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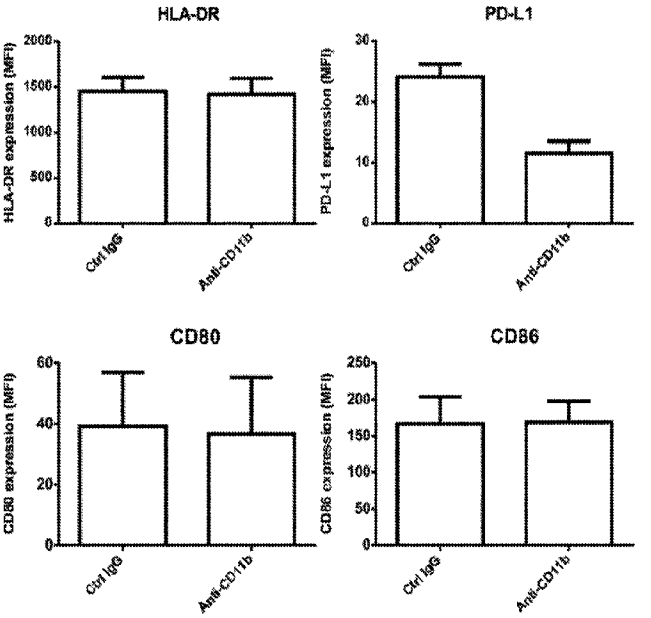

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(54) Title: METHODS AND ANTIBODIES FOR MODULATION OF IMMUNORESPONSE

(57) Abstract: Provided are an anti-CD11b antibody or an antigen-binding portion thereof, and methods and use of the antibody for modulating immunoresponses by regulating CD11b expression on cells

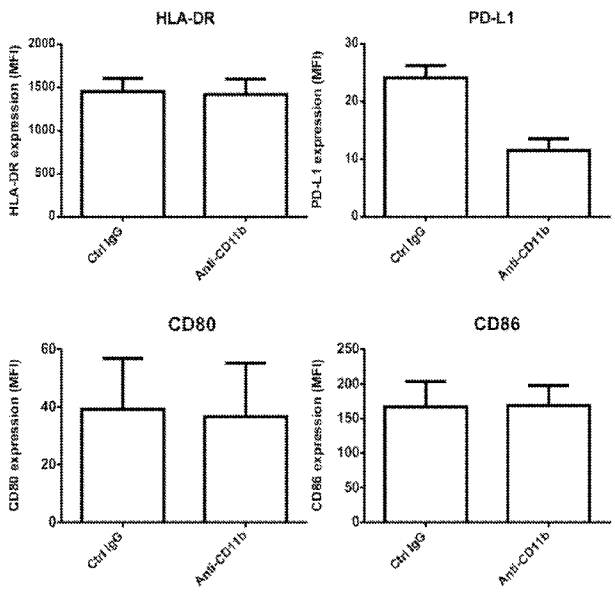

Fig. 1

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METHODS AND ANTIBODIES FOR MODULATION OF IMMUNORESPONSE

Field of the Invention

[0001] The present invention relates to the field of immunotherapy. Particularly, the present invention relates to methods and antibodies for modulating immunoresponses by regulating CD11b expression on cells.

Background of the Invention

[0002] It is widely believed that cancer cells express immunogenic antigens can induce effective immune response against tumor formation. In additions, the tumor microenvironment is rich in components that may trigger TLR signaling to activate anti-tumor response (*Standiford TJ, Keshamouni VG (2012) Breaking the tolerance for tumor: Targeting negative regulators of TLR signaling. Oncoimmunology 1: 340-345*). It means that, at initial stages of disease, cancer cells may have chance to be recognized and rejected by the immune system which exerts both host-protective- and tumor-modeling actions on developing tumors. Nonetheless, cancer cells also have numerous negative regulatory mechanisms to evade immune surveillance, such as downregulation of MHC molecules or the antigen processing and presentation machinery, increasing the secretion of inhibitory cytokines, and expressing inhibitory molecules to induce immune tolerance to cancer cells. Thus, cancer patients are often considered to have poor immunity. Thus, there is still a need to develop an agent or therapy for reversion of cancer associated immunosuppression.

[0003] Integrin alpha M (CD11b, CR3A, and ITGAM) is one protein subunit that forms the heterodimeric integrin αMβ2 molecule that expressed on the surface of many immune cells, including monocytes, granulocytes, macrophage, dendritic cells, natural killer cells, and myeloid-derived suppressor cells. Integrin αMβ2 mediates inflammation, by regulating cell

adhesion, migration, chemotaxis, and phagocytosis through its promiscuous ligand repertoire. Recent research has indicated a critical role for inflammation by modulating TLR4 response (*Han C, Jin J, Xu S, Liu H, Li N, et al. (2010) Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. Nat Immunol 11: 734-742*). A variety of endogenous integrin αMβ2 ligands within the luminal side of blood vessels, such as fibrinogen, can trigger TLR4 signaling. High avidity ligation of ITAM coupled with β2 integrin transiently induces TLR activation, but rapidly inhibits TLR signaling through targeting MyD88 and TRIF for Cbl-b-mediated proteolytic degradation. Thus integrin αMβ2 may serve as a negative regulator of that selectively inhibits components of TLR-signaling pathway to block the effects of the TLR family (*Wang L, Gordon RA, Huynh L, Su X, Park Min KH, et al. (2010) Indirect inhibition of Toll-like receptor and type I interferon responses by ITAM-coupled receptors and integrins. Immunity 32: 518-530*).

[0004] PD-L1 is one of the co-inhibitory proteins that is expressed on many types of immune cells at varying levels and is constitutively expressed on monocytes, macrophages and dendritic cells, T-cells, B-cells, epithelial cells, and vascular endothelial cells. Upon positive inductions such as IFN-γ and mitogenic stimulation, PD-L1 would be further up-regulated. PD-L1 binds to its receptor, PD-1, found on activated T cells, generating a potent immunosuppression by inducing a co-inhibitory signal in activated T-cells that promotes T-cell apoptosis and anergy (*Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ (2007) Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity 27: 111-122; Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, et al. (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T*

cells. J Exp Med 206: 3015-3029). The integrity of PD-L1/PD-1 interaction is also important to avoid excessive immune responses. Defects in the interaction between PD-L1 and PD-1 may result in uncontrollable propagation of immune responses leading to conditions such as autoimmune diseases, hypersensitivity, transplantation rejection and graft versus host disorders.

[0005] US 8,008,449 provides isolated monoclonal antibodies, particularly human monoclonal antibodies, that specifically bind to PD-1. US 8,354,509 relates to antibodies which block the binding of human Programmed Death Receptor 1 (hPD-1) to its ligands (hPD-L1 or hPD-L2). US 8,900,587 discloses antibodies which block binding of hPD-1 to hPD-L1 or hPD-L2 and a method of increasing the activity (or reducing downmodulation) of an immune cell through the PD-1 pathway. US 9,067,999 and US 9,073,994 provide compositions for cancer or infection treatment via immunopotentiation caused by inhibition of immunosuppressive signal induced by PD-1, PD-L1, or PD-L2 and therapies using them. However, the antibodies mentioned in the above patents have low response rate to therapy. US 20140099254A1 provides a method of inducing an immune response to cancer or infectious disease comprising administering to a subject with cancer or infectious disease a combination of two or more agents selected from the group consisting of (i) a leukocyte redirecting bispecific antibody including ADAM17, CD2, CD3, CD4, CD5, CD6, CD8, CD11a, CD11b, CD14, CD16, CD16b, CD25, CD28, CD30, CD32a, CD40, CD40L, CD44, CD45, CD56, CD57, CD64, CD69, CD74, CD89, CD90, CD137, CD177, CEACAM6, CEACAM8, HLA-DR alpha chain, KIR and SLC44A2; (ii) an interferon; (iii) a checkpoint inhibitor antibody including CTLA4, PD1, PD-L1, LAG3, B7-H3, B7-H4. KIR and TIM3; and (iv) an antibody-drug conjugate (ADC). However, this reference only combines a number of known immune related ingredients, while it is silent on the interplay between the ingredients.

Summary of the Invention

[0006] The present invention unexpectedly found that the expression of PD-L1 can be suppressed by CD11b modulators bound to CD11b on immune cells and/or other cells. Binding of CD11b modulator to CD11b would reduce the PD-L1 expression on LPS-primed monocytes. In LPS-induced immunosuppressed monocytes or monocytes from patients with septic shock, binding of CD11b modulator to CD11b also reduces PD-L1 expression when cells are challenged with LPS.

[0007] The invention provides a method for inhibiting PD-L1 expression in an immune cell, comprising contacting the said immune cell with a CD11b modulator that binds to CD11b on the cell, hereby regulating PD-L1 expression of the immune cells.

[0008] The invention provides a method for reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell, comprising contacting the said immune cell with a CD11b modulator that binds to CD11b on the cell.

[0009] The invention provides a method for determining a subject responsive to a CD11b modulator, said method comprising detecting whether PD-L1 is inhibited in a biological sample or a subject by contacting an immune cell in the biological sample or the subject with a CD11b modulator and detecting the inhibition of PD-L1 on the immune cell by the CD11b modulator, wherein the PD-L1 is inhibited indicates that the subject is responsive to a CD11b modulator.

[0010] In some embodiments, the CD11b modulator described herein is an RNAi agent inhibiting CD11b expression, an anti-CD11b antibody or a small molecular compound modulating CD11b.

[0011] In one embodiment, the immune cell is a T cell or monocyte or granulocyte or macrophage or myeloid-derived suppressor cell or natural killer cell. In one embodiment, the

CD11b binding increases IFN-γ, IL-12 or CD8 T cells. In another embodiment, the binding of a CD11b modulator to CD11b on a cell treats and/or prevents a disease associated with immunosuppression. In a further embodiment, the disease associated with immunosuppression or immune exhaustion is an immune cell T-cell exhaustion in an acute and/or chronic infection, a sepsis, an immunodeficiency in cancer or an immunosenescence in aging.

[0012] In one embodiment, the method of prevention and/or treatment of a cancer comprises administering an additional active agent or therapy. In some embodiments, the additional active agent is an immune checkpoint therapy, radiotherapy or chemotherapy.

[0013] The invention also provides an anti-CD11b antibody or an antigen-binding portion thereof, comprising at least one of a heavy chain complementarity determining region 1 (H-CDR1) consisting of the amino acid residues of NYWIN (SEQ ID NO:1) or GFSLTSNSIS (SEQ ID NO:2) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:1 or 2; a heavy chain CDR2 (H-CDR2) consisting of the amino acid residues of NIYPSDTYINHNQKFKD (SEQ ID NO:3) or AIWSGGGTDYNSDLKS (SEQ ID NO:4) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:3 or 4; and a heavy chain CDR3 (H-CDR3) consisting of the amino acid residues of SAYANYFDY (SEQ ID NO:5) or RGGYPYYFDY (SEQ ID NO:6) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:5 or 6; and

at least one of a light chain CDR1 (L-CDR1) consisting of the amino acid residues of RASQNIGTSIH (SEQ ID NO:7) or KSSQSLLYSENQENYLA (SEQ ID NO:8) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99% identity to SEQ ID NO:7 or 8; a light chain CDR2 (L-CDR2) consisting of the amino acid residues of YASESIS (SEQ ID NO:9) or WASTRQS (SEQ ID NO:10) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any of SEQ ID NO:9 or 10; and a light chain CDR3 (L-CDR3) consisting of the amino acid residues QQSDSWPTLT (SEQ ID NO:11) or QQYYDTPLT (SEQ ID NO:12) or a variant having amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any of SEQ ID NO:11 or 12; such that said isolated antibody or antigen-binding portion thereof binds to CD11b.

[0014] In some embodiments, the CDRs described herein comprise one or more insertion, substitution and/or deletion.

[0015] In a further embodiment, the present invention provides an anti-CD11b antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 comprising SEQ ID NO:1, H-CDR2 comprising SEQ ID NO:3 and H-CDR3 comprising SEQ ID NO:5, and (ii) light chain variable regions comprising L-CDR1 comprising SEQ ID NO:7, L-CDR2 comprising SEQ ID NO:9 and L-CDR3 comprising SEQ ID NO:11; or (iii) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 comprising SEQ ID NO:2, H-CDR2 comprising SEQ ID NO:4 and H-CDR3 comprising SEQ ID NO:6, and (iv) light chain variable regions comprising L-CDR1 comprising SEQ ID NO:8, L-CDR2 comprising SEQ ID NO:10 and L-CDR3 comprising SEQ ID NO:12. In a further embodiment, H-CDR1 has the amino acid sequence consisting of SEQ ID NO:1 or 2; H-CDR2 has the amino acid sequence consisting of SEQ ID NO:3 or 4; H-CDR3 has the amino acid sequence consisting of SEQ ID NO:5 or 6; L-CDR1 has the amino acid sequence consisting of SEQ ID NO:7 or 8; L-CDR2 has the amino

acid sequence consisting of SEQ ID NO:9 or 10 and L-CDR3 has the amino acid sequence consisting of SEQ ID NO:11 or 12.

[0016] Further, the present invention provides a humanized anti-CD11b antibody or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:13, and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:23;

(c) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:14, and (ii) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:24;

(e) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:15, and (f) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:25;

(g) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:16, and (h) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:26;

(i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:17, and (j) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:27;

(k) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:18, and (l) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:28;

(m) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:19, and (n) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:29;

(o) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:20, and (p) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:30;

(q) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:21, and (r) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:31; or

[0017] (s) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:22, and (t) a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:32.

[0018] The invention also provides compositions comprising the anti-CD11b antibody or an antigen-binding portion thereof. The invention also provides methods that comprise administering the humanized anti-CD11b antibody of the invention to a subject. Such methods include methods for inhibiting PD-L1 expression in an immune cell, reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell, determining PD-L1 in a subject, and treating or preventing an acute and/or chronic infection, a in sepsis, an immunodeficiency in cancer or an immunosenescence in aging. The anti-CD11b antibodies of the invention can be used in the above-mentioned methods.

Brief Description of the Drawings

[0019] Figure 1 shows that binding CD11b with anti-CD11b antibody alters surface expression of PD-L1. Human monocytes were stimulated with LPS (100 ng/ml) in the presence

of either an isotype control IgG, or anti-CD11b antibody (ICRF44) for 18 hr. The cells were harvested and HLA-DR, PD-L1, CD80 and CD86 molecules were analyzed using flow cytometry. Surface molecule expression is presented as the MFI. Values are presented as the mean ± SEM from 3 independent experiments.

[0020] Figures 2 A and B show the effect of cell adhesion to fibrinogen and reduction of PD-L1 expression by binding CD11b, respectively. Figure 2A shows the effect of ML-C19-A on K562/CD11b cell adhesion to fibrinogen. 25000 of K562/CD11b cells adhered to the bottom of fibrinogen (20 μg/ml)-coated wells in the presence of 10 μM ML-C19-A or DMSO at 37°C for 20 min. The results were quantitated by luciferase-based CellTiter-Glo (Promega™ CO.). Each bar represents mean ± SEM of triplicate determinations from a representative experiment. Figure 2B shows that binding CD11b with CD11b antagonist reduces the PD-L1 expression on monocytes. Human monocytes were stimulated with LPS (100 ng/ml) in the presence of either DMSO control or 10 μM of ML-C19-A for 18 hr. The cells were harvested and PD-L1 molecules were analyzed using flow cytometry. Surface molecule expression is presented as the MFI. Values are presented as the mean ± SEM from 10 independent experiments.

[0021] Figure 3 shows the effect of anti-CD11b antibody monotherapy on the growth of B16F10 tumor. C57BL/6 mice were subcutaneously injected with 2 x 10^5 B16F10 cells at Day 0. On day 7, mice (n=5/group) were injected ip with either control IgG (5 mg/kg) or Rat anti-mouse CD11b antibody. Injections were repeated every three to four days. On Day 18, mice were sacrificed. Tumor volumes and were measured and the results are presented as the mean ± SEM.

[0022] Figure 4 shows MDSCs and CD8 T cells population in tumor-infiltrating leukocytes after anti-CD11b antibody treatment. C57BL/6 mice were subcutaneously injected with 2 x 10^5 B16F10 cells at Day 0. On day 7, mice (n=5/group) were injected ip with either control IgG (5

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mg/kg) or Rat anti-mouse CD11b antibody. Injections were repeated every three to four days. On Day 18, mice were sacrificed. Tumors were digested with collagenase and tumor-infiltrating leukocytes were analyzed by flow cytometry.

[0023] Figure 5 shows PD-L1 expressions on WBCs and IAIE+/CD8 T cells in the blood after anti-CD11b treatment. $2x10^5$ B16F10 cells were injected into each mouse via tail vein on day 0. On day 1, mice (n=3/group) were injected ip with either control IgG (5 mg/kg), or anti-mouse CD11b antibody (5 mg/kg). Injections were repeated every three to four days. On day15, mice were sacrificed. The WBCs cells were harvested and PD-L1 molecules and IAIE+/CD8 T cells were analyzed using flow cytometry.

[0024] Figure 6 shows that production of IFN-γ, IL-12 and TNF-α in tumor-bearing mice is reversed by treatment with anti-CD11b antibody. $2x10^5$ B16F10 cells were injected into each mouse via tail vein on day 0. On day1, mice (n=3/group) were injected ip with either control IgG (5mg/kg) or Rat anti-mouse CD11b antibody (5 mg/kg). Injections were repeated every three to four days. On day 9, mice were sacrificed. Plasma cytokines were quantified by BD CBA mouse inflammation kit.

[0025] Figure 7 shows the effect of anti-CD11b antibody monotherapy on the growth of LLC1 tumor. C57BL/6 mice were subcutaneously injected with 1 x 10^6 LLC1 cells at Day 0. On day 7, mice (n=5/group) were injected ip with either control IgG (5 mg/kg) or Rat anti-mouse CD11b antibody. Injections were repeated every three to four days. Tumor volumes were measured and the results are presented as the mean ± SEM.

[0026] Figure 8 shows the effects of anti-CD11b antibody monotherapy on survival in LLC1 tumor model. C57BL/6 mice were subcutaneously injected with 1 x 10^6 LLC1 cells at Day 0. On day 7, mice (n=5/group) were injected ip with either control IgG (5 mg/kg) or Rat anti-mouse

CD11b antibody. Injections were repeated every three to four days. Mice were analyzed for the effects of anti-CD11b antibody for the long term survival of treated mice in each of the groups.

[0027] Figure 9 shows the effect of anti-CD11b antibody and anti-PD1 combination therapy on LLC1 lung metastases model. $1x10^6$ LLC1 cells were injected into each mouse via tail vein on day 0. On day 1, mice (n=3/group) were injected ip with either control IgG (10 mg/kg), anti-mouse CD11b antibody (10 mg/kg), anti-PD1 antibody (10 mg/kg), or anti-CD11b (10 mg/kg) + anti-PD1 (10 mg/kg). Injections were repeated every three to four day. On day15, mice were sacrificed and the amount of tumor seeding was counted as total numbers of nodules presented in the lungs under microscopy.

[0028] Figure 10 shows the effect of anti-CD11b antibody and anti-PD1 combination therapy on survival in lung metastases model. $1x10^6$ LLC1 cells were injected into each mouse via tail vein on day 0. On day 1, mice (n=4-5/group) were injected ip with either control IgG (10 mg/kg), anti-mouse CD11b antibody (10 mg/kg), anti-PD1 antibody (10 mg/kg), or anti-CD11b (10 mg/kg) + anti-PD1 (10 mg/kg). Injections were repeated every three to four day. Mice were analyzed for the effects of combination therapy for the long term survival of treated mice in each of the groups.

[0029] Figure 11 shows the effect of anti-CD11b antibody and Taxol combination therapy on the growth of B16F10 tumor. C57BL/6 mice were injected subcutaneously with $2 x10^5$ B16F10 cells on day 0. On day7, mice (n=5/group) were injected ip with either control IgG (5 mg/kg), anti-mouse CD11b antibody (5 mg/kg), Taxol (10 mg/kg) + control IgG (5 mg/kg), or Taxol (10 mg/kg) + anti-CD11b antibody (5 mg/kg). Injections were repeated every three to four days. Tumor volumes were measured and the results are presented as the mean ± SEM.

[0030] Figure 12 shows the effect of anti-CD11b antibody and Taxol combination therapy on survival in B16F10 model. C57BL/6 mice were injected subcutaneously with 2 x10^5 B16F10 cells on day 0. On day7, mice (n=5/group) were injected ip with either control IgG (5 mg/kg), anti-mouse CD11b antibody (5 mg/kg), Taxol (10 mg/kg) + control IgG (5 mg/kg), or Taxol (10 mg/kg) + anti-CD11b (5 mg/kg). Injections were repeated every three to four days. Mice were analyzed for the effects of combination therapy for the long term survival of treated mice in each of the groups.

[0031] Figure 13 shows that binding CD11b with anti-CD11b antibody reduces PD-L1 expression in LPS-induced immunosuppressed monocytes challenged with 1 μg/ml LPS. (A) Human monocytes were isolated from healthy volunteers and pre-treated with 100 ng/ml LPS for 2 days to induce immunosuppression. (B) LPS-induced immunosuppressed monocytes were challenge with 1 μg/ml LPS for 18 hr in the presence of 10 μg/ml IgG1 or anti-CD11b antibody (ICRF44). Treated cells were washed and analyzed by flow cytometry. Surface PD-L1 expression is presented as the MFI. "-" indicates no antibody treatment.

[0032] Figure 14 shows that binding CD11b with anti-CD11b antibody reduces PD-L1 expression in human monocytes from patients with septic shock when challenged with 1 μg/ml LPS. Human monocytes were isolated from patient with septic shock and challenged with 1 μg/ml LPS for 18 hr in the presence of 10 μg/ml IgG1 or anti-CD11b antibody. Treated cells were washed and analyzed by flow cytometry. Surface PD-L1 expression is presented as the MFI. "-" indicates no antibody treatment.

[0033] Figure 15 shows the amino acid sequences of the light chain variable region of humanized CD11b antibodies. CDRs are shown in bold letters.

[0034] Figure 16 shows the amino acid sequences of the heavy chain variable region of humanized CD11b antibodies. CDRs are shown in bold letters.

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[0035] Figure 17 shows the binding activities of humanized anti-CD11b antibodies. K562 cells or cells transfected with human CD11b (K562/CD11b) were incubated with 10 μg/ml humanized anti-CD11b antibodies for 30 mins. Bound Ab was detected by FITC-conjugated mouse anti-human IgG. The cells were analyzed by flow cytometry. Dash line represents antibodies bound the K562 cells. Solid line represents antibodies bind to K562/CD11b cells.

[0036] Figure 18 shows binding CD11b with anti-CD11b antibodies reduces PD-L1 expression in LPS-primed human monocytes. Primed-monocytes were incubated in the presence of either an isotype control IgG, anti-CD11b antibody (ICRF44) or humanized anti-CD11b antibodies for 18hr. The cells were harvested and PD-L1 expression on monocytes was analyzed using flow cytometry.

Detailed Description of the Invention

[0037] Before the present composition, methods, and isolation methodologies are described, it is to be understood that this invention is not limited thereto, since such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting

[0038] The present invention surprisingly found that the expression of PD-L1 can be suppressed by the engagement of modulators to CD11b on immune cells and/or other cells, thereby treating and/or preventing diseases associated with immunosuppression such as chronic infections, sepsis, immunodeficiency in cancer and immunosenescence in aging.

Definitions

[0039] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Any methods and materials similar or equivalent to those described herein can be used

in the practice or testing of the invention, as it will be understood that modifications and variations are encompassed within the spirit and scope of the instant disclosure.

[0040] Unless otherwise specified, "a" or "an" means one or more.

[0041] As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

[0042] As used herein, the term "CD11b" refers to integrin alpha M (ITGAM), which is one subunit of the heterodimeric integrin αMβ2. The second subunit of integrin αMβ2 is the common integrin β2 subunit known as CD18. Integrin αMβ2 is also call macrophage-1 antigen (Mac-1) or complement receptor 3 (CR3) which is expressed on the surface of leukocytes including monocytes, granulocytes, macrophages, and nature killer cells.

[0043] As used herein, the term "PD-L1" refers to programmed death-ligand 1 (PD-L1), cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1). PD-L1 is a 40kDa type 1 transmembrane protein that plays a major role in suppressing the immune system during particular events such as pregnancy, autoimmune disease, cancer, sepsis, and other infectious diseases such as mycobacterium tuberculosis, cytomegalovirus, and hepatitis.

[0044] As used herein, the term "monocyte, " also called mononuclear white cell, belongs to a type of white blood cell involved in first-line defensive mechanism and is recognized as able to differentiate into a dendritic cell or macrophage precursor. Monocytes normally move in the blood system. In response to external stimulating signals, monocytes secrete many immuno-

regulatory cytokines, move to the site of infection in the tissues and differentiate into macrophages.

[0045] As used herein, the term "modulating" includes "increasing" or "stimulating," as well as "decreasing" or "reducing," typically in a statistically significant or a physiologically significant amount as compared to a control.

[0046] As used herein, the term "subject means a human or non-human animal selected for treatment or therapy.

[0047] As used herein, "identity" refers to a relationship between two or more polypeptide or protein sequences, as determined by comparing the sequences. In the art, "identity" also refers to the degree of sequence relatedness between polypeptides or proteins, as determined by the match between strings of such sequences. "Identity" can be easily calculated by known bioinformational methods. The "percent identity" of two polynucleotide or two polypeptide sequences is determined by comparing the sequences using the GAP computer program (a part of the GCG Wisconsin Package, version 10.3 (Accelrys™, San Diego, Calif.)) using its default parameters.

[0048] As used herein, the terms "peptide," "polypeptide" and "protein" each refer to a molecule comprising two or more amino acid residues joined to each other by peptide bonds. These terms encompass, e.g., native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins. A peptide, polypeptide, or protein may be monomeric or polymeric.

[0049] As used herein, the term "affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its

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binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described below.

[0050] As used herein, the term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), monovalent antibodies, multivalent antibodies, and antibody fragments so long as they exhibit the desired biological activity (e.g., Fab and/or single-armed antibodies).

[0051] As used herein, the term "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments.

[0052] As used herein, the term "antigen-binding fragment" of an antibody, refers to one or more portions of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the

V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). These antibody fragments are obtained using conventional procedures, such as proteolytic fragmentation procedures, as described in J. Goding, Monoclonal Antibodies: Principles and Practice, pp 98-118 (N.Y. Academic Press 1983). The fragments are screened for utility in the same manner as are intact antibodies.

[0053] As used herein, the term "complementarity determining regions" (CDRs) refers to the regions within antibodies where these proteins complement an antigen's shape. The acronym CDR is used herein to mean "complementarity determining region."

[0054] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three CDRs also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. Exemplary conventions that can be used to identify the boundaries of CDRs include, e.g., the Kabat definition and the Chothia definition. The Kabat definition is based on sequence variability (see Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C.), the Chothia definition is based on the location of the structural loop regions (Chothia et al., 1989, Nature 342:877-883). Other approaches to CDR identification include the "IMGT definition" (Lefranc, M.-P. et al., 1999, Nucleic Acids Res. 27:209-212) and the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software, or the "contact definition" of CDRs based on observed antigen

contacts, set forth in MacCallum et al., 1996, J. Mol. Biol. 262:732-745. As used herein, a CDR may refer to CDRs defined by Kabat numbering system.

[0055] As used herein, the term "humanized antibody" or a "humanized antibody fragment" is a specific type of chimeric antibody which includes an immunoglobulin amino acid sequence variant, or fragment thereof, which is capable of binding to a predetermined antigen and which, comprises one or more frameworks (FRs) having substantially the amino acid sequence of a human immunoglobulin and one or more complementarity determining regions (CDRs) having substantially the amino acid sequence of a non-human immunoglobulin. This non-human amino acid sequence often referred to as an "import" sequence is typically taken from an "import" antibody domain, particularly a variable domain. In general, a humanized antibody includes at least the CDRs or hypervariable region (HVLs) of a non-human antibody, inserted between the FRs of a human heavy or light chain variable domain.

[0056] As used herein, a "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0057] As used herein, the term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies.

[0058] As used herein, the term "heavy chain" includes a full-length heavy chain and fragments thereof having sufficient variable region sequence to confer specificity to an epitope. A full-length heavy chain includes a variable region domain, V_H, and three constant region

domains, CH_1, CH_2, and CH_3. The VH domain is at the amino-terminus of the polypeptide, and the CH_3 domain is at the carboxyl-terminus.

[0059] As used herein, the term "light chain" includes a full-length light chain and fragments thereof having sufficient variable region sequence to confer specificity to an epitope. A full-length light chain includes a variable region domain, V_L, and a constant region domain. C_L. Like the heavy chain, the variable region domain of the light chain is at the amino-terminus of the polypeptide.

[0060] As used herein, the term "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0061] As used herein, the term "subject" refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, humans, farm animals, sport animals, and pets.

[0062] As used herein, the term "effective amount" refers to an amount sufficient to effect beneficial or desired clinical results. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount is an amount that is sufficient to diagnose, palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

[0063] As used herein, the terms "treatment," "treating," "treat" and the like generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse

effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, i.e., arresting its development; or (c) relieving the disease symptom, i.e., causing regression of the disease or symptom.

[0064] The term "preventing" as used herein refers to a preventative or prophylactic measure that stops a disease state or condition from occurring in a patient or subject. Prevention can also include reducing the likelihood of a disease state or condition from occurring in a patient or subject and impeding or arresting the onset of said disease state or condition.

[0065] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

The Binding of CD-11b Modulators Affects PD-L1 Expression

[0066] The present invention surprisingly found reversion of symptoms associated with immunosuppressed state involved in the sepsis, chronic infection, and cancer through treatment with a CD11b modulator reactive with CD11b molecule expressed on the surface of immune cells.

[0067] In one aspect, the invention provides a method for inhibiting PD-L1 expression in an immune cell, comprising contacting the said immune cell with a CD11b modulator that binds CD11b on the cell, hereby inhibiting PD-L1 expression of the immune cell. Alternatively, the invention provides a use of a CD11b modulator in manufacture of a preparation for inhibiting PD-L1 expression in an immune cell. The invention also provides a CD11b modulator for inhibiting PD-L1 expression in an immune cell.

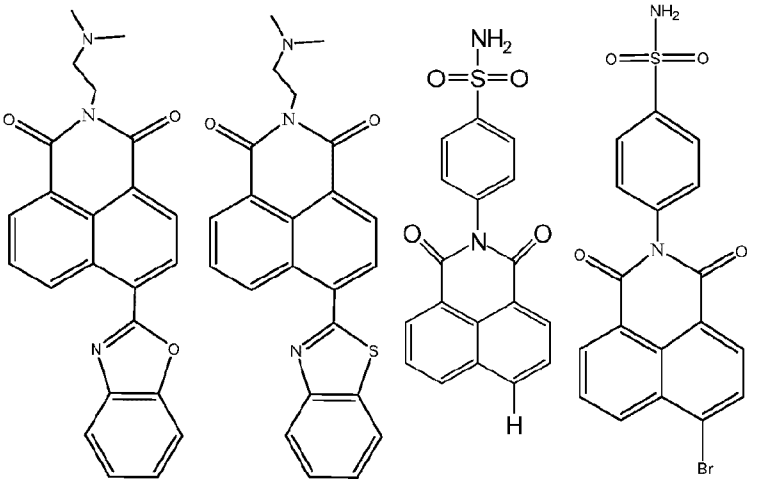
[0068] In another aspect, the invention provides a method for reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell, comprising contacting the said immune cells with a CD11b modulator that binds CD11b on the cells. Alternatively, the invention provides a use of a CD11b modulator in manufacture of a preparation for reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell. The invention also provides a CD11b modulator for reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell.

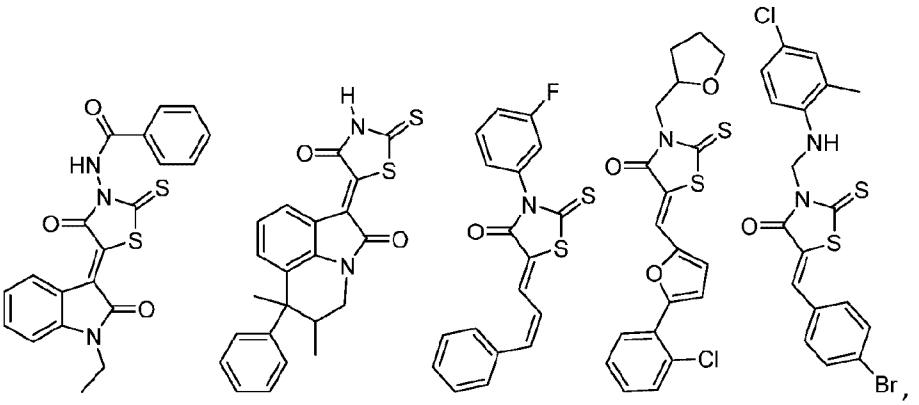
[0069] In another aspect, the invention provides a method for determining a subject responsive to a CD11b modulator, said method comprising detecting whether PD-L1 is inhibited in a biological sample or a subject by contacting an immune cell in the biological sample or the subject with a CD11b modulator and detecting the inhibition of PD-L1 on the immune cells by the CD11b modulator, wherein the PD-L1 is inhibited indicates that the subject is responsive to a CD11b modulator.

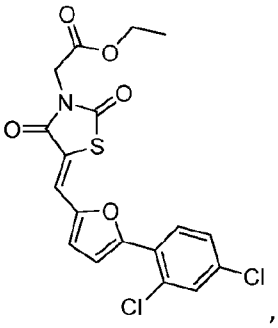
[0070] In one embodiment, the CD11b modulator described herein is an RNAi agent inhibiting CD11b expression, an anti-CD11b antibody or a small molecular compound modulating CD11b.

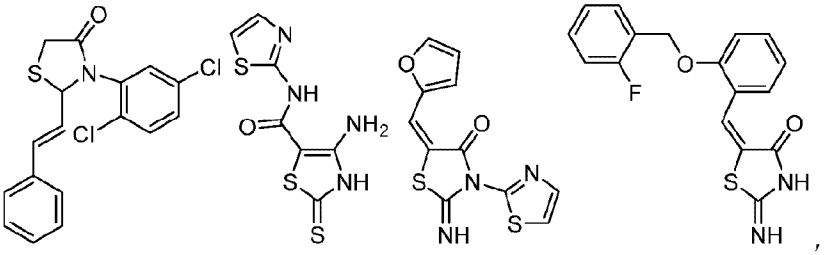
[0071] In some embodiments, the RNAi agent inhibiting CD11b expression is a microRNA (miRNA) or small interfering RNA (siRNA) inhibiting CD11b expression. In some embodiments, the anti-CD11b antibody is a monoclonal, chimeric, humanized, human or bispecific anti-CD11b antibody.

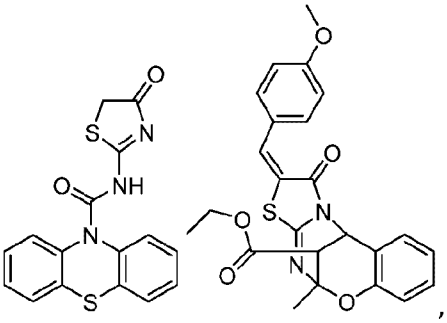
[0072] In some embodiments, examples of the small molecular compound modulating CD11b include, but are not limited to, the compounds described in US 8,268,816, US 20120035154, WO002007039616, WO002006111371, WO002007054128, WO00199901258, J Immunol 2010, 184, pp.3917-26, and Cancer Discov, 2012, 2, pp.1091-99. Preferably, the compound is selected from the group consisting of the following:

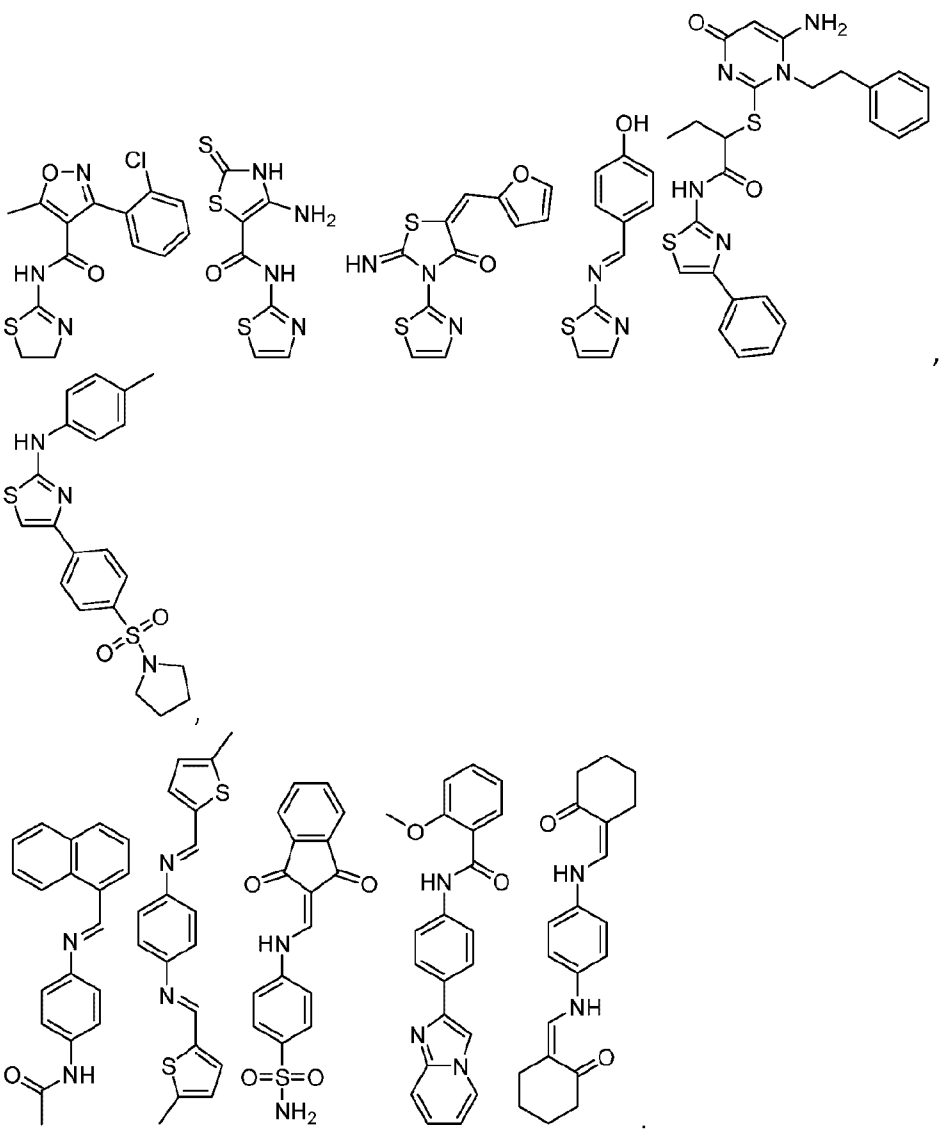












[0073] In one embodiment, the immune cell is a monocyte, granulocyte, macrophage, myeloid-derived suppressor cell or natural killer cell or T cell.

[0074] In one embodiment, the CD11b binding increases IFN-γ, IL-12 or CD8 T cells. In another embodiment, the binding of a CD11b modulator to CD11b on a cell treats and/or prevents a disease associated with immunosuppression.

[0075] In a further embodiment, the disease associated with immunosuppression or immune exhaustion is T-cell exhaustion in an acute and/or chronic infection, a sepsis, an immunodeficiency in cancer or an immunosenescence in aging. Accordingly, the invention provides a method for treating or preventing in a subject an acute and/or chronic infection, a sepsis, an immunodeficiency in cancer or an immunosenescence in aging, comprising administering an effective amount of CD11b modulator to a subject.

[0076] In one embodiment, the cancer described herein is a cancer responsive to immunotherapy. Examples of the cancer responsive to immunotherapy include, but are not limited to, melanoma, lung cancer, squamous cell carcinomas of the lung, head and neck cancer, breast cancer, ovarian cancer, uterine cancer, prostate cancer, gastric carcinoma, cervical cancer, esophageal carcinoma, bladder cancer, kidney cancer, brain cancer, liver cancer, colon cancer, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular malignant melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, esophagus cancer, small intestine cancer, endocrine system cancer, thyroid gland cancer, parathyroid gland cancer, adrenal gland cancer, sarcoma of soft tissue, urethra cancer, penis cancer, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, and T-cell lymphoma.

[0077] In one embodiment, the cancer is a cancer metastasis, refractory cancer, relapsed cancer or advanced cancer.

[0078] In one embodiment, the method of prevention and/or treatment of a cancer comprises administering an additional active agent or therapy. In some embodiments, the additional active agent is an immune checkpoint therapy, radiotherapy or chemotherapy.

[0079] In one embodiment, the CD11b modulator and the immune checkpoint therapy, radiotherapy or chemotherapy are administered simultaneously, sequentially or separately. In a further embodiment, the immune checkpoint therapy comprises administering an immune checkpoint protein. Preferably, the immune checkpoint protein is an anti-PD-1 ligand or anti-CTLA-4 antibody or anti-PD-L1 antibody, or an antigen binding fragment thereof or any combination thereof. Examples of the anti-PD-1 ligand include, but are not limited to, an anti-PD-1 antibody (such as nivolumab and pembrolizumab) and the anti-CTLA-4 antibody (such as ipilimumab).

[0080] In another embodiment, the chemotherapy comprises administering a chemotherapeutic agent. Examples of the chemotherapeutic agent include, but are not limited to, an alkylating agent, an antimetabolite, an anti-microtubule agent, a topoisomerase inhibitor or a cytotoxic antibiotic. Preferably, the chemotherapeutic agent is cisplatin, 5-Fu, taxol, docetaxel, vinorelbine, vindesine, vinflunine, gemcitabine, methotrexate, gefitinib, lapatinib or erlotinib.

[0081] The CD11b modulator and other agents described herein can be formulated as a formulation or composition. The formulations or pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be oral or parenteral.

[0082] In certain embodiments, the compounds and compositions as described herein are administered parenterally. Parenteral administration includes intravenous, intra-arterial, subcutaneous, intraperitoneal or intramuscular injection or infusion.

[0083] In certain embodiments, formulations or compositions for parenteral administration can include sterile aqueous solutions which can also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0084] In certain embodiments, formulations or compositions for oral administration can include, but are not limited to, pharmaceutical carriers, excipients, powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders can be desirable.

[0085] Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Dosing is also dependent on drug potency and metabolism.

[0086] The level of PD-L1 expression in an immune cell may serve as a new therapeutic target for reversing immunosuppression and immune exhaustion and inducing pre-existing immunity.

Anti-CD11b Antibodies of the Present Invention

[0087] Provided herein are novel anti-CD11b antibodies and methods of their use in treatment and/or prevention of diseases associated with immunosuppression and immune

exhaustion, such as cancer immunotherapy, T-cell exhaustion in chronic infections, sepsis, immunodeficiency in cancer and immunosenescence in aging.

[0088] In one aspect, the present invention provides an anti-CD11b antibody or an antigen-binding portion thereof, comprising at least one of a heavy chain complementarity determining region 1 (H-CDR1) consisting of the amino acid residues of NYWIN (SEQ ID NO:1) or GFSLTSNSIS (SEQ ID NO:2) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:1 or 2; a heavy chain CDR2 (H-CDR2) consisting of the amino acid residues of NIYPSDTYINHNQKFKD (SEQ ID NO:3) or AIWSGGGTDYNSDLKS (SEQ ID NO:4) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:3 or 4; and a heavy chain CDR3 (H-CDR3) consisting of the amino acid residues of SAYANYFDY (SEQ ID NO:5) or RGGYPYYFDY (SEQ ID NO:6) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:5 or 6; and

at least one of a light chain CDR1 (L-CDR1) consisting of the amino acid residues of RASQNIGTSIH (SEQ ID NO:7) or KSSQSLLYSENQENYLA (SEQ ID NO:8) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO:7 or 8; a light chain CDR2 (L-CDR2) consisting of the amino acid residues of YASESIS (SEQ ID NO:9) or WASTRQS (SEQ ID NO:10) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any of SEQ ID NO:9 or 10; and a light chain CDR3 (L-CDR3) consisting of the amino acid residues QQSDSWPTLT (SEQ ID NO:11) or QQYYDTPLT (SEQ ID NO:12) or a variant having an amino acid sequence with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98%, 99% identity to any of SEQ ID NO:11 or 12; such that said isolated antibody or antigen-binding portion thereof binds to CD11b.

[0089] In some embodiments, the CDRs described herein comprise one or more insertion, substitution and/or deletion.

[0090] In a further embodiment, the present invention provides an anti-CD11b antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 comprising SEQ ID NO:1, H-CDR2 comprising SEQ ID NO:3 and H-CDR3 comprising SEQ ID NO:5, and (ii) light chain variable regions comprising L-CDR1 comprising SEQ ID NO:7, L-CDR2 comprising SEQ ID NO:9 and L-CDR3 comprising SEQ ID NO:11; or (iii) a heavy chain variable region comprising a heavy chain variable region comprising H-CDR1 comprising SEQ ID NO:2, H-CDR2 comprising SEQ ID NO:4 and H-CDR3 comprising SEQ ID NO:6, and (iv) light chain variable regions comprising L-CDR1 comprising SEQ ID NO:8, L-CDR2 comprising SEQ ID NO:10, and L-CDR3 comprising SEQ ID NO:12. In a further embodiment, H-CDR1 has the amino acid sequence consisting of SEQ ID NO:1 or 2; H-CDR2 has the amino acid sequence consisting of SEQ ID NO:3 or 4; H-CDR3 has the amino acid sequence consisting of SEQ ID NO:5 or 6; L-CDR1 has the amino acid sequence consisting of SEQ ID NO:7 or 8; L-CDR2 has the amino acid sequence consisting of SEQ ID NO:9 or 10; and L-CDR3 has the amino acid sequence consisting of SEQ ID NO:11 or 12.

[0091] In one aspect, the present invention provides a heavy chain variable region or an antigen-binding portion thereof, comprising a heavy chain variable region comprising H-CDR1 having an amino acid sequence consisting of SEQ ID NO:1 or 2, H-CDR2 having an amino acid

sequence consisting of SEQ ID NO:3 or 4 and H-CDR3 having an amino acid sequence consisting of SEQ ID NO:5 or 6.

[0092] In one aspect, the present invention provides a light chain variable region or an antigen-binding portion thereof, comprising L-CDR1 having an amino acid sequence consisting of SEQ ID NO:7 or 8, L-CDR2 having an amino acid sequence consisting of SEQ ID NO:9 or 10, and L-CDR3 having an amino acid sequence consisting of SEQ ID NO:11 or 12.

[0093] In one embodiment, the present invention provides a humanized anti-CD11b antibody or an antigen-binding portion thereof, comprising (i) a heavy chain variable region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any of the amino acid sequences of SEQ ID NOs:13 to 22, and (ii) a light chain variable region comprising an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to any of the amino acid sequences of SEQ ID NOs:23 to 32.

[0094] In a further embodiment, the present invention provides a humanized anti-CD11b antibody or an antigen-binding portion thereof, comprising a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:13 to 22, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:23 to 32.

[0095] Preferably, the present invention provides a humanized anti-CD11b antibody or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:13, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:23;

(b) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:14, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:24;

(c) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:15, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:25;

(d) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:16, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:26;

(e) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:17, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:27;

(f) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:18, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:28;

(g) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:19, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:29;

(h) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:20, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:30;

(i) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:21, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:31; or

(j) a heavy chain variable region comprising an amino acid sequence consisting of SEQ ID NO:22, and a light chain variable region comprising an amino acid sequence consisting of SEQ ID NO:32.

[0096] The amino acid sequences of SEQ ID NOs: 13 to 32 are listed as follows:

Heavy chain variable region of the humanized anti-CD11b antibodies of the invention: (SEQ ID NOs:13 to 22)

VH1
QVQLVQSGAEVKKPGASVKVSCKASGYTFT**NYWIN**WVRQAPGQGLEWMG**NIYPSDT YINHNQKFKD**RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR**SAYANYFDY**WGQGTL VTVSS (SEQ ID NO:13)

VH2
QVQLVQSGAEVKKPGSSVKVSCKASGGTFS**NYWIN**WVRQAPGQGLEWMG**NIYPSDTY INHNQKFKD**RVTITADKSTSTAYMELSSLRSEDTAVYYCAT**SAYANYFDY**WGQGTLVT VSS (SEQ ID NO:14)

VH3
QVQLVQSGAEVKKPGASVKVSCKASGYTFT**NYWIN**WVRQATGQGLEWMG**NIYPSDT YINHNQKFKD**RVTMTRNTSISTAYMELSSLRSEDTAVYYCAR**SAYANYFDY**WGQGTL VTVSS (SEQ ID NO:15)

VH4
QVQLVQSGAEVKKPGASVKVSCKASGYTFT**NYWIN**WVRQAPGQRLEWMG**NIYPSDT YINHNQKFKD**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**SAYANYFDY**WGQGTL VTVSS (SEQ ID NO:16)

VH5
QVQLVQSGAEVKKPGATVKISCKVSGYTFT**NYWIN**WVQQAPGKGLEWMG**NIYPSDTY INHNQKFKD**RVTITADTSTDTAYMELSSLRSEDTAVYYCAR**SAYANYFDY**WGQGTLV TVSR (SEQ ID NO:17)

HC1

QVQLQESGPGLVKPSETLSLTCTVS**GFSLTSNSIS**WIRQPPGKGLEWIG**AIWSGGGTDY NSDLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCA**RGGYPYYFDY**WGQGTLVTVSS (SEQ ID NO:18)

HC2
QVQLQESGPGLVKPSGTLSLTCAVY**GFSLTSNSIS**WIRQPPGKGLEWIG**AIWSGGGTDY NSDLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCA**RGGYPYYFDY**WGQGTMVTVSS (SEQ ID NO:19)

HC3
QVQLQQWGAGLLKPSETLSLTCAVY**GFSLTSNSIS**WIRQPPGKGLEWIG**AIWSGGGTD YNSDLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCA**RGGYPYYFDY**WGQGTLVTV SS (SEQ ID NO:20)

HC4
EVQLVESGGGLVQPGGSLRLSCAAS**GFSLTSNSIS**WVRQAPGKGLEWVS**AIWSGGGTD YNSDLKS**RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA**RGGYPYYFDY**WGQGTLVTV SS (SEQ ID NO:21)

HC5
EVQLVETGGGLIQPGGSLRLSCAAS**GFSLTSNSIS**WVRQAPGKGLEWVS**AIWSGGGTD YNSDLKS**RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA**RGGYPYYFDY**WGQGTLVTV SS (SEQ ID NO:22)

Light chain variable region of the humanized anti-CD11b antibodies of the invention: (SEQ ID NOs:23 to 32)

VL1
EIVLTQSPDFQSVTPKEKVTITC**RASQNIGTSIH**WYQQKPDQSPKLLIK**YASESIS**GVPSR FSGSGSGTDFTLTINSLEAEDAATYYC**QQSDSWPTLT**FGQGTKVEIK (SEQ ID NO:23)

VL2
EIVMTQSPATLSVSPGERATLSC**RASQNIGTSIH**WYQQKPGQAPRLLIY**YASESIS**GIPAR FSGSGSGTEFTLTISSLQSEDFAVYYC**QQSDSWPTLT**FGQGTKLEIK (SEQ ID NO:24)

VL3
DIQMTQSPSSLSASVGDRVTITC**RASQNIGTSIH**WYQQKPGKAPKLLIY**YASESIS**GVPS RFSGSGSGTDFTLTISSLQPEDFATYYC**QQSDSWPTLT**FGGGTKVEIK (SEQ ID NO:25)

VL4
EIVLTQSPATLSLSPGERATLSC**RASQNIGTSIH**WYQQKPGQAPRLLIY**YASESIS**GIPARF SGSGSGTDFTLTISSLEPEDFAVYYC**QQSDSWPTLT**FGGGTKVEIK (SEQ ID NO:26)

VL5

EIVLTQSPGTLSLSPGERATLSC**RASQNIGTSIH**WYQQKPGQAPRLLIY**YASESIS**GIPDRF SGSGSGTDFTLTISRLEPEDFAVYYC**QQSDSWPTLT**FGQGTKLEIK (SEQ ID NO:27)

LC1
DIVMTQSPDSLAVSLGERATINC**KSSQSLLYSENQENYLA**WYQQKPGQPPKLLIY**WAS TRQS**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQYYDTPLT**FGQGTKVEIK (SEQ ID NO:28)

LC2
DIVMTQSPLSLPVTPGEPASISC**KSSQSLLYSENQENYLA**WYLQKPGQSPQLLIY**WAST RQS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**QQYYDTPLT**FGGGTKVEIK (SEQ ID NO:29)

LC3
DIVMTQSPLSLSVTPGQPASISC**KSSQSLLYSENQENYLA**WYLQKPGQSPQLLIY**WAST RQS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**QQYYDTPLT**FGQGTKVEIK (SEQ ID NO:30)

LC4
DVVMTQSPLSLPVTLGQPASISC**KSSQSLLYSENQENYLA**WFQQRPGQSPRRLIY**WAST RQS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**QQYYDTPLT**FGQGTKLEIK (SEQ ID NO:31)

LC5
DIVMTQTPLSSPVTLGQPASISC**KSSQSLLYSENQENYLA**WLQQRPGQPPRLLIY**WAST RQS**GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC**QQYYDTPLT**FGQGTKLEIK (SEQ ID NO:32)

[0097] Techniques for preparing monoclonal antibodies against virtually any target antigen are well known in the art. See, for example, Kohler and Milstein, Nature 256: 495 (1975), and Coligan et al. (eds.), Current Protocols In Immunology, Vol. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991). Monoclonal antibodies can be obtained by injecting mice or chicken with a composition comprising an antigen, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

[0098] Various techniques, such as production of chimeric or humanized antibodies, may involve procedures of antibody cloning and construction. The antigen-binding variable light chain and variable heavy chain sequences for an antibody of interest may be obtained by a variety of molecular cloning procedures. A chimeric antibody is a recombinant protein in which the variable regions of a human antibody have been replaced by the variable regions of, for example, a mouse antibody, including the complementarity-determining regions (CDRs) of the mouse antibody. Chimeric antibodies exhibit decreased immunogenicity and increased stability when administered to a subject. Methods for constructing chimeric antibodies are well known in the art. A chimeric monoclonal antibody may be humanized by transferring the mouse CDRs from the heavy and light variable chains of the mouse immunoglobulin into the corresponding variable domains of a human antibody. The mouse framework regions (FRs) in the chimeric monoclonal antibody are also replaced with human FR sequences.

[0099] For example, a nucleic acid encoding a VL and/or VH of a humanized antibody that specifically binds CD11b can be cloned or amplified by in vitro methods, such as the polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS) etc. For example, a polynucleotide encoding the protein can be isolated by polymerase chain reaction of cDNA using primers based on the DNA sequence of the molecule. A wide variety of cloning and *in vitro* amplification methodologies are well known to persons skilled in the art. Polynucleotides can also be isolated by screening genomic or cDNA libraries with probes selected from the sequences of the desired polynucleotide under stringent hybridization conditions.

[00100] The polynucleotides include a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or

eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences. The nucleotides of the invention can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

[00101] DNA sequences encoding a VL and/or VH of a humanized antibody that specifically binds CD11b can be expressed in vitro by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

[00102] Polynucleotide sequences encoding a VL and/or VH of a humanized antibody that specifically binds CD11b can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. The expression control sequences include, but are not limited to, appropriate promoters, enhancers, transcription terminators, a start codon (for instance, ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons.

[00103] The polynucleotide sequences encoding a VL and/or VH of a humanized antibody that specifically binds CD11b can be inserted into an expression vector. Examples of the expression vector include, but are not limited to, a plasmid, virus or other vehicle that can be manipulated to allow insertion or incorporation of sequences and can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are

well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Transformation of a host cell with recombinant DNA may be carried out by conventional techniques well known to those skilled in the art.

[00104] Isolation and purification of recombinantly expressed polypeptides may be carried out by conventional means including preparative chromatography and immunological separations.

[00105] Humanization can be performed generally following conventional methods known in the art, by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human, for example, rodent antibodies.

[00106] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. The sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies.

[00107] Antibody binding portions include, for example, Fab, Fab', $F(ab)_2$, $F(ab')_2$, Fv, scFv and the like. These fragments are produced from intact antibodies using methods well known in

the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

[00108] Modifications can be made to a nucleic acid encoding a polypeptide described herein without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation, site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids to aid in purification steps. In addition to recombinant methods, the antibodies of the present disclosure can also be constructed in whole or in part using standard peptide synthesis well known in the art.

[00109] In another aspect, the present invention provides compositions comprising an anti-CD11b antibody of the invention. In some embodiments, such compositions may be administered to subjects. In some embodiments, the anti-CD11b antibody of the invention may be provided in a composition that comprises one or more other components, including, but not limited to, pharmaceutically acceptable carriers, adjuvants, wetting or emulsifying agents, pH buffering agents, preservatives, and/or any other components suitable for the intended use of the compositions. Such compositions can take the form of solutions, suspensions, emulsions and the like. The term "pharmaceutically acceptable carrier" includes various diluents, excipients and/or vehicles. The pharmaceutically acceptable carrier includes, but is not limited to, carriers known to be safe for delivery to human and/or other animal subjects, and/or approved by a regulatory agency of the federal or a state government, and/or listed in the U.S. Pharmacopeia, and/or other generally recognized pharmacopeia, and/or receiving specific or individual approval from one or

more generally recognized regulatory agencies for use in humans and/or other animals. Such pharmaceutically acceptable carriers, include, but are not limited to, water, aqueous solutions (such as saline solutions, buffers, and the like), organic solvents (such as certain alcohols and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil) and the like.

[00110] In one embodiment, the humanized anti-CD11b antibody of the invention may be provided in a composition that comprises one or more "chemotherapeutic agents" that are chemical compounds used in the treatment of a cancer, also called anti-neoplastic drugs. An anti-nucleoplastic drug is usually classified, according to differences in the chemical structure and origin of the drug, into alkylating agents, anti-metabolic drugs, anti-neoplastic antibiotics, anthracycline antibiotics, anti-neoplastic herbal drugs, and hormones. Depending on the cycle or phase specificity, the chemotherapeutic drugs against tumor can be classified into (1) cell cycle non-specific agents (CCNSA), such as alkylating agents, anti-neoplastic antibiotics and platinum coordination complexes, etc., and (2) cell cycle specific agents (CCSA), such as anti-metabolic drugs, vinca alkaloids, etc.

[00111] In some embodiments, the compositions of the invention comprise an "effective amount" of an anti-CD11b antibody of the invention. An "effective amount" is an amount required to achieve a desired end result. The amount of a humanized anti-CD11b antibody of the invention that is effective to achieve the desired end result will depend on a variety of factors including, but not limited to, the species of the intended subject (e.g. whether human or some other animal species), the age and/or sex of the intended subject, the planned route of administration, the planned dosing regimen, the seriousness of any ongoing diseases or conditions, and the like. The effective amount—which may be a range of effective amounts—

can be determined by standard techniques without any undue experimentation, for example using in vitro assays and/or in vivo assays in the intended subject species or any suitable animal model species. Suitable assays include, but are not limited to, those that involve extrapolation from dose-response curves and/or other data derived from in vitro and/or in vivo model systems. In some embodiments the effective amount may be determined according to the judgment of a medical or veterinary practitioner based on the specific circumstances.

[00112] In one embodiment, an effective amount of the humanized anti-CD11b antibody ranges from about 0.01 mg/kg to about 40 mg/kg of body weight per administration; preferably, about 0.01 mg/kg to about 30 mg/kg, about 0.01 mg/kg to about 20 mg/kg, about 0.01 mg/kg to about 10 mg/kg, about 1 mg/kg to about 40 mg/kg, about 1 mg/kg to about 30 mg/kg, about 1 mg/kg to about 20 mg/kg, about 1 mg/kg to about 10 mg/kg, about 2 mg/kg to about 40 mg/kg, about 2 mg/kg to about 30 mg/kg, about 2 mg/kg to about 20 mg/kg, about 2 mg/kg to about 10 mg/kg, about 5 mg/kg to about 40 mg/kg, about 5 mg/kg to about 30 mg/kg, about 5 mg/kg to about 20 mg/kg or about 5 mg/kg to about 10 mg/kg or about 1 mg/kg to about 5 mg/kg.

[00113] In some embodiments, the present invention provides methods that comprise administering the humanized anti-CD11b antibody of the invention to a subject. Such methods include methods for inhibiting PD-L1 expression in an immune cell, reversing immune suppression or immune exhaustion or inducing pre-existing immunity in an immune cell, detecting PD-L1 in a subject, and treating or preventing an acute and/or chronic infection, a sepsis, an immunodeficiency in cancer or an immunosenescence in aging. The anti-CD11b antibodies of the invention can be used in the above-mentioned methods.

[00114] The cancer described herein is a cancer responsive to immunotherapy and the examples of the cancers are as described herein. The method of prevention and/or treatment of a

cancer comprises administering an additional active agent or therapy. The additional active agents, their embodiments and administrations are as described herein.

[00115] Subjects to which the anti-CD11b antibody of the invention, or compositions comprising the anti-CD11b antibody, can be administered (for example in the course of a method of treatment) include any and all animal species. In some embodiments, the subjects are mammalian species. Mammalian subjects include, but are not limited to, humans, non-human primates, rodents, rabbits, and ferrets.

[00116] Various delivery systems are known in the art and any suitable delivery system can be used to administer the compositions of the present invention to subjects. Such delivery systems include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral delivery systems. The compositions of the present invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00117] In some such embodiments, administration of a single dose is preferred. However, in other embodiments, additional dosages can be administered, by the same or different route to achieve the desired effect. In some embodiments, dosing regimens may comprise a single administration. In other embodiments, dosing regimens may comprise multiple administrations.

Examples

[00118] The materials and methods used in the following examples are described below.

[00119] Materials and Methods

[00120] Human cell isolation and cell culture

[00121] White blood cell concentrates from healthy volunteers were obtained from the Taiwan Blood Service Foundation (Taipei, Taiwan). Written informed consent was obtained for participation in the study, which was approved by the Institutional Review Board of the Mackay Memorial Hospital. Human monocytes were isolated as previous described. In brief, peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque™ Plus (GE™ Healthcare) gradient centrifugation. The monocytes were further purified by conducting CD14 selection using CD14 MACS™ microbeads (Miltenyi Biotec). The purity of monocytes confirmed using flow cytometry analysis was approximately 90%.

[00122] Animal and tumor cell line.

[00123] C57BL/6 mice (6 to 8 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All animal experiments were performed under specific pathogen-free conditions and in accordance with guidelines approved by the Animal Care and Usage Committee of Mackay memorial hospital (Taipei, Taiwan). The body weight of each mouse was measured at the beginning of treatment and every day during the treatment period. B16F10 are murine melanoma cells and LLC1 are murine Lewis lung carcinoma. All cells were derived from C57BL/6 mice. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM), 10% heat-inactivated fetal calf serum, 2mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37C in a 5% CO_2 humidified atmosphere.

[00124] Antibodies and reagents

[00125] For human monocytes study

[00126] LPS from *E. coli* (O111:B4) was obtained from Sigma™. Murine binding antibodies specific to human CD11b (ICRF44) and mouse IgG1 used for a control antibody were purchased from Biolegend.

Date Reçue/Date Received 2020-10-08

[00127] For murine cancer model

[00128] Rat binding antibody specific to murine CD11b (M1/70), rat control IgG2b antibody (LTF-2), Armenian hamster anti-murine PD1 (J43), and Armenian hamster control IgG were purchased from BioXcell. Taxol is a chemotherapy drug obtained from MacKay Memorial hospital

[00129] Protocol of cancer treatment

[00130] Subcutaneous tumor model

[00131] C57BL/6 mice were inoculated subcutaneously with 2×10^5 B16F10 cells or $1x10^6$ LLC1 cells. 7 days after tumor inoculation, treatment was started. Tumor-bearing mice were treated intraperitoneally (ip) with different antibodies and a chemotherapy drug twice per week. Mice were monitored and scored for the formation of palpable tumors twice weekly and sacrificed if tumors exceeded the predetermined size of 3000 mm^3. Tumor volumes were measured with calipers and calculated with the following formula: $A\times B^2 \times 0.54$, where A is the largest diameter, and B is the smallest diameter.

[00132] Lung metastasis model

[00133] $2x10^5$ B16F10 cells or LLC1 cells were injected into each mouse via tail vein on day 0. On day1, mice were injected ip with various antibodies. Injections were repeated every three to four day. On day15, mice were sacrifice and the amount of tumor seeding was counted as total numbers of nodules presented in the lungs under microscopy. In other experiments, mice were analyzed for the effects of combination therapy for the long term survival of treated mice in each of the groups.

[00134] Flow cytometric analysis

[00135] For human monocyte study

[00136] Monocytes were pre-incubated anti-CD11b (ICRF44), or appropriate isotype control antibodies for 1 hour. The cells were subsequently added with 100 ng/ml LPS and incubated overnight. To analyze the surface phenotype of the LPS primed monocytes, the cells were incubated for 30 minutes on ice in the dark with the following mAbs diluted in phosphate-buffered saline (PBS) containing 1% BSA: PD-L1-FITC, CD80-PE, CD86-PE, HLA-DR-PE, and CD14-PerCP (BD Biosciences). Monocytes, polymorphonuclear leukocytes (PMNs), and lymphocytes are gated based on their FSC/SSC properties. The fluorescence was detected using FACS Calibur, and data analysis was performed using FCS Express version 3 (De Novo Software).

[00137] For murine cancer study

[00138] To obtain tumor-infiltrating leukocytes, tumor tissues were digested by collagenase IV (Sigma). Single-cell suspensions were stained with following antibodies: CD45-PE, Ly-6G-FITC, Ly-6C-APC, and CD8b.2-FITC. Tumor-infiltrating leukocytes were gated from CD45+ populations. The fluorescence was detected using FACS Calibur, and data analysis was performed using FCS Express version 3 (De Novo Software).

[00139] To isolate white blood cells (WBCs) from each experiment, whole blood cells were lysed by RBC lysis buffer. Single-cell suspensions were stained with following antibodies: PD-L1-APC, IAIE-APC, and CD8b.2-FITC (Biolegend). Monocytes, polymorphonuclear leukocytes (PMNs), and lymphocytes were based on their FSC/SSC properties. The fluorescence was detected using FACS Calibur, and data analysis was performed using FCS Express version 3 (De Novo Software).

[00140] Cytokine quantification

[00141] Human IL-6, IL-10, IL-12, and TNF-α in the culture supernatant were detected by a commercial enzyme-linked immunosorbent assay (ELISA; R&D Systems) according to the manufacturer instructions. Murine IL-12, IFN-γ, and TNF-α in the plasma were quantified by BD CBA mouse inflammation kit.

Example 1 Binding CD11b would reduce the PD-L1 expression on LPS-primed monocytes

[00142] In this example, we investigated whether blockade of the integrin αMβ2 (Mac-1), could functionally increase the TLR response. As shown in Figure 1, administration of CD11b binding agent such as anti-CD11b antibody (ICRF44) can reduce the LPS induced PD-L1expression on monocytes. By contrast, anti-CD11b antibody treatment did not alter the levels of HLA-DR, CD80, and CD86 expression on LPS-primed monocytes. Binding CD11b with ML-C19-A, a small molecule of CD11b antagonist (Figure 2A), also demonstrated inhibitory PD-L1 expression in LPS-primed monocytes (Figure 2B). Together, these results suggest that CD11b plays a crucial role in the induction of PD-L1 expression on LPS-primed monocytes.

Example 2 Effect of CD11b binding in antitumor immunity

[00143] To examine the effect of CD11b binding in antitumor immunity, anti-mouse CD11b (M1/70) antibody was tested as a monotherapy in B16F10 murine tumor model. C57BL/6 mice were subcutaneously injected with B16F10 cells at Day 0. On day 7, mice were injected intraperitoneally (ip) with either control IgG (5 mg/kg) or anti-mouse CD11b antibody (5 mg/kg). Injections were repeated every three to four days. Efficiency was determined by monitoring tumor volumes and long term survival for each group. As shown in Figure 3, binding CD11b with anti-mouse CD11b antibody potently inhibited the subcutaneous growth of B16F10 tumors (control IgG vs. anti-CD11b = 1054 ± 385.4 mm^3 vs. 502.7 ± 268.2 mm^3 on day 18). We examined the proportion of immune cell populations in the tumor. On day 18 after tumor

inoculation, binding CD11b with anti-CD11b antibody reduced the local accumulation of tumor-infiltrating myeloid-derived suppressor cells (MDSCs), which suppress T cells and resulted in an increase in tumor infiltrated CD8 T cells (Figure 4). Together, binding CD11b with anti-CD11b antibody shifted an immunosuppressive tumor microenvironment to an immunostimulatory state, which favorably contributes to an antitumor effect. We further examined the proportion of immune cell populations in the periphery after anti-CD11b antibody treatment. On day 15 after tumor injection, anti-CD11b treatment resulted in a decrease PD-L1 expression in CD11b positive white blood cells, while the percentages of IAIE positive CD8 T cells, activated T cells, in CD8 T cells were increased (Figure 5). Plasma levels of IFN-γ, IL-12, and TNF-αreflect immunostimulatory state in various inflammatory or malignant diseases. We measured plasma IFN-γ, IL-12, and TNF-α levels in tumor-bearing mice with anti-CD11b antibody treatment. In comparison to control IgG treatment, anti-CD11b antibody treated mice showed elevated plasma IFN-γ, IL-12, and TNF-α levels (Figure 6).

[00144] CD11b binding also demonstrated efficiency in the distinct syngeneic LLC1 tumor model. Treatment with 5 mg/kg of anti-CD11b antibody potently inhibited tumor growth of LLC1 tumor (Figure 7) and prolong animal survival (Figure 8) (median survival day Ctrl IgG: 31 day; Anti-CD11b: 42 day).

Example 3 Synergistic effect of CD11b binding and immune checkpoint therapy in antitumor immunity

[00145] The combined treatment demonstrated efficiency in the distinct syngeneic LLC1 lung metastasis model. Treatment with anti-CD11b (10 mg/kg) + anti-PD-1 (10 mg/kg) antibody potently reduced tumor nodule of LLC1 tumor (Figure 9) (Ctrl IgG vs. anti-CD11b vs. anti-PD-1 vs. anti-CD11b+anti-PD-1 = 200 ± 13 vs. 167 vs. 164 ± 11 vs. 131 ± 2 on day 15) and prolong

animal survival (Figure 10) (median survival day Ctrl IgG: 24 day; anti-CD11b: 24 day; anti-PD-1: 22 day; anti-CD11b+anti-PD-1: 26 day).

Example 4 Synergistic effect of CD11b binding and chemotherapy in antitumor immunity

[00146] CD11b binding also enhances chemotherapy. In this example, B16F10 cells were implanted on day 0. On day7, mice were injected ip with either control IgG (5 mg/kg), anti-mouse CD11b antibody (5 mg/kg), Taxol (10 mg/kg) + control IgG (5 mg/kg), or Taxol (10 mg/kg) + anti-CD11b (5 mg/kg). Injections were repeated every three to four days. As shown in Figure 11, treatment with a combination of taxol plus anti-CD11b antibody effectively controlled tumor growth. The effectiveness of the combination treatment was also confirmed in the long term survival (Figure 12) (median survival day Ctrl IgG: 25 day; anti-CD11b: 32 day; Taxol + Ctrl IgG: 25 day; Taxol + anti-CD11b: 32 day).

Example 5 In LPS-induced immunosuppressed monocytes or monocytes from patients with septic shock, binding CD11b with anti-CD11b antibody also reduces PD-L1 expression when cells are challenged with LPS.

[00147] Sepsis, a systematic inflammatory response syndrome caused by severe infection, remains a worldwide healthcare problem and a life-threatening disease. It is becoming increasingly clear that sepsis initiates a biphasic immunological reaction that varies over time. During the initial phase of sepsis, a systematic hyperinflammatory immune response can systematically produce inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, which may cause hemodynamic instability, multiorgan dysfunction, coagulation abnormalities, and shock. Concomitant with the hyperinflammatory immune response is a nearly simultaneous production of anti-inflammatory cytokines, including IL-10, and tumor growth factor (TGF)-β; the immune system rapidly enters an immune hyporeactivity

state, termed immunoparalysis, which is manifested in an inability to eradicate the primary infection and the development of late nosocomial infections. The indicators of immunoparalysis observed in patients with sepsis include lymphocyte abnormalities, monocytic deactivation with diminished human leukocyte antigen-DR (HLA-DR) surface expression, and low TNF-α production under *ex vivo* stimulation. Sustained reductions in monocyte HLA-DR expression indicate a high risk for nosocomial infection and death in patients with sepsis. Recently, elevated program death ligand-1 (PD-L1) expression in monocytes in patients with septic shock was observed and was associated with an increased occurrence of secondary nosocomial infections and mortality (*Guignant C, Lepape A, Huang X, Kherouf H, Denis L, et al. (2011) Programmed death-1 levels correlate with increased mortality, nosocomial infection and immune dysfunctions in septic shock patients. Crit Care 15: R99*). Therefore, the level of PD-L1 expression in monocytes may serve as a new marker for immunoparalysis.

It has been report that prior exposure of monocytes to LPS over 2 days would cause them to become immunosuppressed monocytes (*Wolk K, Docke WD, von Baehr V, Volk HD, and Sabat R. (2000) Impaired antigen presentation by human monocytes during endotoxin tolerance. Blood 96: 218*). Clinically, these cells are associated with immunoparalysis and mortality. We established reproducible LPS-induced immunosuppressed monocytes, in which human monocytes are preincubated with 100 ng/ml LPS for 2 days. Compare with fresh isolated human monocytes, LPS-induced immunosuppressed monocytes expressed higher PD-L1 levels on the cell surface (figure 13A). To examine the effect of CD11b modulators in LPS-induced immunosuppressed monocytes, cells were exposed to 1 μg/ml LPS for 18 hr in the presence of IgG1 or anti-CD11b antibody (ICRF44). As shown in Figure 13B, binding CD11b with anti-CD11b antibody (ICRF44) reduced the PD-L1 expression in LPS-induced immunosuppressed monocytes when

cells challenged with LPS. Moreover, anti-CD11b antibody (ICRF44) treatment also reduced PD-L1 expression in monocytes from patients with septic shock upon *in vitro* LPS stimulation (Figure 14).

Example 6 Humanized antibodies that bind human CD11b

[00148] The variable domain sequences of murine anti-human CD11b antibody were searched against the human antibody database. 10 sets of human framework sequences with high homology to murine anti-human CD11b were chosen as human acceptors for both light and heavy chains. Meanwhile, N-glycosylation motifs were analyzed. Potential glycosylation sites in the candidate human variable regions should therefore be avoided. The humanized variable domains of 10 light chains were denoted as VL1, VL2, VL3, VL4, VL5, LC1, LC2, LC3, LC4, and LC5 (Figure 15); while the humanized variable domains of 10 heavy chains were denoted as VH1, VH2, VH3, VH4, VH5, HC1, HC2, HC3, HC4, and HC5 (Figure 16). These light chain and heavy chain peptide sequence may provide humanized antibodies or antigen-binding portions that bind to human anti-CD11b with high affinity.

Example 7 Functional activity of humanized CD11b antibody

[00149] The specificity of humanized anti-CD11b antibodies were determined by flow cytometry using K562 cells expressing CD11b. As shown in Figure 17, all humanized anti-CD11b antibodies in this example were able to bind to the CD11b transfected K562 cells. In contrast, these antibodies did not bind to K562 cells. Taken together, these results demonstrate that the humanized anti-CD11b antibodies can specifically bind to the CD11b epitope.

[00150] To examine the functional activity of humanized anti-CD11b antibody, the antibody was used in LPS-primed monocytes that measure the ability of the antibody to inhibit PD-L1

expression on the surface of monocytes. As shown in Figure 18, the upregulation of PD-L1 by LPS can be significantly reduced by the humanized anti-CD11b antibodies.

[00151] In summary, we described a series of humanized anti-CD11b antibodies directed against the human αM domain. Binding of humanized anti-CD11b antibodies was able to reduce PD-L1 expression on LPS-primed monocytes.

CLAIMS:

1. An antibody or an antigen-binding portion thereof for use in inhibiting PD-L1 expression, wherein the antibody or the antigen-binding portion thereof comprises:

(i) a heavy chain variable region comprising H-CDR1 having the sequence of SEQ ID NO:1, H-CDR2 having the sequence of SEQ ID NO:3, and H-CDR3 having the sequence of SEQ ID NO:5; and

(ii) a light chain variable region comprising L-CDR1 having the sequence of SEQ ID NO:7, L-CDR2 having the sequence of SEQ ID NO:9, and L-CDR3 having the sequence of SEQ ID NO:11.

2. An antibody or an antigen-binding portion thereof for use in inhibiting PD-L1 expression, wherein the antibody or the antigen-binding portion thereof comprises:

a heavy chain variable region comprising the amino acid sequence of one of SEQ ID NO:13 – SEQ ID NO:17, and

a light chain variable region comprising the amino acid sequence of one of SEQ ID NO:23 – SEQ ID NO:27.

3. An antibody or an antigen-binding portion thereof for use in inhibiting PD-L1 expression, wherein the antibody or the antigen-binding portion thereof comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:23; or

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:14, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:24; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:25; or

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(d) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:16, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:26; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:27.

4. The antibody or the antigen-binding portion thereof for use according to any one of claims 1-3, wherein the antibody or the antigen-binding portion thereof is a chimeric, humanized, or human antibody, or an antigen-binding portion thereof.

5. An antibody binding specifically to CD11b or a binding fragment thereof, comprising:

a heavy chain variable region comprising H-CDR1 having the sequence of SEQ ID NO:1, H-CDR2 having the sequence of SEQ ID NO:3, and H-CDR3 having the sequence of SEQ ID NO:5; and

a light chain variable region comprising L-CDR1 having the sequence of SEQ ID NO:7, L-CDR2 having the sequence of SEQ ID NO:9, and L-CDR3 having the sequence of SEQ ID NO:11.

6. The antibody binding specifically to CD11b or a binding fragment thereof according to claim 5, wherein the antibody or the binding fragment thereof comprises:

a heavy chain variable region comprising the amino acid sequence of one of SEQ ID NO:13 – SEQ ID NO:17, and

a light chain variable region comprising the amino acid sequence of one of SEQ ID NO:23 – SEQ ID NO:27.

7. The antibody binding specifically to CD11b or a binding fragment thereof according to claim 5, wherein the antibody or the binding fragment thereof comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:13, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:23; or

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(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:14, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:24; or

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:15, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:25; or

(d) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:16, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:26; or

(e) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:17, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:27.

8. A use of a CD11b modulator for inhibition of PD-L1 expression, wherein the CD11b modulator is an antibody binding specifically to CD11b or the binding fragment thereof as defined in any one of claims 5-7.

9. A use of a composition in the manufacture of a medicament for treating a disease or pathophysiology of immune suppression or immune exhaustion associated with PD-L1 expression, wherein the composition comprises the antibody binding specifically to CD11b, or the binding fragment thereof as defined in any one of claims 5-7, and a pharmaceutically acceptable carrier.

10. A use of a composition for treating a disease or pathophysiology of immune suppression or immune exhaustion associated with PD-L1 expression, wherein the composition comprises the antibody binding specifically to CD11b, or the binding fragment thereof as defined in any one of claims 5-7, and a pharmaceutically acceptable carrier.

11. The use according to claim 9 or 10, wherein the composition further comprises an immune checkpoint modulator or a chemotherapeutic agent.

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12. The use according to claim 11, wherein the immune checkpoint modulator is an anti-PD-1 antibody, a PD-1 ligand, an anti-PD-L1 antibody, a PD-L1 ligand, an anti-CTLA-4 antibody, a CTLA-4 ligand, or a binding fragment thereof.

13. The use according to claim 11, wherein the chemotherapeutic agent is an alkylating agent, an antimetabolite, an anti-microtubule agent, a topoisomerase inhibitor, or a cytotoxic antibiotic.

14. The use according to any one of claims 9-13, wherein the disease or pathophysiology is selected from the group consisting of melanoma, lung cancer, squamous cell carcinomas of the lung, head and neck cancer, breast cancer, ovarian cancer, uterine cancer, prostate cancer, gastric carcinoma, cervical cancer, esophageal carcinoma, bladder cancer, kidney cancer, brain cancer, liver cancer, colon cancer, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular malignant melanoma, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, esophagus cancer, small intestine cancer, endocrine system cancer, thyroid gland cancer, parathyroid gland cancer, adrenal gland cancer, sarcoma of soft tissue, urethra cancer, penis cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphoma, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, and T-cell lymphoma.

15. The use according to claim 14, wherein the chronic or acute leukemia is acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, or chronic lymphocytic leukemia.

16. A composition comprising the antibody or the antigen-binding portion thereof as defined in any one of claims 1-4 and a carrier.

17. A pharmaceutical composition comprising the antibody or the antigen-binding portion thereof as defined in any one of claims 1-4 and a pharmaceutically acceptable carrier.

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18. A composition comprising the antibody binding specifically to CD11b or the binding fragment thereof as defined in any one of claims 5-7 and a carrier.

19. A pharmaceutical composition comprising the antibody binding specifically to CD11b or the binding fragment thereof as defined in any one of claims 5-7 and a pharmaceutically acceptable carrier.

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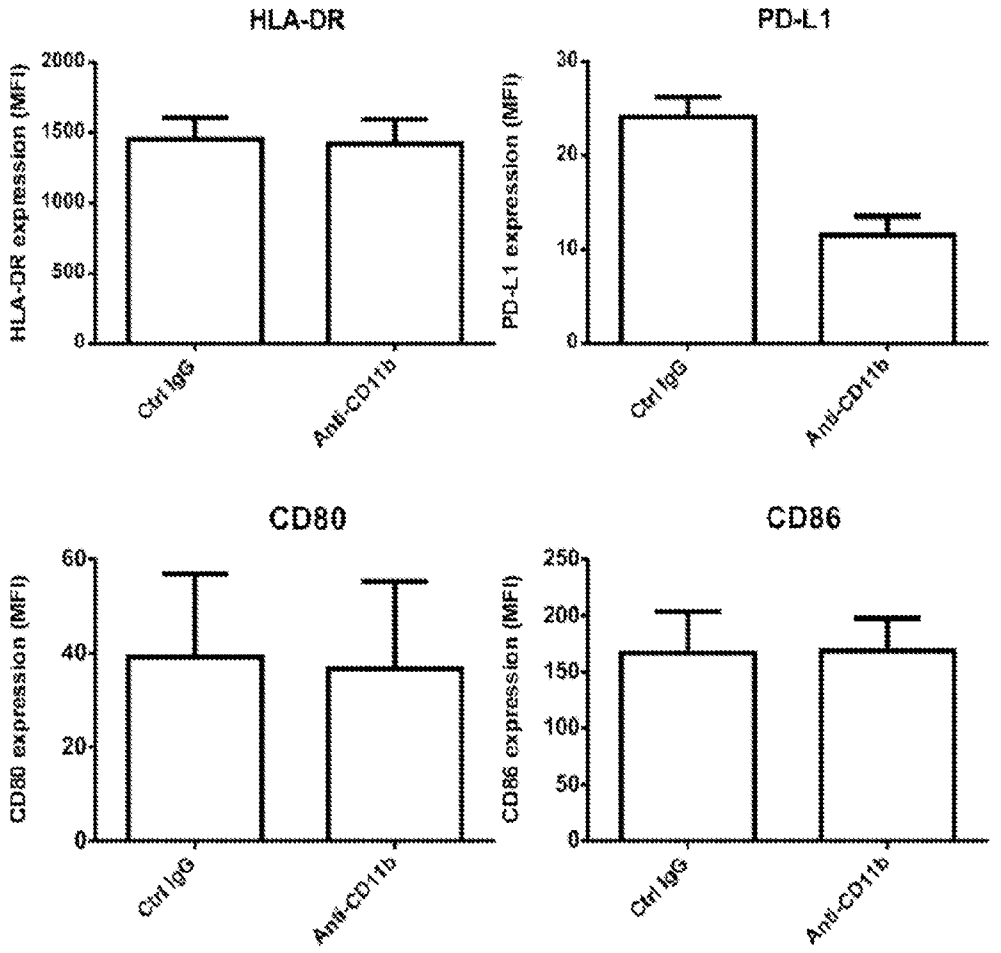
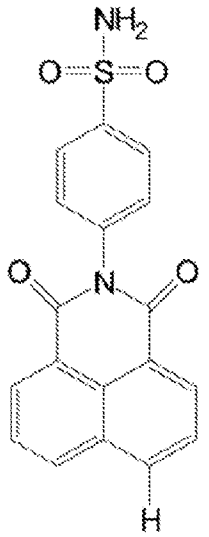
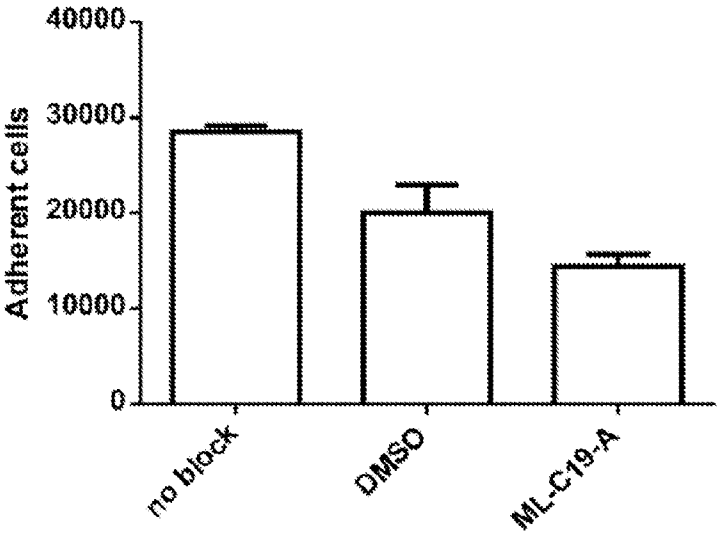

HLA-DR
HLA-DR expression (MFI)
2000
1500
1000
500
0
Ctrl IgG
Anti-CD11b
PD-L1
PD-L1 expression (MFI)
30
20
10
0
Ctrl IgG
Anti-CD11b
CD80
CD80 expression (MFI)
60
40
20
0
Ctrl IgG
Anti-CD11b
CD86
CD86 expression (MFI)
250
200
150
100
50
0
Ctrl IgG
Anti-CD11b

Fig. 1

A



ML-C19-A



B

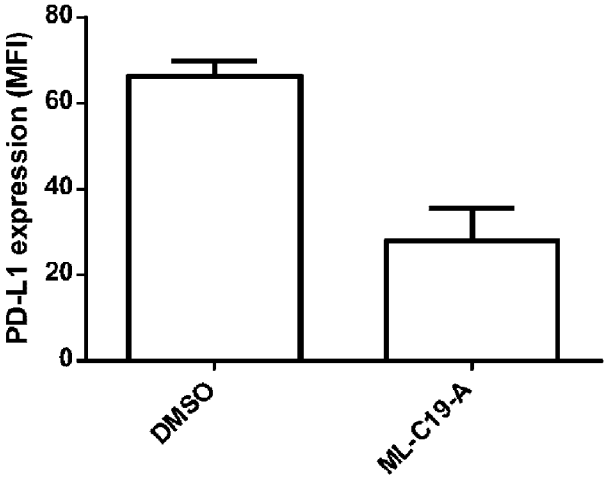


Fig. 2

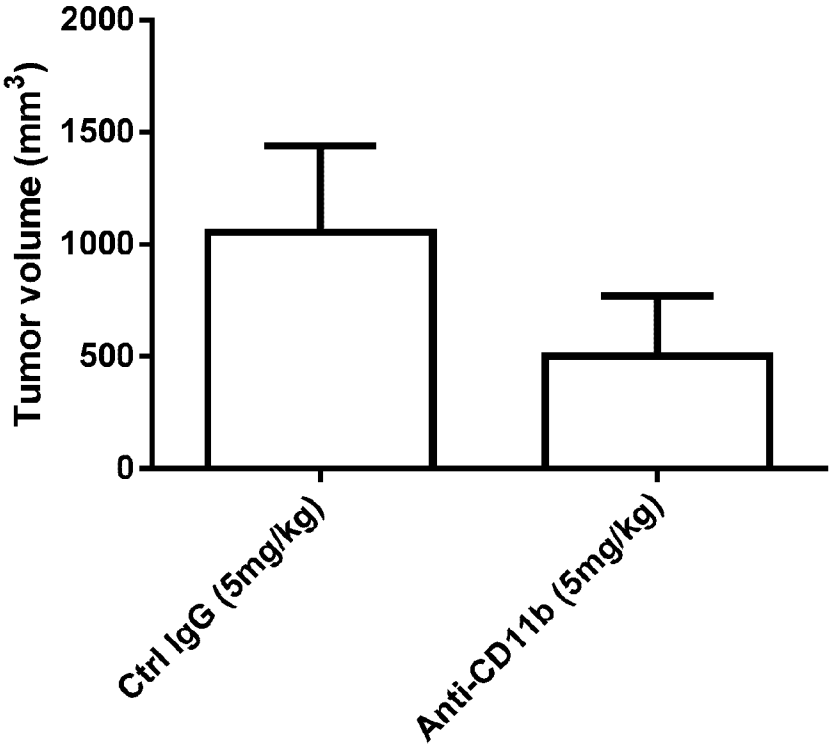

2000
1500
1000
500
0
Tumor volume (mm3)
Ctrl IgG (5mg/kg)
Anti-CD11b (5mg/kg)

Fig. 3

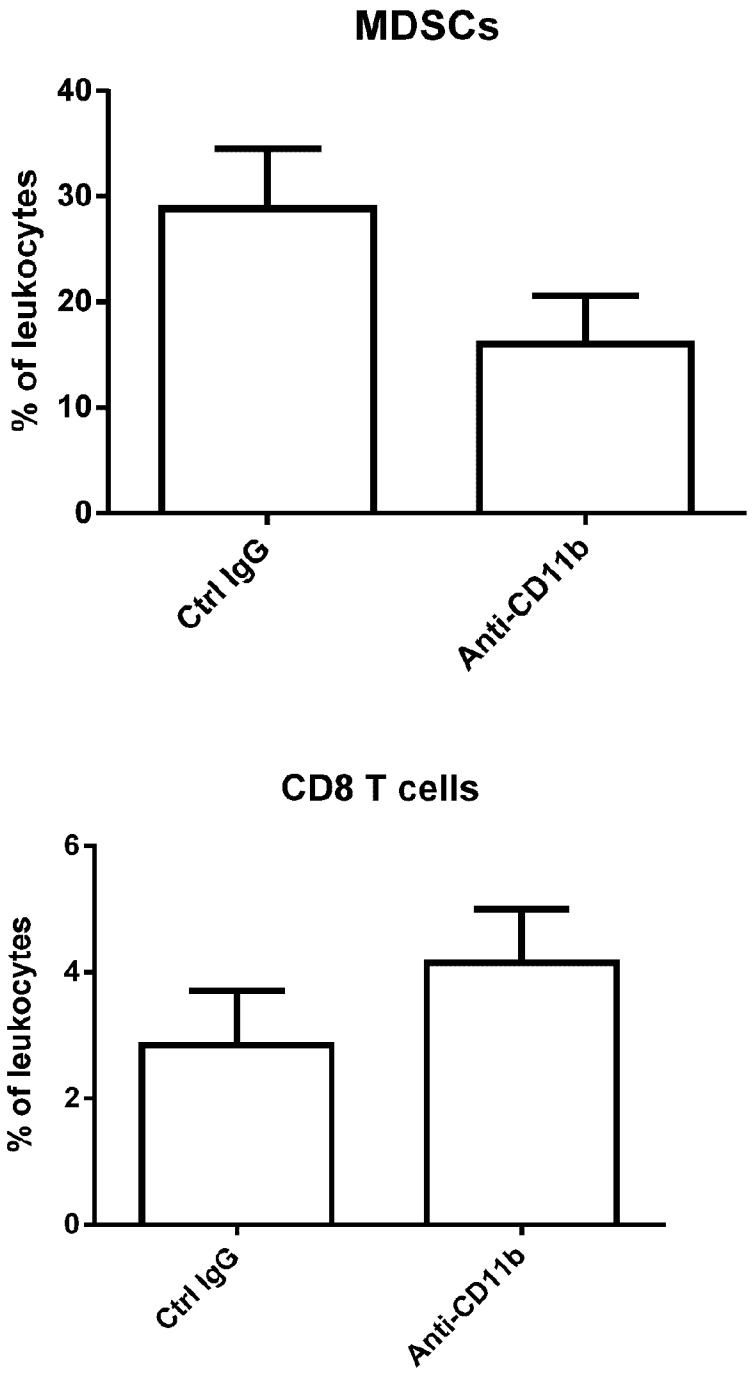

MDSCs
% of leukocytes
40
30
20
10
0
Ctrl IgG
Anti-CD11b
CD8 T cells
% of leukocytes
6
4
2
0
Ctrl IgG
Anti-CD11b

Fig. 4

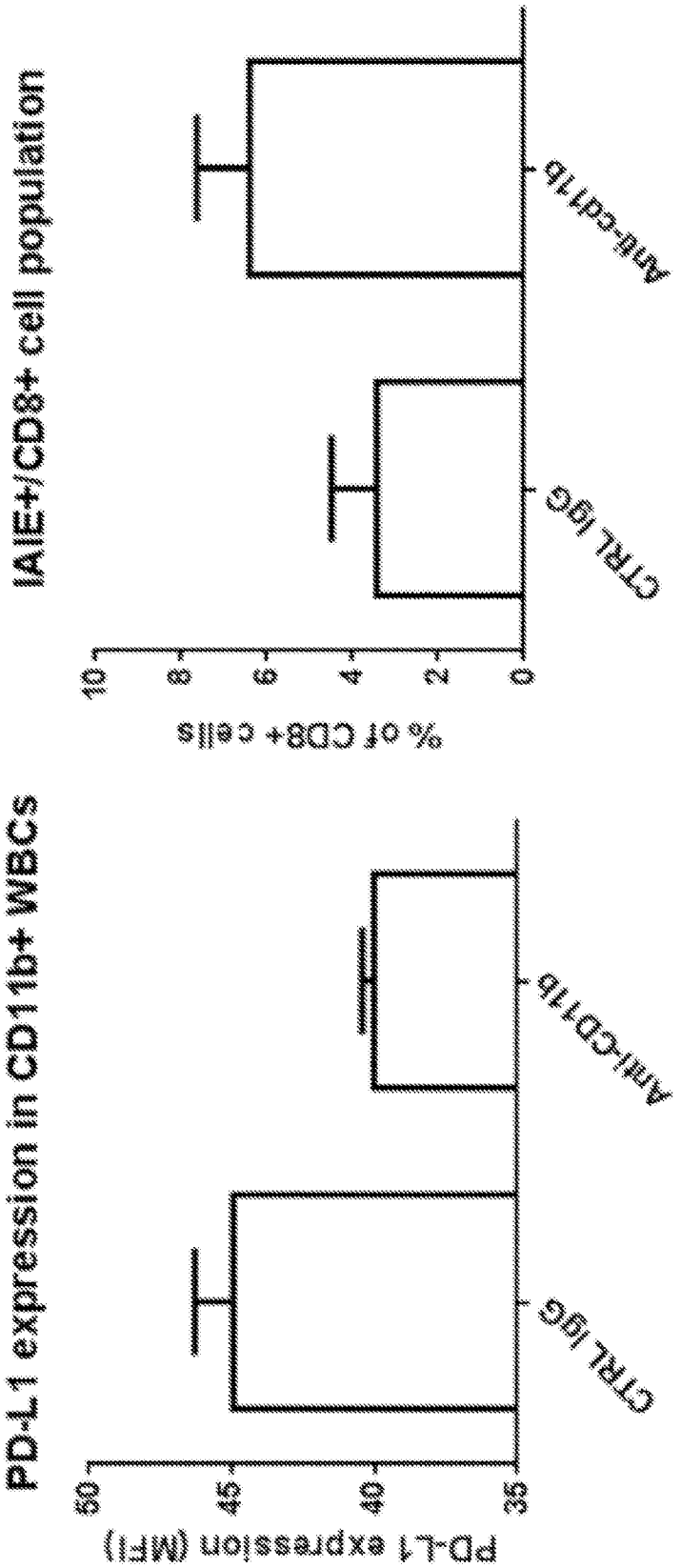

IAIE+/CD8+ cell population
% of CD8+ cells
0
2
4
6
8
10
CTRL IgG
Anti-cd11b
PD-L1 expression in CD11b+ WBCs
PD-L1 expression (MFI)
35
40
45
50
CTRL IgG
Anti-CD11b

Fig. 5

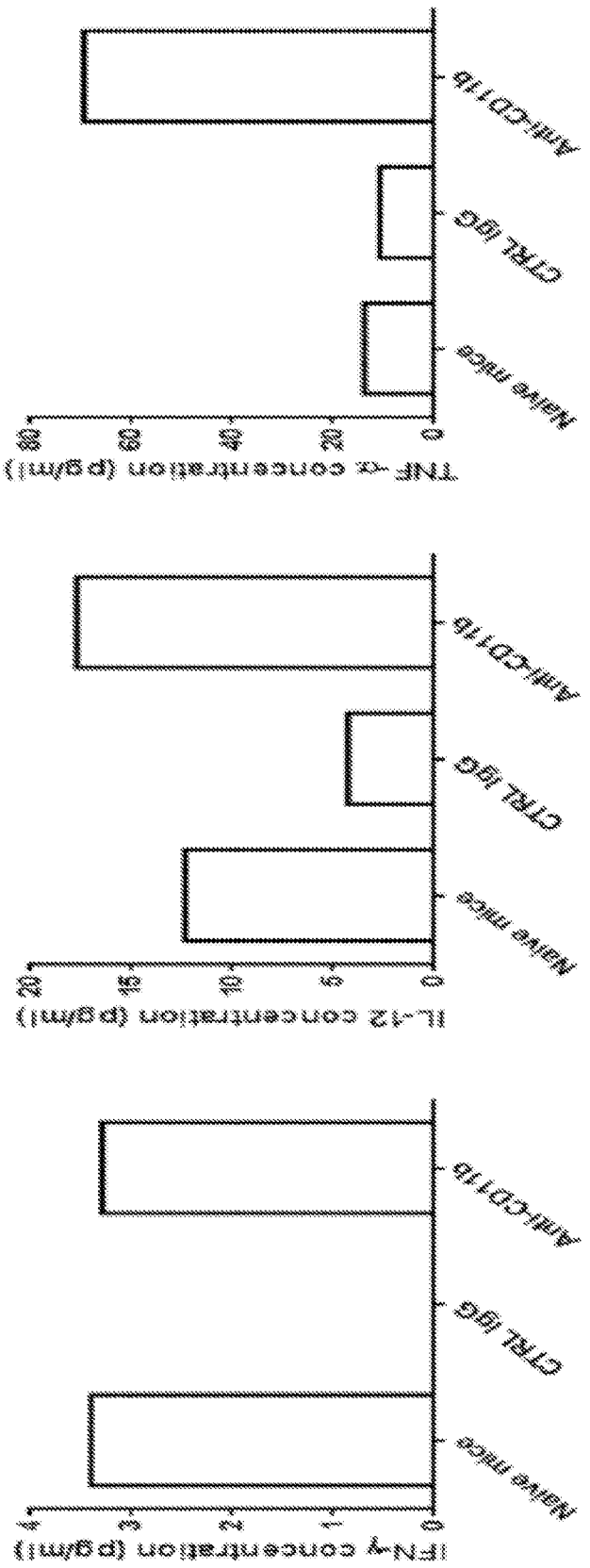

TNF-α concentration (pg/ml)
0
20
40
60
80
Naive mice
CTRL IgG
Anti-CD11b
IL-12 concentration (pg/ml)
0
5
10
15
20
Naive mice
CTRL IgG
Anti-CD11b
IFN-γ concentration (pg/ml)
0
1
2
3
4
Naive mice
CTRL IgG
Anti-CD11b

Fig. 6

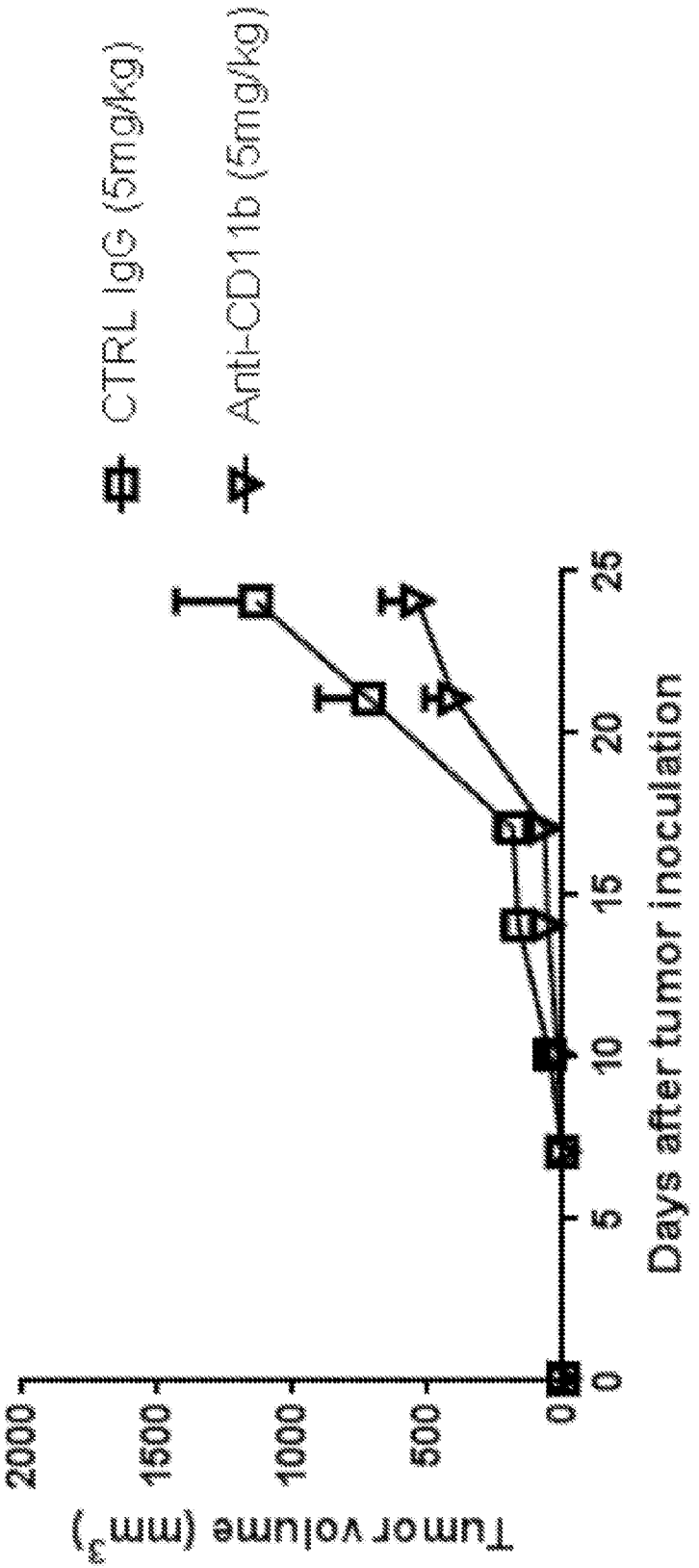

CTRL IgG (5mg/kg)
Anti-CD11b (5mg/kg)
Tumor volume (mm3)
2000
1500
1000
500
0
0
5
10
15
20
25
Days after tumor inoculation

Fig. 7

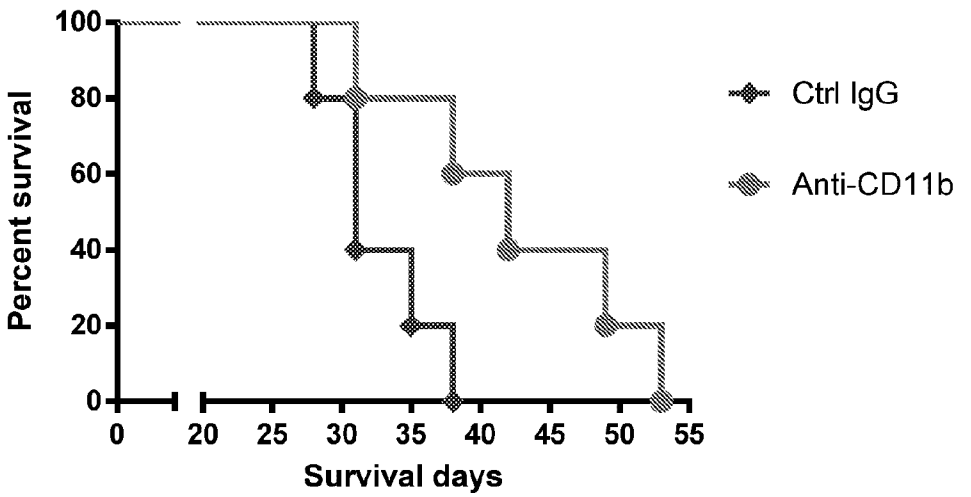

100
80
60
40
20
0
Percent survival
0
20
25
30
35
40
45
50
55
Survival days
Ctrl IgG
Anti-CD11b

Fig. 8

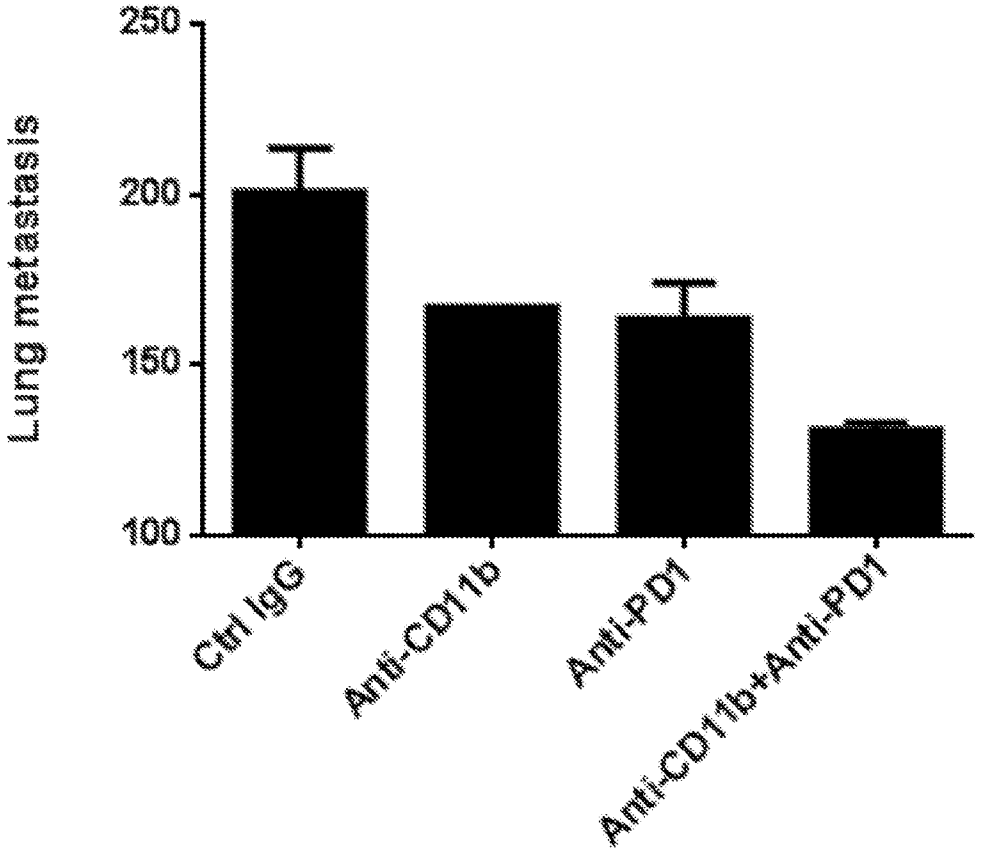

Lung metastasis
250
200
150
100
Ctrl IgG
Anti-CD11b
Anti-PD1
Anti-CD11b+Anti-PD1

Fig. 9

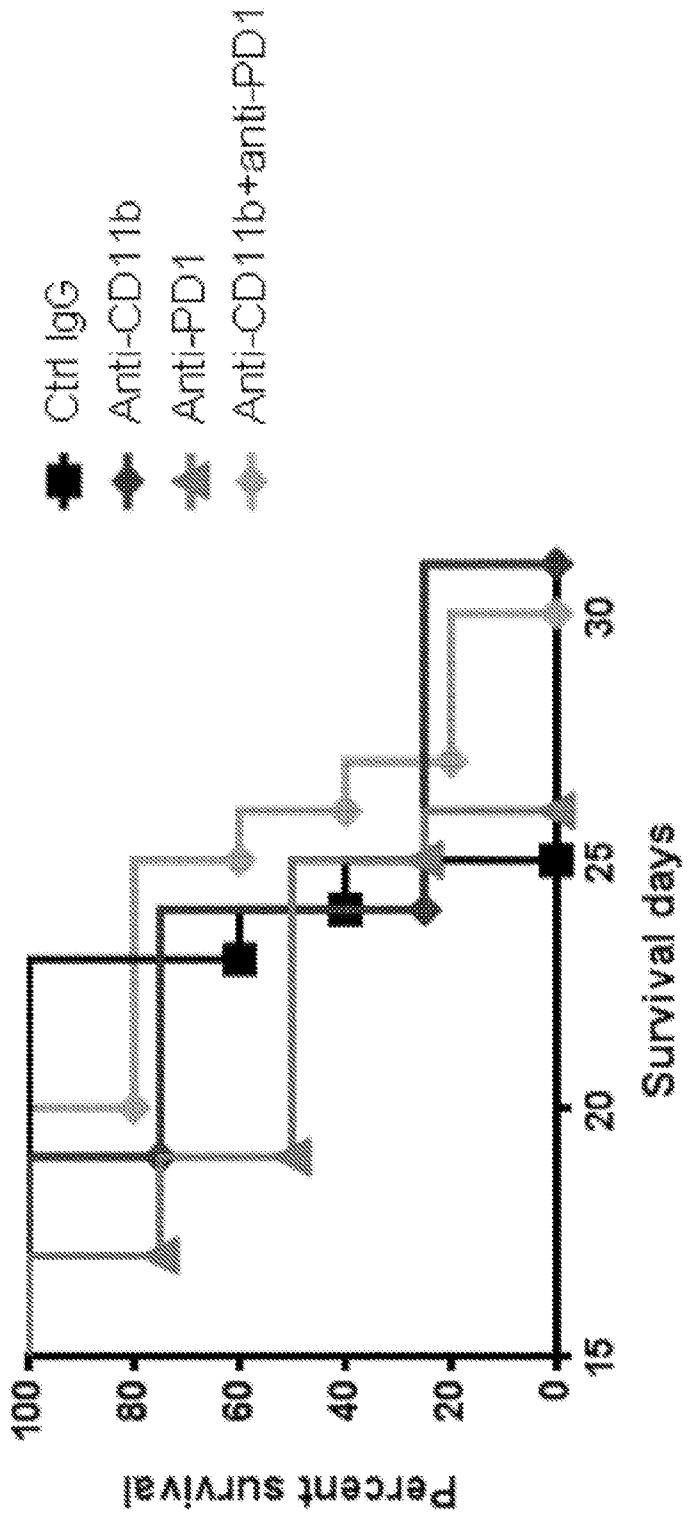

Ctrl IgG
Anti-CD11b
Anti-PD1
Anti-CD11b+anti-PD1
Percent survival
100
80
60
40
20
0
15
20
25
30
Survival days

Fig. 10

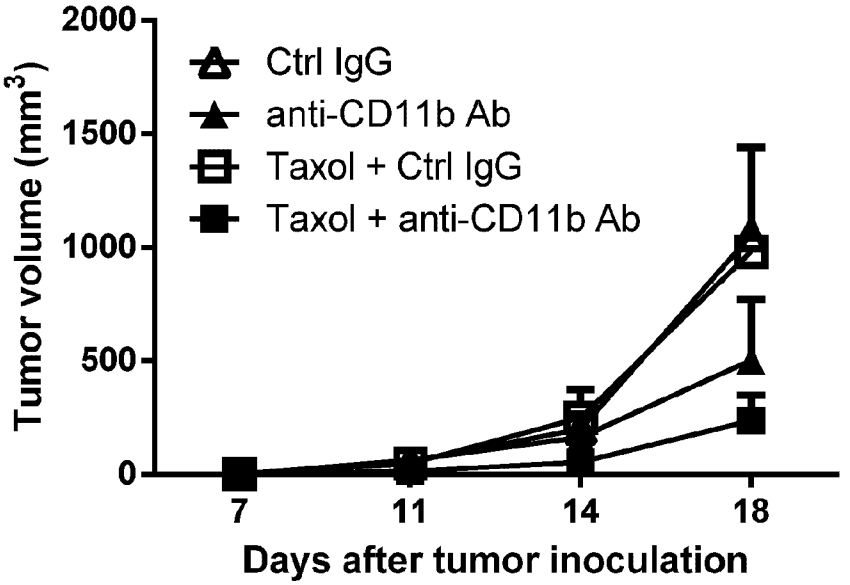

2000
1500
1000
500
0
Tumor volume (mm3)
Ctrl IgG
anti-CD11b Ab
Taxol + Ctrl IgG
Taxol + anti-CD11b Ab
7
11
14
18
Days after tumor inoculation

Fig. 11

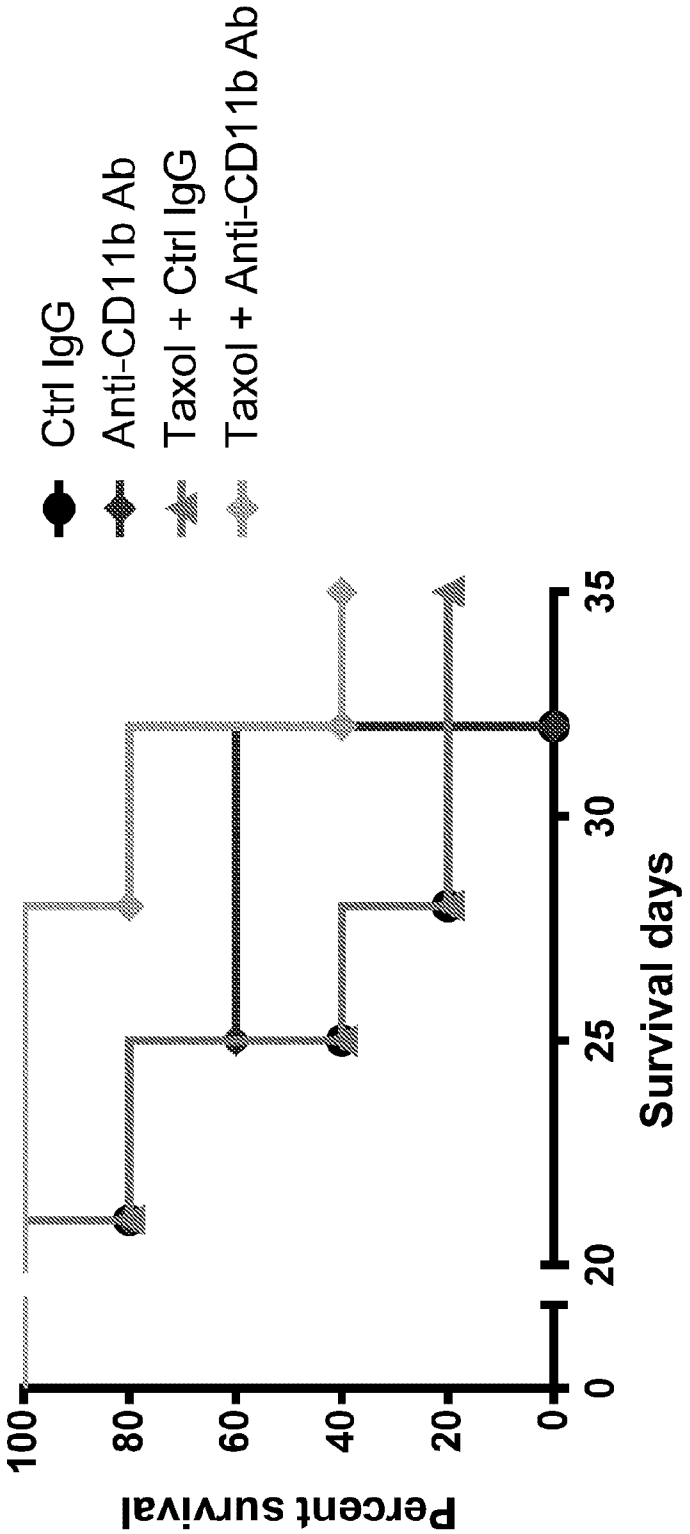

Ctrl IgG
Anti-CD11b Ab
Taxol + Ctrl IgG
Taxol + Anti-CD11b Ab
Percent survival
100
80
60
40
20
0
0
20
25
30
35
Survival days

Fig. 12

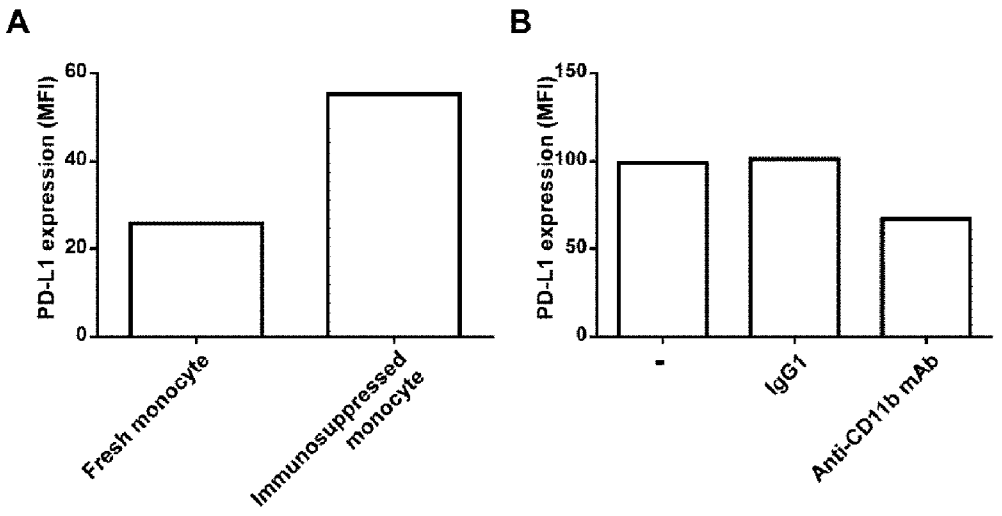

A
PD-L1 expression (MFI)
60
40
20
0
Fresh monocyte
Immunosuppressed monocyte
B
PD-L1 expression (MFI)
150
100
50
0
-
IgG1
Anti-CD11b mAb

Fig. 13

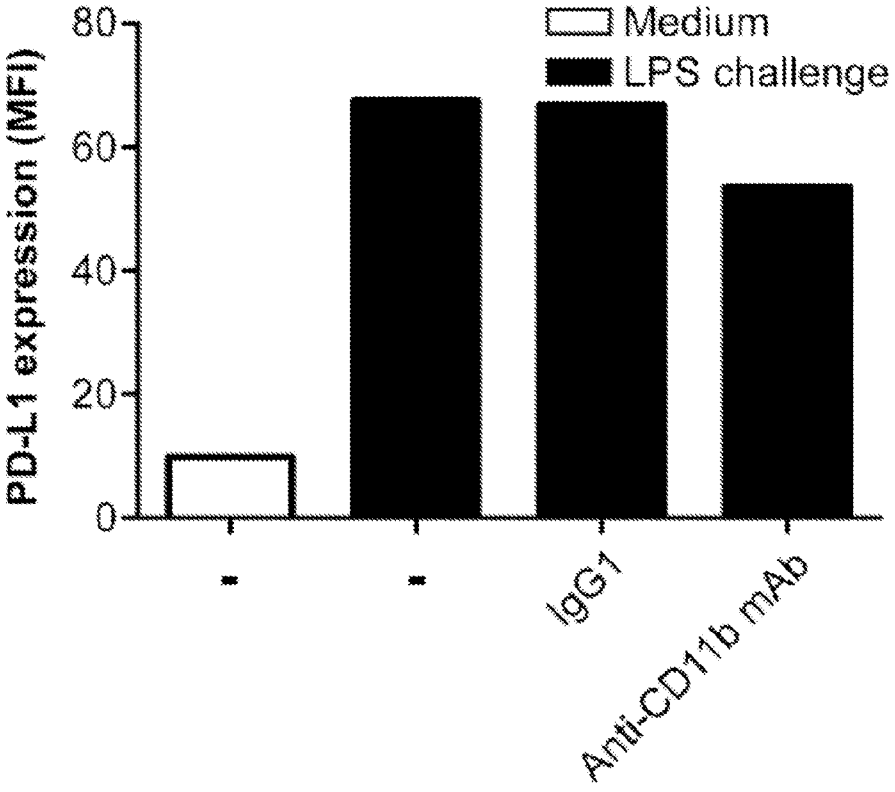

Medium
LPS challenge
PD-L1 expression (MFI)
80
60
40
20
0
-
-
IgG1
Anti-CD11b mAb

Fig. 14

```
                                CDR1                    CDR2
VL1   1 EIVLTQSPDFQSVTPKEKVTITCRASQNIGTSIHWYQQKPDQSPKLLIKYASESISGVPS 60
VL2   1 EIVMTQSPATLSVSPGERATLSCRASQNIGTSIHWYQQKPDQAPRLLIYYASESISGIPA 60
VL3   1 DIQMTQSPSSLSASVGDRVTITCRASQNIGTSIHWYQQKPGKAPKLLIYYASESISGVPS 60
VL4   1 EIVLTQSPATLSLSPGERATLSCRASQNIGTSIHWYQQKPDQAPRLLIYYASESISGIPA 60
VL5   1 EIVLTQSFGTLSLSPGERATLSCRASQNIGTSIHWYQQKPGQAPRLLIYYASESISGIPD 60
                                CDR3
VL1  61 RFSGSGSGTDFTLTINSLEAEDAATYYCQQSDSWPTLTFGQGTKVEIK 108
VL2  61 RFSGSGSGTEFTLTISSLQSEDFAVYYCQQSDSWPTLTFGQGTKLEIK 108
VL3  61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSDSWPTLTFGGGTKVEIK 108
VL4  61 RFSGSGSGTDFTLTISSLEPEDFAVYYCQQSDSWPTLTFGQGTKVEIK 108
VL5  61 RFSGSGSGTDFTLTISRLEPEDFAVYYCQQSDSWPTLTFGQGTKLEIK 108

                        CDR1                    CDR2
LC1   1 DIVMTQSPDSLAVSLGERATINCKSSQSLLYSENQENYLAWYQQKPGQPPKLLIYWASTR 60
LC2   1 DIVMTQSPLSLPVTPGEPASISCKSSQSLLYSENQMTLAWYLQKPGQSPQLLIYWASTR 60
LC3   1 DIVMTQSPLSLSVTPGQPASISCKSSQSLLYSENQENYLAWYLQKPGQSPQLLIYWASTR 60
LC4   1 DVVMTQSPLSLPVTLGQPASISCKSSQSLLYSENQMTLAWFQQRPGQSPRRLIYWASTR 60
LC5   1 DIVMTQFPLSSPVTLGQPASISCKSSQSLLYSENQENYLAWLQQRPGQPPRLLIYWASTR 60
                                CDR3
LC1  61 QSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYDTPLTFGQGTKVEIK 113
LC2  61 QSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQYYDTPLTFGGGTKVEIK 113
LC3  61 QSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQYYDTPLTFGQGTKVEIK 113
LC4  61 QSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQQYYDTPLTFGQGTKLEIK 113
LC5  61 QSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCQQYYDTPLTFGQGTKLEIK 113
```

Fig. 15

```
                                CDR1                                CDR2
VH1   1 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIMWVRQAPGQGLEWMGMIYPSDTYIMM 60
VH2   1 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSNYWIMWVRQAPGQGLEWMGMIYPSDTYIMM 60
VH3   1 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIMWVRQATGQGLEWMGMIYPSDTYIMM 60
VH4   1 QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIMWVRQAPGQRLEWMGMIYPSDTYIMM 60
VH5   1 QVQLVQSGAEVKKPGATVKISCKVSGYTFTNYWIMWVQQAPGKGLEWMGMIYPSDTYIMM 60
                                                CDR3
VH1  61 MQKFKDRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSAYANYYDYWGQGTLVTVSS 118
VH2  61 MQKFKDRVTITADKSTSTAYMELSSLRSEDTAVYYCATSAYANYEDYWGQGTLVTVSS 118
VH3  61 MQKFKDRVTMTRNTSISTAYMELSSLRSEDTAVYYCARSAYANYTDYWGQGTLVTVSS 118
VH4  61 MQKFKDRVTITRDTSASTAYMELSSLRSEDTAVYYCARSAYANYEDYWGQGTLVTVSS 118
VH5  61 MQKFKDRVTITADTSTDTAYMELSSLRSEDTAVYYCARSAYANYEDYWGQGTLVTVSR 118

                                CDR1                                CDR2
HC1   1 QVQLQESGPGLVKPSETLSLTCTVSGFSLTSMSISWIRQPPGKGLEWIGAIWSGGSTDYN 60
HC2   1 QVQLQESGPGLVKPSGTLSLTCAVYGFSLTSMSISWIRQPPGKGLEWIGAIWSGGSTDYN 60
HC3   1 QVQLQQWGAGLLKPSETLSLTCAVYGFSLTSMSISWIRQPPGKGLEWIGAIWSGGSTDYN 60
HC4   1 EVQLVESGGGLVQPGGSLRLSCAASGFSLTSMSISWVRQAPGKGLEWVSAIWSGGSTDYN 60
HC5   1 EVQLVETGGGLIQPGGSLRLSCAASGFSLTSMSISWVRQAPGKGLEWVSAIWSGGSTDYN 60
                                                CDR3
HC1  61 SDLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGYEYYEYDYWGQGTLVTVSS 117
HC2  61 SDLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGYEYYEYDYWGQGTMVTVSS 117
HC3  61 SDLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGYEYYEYDYWGQGTLVTVSS 117
HC4  61 SDLKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGYEYYEYDYWGQGTLVTVSS 117
HC5  61 SDLKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGGYEYYEYDYWGQGTLVTVSS 117
```

Fig. 16

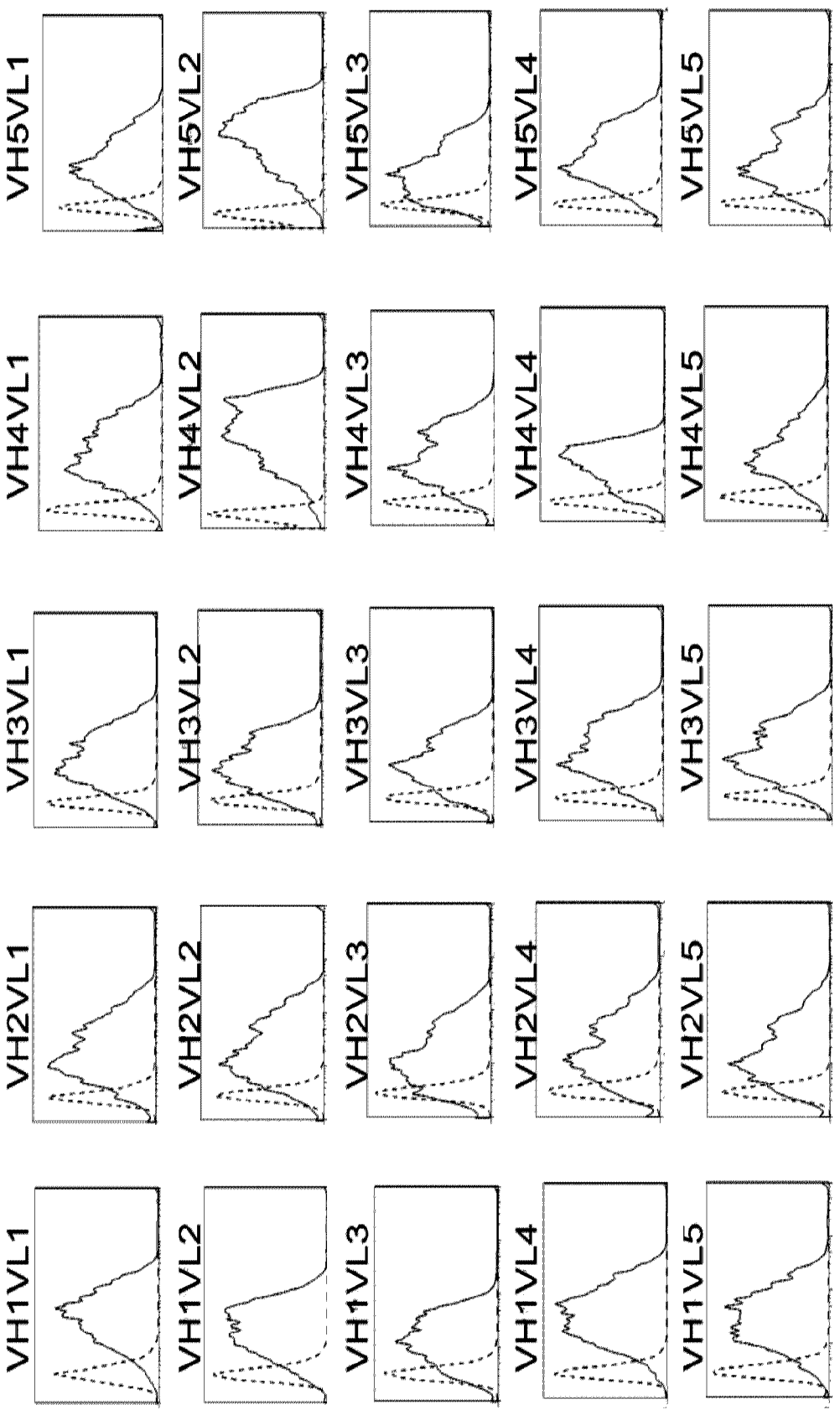

VH1VL1
VH2VL1
VH3VL1
VH4VL1
VH5VL1
VH1VL2
VH2VL2
VH3VL2
VH4VL2
VH5VL2
VH1VL3
VH2VL3
VH3VL3
VH4VL3
VH5VL3
VH1VL4
VH2VL4
VH3VL4
VH4VL4
VH5VL4
VH1VL5
VH2VL5
VH3VL5
VH4VL5
VH5VL5
- - - - K562
——— K562/CD11b

Fig. 17

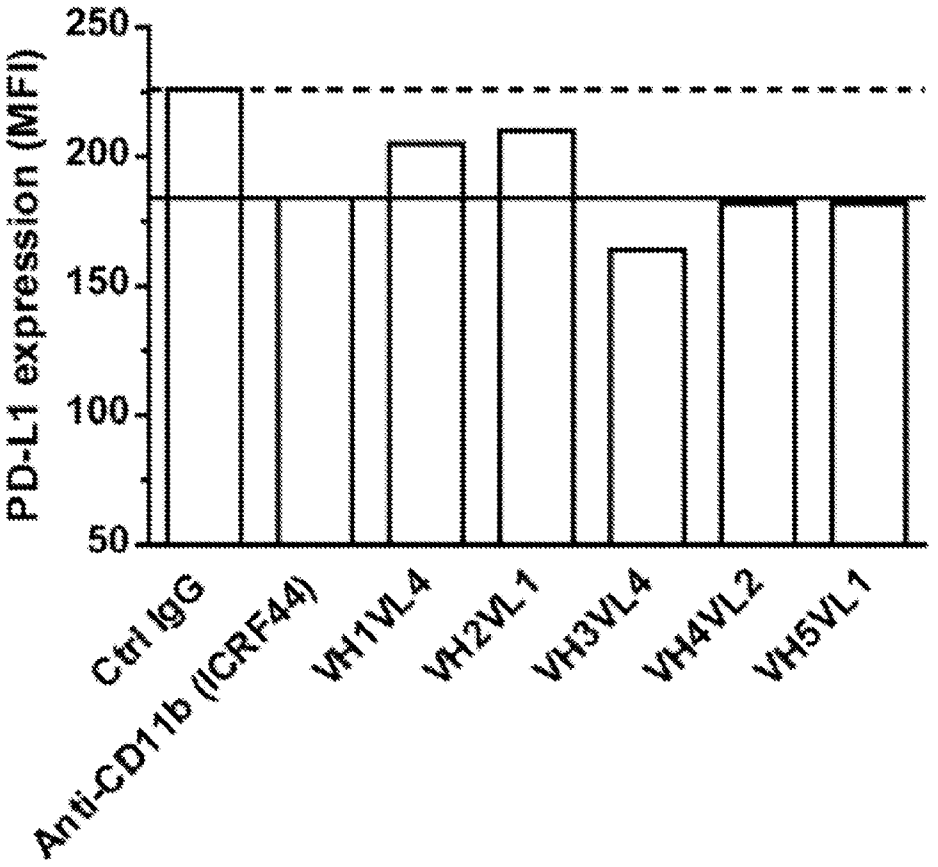
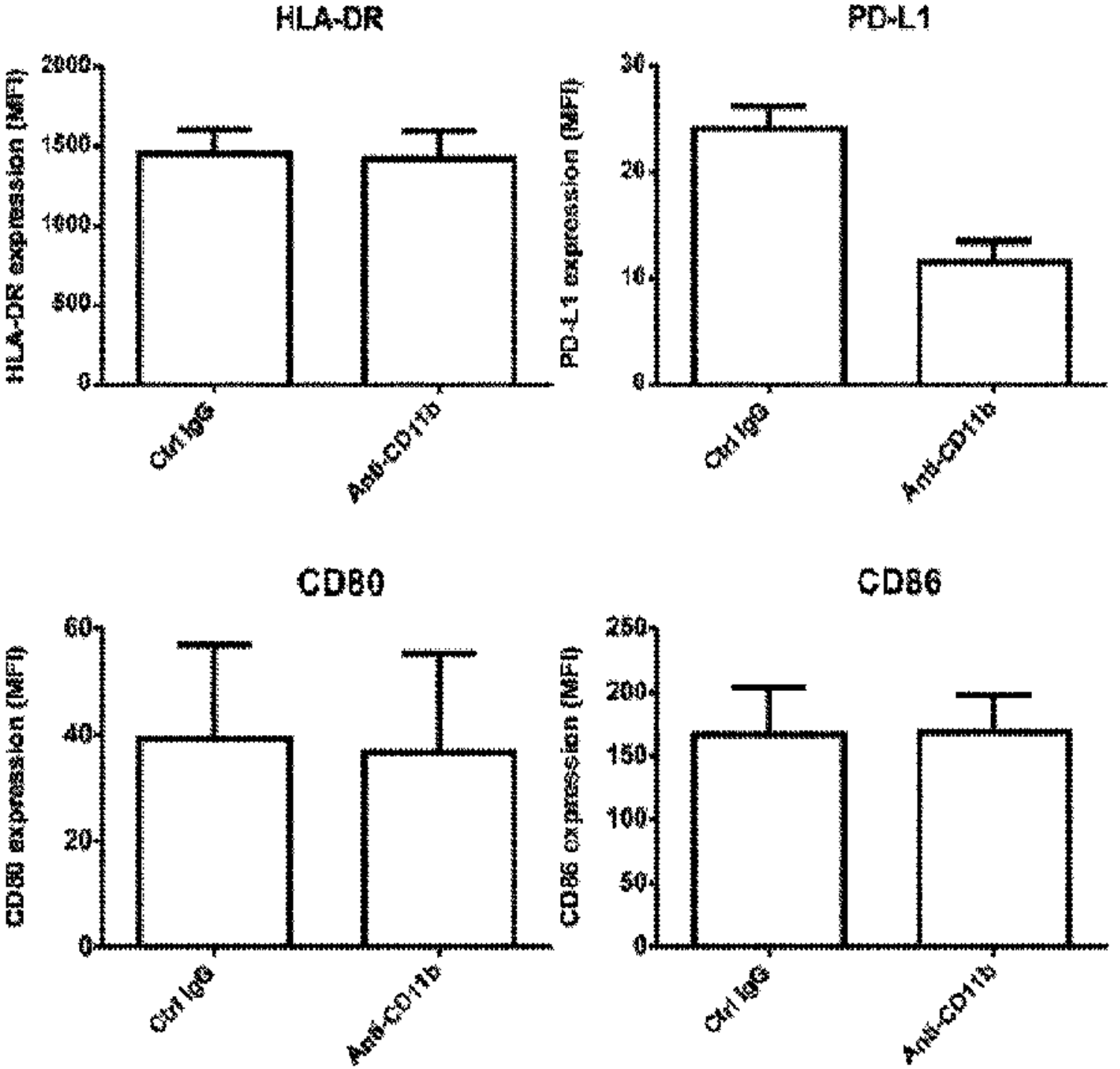

PD-L1 expression (MFI)
250
200
150
100
50
Ctrl IgG
Anti-CD11b (ICRF44)
VH1VL4
VH2VL1
VH3VL4
VH4VL2
VH5VL1

Fig. 18


HLA-DR
HLA-DR expression (MFI)
2000
1500
1000
500
0
Ctrl IgG
Anti-CD11b
PD-L1
PD-L1 expression (MFI)
30
20
10
0
Ctrl IgG
Anti-CD11b
CD80
CD80 expression (MFI)
60
40
20
0
Ctrl IgG
Anti-CD11b
CD86
CD86 expression (MFI)
250
200
150
100
50
0
Ctrl IgG
Anti-CD11b