcs, the term “Fc domain” includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0056] Fc sequences are known in the art and are contemplated for use in the invention. For example, Fc IgG1 (GenBank Accession No. POI 857), Fc IgG2 (GenBank Accession No. POI 859), Fc IgG3 (GenBank Accession No. POI 860), Fc IgG4 (GenBank Accession No. POI 861), Fc IgA1 (GenBank Accession No. PO 1876), Fc IgA2 (GenBank
5 Accession No. POI 877), Fc IgD (GenBank Accession No. POI 880), Fc IgM (GenBank Accession No. P01871), and Fc IgE (GenBank Accession No. POI 854) are some Fc sequences contemplated for use herein.

[0057] The term “multimer” as applied to Fc domains or molecules comprising Fc domains refers to molecules having two or more polypeptide chains associated covalently,
10 noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers, trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing such a native Fc.

[0058] Embodiments provide peptibodies against MDSC-specific antigens,
15 polypeptides and peptides that are linked to at least one agent to form a peptibody conjugate or payload. In order to increase the efficacy of peptibody molecules as diagnostic or therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one effector or reporter molecule. Effector molecules comprise molecules having a desired
20 activity, *e.g.*, cytotoxic activity. Non-limiting examples of effector molecules that may be attached to peptibodies include toxins, therapeutic enzymes, antibiotics, radio-labeled nucleotides and the like. By contrast, a reporter molecule is defined as any moiety that may be detected using an assay. Non-limiting examples of reporter molecules that may be conjugated to peptibodies include enzymes, radiolabels, haptens, fluorescent labels,
25 phosphorescent molecules, chemiluminescent molecules, chromophores, luminescent molecules, photoaffinity molecules, colored particles or ligands, such as biotin.

[0059] Several methods are known in the art for the attachment or conjugation of a peptibody to a conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a
30 diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3-6-diphenylglycouril attached to the peptibody. Peptibodies may also be reacted with an enzyme in the presence of a coupling

agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate.

[0060] As used herein the term “peptide” refers to molecules of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more amino acids linked by peptide bonds. Peptides typically contain random and/or flexible conformations, such as random coils; and typically lack stable conformations, such as those observed in larger proteins/polypeptides, typically via secondary and tertiary structures. In particular embodiments, numerous size ranges of peptides are contemplated herein, such from about: 3-90, 3-80, 3-70, 3-60, 3-50; 5-90, 5-80, 5-70, 5-60, 5-50, 5-40, 5-30; 10-90, 10-80, 10-70, 10-60, 10-50, 10-40, 10-30; 10-20 amino acids in length, and the like. In further embodiments, the peptides used herein are no more than 100, 90, 80, 70, 60, 50, 40, 30, or 20 amino acids in length. Exemplary peptides may be generated by any of the methods set forth herein, such as carried in a peptide library (*e.g.*, a phage display library), generated by chemical synthesis, derived by digestion of proteins, or generated using recombinant DNA techniques. Peptides include D and L form, either purified or in a mixture of the two forms.

[0061] As used herein the terms “protein” and “polypeptide” refer to compounds comprising amino acids residues joined *via* peptide bonds and are used interchangeably. As used herein, a protein or peptide generally refers, but is not limited to, a protein of greater than about 200 amino acids, up to a full length sequence translated from a gene; a polypeptide of greater than about 100 amino acids; and/or a peptide of from about 3 to about 100 amino acids. For convenience, the terms “protein,” “polypeptide,” and “peptide” are used interchangeably herein.

[0062] As used herein, an “amino acid residue” refers to any naturally occurring amino acid, any amino acid derivative, or any amino acid mimic known in the art. In certain embodiments, the residues of the protein or peptide are sequential, without any non-amino acids interrupting the sequence of amino acid residues. In other embodiments, the sequence may comprise one or more non-amino acid moieties. In particular embodiments, the sequence of residues of the protein or peptide may be interrupted by one or more non-amino acid moieties. Accordingly, the term “protein or peptide” encompasses amino acid sequences

comprising at least one of the 20 common amino acids found in naturally occurring proteins, or at least one modified or unusual amino acid.

[0063] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

[0064] Proteins or peptides may be made by any technique known to those of skill in the art, including the expression of proteins, polypeptides, or peptides through standard molecular biological techniques, the isolation of proteins or peptides from natural sources, or the chemical synthesis of proteins or peptides. Proteins may be recombinant, or synthesized *in vitro*. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that a bacterium containing such a variant may be implemented in compositions and methods. Consequently, a protein need not be isolated.

[0065] The nucleotide and protein, polypeptide, and peptide sequences corresponding to various genes have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (available on the world wide web at ncbi.nlm.nih.gov/). The coding regions for known genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art. Alternatively, various commercial preparations of proteins, polypeptides, and peptides are known to those of skill in the art.

[0066] Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the homogenization and crude fractionation of the cells, tissue, or organ to polypeptide and non-polypeptide fractions. The protein or polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity) unless otherwise specified. Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, gel exclusion chromatography, polyacrylamide gel electrophoresis, affinity chromatography, immunoaffinity chromatography, and isoelectric focusing. A particularly efficient method of purifying peptides is fast-performance liquid chromatography (FPLC) or even high-performance liquid chromatography (HPLC).

[0067] A purified protein or peptide is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally-obtainable state. An isolated or purified protein or peptide, therefore, also refers to a protein or peptide free from the environment in which it may naturally occur. Generally, “purified” will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term “substantially purified” is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, or more of the proteins in the composition.

[0068] Various techniques suitable for use in protein purification are well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like, or by heat denaturation, followed by centrifugation; chromatography steps, such as ion exchange, gel filtration, reverse phase, hydroxyapatite, and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of these and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

[0069] Various methods for quantifying the degree of purification of the protein or peptide are known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of

polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity therein, assessed by a “-fold purification number.” The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification, and whether or not the expressed protein or peptide exhibits a detectable activity.

[0070] There is no general requirement that the protein or peptide will always be provided in its most purified state. Indeed, it is contemplated that less substantially purified products may have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater “-fold” purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

[0071] In certain embodiments a protein or peptide may be isolated or purified, for example, a peptibody or a fusion protein containing the peptibody. For example, a His tag or an affinity epitope may be comprised in such a peptibody to facilitate purification. Affinity chromatography is a chromatographic procedure that relies on the specific affinity between a substance to be isolated and a molecule to which it can specifically bind. This is a receptor-ligand type of interaction. The column material is synthesized by covalently coupling one of the binding partners to an insoluble matrix. The column material is then able to specifically adsorb the substance from the solution. Elution occurs by changing the conditions to those in which binding will not occur (*e.g.*, altered pH, ionic strength, temperature, *etc.*). The matrix should be a substance that does not adsorb molecules to any significant extent and that has a broad range of chemical, physical, and thermal stability. The ligand should be coupled in such a way as to not affect its binding properties. The ligand should also provide relatively tight binding. It should be possible to elute the substance without destroying the sample or the ligand.

[0072] Size exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated based on their size, or in more technical terms, their

hydrodynamic volume. It is usually applied to large molecules or macromolecular complexes, such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as gel filtration chromatography, versus the name gel permeation chromatography, which is used when an
5 organic solvent is used as a mobile phase.

[0073] The underlying principle of SEC is that particles of different sizes will elute (filter) through a stationary phase at different rates. This results in the separation of a solution of particles based on size. Provided that all the particles are loaded simultaneously or near simultaneously, particles of the same size should elute together. Each size exclusion
10 column has a range of molecular weights that can be separated. The exclusion limit defines the molecular weight at the upper end of this range and is where molecules are too large to be trapped in the stationary phase. The permeation limit defines the molecular weight at the lower end of the range of separation and is where molecules of a small enough size can penetrate into the pores of the stationary phase completely and all molecules below this
15 molecular mass are so small that they elute as a single band.

[0074] High-performance liquid chromatography (or high-pressure liquid chromatography, HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that
20 moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

[0075] Compositions and methods of the present invention may comprise peptibodies conjugated with heterologous peptide segments or polymers, such as polyethylene glycol. In
25 further aspects, the peptibodies may be linked to PEG to increase the hydrodynamic radius of the enzyme and hence increase the serum persistence.

[0076] In certain aspects of the invention, methods and compositions relating to PEGylation of peptibodies are disclosed. For example, the peptibodies may be PEGylated in accordance with the methods disclosed herein.

30 [0077] The term "PEGylated" refers to conjugation with polyethylene glycol (PEG), which has been widely used as a drug carrier, given its high degree of biocompatibility and

ease of modification. PEG can be coupled (*e.g.*, covalently linked) to active agents through the hydroxy groups at the end of the PEG chain *via* chemical methods; however, PEG itself is limited to at most two active agents per molecule. In a different approach, copolymers of PEG and amino acids have been explored as novel biomaterial that would retain the biocompatibility of PEG, but that would have the added advantage of numerous attachment points per molecule (thus providing greater drug loading), and that can be synthetically designed to suit a variety of applications.

[0078] PEGylation is the process of covalent attachment of poly(ethylene glycol) polymer chains to another molecule, normally a drug or therapeutic protein. PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target macromolecule. The covalent attachment of PEG to a drug or therapeutic protein can “mask” the agent from the host's immune system (reduced immunogenicity and antigenicity) or increase the hydrodynamic size (size in solution) of the agent, which prolongs its circulatory time by reducing renal clearance. PEGylation can also provide water solubility to hydrophobic drugs and proteins.

[0079] It is contemplated that in compositions there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. Thus, the concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% may be a MDSC antigen-specific peptibody.

III. Nucleic Acids and Vectors

[0080] In certain aspects of the invention, nucleic acid sequences encoding a peptide or peptibody may be disclosed. Depending on which expression system is used, nucleic acid sequences can be selected based on conventional methods. For example, if the peptibody is derived from a mammalian Fc and contains multiple codons that are rarely utilized in *E. coli*, then that may interfere with expression. Therefore, the respective genes or variants thereof

may be codon optimized for *E. coli* expression. Various vectors may be also used to express the protein of interest, such a peptibody. Exemplary vectors include, but are not limited, plasmid vectors, viral vectors, transposon, or liposome-based vectors.

IV. Host Cells

5 [0081] Host cells may be any that may be transformed to allow the expression and secretion of peptibody and conjugates thereof. The host cells may be bacteria, mammalian cells, yeast, or filamentous fungi. Various bacteria include *Escherichia* and *Bacillus*. Yeasts belonging to the genera *Saccharomyces*, *Kluyveromyces*, *Hansenula*, or *Pichia* would find use as an appropriate host cell. Various species of filamentous fungi may be used as
10 expression hosts, including the following genera: *Aspergillus*, *Trichoderma*, *Neurospora*, *Penicillium*, *Cephalosporium*, *Achlya*, *Podosporea*, *Endothia*, *Mucor*, *Cochliobolus*, and *Pyricularia*.

[0082] Examples of usable host organisms include bacteria, *e.g.*, *Escherichia coli* MC1061, derivatives of *Bacillus subtilis* BRB1 (Sibakov *et al.*, 1984), *Staphylococcus aureus*
15 SAI123 (Lordanescu, 1975) or *Streptococcus lividans* (Hopwood *et al.*, 1985); yeasts, *e.g.*, *Saccharomyces cerevisiae* AH 22 (Mellor *et al.*, 1983) or *Schizosaccharomyces pombe*; and filamentous fungi, *e.g.*, *Aspergillus nidulans*, *Aspergillus awamori* (Ward, 1989), or *Trichoderma reesei* (Penttila *et al.*, 1987; Harkki *et al.*, 1989).

[0083] Examples of mammalian host cells include Chinese hamster ovary cells
20 (CHO-K1; ATCC CCL61), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548), SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650), and murine embryonic cells (NIH-3T3; ATCC CRL 1658). The foregoing being illustrative but not limitative of the many possible host organisms known in the art. In principle, all hosts capable of secretion can be used whether
25 prokaryotic or eukaryotic.

[0084] Mammalian host cells expressing the peptibody and/or a fusion protein thereof are cultured under conditions typically employed to culture the parental cell line. Generally, cells are cultured in a standard medium containing physiological salts and nutrients, such as standard RPMI, MEM, IMEM, or DMEM, typically supplemented with 5%-10% serum, such
30 as fetal bovine serum. Culture conditions are also standard, *e.g.*, cultures are incubated at 37 °C in stationary or roller cultures until desired levels of the proteins are achieved.

V. Treatment and Diagnosis of Diseases

[0085] Certain aspects of the present embodiments can be used to prevent or treat a disease or disorder associated with MDSC-mediated immune suppression. MDSCs may be depleted by any suitable means to induce, for example, tumor regression. Preferably, such substances would be a peptibody against a MDSC-specific epitope, such as, for example, an anti-cell surface S100 peptibody.

[0086] “Treatment” and “treating” refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration of a pharmaceutically effective amount of an MDSC-depleting peptibody.

[0087] “Subject” and “patient” refer to either a human or non-human, such as primates, mammals, and vertebrates. In particular embodiments, the subject is a human.

[0088] The term “therapeutically effective amount” as used herein refers to an amount of a therapeutic composition (such as a therapeutic polynucleotide and/or therapeutic polypeptide) that is employed in methods to achieve a therapeutic effect. The term “therapeutic benefit” or “therapeutically effective” as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the size of a tumor, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

[0089] An MDSC-depleting peptibody may be administered to treat a cancer. The cancer may be a solid tumor, metastatic cancer, or non-metastatic cancer. In certain embodiments, the cancer may originate in the bladder, blood, bone, bone marrow, brain, breast, colon, esophagus, duodenum, small intestine, large intestine, colon, rectum, anus, gum, head, kidney, liver, lung, nasopharynx, neck, ovary, pancreas, prostate, skin, stomach, testis, tongue, or uterus.

[0090] The cancer may specifically be of the following histological type, though it is not limited to these: neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; small cell lung cancer; non-small cell lung cancer; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant; sertoli cell carcinoma; leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma; fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant;

mesenchymal chondrosarcoma; giant cell tumor of bone; ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma; hodgkin's disease; hodgkin's; paraganuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; and hairy cell leukemia.

[0091] A peptibody may be used herein as an antitumor agent in a variety of modalities for depleting and/or detecting MDSC where MDSC depletion and/or detection are considered desirable. In one embodiment, the depletion is accomplished by administering, by intravenous or intraperitoneal injection, a therapeutically effective amount of a physiologically tolerable composition comprising a peptibody of this invention to a patient, thereby depleting MDSC in the patient.

[0092] A therapeutically effective amount of a peptibody is a predetermined amount calculated to achieve the desired effect, *i.e.*, to deplete MDSC. Thus, the dosage ranges for the administration of peptibodies of the invention are those large enough to produce the desired effect in which the symptoms of tumor cell division and cell cycling are reduced. The dosage should not be so large as to cause adverse side effects, such as hyperviscosity syndromes, pulmonary edema, congestive heart failure, and the like. Generally, the dosage will vary with age of, condition of, sex of, and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication.

[0093] For example, a therapeutically effective amount of a peptibody may be an amount such that when administered in a physiologically tolerable composition is sufficient

to achieve an intravascular (plasma) or local concentration of from about 0.001 to about 100 units (U) per mL, preferably above about 0.1 U, and more preferably above 1 U peptibody per mL. Typical dosages can be administered based on body weight, and are in the range of about 5-1000 U/kilogram (kg)/day, preferably about 5-100 U/kg/day, more preferably about 10-50 U/kg/day, and more preferably about 20-40 U/kg/day.

[0094] The peptibody can be administered parenterally by injection or by gradual infusion over time. The peptibody can be administered intravenously, intraperitoneally, orally, intramuscularly, subcutaneously, intracavity, transdermally, dermally, can be delivered by peristaltic means, can be injected directly into the tissue containing the tumor cells, or can be administered by a pump.

[0095] The therapeutic compositions containing peptibodies are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent, *i.e.*, carrier, or vehicle.

[0096] The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for initial administration and booster shots are also contemplated and are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Exemplary multiple administrations are described herein and are particularly preferred to maintain continuously high serum and tissue levels of peptibodies and conversely low serum and tissue levels of MDSC. Alternatively, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for *in vivo* therapies are contemplated.

[0097] Some embodiments disclosed herein concern diagnostic and prognostic methods for the detection of inflammation and/or cancer. Such detection methods may be used, for example, for early diagnosis of the disease, to determine whether a tumor is malignant or benign, to monitor the progress of the disease or the progress of treatment protocols, or to assess the grade of the cancer. The detection can occur *in vitro* or *in vivo*. The presence of a cancer is determined when the percentage of myeloid cells is at least 2% MDSC, at least 3% MDSC, at least 4% MDSC, at least 5% MDSC, at least 6% MDSC, at least 7% MDSC, at least 8% MDSC, at least 9% MDSC, at least 10% MDSC, at least 11% MDSC, at least 12% MDSC, at least 13% MDSC, at least 14% MDSC, or at least 15% MDSC. Useful methods include, but are not limited to, flow cytometry, an enzyme immune assay (EIA) such as enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay, or Western blot detection.

A. Pharmaceutical Preparations

[0098] Where clinical application of a therapeutic composition containing a polypeptide is undertaken, it will generally be beneficial to prepare a pharmaceutical or therapeutic composition appropriate for the intended application. This will typically entail preparing a pharmaceutical composition that is essentially free of pyrogens, as well as any other impurities that could be harmful to humans or animals. One may also employ appropriate buffers to render the complex stable and allow for uptake by target cells. In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein.

[0099] The therapeutic compositions of the present embodiments are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified.

[00100] The phrases “pharmaceutical or pharmacologically acceptable” refers to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such as a human, as appropriate. The preparation of a pharmaceutical composition comprising an antibody or additional active

ingredient will be known to those of skill in the art in light of the present disclosure. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biological Standards.

5 **[00101]** As used herein, “pharmaceutically acceptable carrier” includes any and all aqueous solvents (*e.g.*, water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles, such as sodium chloride, Ringer's dextrose, *etc.*), non-aqueous solvents (*e.g.*, propylene glycol, polyethylene glycol, vegetable oil, and injectable organic esters, such as ethyloleate), dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*,
10 antibacterial or antifungal agents, anti-oxidants, chelating agents, and inert gases), isotonic agents, absorption delaying agents, salts, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, fluid and nutrient replenishers, such like materials and combinations thereof, as would be known to one of ordinary skill in the art. The pH and exact concentration of the various components in a
15 pharmaceutical composition are adjusted according to well-known parameters.

[00102] The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the therapeutic composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be
20 administered, both according to number of treatments and unit dose, depends on the effect desired.

[00103] The actual dosage amount of a composition of the present embodiments administered to a patient or subject can be determined by physical and physiological factors, such as body weight, the age, health, and sex of the subject, the type of disease being treated,
25 the extent of disease penetration, previous or concurrent therapeutic interventions, idiopathy of the patient, the route of administration, and the potency, stability, and toxicity of the particular therapeutic substance. For example, a dose may also comprise from about 1 $\mu\text{g/kg/body weight}$ to about 1000 mg/kg/body weight (this such range includes intervening doses) or more per administration, and any range derivable therein. In non-limiting examples
30 of a derivable range from the numbers listed herein, a range of about 5 $\mu\text{g/kg/body weight}$ to about 100 mg/kg/body weight , about 5 $\mu\text{g/kg/body weight}$ to about 500 mg/kg/body weight , *etc.*, can be administered. The practitioner responsible for administration will, in any event,

determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[00104] The active compounds can be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even
5 intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[00105] The pharmaceutical forms suitable for injectable use include sterile
10 aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such
15 as bacteria and fungi.

[00106] The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic,
20 and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00107] A pharmaceutical composition can include a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene
25 glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,
30 sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable

compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00108] Solutions of therapeutic compositions can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00109] The therapeutic compositions of the present invention are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like.

[00110] Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters, such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles, such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to well known parameters.

[00111] Additional formulations are suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders.

[00112] The therapeutic compositions of the present invention may include classic pharmaceutical preparations. Administration of therapeutic compositions according to the present invention will be via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Topical

administration may be particularly advantageous for the treatment of skin cancers, to prevent chemotherapy-induced alopecia or other dermal hyperproliferative disorder. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered
5 as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients. For treatment of conditions of the lungs, or respiratory tract, aerosol delivery can be used. Volume of the aerosol is between about 0.01 mL and 0.5 mL.

[00113] An effective amount of the therapeutic composition is determined based on the intended goal. For example, one skilled in the art can readily determine an
10 effective amount of an antibody of the invention to be administered to a given subject, by taking into account factors such as the size and weight of the subject; the extent of the neovascularization or disease penetration; the age, health and sex of the subject; the route of administration; and whether the administration is regional or systemic. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit
15 containing a predetermined-quantity of the therapeutic composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection or effect desired.

[00114] Precise amounts of the therapeutic composition also depend on the
20 judgment of the practitioner and are particular to each individual. Factors affecting the dose include the physical and clinical state of the patient, the route of administration, the intended goal of treatment (*e.g.*, alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular therapeutic substance.

B. Combination Treatments

25 [00115] In certain embodiments, the compositions and methods of the present embodiments involve a peptibody against a MDSC-specific epitope to deplete MDSCs, in combination with a second or additional therapy. Such therapy can be applied in the treatment of any disease that is associated with MDSCs. For example, the disease may be cancer.

30 [00116] The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another

anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. This process may involve contacting the cells with both a peptibody and a second therapy. A
5 tissue, tumor, or cell can be contacted with one or more compositions or pharmacological formulation(s) comprising one or more of the agents (*i.e.*, peptibody or an anti-cancer agent), or by contacting the tissue, tumor, and/or cell with two or more distinct compositions or formulations, wherein one composition provides 1) a peptibody, 2) an anti-cancer agent, or 3) both a peptibody and an anti-cancer agent. Also, it is contemplated that such a combination
10 therapy can be used in conjunction with chemotherapy, radiotherapy, surgical therapy, or immunotherapy.

[00117] The terms “contacted” and “exposed,” when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with
15 the target cell. To achieve cell killing, for example, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

[00118] A peptibody may be administered before, during, after, or in various combinations relative to an anti-cancer treatment. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the peptibody
20 is provided to a patient separately from an anti-cancer agent, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the peptibody therapy and the anti-cancer therapy within about 12 to 24 or 72 h of each other and, more
25 particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[00119] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that one agent may be given
30 on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another agent is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the

patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no anti-cancer treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, *etc.* It is expected that the treatment cycles would be repeated as necessary.

[00120] Various combinations may be employed. For the example below a peptibody therapy is “A” and an anti-cancer therapy is “B”:

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B
 B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A
 B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[00121] Administration of any compound or therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

1. Chemotherapy

[00122] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[00123] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic

analogue topotecan); bryostatin; callistatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics, such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine;

arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel; gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

2. Radiotherapy

[00124] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

3. Immunotherapy

[00125] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, *etc.*) and serve merely as a targeting agent. Alternatively, the effector may be

a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

[00126] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[00127] Examples of immunotherapies currently under investigation or in use are immune adjuvants, *e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998); cytokine therapy, *e.g.*, interferons α , β , and γ , IL-1, GM-CSF, and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

4. Surgery

[00128] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[00129] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, 5 and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

[00130] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

VI. Kits and Diagnostics

[00131] In various aspects of the embodiments, a kit is envisioned containing therapeutic agents and/or other therapeutic and delivery agents. In some embodiments, a kit is contemplated for preparing and/or administering a therapy of the embodiments. The kit may comprise one or more sealed vials containing any of the pharmaceutical compositions of the present embodiments. The kit may include, for example, at least one MDSC-specific peptide and/or peptibody as well as reagents to prepare, formulate, and/or administer the components of the embodiments or perform one or more steps of the inventive methods. In some embodiments, the kit may also comprise a suitable container, which is a container that

will not react with components of the kit, such as an eppendorf tube, an assay plate, a syringe, a bottle, or a tube. The container may be made from sterilizable materials such as plastic or glass.

[00132] The kit may further include an instruction sheet that outlines the procedural steps of the methods set forth herein, and will follow substantially the same procedures as described herein or are known to those of ordinary skill in the art. The instruction information may be in a computer readable media containing machine-readable instructions that, when executed using a computer, cause the display of a real or virtual procedure of delivering a pharmaceutically effective amount of a therapeutic agent.

VII. Examples

[00133] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Generation of novel therapeutic peptides that deplete MDSC in tumor-bearing mice

[00134] *Identification of mouse MDSC-binding peptides.* MDSC frequency is low in naïve C57BL/6 mice; however, after transplantation of syngeneic EL4 thymomas, MDSC are increased accounting for approximately 10% of total splenocytes (Gabrilovich and Nagaraj, 2009). Splenic MDSC from EL4-bearing mice consist of two distinct subpopulations characterized by Gr-1^{high}CD11b⁺ granulocytic (P7) and Gr-1^{int}CD11b⁺ monocytic (P10) staining (FIG. 1a). With the goal of selecting specifically binding peptide ligands by phage display, Gr-1 and CD11b labeled MDSC were separated from non-MDSC by cell sorting after incubation with a Ph.D.-12 peptide phage library. Phage eluted from granulocytic or monocytic MDSC were then expanded by three rounds of competitive biopanning. Enrichment was analyzed by the number of phage eluted from 10⁶ MDSC (FIG. 1b) and by phage output, normalized to the initial input of 2 × 10¹⁰ phage (FIG. 1c).

[00135] Sequencing of enriched phage revealed over-represented peptide sequences, and two predominant peptides (H6 and G3) were selected for further study (Table 1). Each clone that was expanded and tested for binding to MDSC revealed the specificity of both H6 and G3 phage for MDSC, without significant binding to non-MDSC. Additional candidate MDSC-binding phages were isolated, but were not given further consideration, because their binding was not specific. Corresponding synthetic FITC-conjugated H6 and G3 peptides bound specifically to Gr-1⁺CD11b⁺ gated MDSC, but not Gr-1⁻CD11b⁻ non-MDSC splenocytes from EL4-bearing mice (FIG. 1d).

Table 1. Amino acid sequences of MDSC-binding peptides identified by phage display

Peptide	Granulocytic MDSC (66 colonies)		Monocytic MDSC (48 colonies)	
	Positive colonies	Percentage	Positive colonies	Percentage
MEWSLEKGYTIK (H6) (SEQ ID NO: 1)	24	36.36%	25	52.08%
WGWSLSHGYQVK (G3) (SEQ ID NO: 2)	5	7.58%	10	20.83%

[00136] *Generation of MDSC-specific peptibodies.* Sequences encoding H6 and G3 peptides were genetically fused with the Fc portion of mouse IgG2b to generate peptibodies (Pep-H6 and -G3, respectively; SEQ ID NOs: 3 and 4, respectively) (FIG. 2a). Control peptibodies (Pep-irrel) were also made using non-specific sequences. Recombinant peptibodies were produced in 293T mammalian cells, followed by purification of the peptibodies by Protein A chromatography and characterization by Western blot using anti-mouse IgG and anti-His tag antibodies (FIG. 2b). These peptibodies were conjugated with fluorescein isothiocyanate (FITC) and analyzed for binding specificity on CD11b⁺Ly6G⁻ Ly6C^{high} monocytic MDSC and CD11b⁺Ly6G⁺Ly6C^{int/low} granulocytic MDSC from EL4-bearing mice. Gating on these well-defined subpopulations, the inventors conclusively showed that Pep-H6 and Pep-G3 bind both monocytic and granulocytic MDSC (FIGs. 2c and 5a). However, the expression of the Pep-G3 target seems to be lower in granulocytic MDSC than in monocytic MDSC.

[00137] In addition to EL4, peptibodies stained splenic MDSC from mice bearing EG.7 thymoma, B16 melanoma, and A20 lymphoma (FIGs. 2d and 5b). Interestingly, neither peptibody stained Ly6G⁻CD11c⁺ dendritic cells (DC), also of myeloid origin (FIG. 2e). Furthermore, peptibodies did not bind lymphocytes including B, T and NK

cells (Pep-G3 bound a small proportion of T and NK cells) (FIG. 6). However, specific staining of Gr-1⁺CD11b⁺ immature myeloid cells was observed in bone marrow with peptibodies in EL4-bearing mice (FIG. 2f). When co-staining MDSC with both peptibodies, there was nearly complete overlap between Pep-H6 and -G3 binding populations (FIG. 2g).

5 **[00138]** *Peptibodies deplete MDSC in vivo and retard tumor growth.* Next, the effect of peptibody treatment on MDSC *in vivo* was determined. Intravenous injection of 50 µg Pep-H6 and Pep-G3 completely depleted MDSC in blood and spleens of EL4 tumor-bearing mice, compared with control peptibody (Pep-irrel) or untreated mice (FIGs. 3a and 3b). Whereas the peptibodies depleted both monocytic and granulocytic MDSC subsets, Gr-1
10 mAb depleted granulocytic, but not monocytic, MDSC in both EL4 and EG.7 models. This distinguishing effect of peptibody treatment on monocytic MDSC was especially apparent when blood was first subjected to Ficoll sedimentation to remove granulocytes and granulocytic MDSC (FIGs. 3a-d). Importantly, peptibody treatment depleted intratumoral MDSC in both EG.7 (FIGs. 3c and 3d) and EL4 models (FIG. 7). Peptibodies also depleted
15 blood and splenic MDSC in mice bearing A20 lymphomas (FIG. 3e).

[00139] Peptibodies specifically depleted MDSC in tumor-bearing mice without affecting other proinflammatory cells including DC and lymphocytes (T, B and NK cells), consistent with their lack of binding, although Pep-G3 treatment was associated with a minor reduction in NK cells (FIG. 3f). Interestingly, although staining of Gr-1⁺CD11b⁺
20 immature myeloid cells from bone marrow was observed with peptibodies, peptibody treatment of tumor-bearing mice did not deplete myeloid precursor cells in this compartment either (FIG. 3f). Analysis of other blood cells subsets revealed associated depletion of elevated numbers of neutrophils in tumor-bearing, compared with naïve mice, but no effect on red blood cells or platelets (Table 2).

Table 2. Peptibody treatment corrected aberrant neutrophilia in EL4-bearing C57BL/6 mice

	Non-tumor bearing			EL4/PBS			EL4/Pep-irrel			EL4/Pep-G3			EL4/Pep-H6		
	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3
WBC ($10^3/\mu\text{L}$)	2.9	8.68	3.72	18.4	14.79	19.86	12.82	19.26	19.92	5.12	3.3	4.05	2.84	3.28	2.78
RBC ($10^6/\mu\text{L}$)	9.35	10.77	10.47	7.98	9.17	8.65	9.55	8.49	8.28	8	9.07	8.77	8.74	9.79	8.89
HGB (g/dL)	13.8	16	15.8	11.6	13.2	12.6	13.5	12.1	12.2	11.5	13	12.5	12.8	14.4	13.4
PLT ($10^3/\mu\text{L}$)	686	1167	975	721	1044	596	896	1125	721	559	506	750	694	1199	933
NEUT (%)	9.9	7.4	8.5	50	51.6	49	44	59.4	58	26	30	18	12	12.3	11
LYMPH (%)	85.3	88.2	85.7	35	40.6	40	45	31.7	33	59	63	79	84	81.9	82
MONO (%)	1.6	1.4	2.2	3	1.3	1	3	1.1	1	0	0	0	0	0.5	1
EOS (%)	2.7	1.3	1.5	7	3.9	3	5	4.7	5	2	0	1	2	2.1	0
BASO (%)	0.1	0.6	0.5	0	0.7	0	0	0.6	0	0	0	0	0	1.6	0

Reference values for blood tests in female mice: WBC: 2.1-7.1; RBC: 7.4-9.9; HGB: 12.1-16.5; Platelets: 659-1427; Neutrophils: 7.4-25.9; Lymphocytes: 60.8-85.0; Monocytes: 0.2-4.4; Eosinophils: 0.0-13.0; Basophils: 0.0-0.7.

[00140] Finally, to test the hypothesis that MDSC depletion can augment antitumor immunity, peptibodies were administered to EL4-bearing mice every other day to achieve a continuous depletion of systemic MDSC. Using this strategy, treatment with Pep-H6 or Pep-G3 alone significantly delayed tumor development, measured by tumor size (FIG. 3g) or tumor mass (FIG. 3h), compared with untreated or Pep-irrel-treated control mice. Importantly, while both peptibodies clearly inhibited tumor growth *in vivo*, Gr-1 mAb treatment was associated with less consistent inhibition of tumor growth. This effect of peptibody treatment correlated with MDSC depletion at the end of the two-week treatment (FIG. 8).

[00141] To evaluate potential off-target activity of our specific peptibodies, the inventors also assessed potential depletion of normal cell types, including those of myeloid origin. With the exception of transient reduction of blood neutrophils, and possibly monocytes, no depletion of DC, T, B, NK cells, or Gr-1⁺CD11b⁺ immature myeloid precursor cells in the bone marrow was observed in tumor-bearing mice. The inventors also observed that in naïve mice, peptibody treatment reduced circulating Gr-1⁺CD11b⁺ splenocytes (FIG. 10a), but did not affect immature myeloid cells in the bone marrow (FIG. 10b), suggesting that peptibody treatment may not have an effect on myeloid-lineage cells other than systemic (or intratumoral) MDSC. Consistent with this possibility, systemic MDSC numbers recovered 3 days after single dose treatment.

[00142] *Cell surface-bound alarmin is a candidate target.* To explore the target of the peptibody, a strategy was developed to identify peptibody-bound proteins on the surface of MDSC (FIG. 4a). Specifically, surface proteins on sorted Gr-1⁺CD11b⁺ MDSC were biotinylated and subsequently captured by monomeric avidin after cell lysis. Then, Pep-H6 that was immobilized on Protein A was used to immunoprecipitate the surface protein of interest. Proteomic analysis of eluted proteins suggested that S100A9 was the source protein, with sequence coverage above 40% (FIG. 4b). Consistent with these results, immunoprecipitation studies (without prior biotinylation) showed that eluted proteins from Pep-H6-bound, sorted MDSC co-migrated with recombinant S100A9 (6 × His tagged) and was recognized by S100A9 antibodies (FIG. 4c), but no signal was detected when using lysates from MDSC without peptibody, excluding contamination by intracellular S100A9 (FIG. 4c). Furthermore, such direct immunoprecipitation of MDSC cell surface proteins with Pep-H6 revealed a protein band that more closely co-migrated with native S100A9 identified

by immunoblotting of MDSC total cell lysates (FIG. 4d). Immunoprecipitation experiments suggested that the peptibody also recognizes S100A8, consistent with the formation of S100A9 and S100A8 dimers (FIG. 4d). Immunoprecipitation with Pep-G3 also suggested binding to S100A9 and S100A8 proteins (FIG. 9). These results suggested either cross-reactivity with S100A8 or perhaps recognition of a combinatorial determinant on the S100A9 and S100A8 complex.

[00143] The peptibodies were then tested in S100A9-deficient mice and it was observed that peptibodies could bind MDSC (FIG. 4e) and also partially deplete MDSC *in vivo* (FIG. 4f). Taking these results together, the most likely explanation is that peptibodies are cross-reactive for S100A9 and S100A8. This explanation is consistent with the fact that S100A9-deficient mice express S100A8 (Vogl *et al.*, 2004). Unfortunately, S100A8-deficient mice show an early embryonic lethal phenotype precluding further analysis for this study.

Example 2 – Generation of a novel therapeutic peptide that depletes human MDSC

[00144] *Identification and characterization of human MDSC.* By staining splenic lymphoma samples, the inventors identified a HLA-DR⁻CD33⁺CD11b⁺ subpopulation that contained both CD15⁺ (92%) and CD14⁺ (8%) cells, suggesting granulocytic and monocytic MDSC, respectively (FIG. 11a). These tumor-infiltrated human MDSC suppressed *in vitro* activation of HLA-matched, allogeneic antigen-specific T cells (FIG. 11b). In this experiment, HLA-A2⁺ lymphoma idiotype-peptide specific T-cell clones were mixed with peptide-pulsed T2 cells, with or without HLA-DR⁻CD11b⁺CD33⁺ sorted human MDSC isolated from (a) at a 1:1 ratio. After a 24 h incubation, culture medium was collected and assayed for INFγ by ELISA. Negative controls included T cells + T2 cells pulsed with irrelevant or no peptide. The FACS data revealed the presence of S100A9 on the surface of these tumor-infiltrated human MDSC (FIG. 11c).

[00145] Expression of S100A9 has also been screened on the cell surface of human MDSC isolated from the blood of five additional patients with a range of hematological tumors, including follicular lymphoma and multiple myeloma, or directly from the tumors as above. Gating on HLA-DR⁻/CD11b⁺/CD33⁺ human MDSC cells, a significant proportion of cells was identified as being positively stained for human S100A9,

compared with minimal to no staining of gated cells from normal human donors, which supports the hypothesis that S100A9 can be targeted for human MDSC depletion therapy.

[00146] *Identification of human MDSC-binding peptides.* With the goal of selecting specifically binding peptide ligands by phage display, human MDSC will be separated from non-MDSC by cell sorting after incubation with a Ph.D.-12 peptide phage library. Phage eluted from the MDSC will then be expanded by competitive biopanning. Enrichment will be analyzed by the number of phage eluted from a set number of MDSC and by phage output, normalized to the initial input of phage.

[00147] Sequencing of enriched phage will reveal over-represented peptide sequences for further study. Each expanded clone will be tested for binding to MDSC to confirm the specificity for binding to MDSC over non-MDSC.

[00148] *Generation of human MDSC-specific peptibodies.* Sequences encoding the identified peptides will be genetically fused with the Fc portion of human IgG to generate peptibodies. Recombinant peptibodies will be produced in 293T mammalian cells, followed by purification of the peptibodies by Protein A chromatography and characterization by Western blot. These peptibodies will be conjugated with fluorescein isothiocyanate (FITC) and analyzed for binding specificity to human MDSC.

Example 3 – Methods

[00149] *Phage display.* Total splenocytes from EL4 tumor-bearing C57BL/6 mice were blocked with formaldehyde-inactivated M13 phage and 2.4G2 antibody (BD Biosciences, San Jose, CA), followed by staining with anti-CD11b-APC and anti-Gr-1-FITC (BD Biosciences). Gr-1- and CD11b-labeled splenocytes were then incubated with 2×10^{10} Ph.D.-12 peptide phage display library (New England BioLabs Inc., Ipswich, MA) for 1 h at 4 °C. Gr-1^{high}CD11b⁺ granulocytic and Gr-1^{int}CD11b⁺ monocytic MDSC subsets were sorted separately and their bound phage was eluted, tittered, and amplified for the next round of biopanning. After the third round of biopanning, predominant MDSC-binding peptides were identified by PCR analysis of 66 and 48 individual phage eluted from granulocytic and monocytic MDSC, respectively. The PCR product of each individual phage, generated using specific primers spanning encoded peptides, was sequenced.

[00150] *MDSC specificity of synthetic peptide.* FITC-conjugated H6 or G3 synthetic peptides were made by Pi Proteomics, LLC (Huntsville, AL). After blocking with 2.4G2 antibody, splenocytes from EL4-bearing *C57BL/6* mice were co-stained with anti-CD11b-APC, anti-Gr-1-PE, and FITC-conjugated peptides. H6 and G3 peptides were
5 analyzed for their binding to Gr-1⁺CD11b⁺ gated MDSC, compared with Gr-1⁻CD11b⁻ gated non-MDSC splenocytes. A non-specific peptide (irrel peptide) was used as a negative control to exclude non-specific binding. FACS analysis was performed using LSRFortessa cell analyzer (BD Biosciences) and results were analyzed using FlowJo software.

[00151] *Generation and production of MDSC-specific peptibody.* Synthetic,
10 complementary double-stranded oligonucleotides encoding H6 or G3 peptide were fused with a human IL-2 signal peptide and 6 × His tag and then cloned into EcoRI and BglII sites of pINFUSE-mIgG2b-Fc2 vector (InvivoGen, San Diego, CA). For initial characterization, recombinant peptibodies were produced by transfecting 293T human embryonic kidney cells with the plasmid constructs using LIPOFECTAMINE® 2000 kit (Life Technologies, Grand
15 Island, NY). Peptibodies excreted into growth media were purified using Protein A chromatography (GE Healthcare Life Sciences, Pittsburgh, PA). The identity of peptibodies was verified by Western blot using anti-His-HRP (BD Biosciences) or anti-mouse IgG-HRP antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) (Table 3). Recombinant peptibodies used in all *in vivo* studies were produced by Aldevron (Madison,
20 WI).

[00152] *Animals used for in vivo studies.* Six-week-old *C57BL/6* and *Balb/c* female mice were purchased from US National Cancer Institute. S100A9-deficient female mice were a generous gift from Dr. Donna Kusewitt (M.D. Anderson Cancer Center, Smithville, TX) by permission of Dr. Johannes Roth (University of Muenster, Germany) who
25 originally generated the model. Mice were maintained in a pathogen-free mouse facility according to institutional guidelines. All animal studies were approved by the Institutional Animal Care and Use Committee at MD Anderson Cancer Center.

[00153] *FACS analysis of binding specificity of peptibodies.* Recombinant peptibodies were conjugated with FITC or APC using FLUOREPORTER® Protein Labeling
30 kits (Life Technologies). All other fluorophore-labeled monoclonal antibodies used for immune staining of cell type-specific markers including CD11b, Gr-1, Ly6C, Ly6G, CD3, CD19, CD49b and CD11c were purchased from BD Biosciences and BioLegend (San Diego,

CA) (Table 3). To determine the binding pattern of Pep-H6 and -G3, splenocytes from EL4-bearing *C57BL/6* mice, EG.7-bearing *C57BL/6* mice, B16-bearing *C57BL/6* mice, and A20-bearing *Balb/c* mice were co-stained with anti-CD11b-APC, anti-Ly6G-PE, anti-Ly6C-PerCP, and FITC-conjugated peptibodies. Peptibody binding was analyzed on

5 CD11b⁺Ly6G⁺Ly6C^{int/low} gated granulocytic MDSC and CD11b⁺Ly6G⁻Ly6C^{high} gated monocytic MDSC, respectively. To further characterize the binding specificity of peptibodies, splenocytes from EL4-bearing *C57BL/6* mice were co-stained with anti-CD11c-PerCP, anti-Ly6G-PE, and peptibody-FITC. CD11c⁺Ly6G⁻ DC were analyzed for peptibody binding. In a separate experiment, splenocytes from EL4-bearing *C57BL/6* mice were co-

10 stained for CD19-APC, CD3-PerCP, CD49b-PE, and peptibody-FITC. CD3⁺ T cells, CD19⁺ B cells, and CD3⁻CD49b⁺ NK cells were gated and analyzed for peptibody binding. Likewise, bone marrow cells from EL4-bearing *C57BL/6* mice were isolated and co-stained with anti-CD11b-APC, anti-Gr-1-PE, and FITC-conjugated peptibodies. CD11b⁺Gr-1⁺ immature myeloid cells were analyzed for their binding to peptibodies. Finally, to determine

15 whether Pep-H6-bound population would be overlapped with Pep-G3-bound populations, splenocytes from EL4-bearing *C57BL/6* mice were co-stained with anti-Gr-1-PE, anti-CD11b-PerCP, and either APC-labeled Pep-G3 or FITC-labeled Pep-H6, or both. CD11b⁺Gr-1⁺ gated MDSC were analyzed for peptibody binding.

[00154] *In vivo MDSC depletion.* Groups of five *C57BL/6* mice were

20 challenged s.c. with 10⁶ EL4 or EG.7 mouse thymoma tumor cells on day 0. Tumor-bearing mice were treated i.v. with 50 µg of peptibodies per day for three consecutive days (days 17, 18 and 19), and then sacrificed on day 20 to harvest blood and spleens. Control mice received Gr-1 monoclonal antibodies (clone 1A8, BioXcell, West Lebanon, NH), irrelevant control peptibody (Pep-irrel), or PBS. Ficoll blood and splenocytes were stained for Gr-1-

25 PE and CD11b-APC to identify MDSC. To prepare single cell suspensions from harvested tumors, EL4 or EG.7 subcutaneous tumors were cut into small pieces of 2-4 mm and digested with the enzyme mix (40 min at 37 °C) provided in "Tumor Dissociation Kit" (Miltenyi Biotec Inc., Auburn, CA). Tumors were dissociated into single cell suspension with a GENTLEMACS™ Dissociator (Miltenyi Biotec Inc.). The tumor cells were then stained for

30 Gr-1-PE and CD11b-APC to identify intratumoral MDSC. To determine whether the *in vivo* depletion effect of peptibodies was MDSC-specific, splenocytes from peptibody-treated, EL4-bearing *C57BL/6* mice were co-stained for CD11b-APC, CD11c-PerCp, and Ly6G-PE

and myeloid cells were gated based on forward and side scatter profile. Ly6G⁻CD11c⁺ DC were enumerated and their representative frequencies were calculated by multiplying the myeloid cell frequency in total splenocytes. Splenocytes were also stained for CD19-APC, CD3-PerCP, and CD49b-PE, and frequencies of CD3⁺ T cells, CD19⁺ B cells, and CD3⁻ CD49b⁺ NK cells in total splenocytes were analyzed. Bone marrow cells were stained for CD11b-APC and Gr-1-PE. Double positive immature myeloid cells were enumerated.

[00155] *In vivo therapeutic studies.* Groups of five C57BL/6 mice were challenged with 10⁶ EL4 tumor cells on Day 0. Twenty-four hours later, mice started to receive 50 µg of peptibodies per day, every other day for two weeks. Control mice received Gr-1 monoclonal antibodies (BioXcell), Pep-irrel, or PBS. Tumor dimensions were measured daily with calipers to monitor growth. On day 14, mice were sacrificed and spleens and subcutaneous tumors were isolated. Tumor mass was measured on an analytical balance. Splenocytes were stained for MDSC using anti-Gr-1-PE and anti-CD11b-APC antibodies.

[00156] *Identification of targets for peptibodies on the surface of MDSC.* Splenocytes from day 21 EL4-bearing C57BL/6 mice were immunolabeled for Gr-1-PE and CD11b-APC and double-positive MDSC were sorted. Cell surface proteins on sorted cells were biotinylated with EZ-LINK™ Amine-PEG-Biotin kit (Thermo Scientific, Rockford, IL) and precipitated by monomeric avidin after cell lysis. A sequential 2nd immunoprecipitation was performed using Pep-H6 immobilized on Protein A-agarose. After washing away unbound proteins, the eluate was analyzed by proteomic sequencing in the Proteomic core at MD Anderson Cancer Center.

[00157] *Immunoprecipitation and Western blot.* To confirm the results of proteomic analysis, total cell lysates prepared from Pep-H6-bound, sorted MDSC (without biotinylation) were loaded onto a protein A column. The eluate was separated by SDS-PAGE followed by immunoblotting with S100A9 antibodies (Abcam, Cambridge, MA) (Table 3). Recombinant mouse S100A9 protein (rprotein) (Fitzgerald, Acton, MA) served as a positive control, and lysates from unbound MDSC were negative controls. Input lysates were blotted with actin as an internal control. Western blot was also performed on the Protein A eluates of Pep-H6-bound MDSC lysates or total cell lysates alone using anti-S100A8 antibodies (Abcam).

Table 3. List of antibodies used in the present studies

Antibody	Clone number	Working concentration	Supplier	Catalog number
Anti-CD3-PerCP	145-2C11	1 µg/mL	BD Bioscience	553067
Anti-CD11b-APC	M1/70	1 µg/mL	BD Bioscience	553312
Anti-CD11b-PerCP	M1/70	1 µg/mL	BD Bioscience	550993
Anti-CD11c-PerCP	HL3	1 µg/mL	BD Bioscience	560584
Anti-CD19-APC	1D3	1 µg/mL	BD Bioscience	550992
Anti-CD49-PE	HMa2	1 µg/mL	BD Bioscience	558759
Anti-Gr-1-FITC	RB6-8C5	1 µg/mL	BD Bioscience	553127
Anti-Gr-1-PE	RB6-8C5	1 µg/mL	BD Bioscience	553128
Anti-Ly6C-PerCP	HK1.4	1 µg/mL	BioLegend	128011
Anti-Ly6G-PE	1A8	1 µg/mL	BD Bioscience	551461
Goat anti-mouse IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	115-035-166
Anti 6xHis-HRP	F24-796	1:1,000	BD Bioscience	552564
Anti-S100A9	2B10	1:1,000	Abcam	Ab105472
Anti-S100A8	EPR3554	1:1,000	Abcam	Ab92331
Goat anti-rat IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	112-035-167
Goat anti-rabbit IgG-HRP	pAb	1:10,000	Jackson ImmunoResearch	112-035-003

[00158] *S100A9-deficient mouse studies.* Groups of three S100A9-deficient *C57BL/6* mice were challenged s.c. with 10^6 EL4 tumors on day 0. Three weeks later, splenocytes were harvested and co-stained with anti-CD11b-APC, anti-Gr-1-PE, and peptibody-FITC. CD11b⁺Gr-1⁺ MDSC were analyzed for peptibody binding. In a separate experiment, EL4-bearing S100A9-deficient *C57BL/6* mice were treated i.p. with 50 µg of peptibodies for three consecutive days (days 17, 18 and 19). Splenocytes were harvested on day 20 and stained for MDSC, as above.

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[00159] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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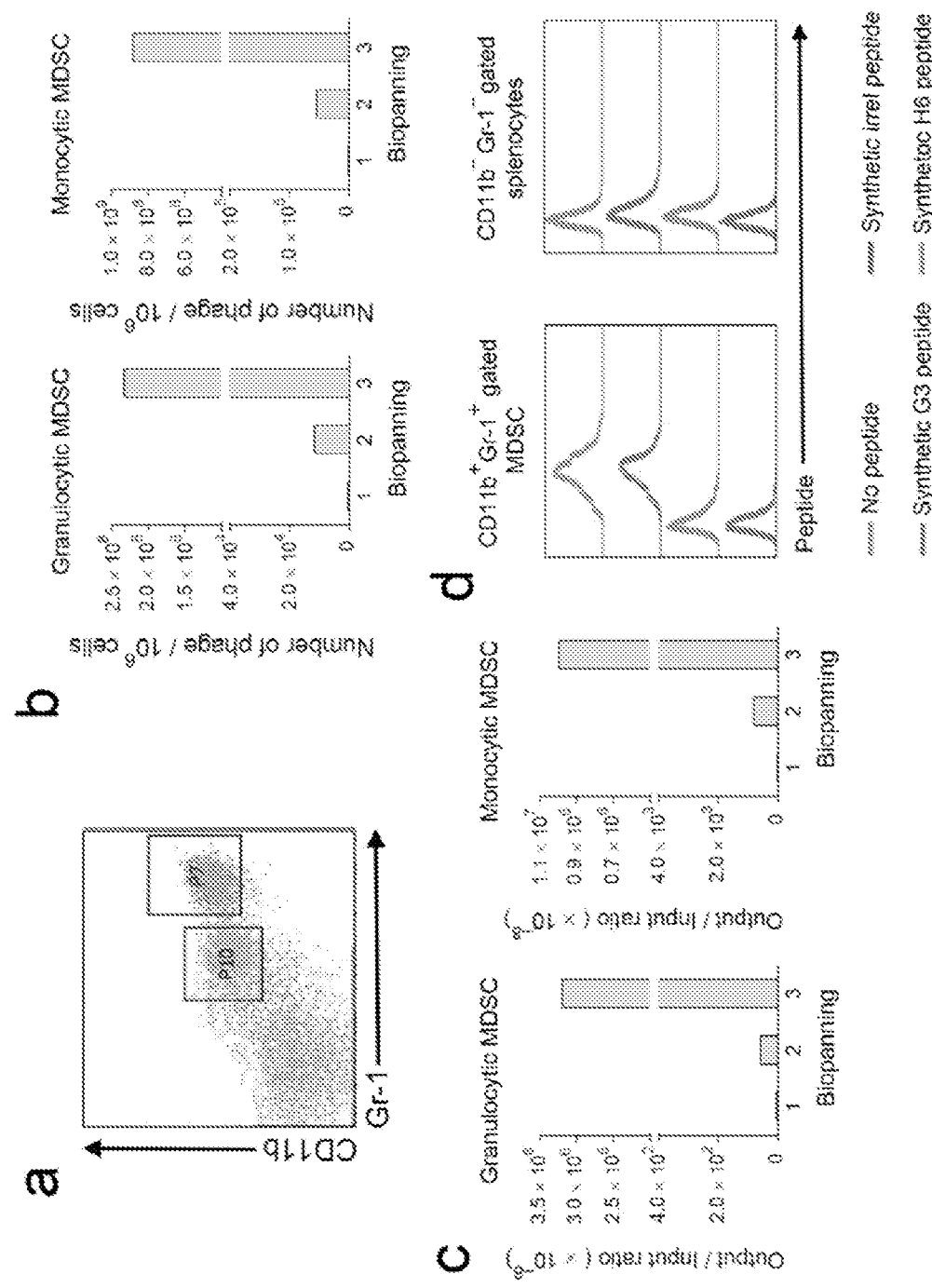
CLAIMS

1. A peptibody comprising a peptide that specifically binds to a myeloid-derived suppressor cell (MDSC) fused to an Fc portion of an IgG antibody.
- 5 2. The peptibody of claim 1, wherein the IgG antibody is a human or a mouse IgG antibody.
3. The peptibody of claim 1, wherein the MDSC is a mouse MDSC.
4. The peptibody of claim 3, wherein the peptide has a sequence according to either SEQ ID NOs: 1 or 2.
- 10 5. The peptibody of claim 2, wherein the peptibody has at least 90% sequence identity to the sequence of either SEQ ID NOs: 3 or 4.
6. The peptibody of claim 5, wherein the peptibody has at least 95% sequence identity to the sequence of either SEQ ID NOs: 3 or 4.
7. The peptibody of claim 6, wherein the peptibody has at least 99% sequence identity to the
15 sequence of either SEQ ID NOs: 3 or 4.
8. The peptibody of claim 7, wherein the peptibody has a sequence according to either SEQ ID NOs: 3 or 4.
9. The peptibody of claim 1, wherein the MDSC is a human MDSC.
10. The peptibody of claim 1, wherein the peptibody is conjugated to an imaging agent or
20 a radioligand.
11. The peptibody of claim 10, wherein the imaging agent is a fluorochrome.
12. A nucleic acid comprising a nucleotide sequence encoding the peptibody of claim 1.
13. An expression vector comprising the nucleic acid of claim 12.
14. A host cell comprising the nucleic acid of claim 12.

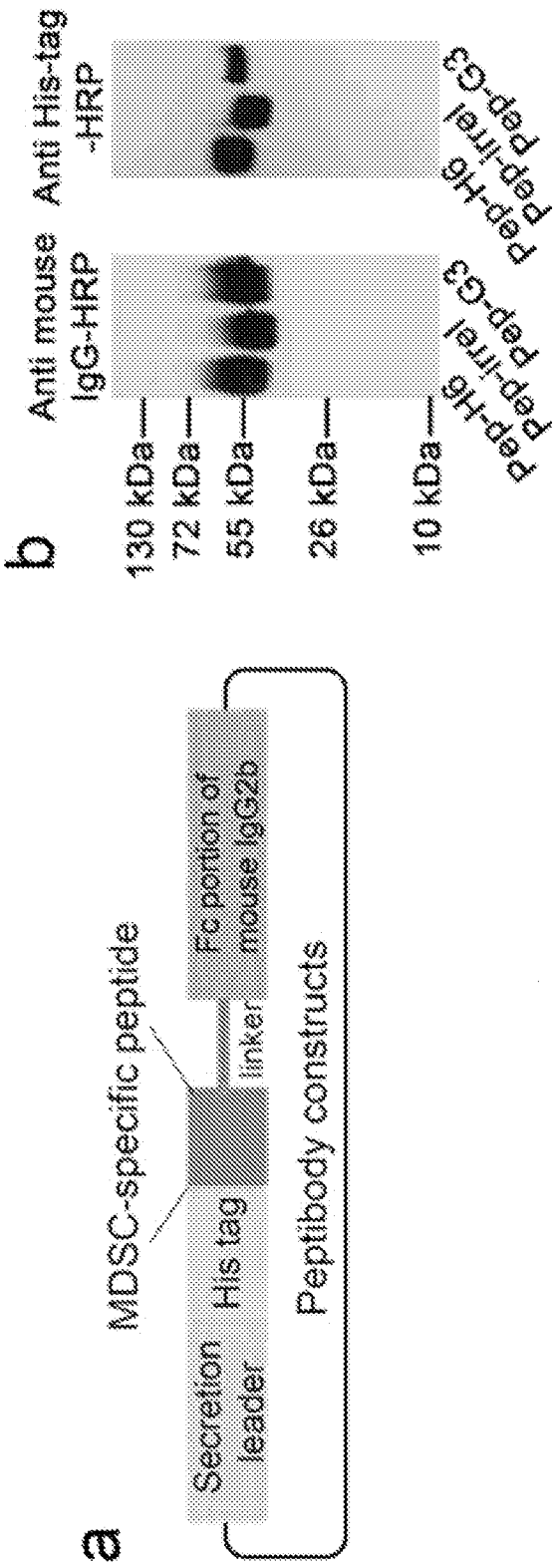
15. The host cell of claim 14, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, or a mammalian cell.
16. A method of producing the polypeptide of claim 1 comprising expressing the peptibody in a host cell of claim 14 and purifying the peptibody therefrom.
- 5 17. A pharmaceutical formulation comprising the peptibody of claim 1 or the nucleic acid of claim 12 in a pharmaceutically acceptable carrier.
18. A method of depleting MDSC in a subject comprising administering to the subject the formulation of claim 17.
19. The method of claim 18, wherein the method is further defined as a method of
10 inhibiting tumor growth.
20. The method of claim 18, wherein the method is further defined as a method of treating cancer.
21. The method of claim 20, wherein the cancer is a breast cancer, lung cancer, head and neck cancer, prostate cancer, esophageal cancer, tracheal cancer, brain cancer, liver cancer,
15 bladder cancer, stomach cancer, pancreatic cancer, adrenal cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer, blood cancer, or skin cancer.
22. The method of claim 20, wherein the cancer is metastatic, recurrent, or multi-drug resistant.
- 20 23. The method of claim 18, wherein the subject is a human patient.
24. The method of claim 18, wherein the subject is a non-human mammal.
25. The method of claim 18, wherein the formulation is administered systemically, intravenously, intradermally, intraperitoneally, intramuscularly, subcutaneously, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, via a
25 catheter, or via a lavage.
26. The method of claim 18, further comprising administering at least a second therapy to the subject, wherein the second therapy is an anticancer therapy.

27. The method of claim 26, wherein the second anticancer therapy is a surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy or cytokine therapy.
28. The method of claim 18, wherein the formulation is administered at least a second
5 time.
29. The method of claim 18, wherein the formulation is administered over a period of 1 week to 6 months.
30. A method of identifying a MDSC comprising (a) obtaining a sample from a subject; (b) contacting the sample with a peptide that specifically binds to a MDSC, wherein said
10 peptide has a sequence according to either SEQ ID NOs: 1 or 2; and (c) detecting binding of the peptide to a cell in the sample, wherein said binding identifies the cell as a MDSC.
31. The method of claim 30, wherein the peptide is conjugated to an imaging agent or radioligand.
32. The method of claim 31, wherein detecting is further defined as detecting binding of
15 the imaging agent or radioligand to the cell.
33. The method of claim 30, wherein the peptide is comprised within a peptibody of claim 1.
34. A method of identifying a MDSC comprising (a) obtaining a sample from a subject; (b) contacting the sample with a peptibody of claim 1; and (c) detecting binding of the
20 peptibody to a cell in the sample, wherein said binding identifies the cell as a MDSC.
35. The method of claim 30 or 34, wherein the subject is a human patient.
36. The method of claim 30 or 34, wherein the sample is a tumor sample, a spleen sample, or a serum sample.
37. A kit comprising the peptibody of claim 1.
- 25 38. A composition comprising a peptibody according to claim 1 or a nucleic acid according to claim 12, for use in the treatment of a cancer in a subject.

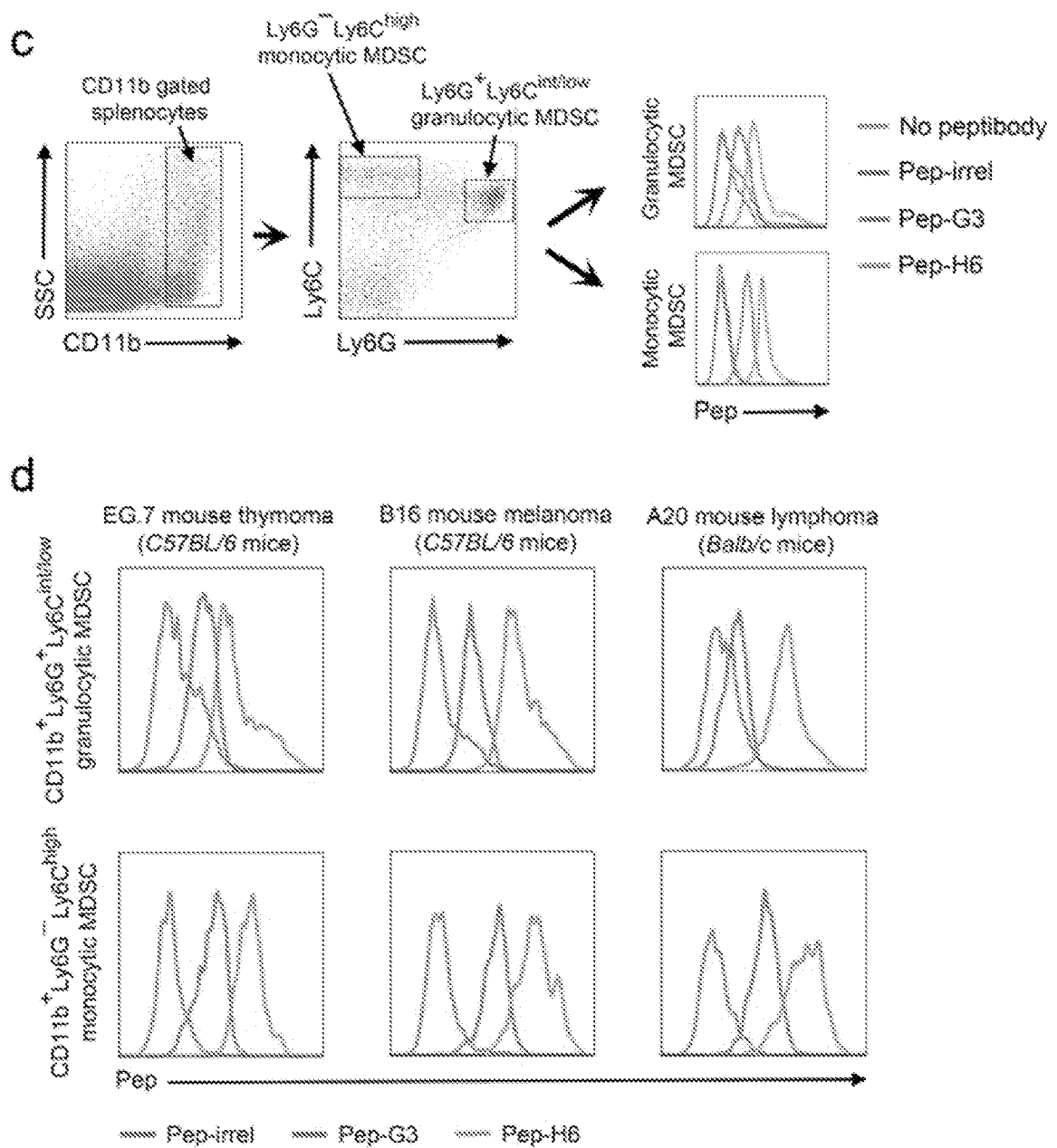
39. The composition of claim 38, wherein the peptibody is coupled to polyethylene glycol (PEG).
40. The composition of claim 38, wherein the nucleic acid is codon optimized for expression in bacteria, fungus, insects, or mammals.
- 5 41. The composition of claim 38, wherein the composition is formulated for intratumoral, intravenous, intradermal, intraarterial, intraperitoneal, intralesional, intracranial, intraarticularly, intraprostatic, intrapleural, intratracheal, intraocular, intranasal, intravitreal, intravaginal, intrarectal, intramuscular, subcutaneous, subconjunctival, intravesicularl, mucosal, intrapericardial, intraumbilical, oral administration.
- 10 42. The composition of claim 38, further comprising at least a second anticancer therapy.
43. The composition of claim 42, wherein the second anticancer therapy is chemotherapy, hormone therapy, immunotherapy or cytokine therapy.
44. Use of a peptibody according to claim 1 or a nucleic acid according to claim 12 in the manufacture of a medicament for the treatment of a cancer.
- 15 45. A composition comprising a peptide comprising a sequence according to either SEQ ID NOs: 1 or 2, for use in identifying a MDSC in a sample from a subject.
46. The composition of claim 45, wherein the peptide is conjugated to an imaging agent or radioligand.
47. The method of claim 45, wherein the peptide is comprised within a peptibody of
20 claim 1.
48. A composition comprising a peptibody according to claim 1, for use in identifying a MDSC in a sample from a subject.
49. The composition of claim 45 or 48, wherein the subject is a human patient.
50. The composition of claim 45 or 48, wherein the sample is a tumor sample, a spleen
25 sample, or a serum sample.



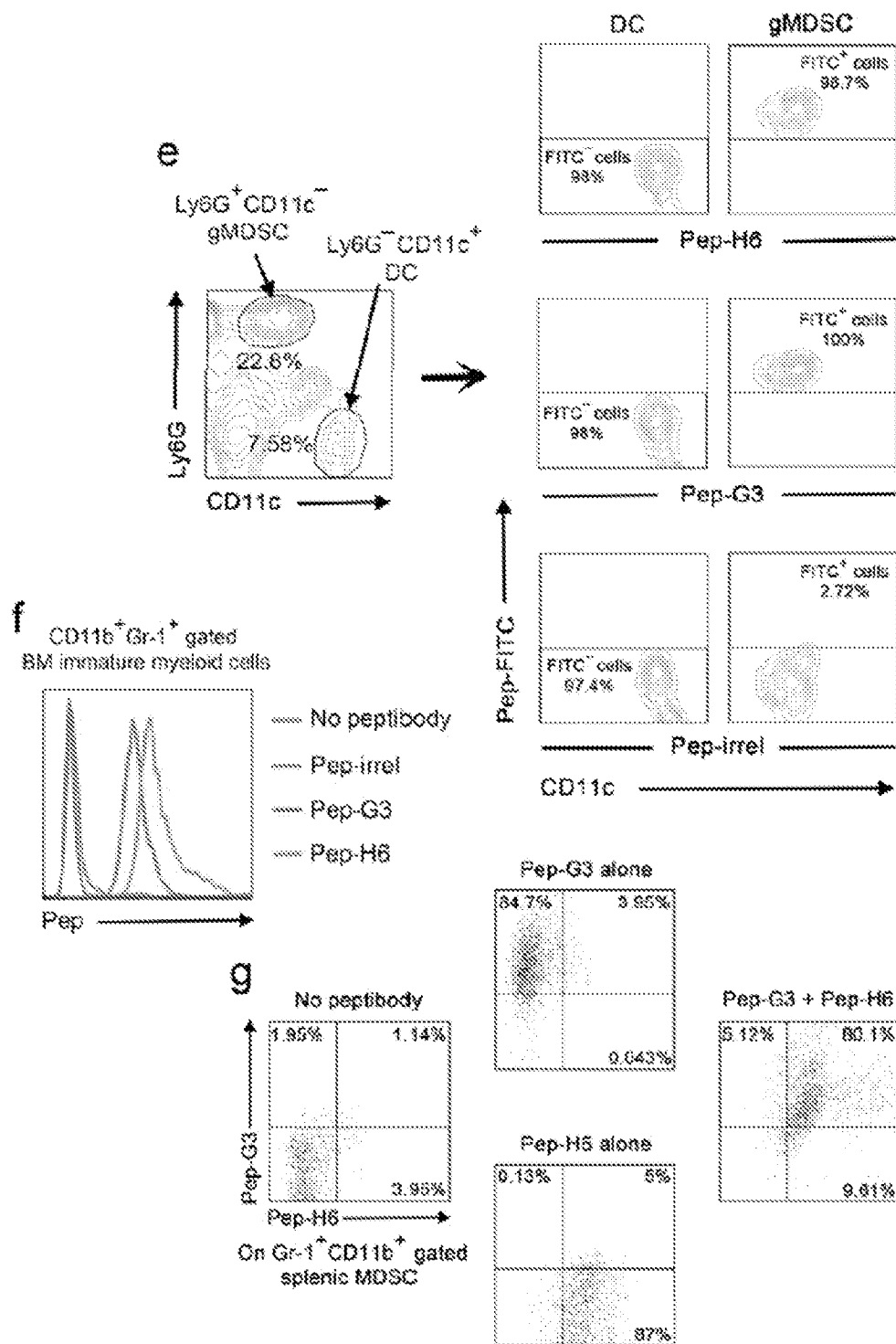
FIGs. 1a-d



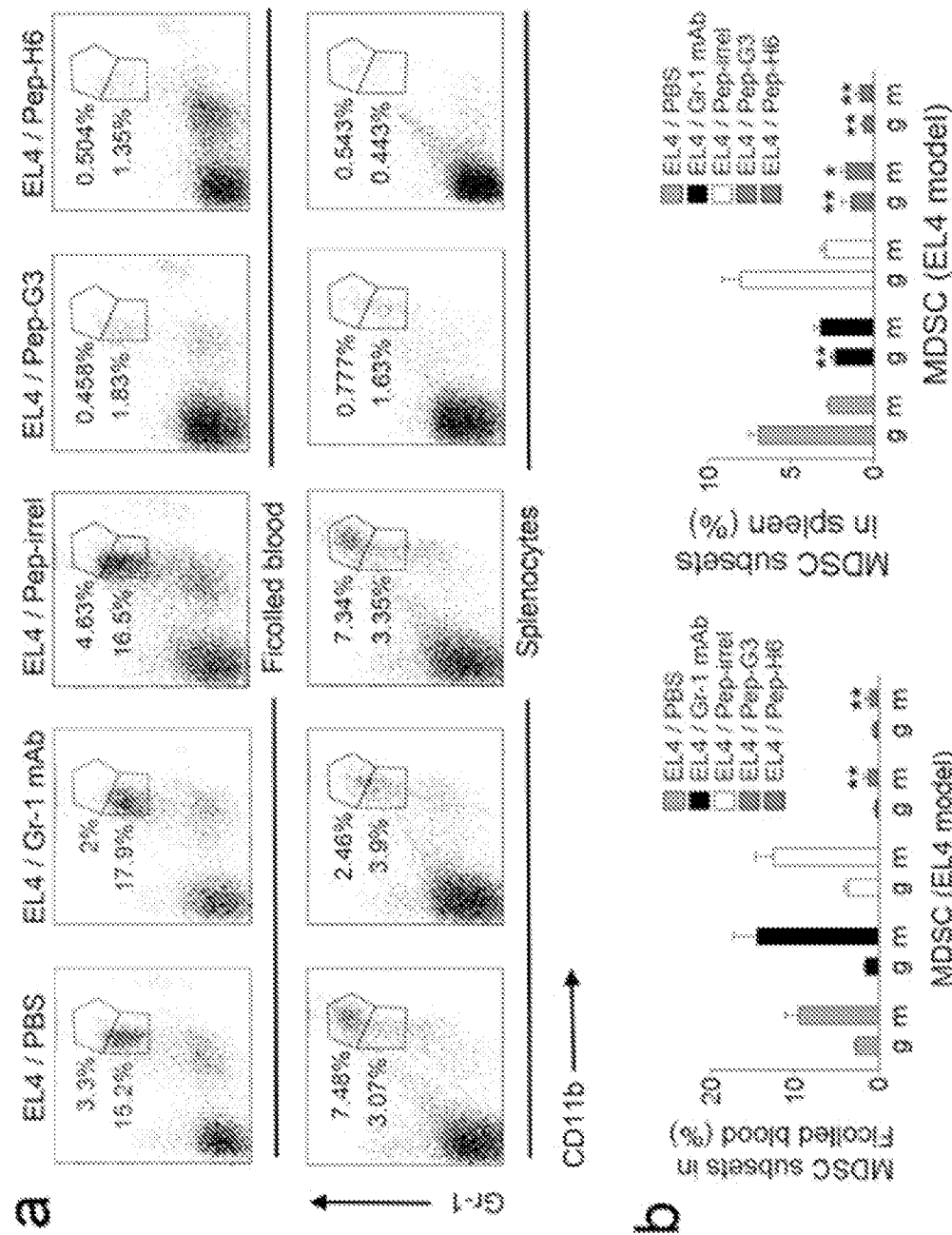
FIGs. 2a-b



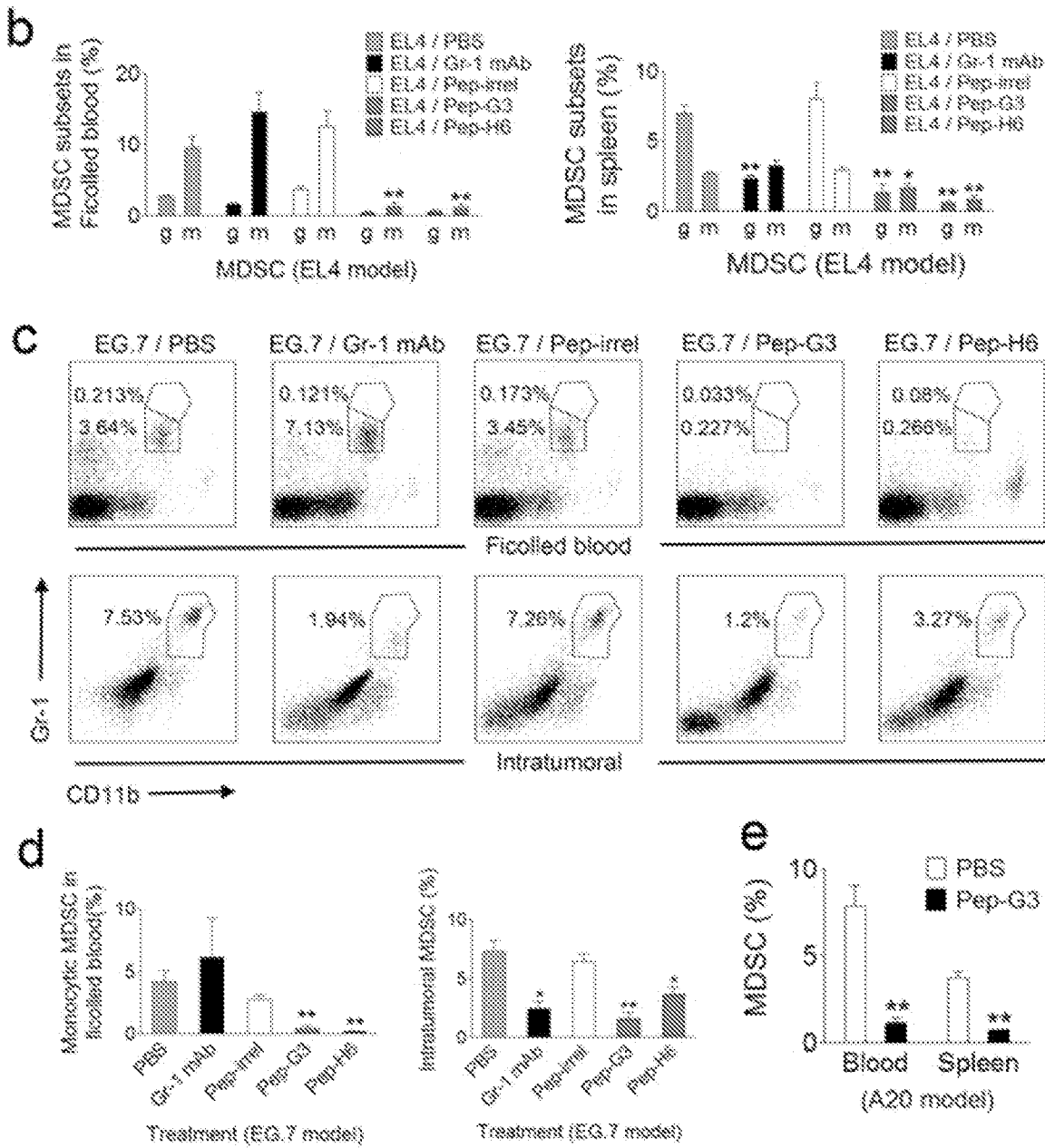
FIGs. 2c-d



FIGs. 2e-g



FIGs. 3a-b



FIGs. 3c-e

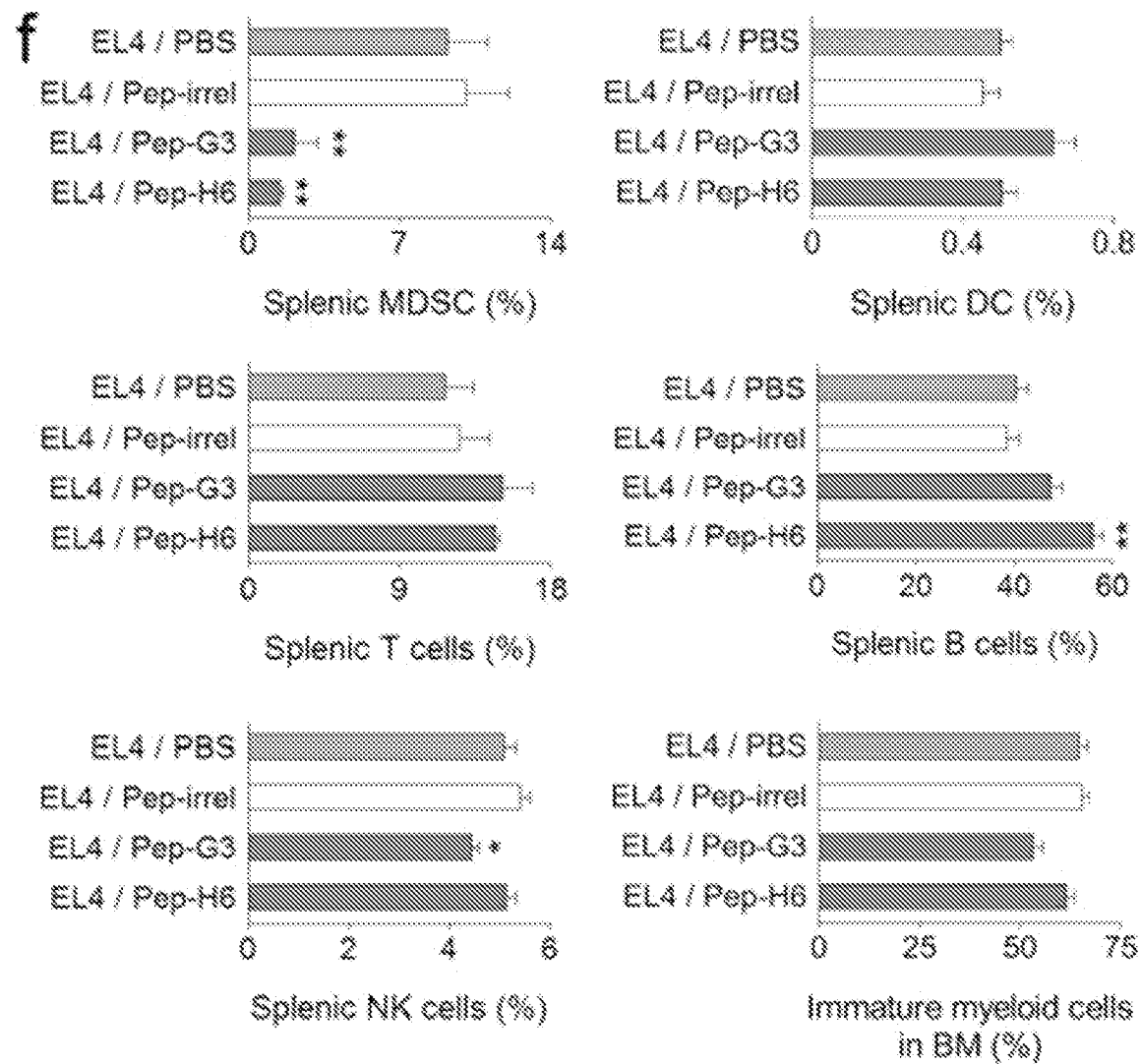
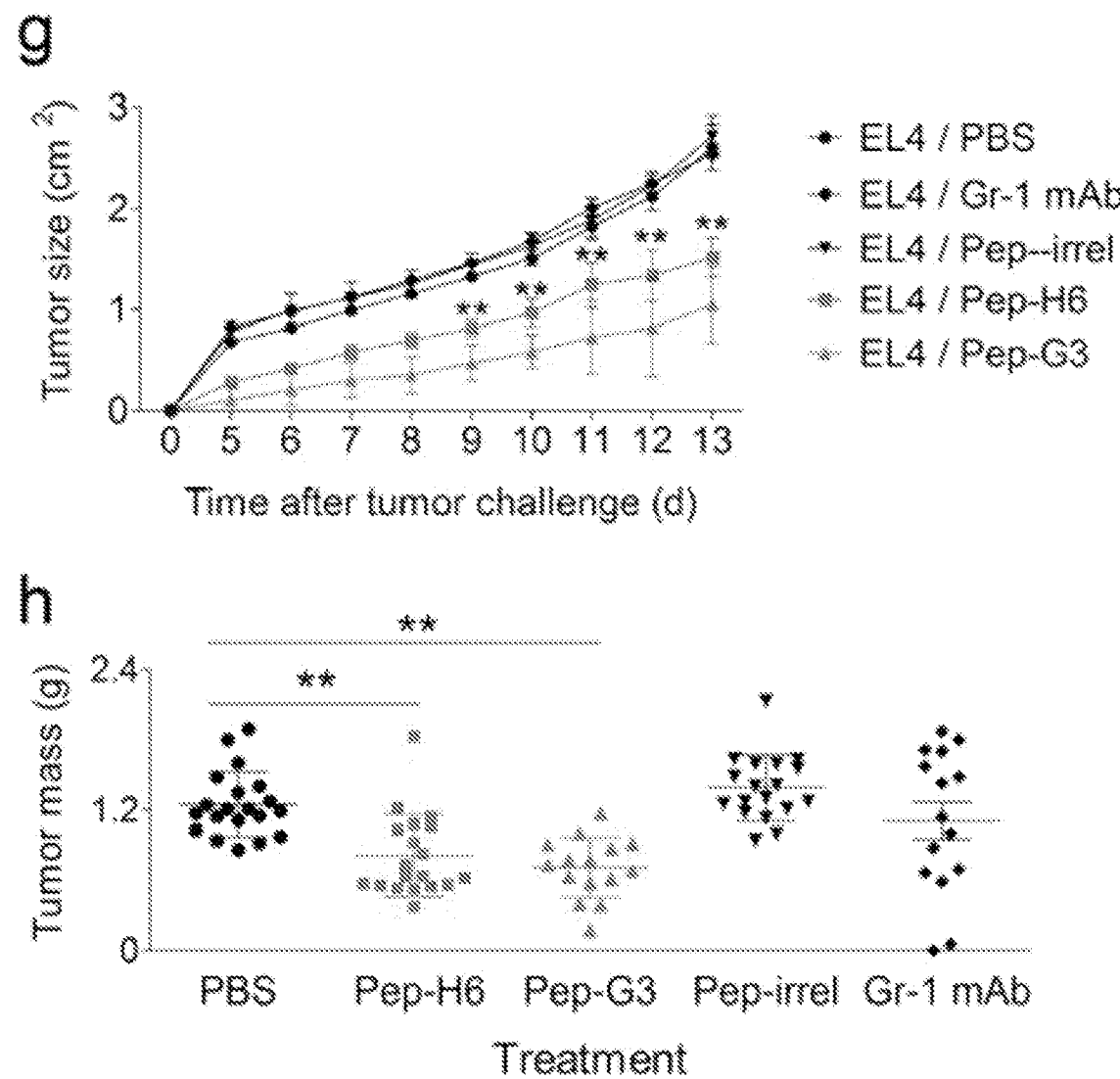
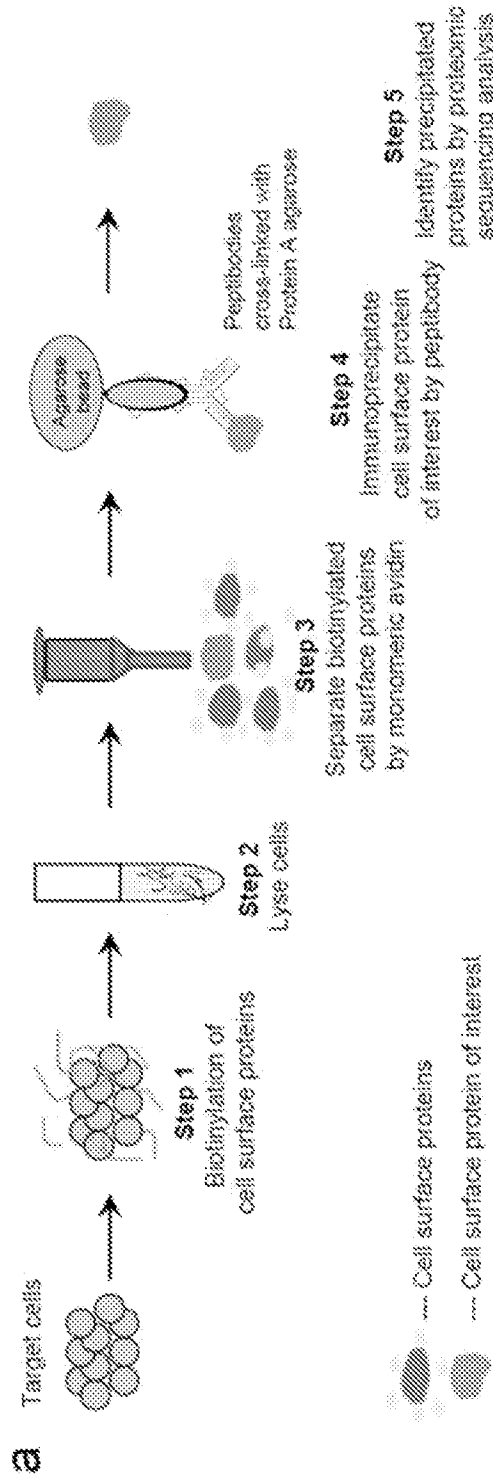


FIG. 3f



FIGs. 3g-h



b

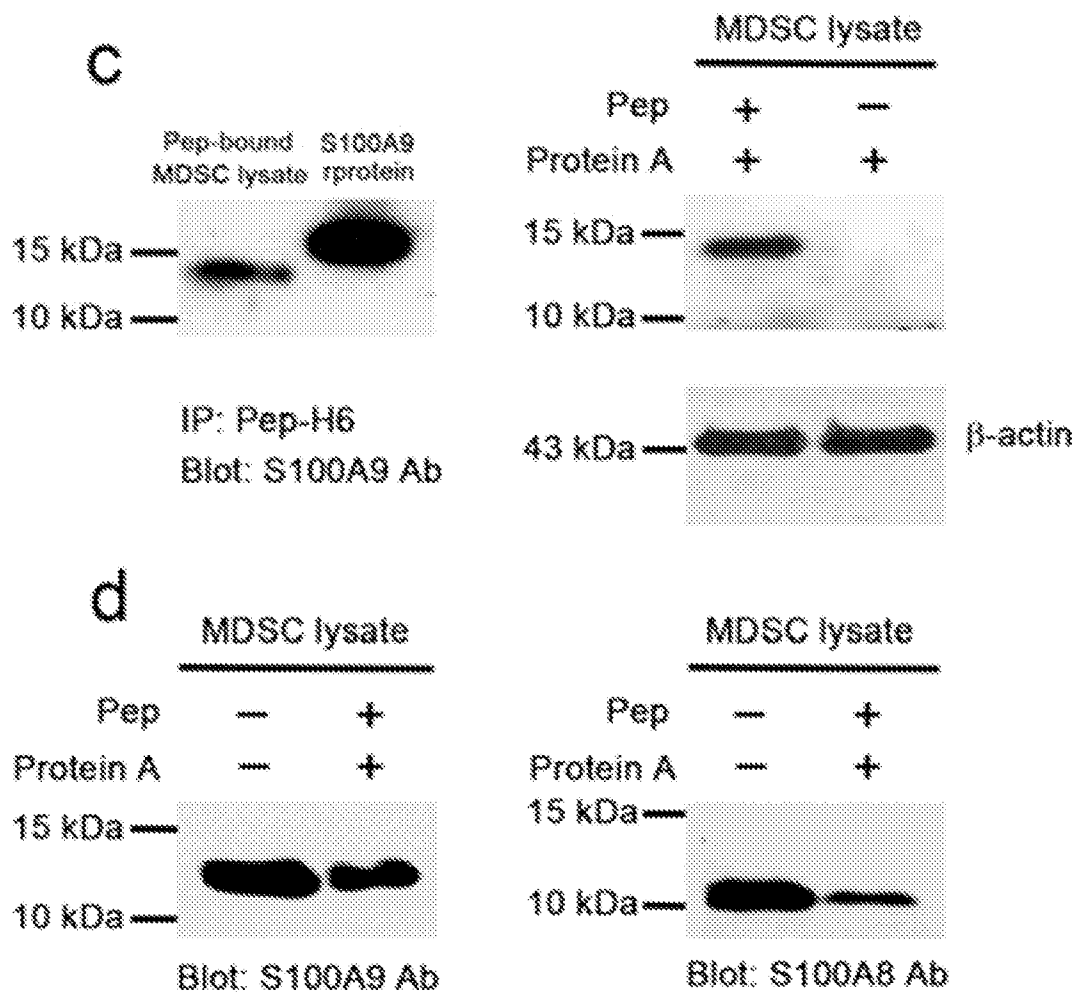
BL003_MOUSE Mass: 13040 Score: 281 Matches: 11(11) Sequences: 5(5) empAI: 5.40
Protein E100-20 US-Mus musculus E100-20 22-1 20%3

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
102	409.6958	917.2770	917.3752	2.24	0	37	0.00002	1	0	K.AVQYK.S 101
103	409.2437	982.4720	982.4720	0.89	0	23	0.000	1	0	K.EQKDTLSK.R
104	556.2907	1110.5668	1110.5668	-0.07	1	27	0.027	1	0	K.KCHPDTLSK.R
105	686.8931	1395.6916	1395.6900	1.98	0	61	1.5e-005	1	0	K.QMVEQLATPMK.K 102
106	706.8496	1411.6846	1411.6838	0.90	0	(46)	0.00028	1	0	K.QMVEQLATPMK.R
107	981.2902	1680.8488	1680.8471	0.97	0	(40)	0.0019	1	0	K.SITTIIDTFHGYR.K 103
108	841.4322	1680.8498	1680.8471	1.61	0	72	9.9e-007	1	0	K.SITTIIDTFHGYR.K 104

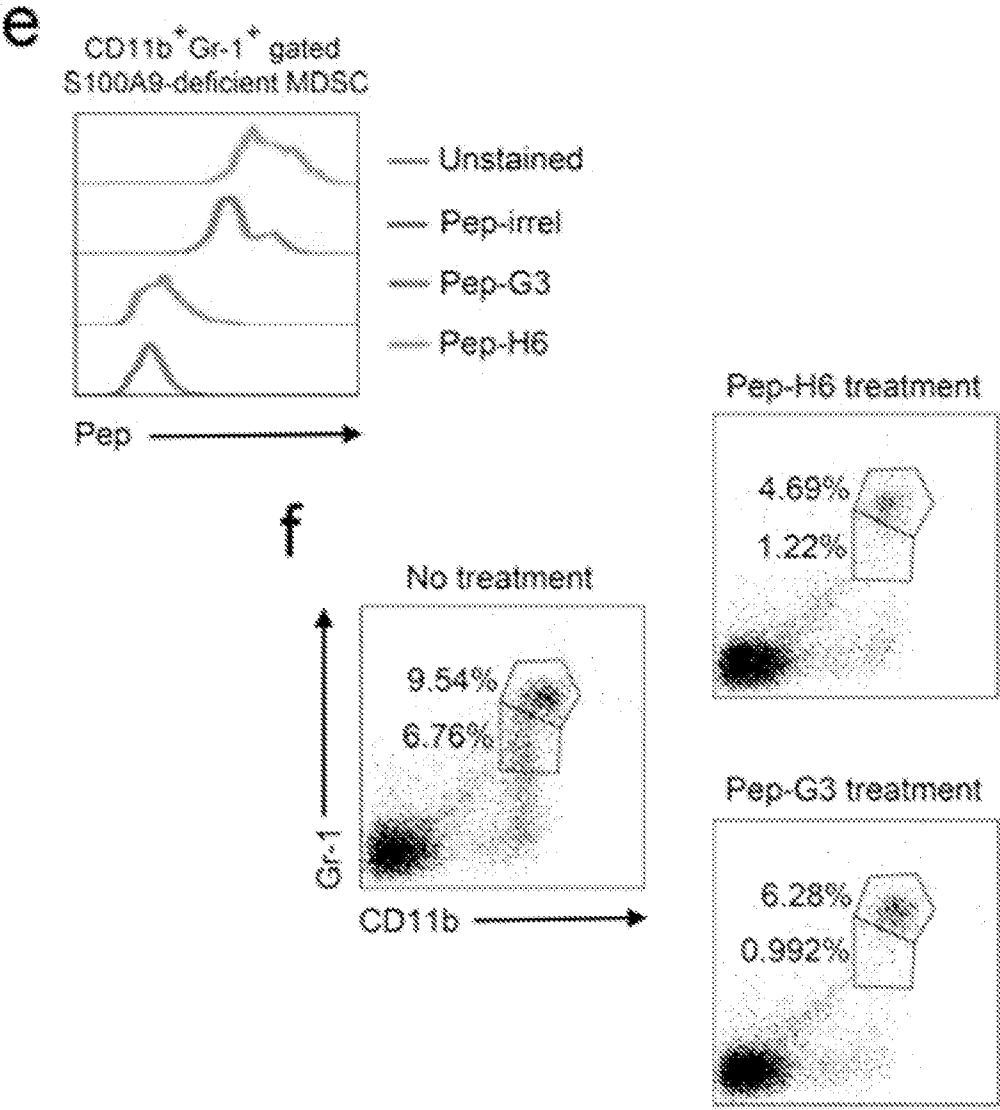
MANCAPQMPSTTTIIDTFHGYRQKCHPDTLSK.EFQMVEQLATPMKPKRNEALI
NDIMEDLTNQDNQLSFECMLMAKLIACEKRLHENNPRGHSHGKGGK

Protein sequence coverage: 41.59%

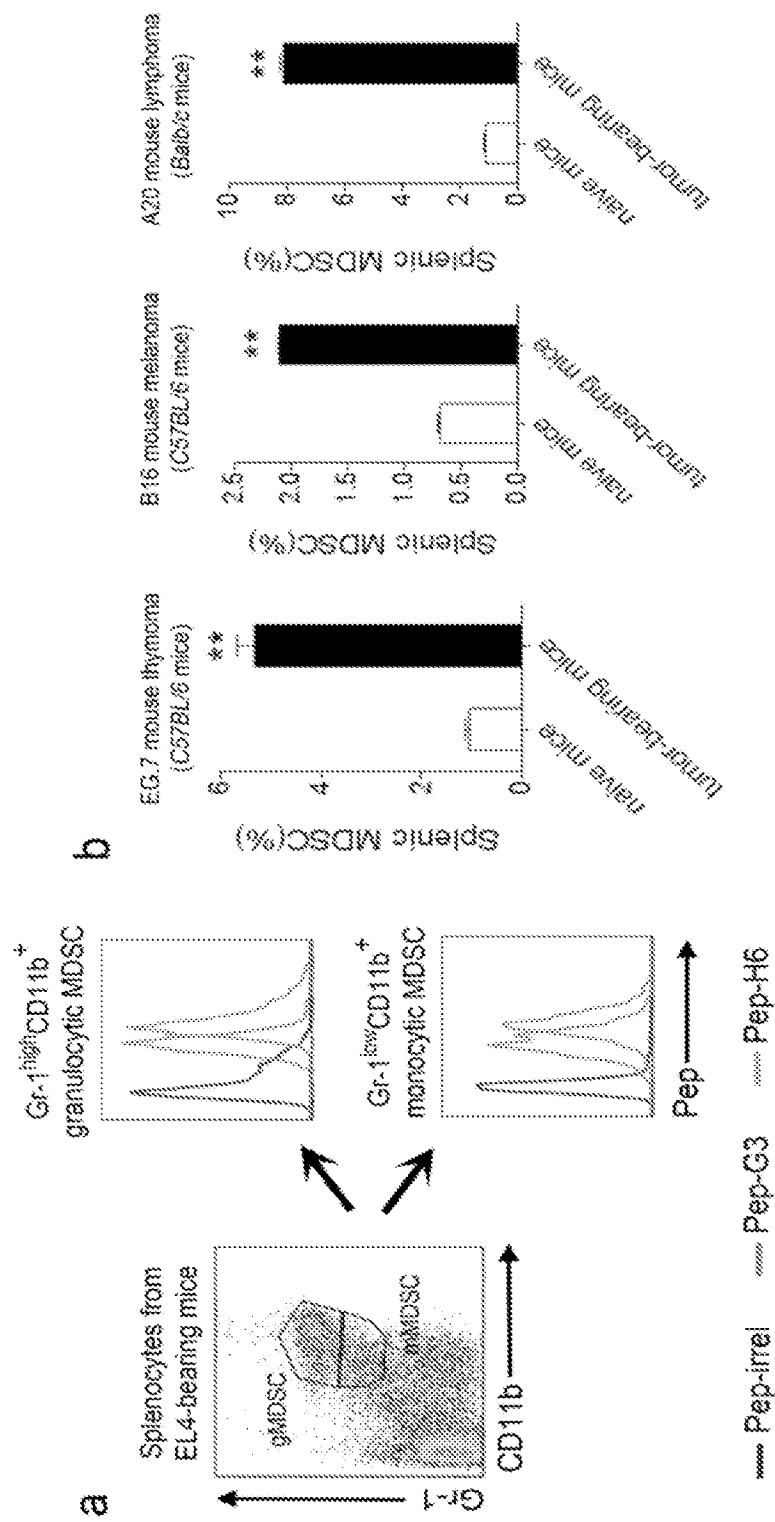
FIGs. 4a-b



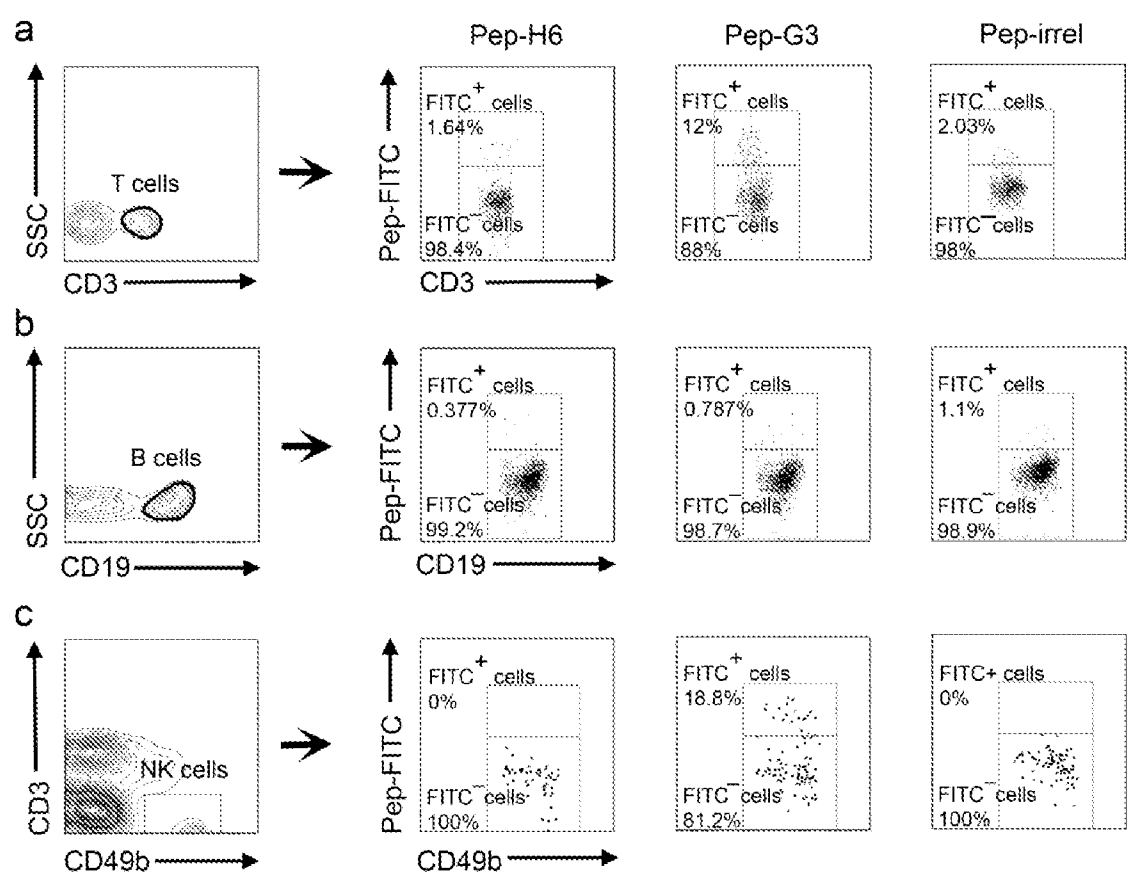
FIGs. 4c-d



FIGs. 4e-f



FIGs. 5a-b



FIGs. 6a-c

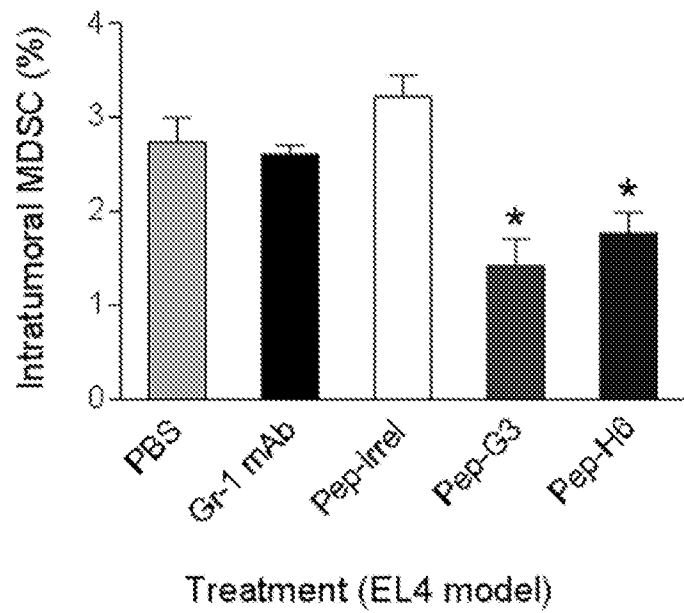


FIG. 7

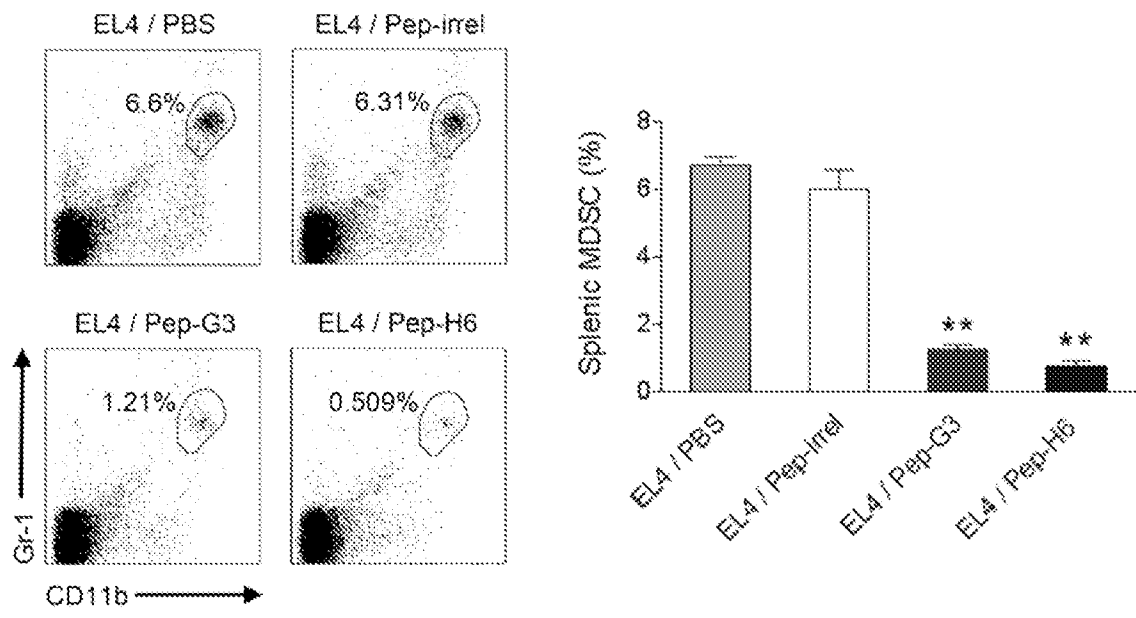
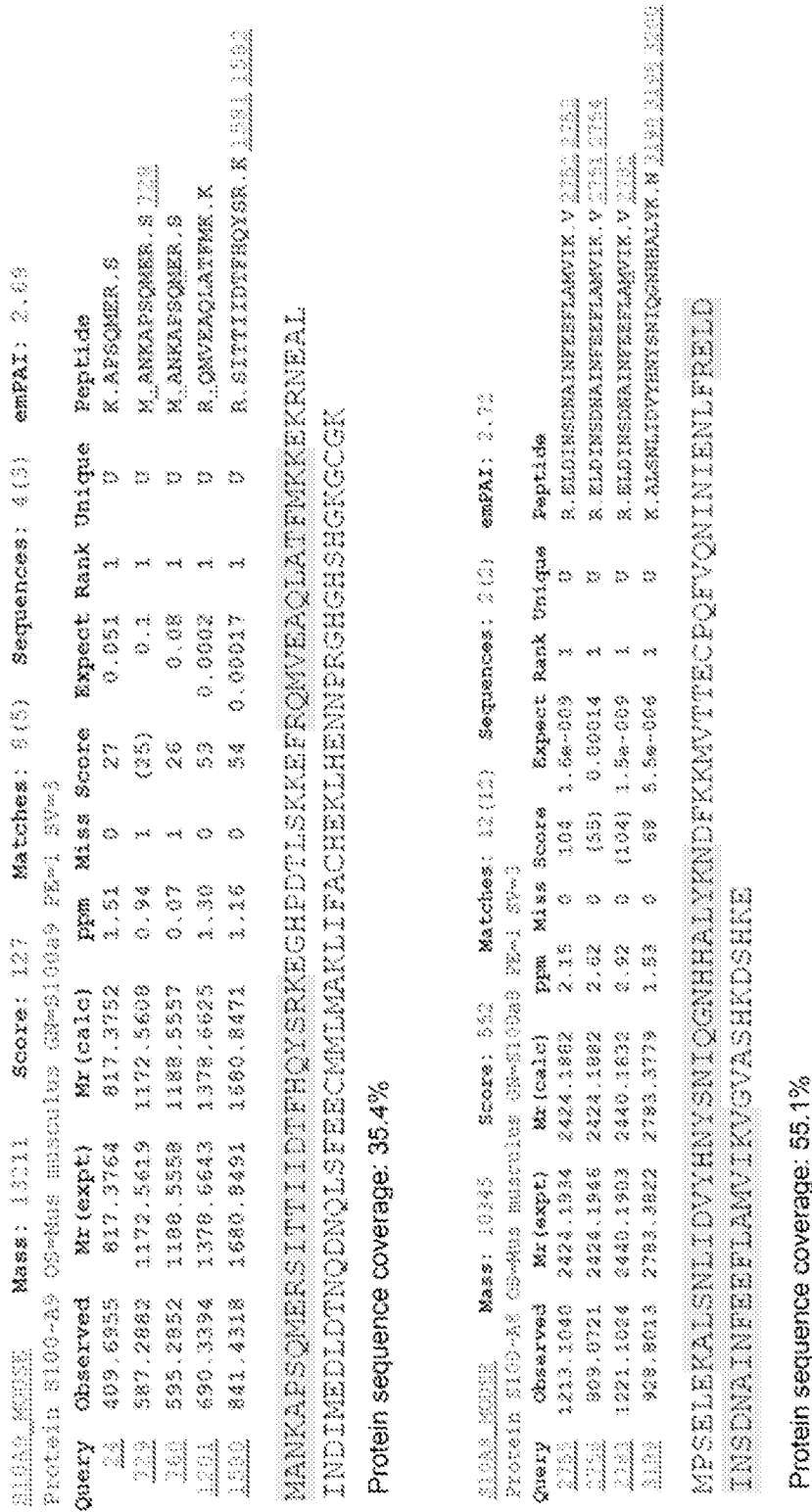


FIG. 8



SL100A_MOUSE

Mass: 10345

Score: 552

Matches: 12(12)

Sequences: 2(2)

emPAI: 2.72

Protein: SL100-A8 OS=Mus musculus GN=Sl100a8 PE=1 SV=3

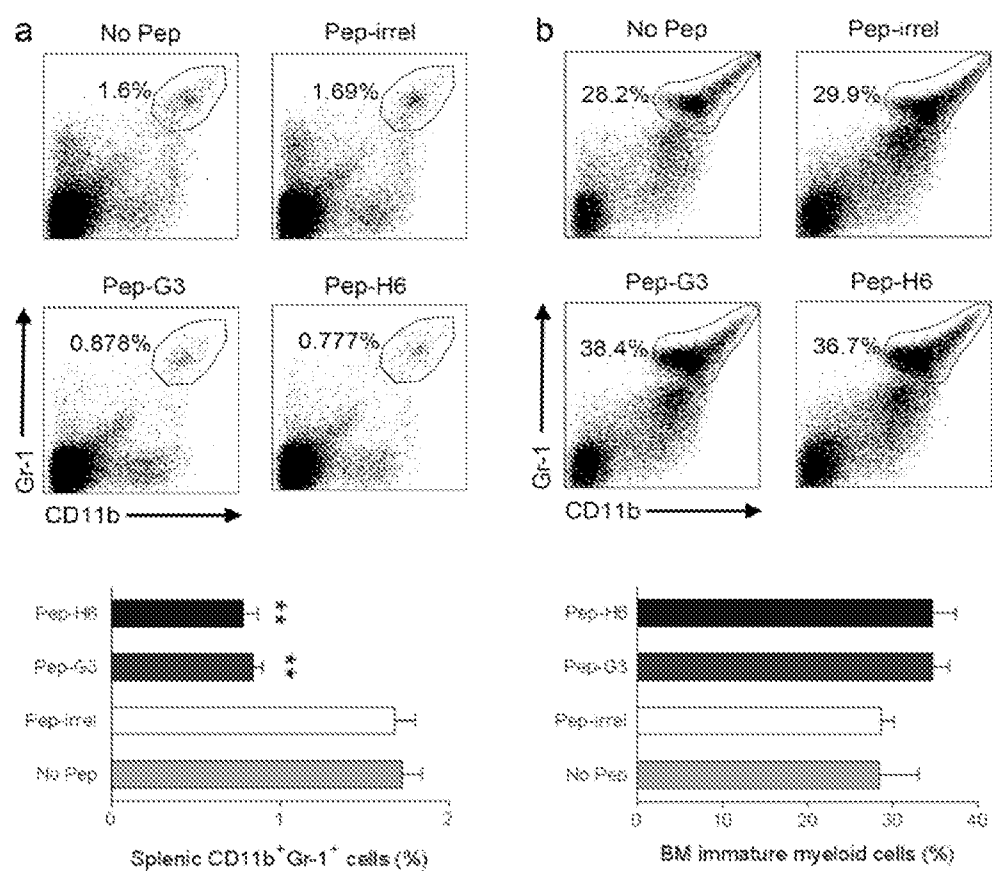
Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss Score	Expect	Rank	Unique	Peptide
222	1213.1040	2424.1934	2424.1862	2.15	0	104	1.5e-009	1	U R.ELDINSDNAINTEFEFLAMVIR.V [222] [222]
222	909.0721	2424.1946	2424.1882	2.62	0	(55)	0.00014	1	U R.ELDINSDNAINTEFEFLAMVIR.V [222] [222]
222	1221.1064	2440.1903	2440.1632	6.92	0	(104)	1.5e-009	1	U R.ELDINSDNAINTEFEFLAMVIR.V [222]
3102	909.8013	2783.3822	2783.3779	1.83	0	69	5.5e-006	1	U K.ALENLIVYHYNTSNIQGNHHAIVK.M [222] [3102] [222]

MPSELEKALSNLIDVYHNYSNIQGNHHAIVKNDFKKMVTTECPQVQNININLENLFRELD

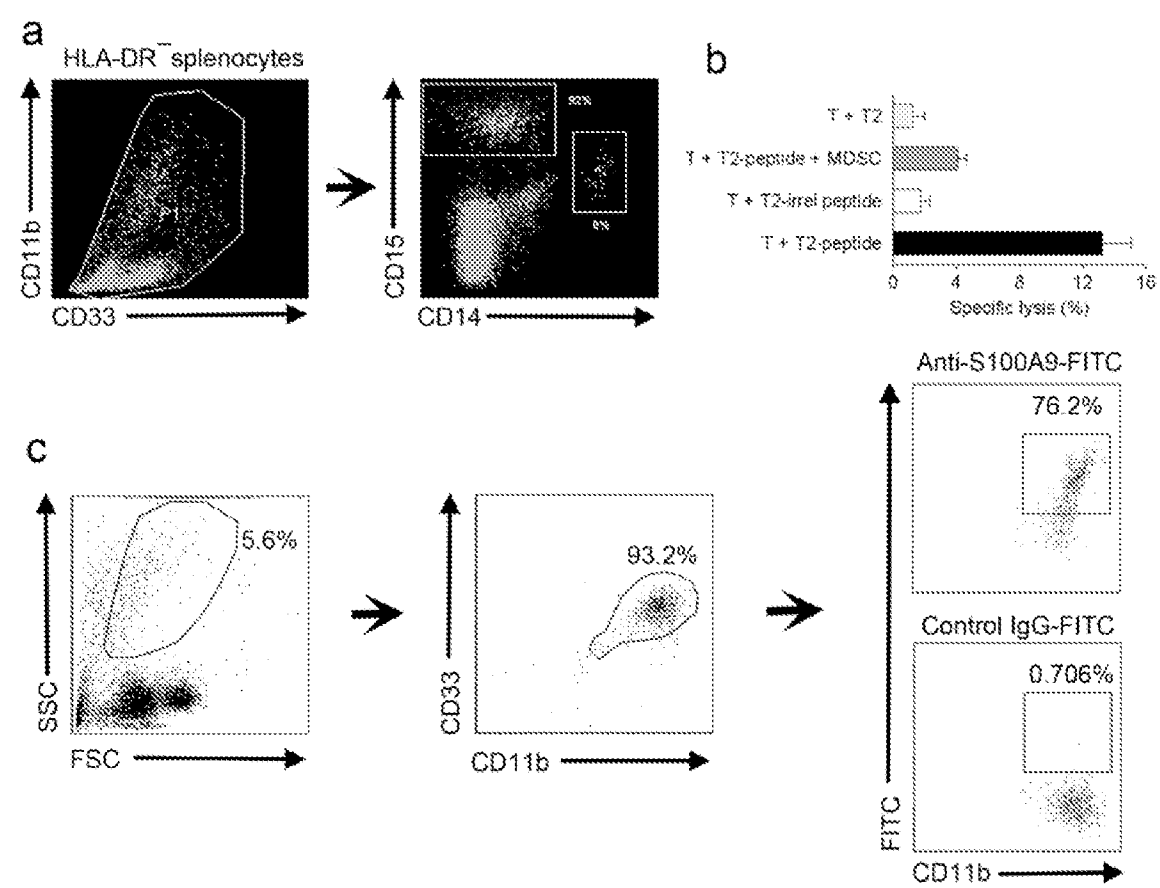
INSDNAINTEFEFLAMVIRKVGVAHKDSHKE

Protein sequence coverage: 55.1%

FIG. 9



FIGs. 10a-b



FIGs. 11a-c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/058318**A. CLASSIFICATION OF SUBJECT MATTER****C07K 19/00(2006.01)i, C07K 16/28(2006.01)i, C12N 15/63(2006.01)i, A61K 39/395(2006.01)i, A61P 35/00(2006.01)i, G01N 33/53(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 19/00; A61K 9/127; A61P 35/00; A61K 39/00; A61K 48/00; A61K 38/16; A61K 38/17; C07K 16/28; C12N 15/63; A61K 39/395; G01N 33/53

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: myeloid-derived suppressor cell, peptibody, tumor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012-054747 A2 (MOUNT SINAI SCHOOL OF MEDICINE) 26 April 2012 See abstract; and claims 1-40.	1-17, 24, 30-50
A	US 2012-0156280 A1 (DOW et al.) 21 June 2012 See abstract; and claims 1-20.	1-17, 24, 30-50
A	WO 2012-149416 A2 (UNIVERSITY OF SOUTHERN CALIFORNIA) 01 November 2012 See the whole document.	1-17, 24, 30-50
A	US 2012-0070461 A1 (SINGH et al.) 22 March 2012 See the whole document.	1-17, 24, 30-50
A	WESOLOWSKI et al., 'Myeloid derived suppressor cells-a new therapeutic target in the treatment of cancer' Journal for Immunotherapy of Cancer, Vol.1, No.10 (internal pages 1-11) (e-pub. 15 July 2013) See the whole document.	1-17, 24, 30-50
PX	QIN et al., 'Generation of a new therapeutic peptide that depletes myeloid-derived suppressor cells in tumor-bearing mice' Nature Medicine, Vol.20, No.6, pp.676-681 (June 2014) See the whole document.	1-17, 24, 30-50



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 December 2014 (29.12.2014)

Date of mailing of the international search report

02 January 2015 (02.01.2015)

Name and mailing address of the ISA/KR

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Republic of Korea

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Authorized officer

KIM, Seung Beom

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/058318**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: 18-23, 25-29
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 18-23 and 25-29 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required 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 an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/058318

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012-054747 A2	26/04/2012	CN 103260650 A EP 2629803 A2 US 2014-0086869 A1 WO 2012-054747 A3	21/08/2013 28/08/2013 27/03/2014 09/08/2012
US 2012-0156280 A1	21/06/2012	CA 2793657 A1 EP 2547366 A2 EP 2547366 A4 WO 2011-116299 A2 WO 2011-116299 A3	22/09/2011 23/01/2013 07/08/2013 22/09/2011 23/02/2012
WO 2012-149416 A2	01/11/2012	CN 103608028 A EP 2701722 A2 US 2012-0276004 A1 WO 2012-149416 A3	26/02/2014 05/03/2014 01/11/2012 10/01/2013
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