EXOSOMES FOR IMMUNO-ONCOLOGY AND ANTI-INFLAMMATORY THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/723,267, filed August 27, 2018; and 62/611,140, filed December 28, 2017, each of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to compositions for interacting and modulating the human immune system, methods of making the compositions, and methods of using the compositions to treat cancer, GvHD, and autoimmune diseases.

BACKGROUND

[0003] Immunotherapy is the treatment of disease by inducing, enhancing, or suppressing the immune response. Immunotherapy can stimulate the patient's own immune system to attack cancer cells. Cancer immunotherapy usually has fewer side effects than traditional cancer therapies, such as chemotherapy and radiation therapy. Anti-inflammatory immunotherapy can down-regulate the patient's immune system for treating autoimmune diseases and graft-versus-host disease (GvHD). What is needed are improved methods for delivering immunomodulatory molecules to cells and tissues of the body.

SUMMARY

[0004] As drug delivery vehicles, extracellular vesicles offer many advantages over traditional drug delivery methods, especially for gene therapy. Systemic delivery of extracellular vesicles results in distribution of these lipid nanoparticles to various tissues. Studies have shown that extracellular vesicles can interact with various cells involved with the modulation of the human immune system. Extracellular vesicles that are selected, enriched, or engineered to deliver therapeutic molecules to activate, suppress, or influence the human immune system can be potent therapeutics for cancer and other immune system related diseases.

[0005] Provided herein are compositions comprising extracellular vesicles selected, enriched, or engineered with immunomodulating components that can up-regulate or down-regulate the human immune system, boosting the patient's immune system to fight cancer or

suppressing the patient's immune system to alleviate the symptoms of GvHD and autoimmune diseases.

[0006] Also provided are methods of producing and utilizing the extracellular vesicles for modulating the human immune system.

[0007] Accordingly, in a first aspect, provided herein is a composition, comprising: an extracellular vesicle comprising a cell membrane bounding an enclosed volume, the cell membrane having an interior surface and an exterior surface; and a first immunomodulating component associated with the cell membrane or enclosed within the enclosed volume.

[0008] In various embodiments, the first immunomodulating component is an inhibitor for a negative checkpoint regulator or an inhibitor for a binding partner of a negative checkpoint regulator. In some of these embodiments, the negative checkpoint regulator is selected from the group consisting of: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte-activated gene 3 (LAG-3), T-cell immunoglobulin mucin-containing protein 3 (TIM-3), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), adenosine A2a receptor (A2aR), killer cell immunoglobulin like receptor (KIR), indoleamine 2,3-dioxygenase (IDO), CD20, CD39, and CD73.

[0009] In various embodiments, the first immunomodulating component is an activator for a positive co-stimulatory molecule or an activator for a binding partner of a positive co-stimulatory molecule. In some embodiments, the positive co-stimulatory molecule is a TNF receptor superfamily member. In some of these embodiments, the TNF receptor superfamily member is selected from the group consisting of: CD120a, CD120b, CD18, OX40, CD40, Fas receptor, M68, CD27, CD30, 4-1BB, TRAILR1, TRAILR2, TRAILR3, TRAILR4, RANK, OCIF, TWEAK receptor, TACI, BAFF receptor, ATAR, CD271, CD269, AITR, TROY, CD358, TRAMP, and XEDAR. In some embodiments, the activator for a positive co-stimulatory molecule is a TNF superfamily member. In some of these embodiments, the TNF superfamily member is selected from the group consisting of: TNFα, TNF-C, OX40L, CD40L, FasL, LIGHT, TL1A, CD27L, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, and EDA-2. In certain embodiments, the TNF superfamily member is CD40L. In certain embodiments, the TNF superfamily member is CD27L. In certain embodiments, the TNF superfamily member is OX40L.

[0010] In some embodiments, the positive co-stimulatory molecule is a CD28-superfamily co-stimulatory molecule. In some of these embodiments, the CD28-superfamily co-stimulatory molecule is ICOS or CD28. In some embodiments, the activator for a positive co-stimulatory molecule is ICOSL, CD80, or CD86. In certain embodiments, the activator for a positive co-stimulatory molecule is CD80.

[0011] In some embodiments, the first immunomodulating component is a cytokine or a binding partner of a cytokine. In some embodiments, the cytokine is selected from the group consisting of: IL-2, IL-7, IL-10, IL-12, and IL-15. In certain embodiments, the cytokine is IL-7. In certain embodiment, the cytokine is IL-12. In certain embodiments, the cytokine is IL-15.

[0012] In some embodiments, the first immunomodulating component is a T-cell receptor (TCR), a T-cell co-receptor, a major histocompatibility complex (MHC), a human leukocyte antigen (HLA), or a derivative thereof.

[0013] In some embodiments, the first immunomodulating component is an activator of a T-cell receptor or co-receptor. In certain embodiments, the activator of a T-cell receptor or co-receptor is an activator of CD3, optionally an agonist antibody of CD3.

[0014] In some embodiments, the first immunomodulating component is a tumor antigen. In some embodiments, the tumor antigen is selected from the group consisting of: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand. In certain embodiments, the tumor antigen is derived from a reference genome sequence. In certain embodiments, the tumor antigen is derived from a genome sequence of a subject.

[0015] In some embodiments, the first immunomodulating component is an agonist or an antagonist of a selected target or activity.

[0016] In some embodiments, the first immunomodulating component is an antibody or an antigen-binding fragment.

[0017] In some embodiments, the first immunomodulating component is a polynucleotide. In some of these embodiments, the polynucleotide is selected from the group consisting of: an mRNA, a miRNA, an siRNA, an antisense RNA, an shRNA, a lncRNA, and a dsDNA.

[0018] In some embodiments, the first immunomodulating component is a protein, a peptide, a glycolipid, or a glycoprotein.

[0019] In some embodiments, the first immunomodulating component is expressed as a fusion protein displayed on the exterior surface of said extracellular vesicle. In some embodiments, the fusion protein comprises PTGFRN or a fragment or a variant thereof. In some embodiments, the sequence of the fusion protein is SEQ ID NO: 3.

[0020] In some embodiments, the extracellular vesicle is an exosome. In some other embodiments, the extracellular vesicle is a nanovesicle.

[0021] In certain embodiments, the composition further comprises a pharmaceutically-acceptable carrier.

[0022] In some embodiments, the extracellular vesicle additionally comprises a second immunomodulating component.

[0023] In various embodiments, the second immunomodulating component is an inhibitor for a negative checkpoint regulator or an inhibitor for a binding partner of a negative checkpoint regulator. In some of these embodiments, the negative checkpoint regulator is selected from the group consisting of: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte-activated gene 3 (LAG-3), T-cell immunoglobulin mucin-containing protein 3 (TIM-3), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), adenosine A2a receptor (A2aR), killer cell immunoglobulin like receptor (KIR), indoleamine 2,3-dioxygenase (IDO), CD20, CD39, and CD73.

[0024] In various embodiments, the second immunomodulating component is an activator for a positive co-stimulatory molecule or an activator for a binding partner of a positive co-stimulatory molecule. In some embodiments, the positive co-stimulatory molecule is a TNF receptor superfamily member. In some of these embodiments, the TNF receptor superfamily member is selected from the group consisting of: CD120a, CD120b, CD18, OX40, CD40, Fas receptor, M68, CD27, CD30, 4-1BB, TRAILR1, TRAILR2, TRAILR3, TRAILR4, RANK, OCIF, TWEAK receptor, TACI, BAFF receptor, ATAR, CD271, CD269, AITR,

TROY, CD358, TRAMP, and XEDAR. In some embodiments, the activator for a positive co-stimulatory molecule is a TNF superfamily member. In some of these embodiments, the TNF superfamily member is selected from the group consisting of: TNFα, TNF-C, OX40L, CD40L, FasL, LIGHT, TL1A, CD27L, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, and EDA-2. In certain embodiments, the TNF superfamily member is CD40L. In certain embodiments, the TNF superfamily member is CD27L. In certain embodiments, the TNF superfamily member is OX40L.

[0025] In some embodiments, the positive co-stimulatory molecule is a CD28-superfamily co-stimulatory molecule. In some of these embodiments, the CD28-superfamily co-stimulatory molecule is ICOS or CD28. In some embodiments, the activator for a positive co-stimulatory molecule is ICOSL, CD80, or CD86. In certain embodiments, the activator for a positive co-stimulatory molecule is CD80.

[0026] In some embodiments, the second immunomodulating component is a cytokine or a binding partner of a cytokine. In some embodiments, the cytokine is selected from the group consisting of: IL-2, IL-7, IL-10, IL-12, and IL-15. In certain embodiments, the cytokine is IL-7. In certain embodiment, the cytokine is IL-12. In certain embodiment, the cytokine is IL-15.

[0027] In some embodiments, the second immunomodulating component is a T-cell receptor (TCR), a T-cell co-receptor, a major histocompatibility complex (MHC), a human leukocyte antigen (HLA), or a derivative thereof.

[0028] In some embodiments, the second immunomodulating component is an activator of a T-cell receptor or co-receptor. In certain embodiments, the activator of a T-cell receptor or co-receptor is an activator of CD3, optionally an agonist antibody of CD3.

[0029] In some embodiments, the second immunomodulating component is a tumor antigen. In some embodiments, the tumor antigen is selected from the group consisting of: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand. In certain

embodiments, the tumor antigen is derived from a reference genome sequence. In certain embodiments, the tumor antigen is derived from a genome sequence of a subject.

[0030] In some embodiments, the second immunomodulating component is an agonist or an antagonist of a selected target or activity.

[0031] In some embodiments, the second immunomodulating component is an antibody or an antigen-binding fragment.

[0032] In some embodiments, the second immunomodulating component is a polynucleotide. In some of these embodiments, the polynucleotide is selected from the group consisting of: an mRNA, a miRNA, an siRNA, an antisense RNA, an shRNA, a lncRNA, and a dsDNA.

[0033] In some embodiments, the second immunomodulating component is a protein, a peptide, a glycolipid, or a glycoprotein.

[0034] In some embodiments, the second immunomodulating component is expressed as a fusion protein displayed on the exterior surface of said extracellular vesicle. In some embodiments, the fusion protein comprises PTGFRN or a fragment or a variant thereof. In some embodiments, the sequence of said fusion protein is SEQ ID NO: 3.

[0035] In some embodiments, the second immunomodulating component is different from said first immunomodulating component.

[0036] In some embodiments, the extracellular vesicle additionally comprises a third immunomodulating component. In some embodiments, the third immunomodulating component is different from said first and second immunomodulating components.

[0037] In another aspect, provided herein is a method of producing the composition. In some embodiments, the method comprises modifying a producer cell with the first, second, and/or third immunomodulating components; obtaining the extracellular vesicle from the producer cell; and optionally isolating the obtained extracellular vesicles. In some other embodiments the method comprises obtaining the extracellular vesicle from a producer cell; isolating the obtained extracellular vesicles; and modifying the isolated extracellular vesicle with the first, second, and/or third immunomodulating components. In certain embodiments, the method further comprises formulating the isolated extracellular vesicles into a pharmaceutical composition.

[0038] In another aspect, provided herein is a method of treating cancer in a subject. The method comprises administering to the subject a therapeutically effective amount of the composition, wherein the composition is capable of up-regulating an immune response in the subject, thereby enhancing the tumor targeting of the subject's immune system.

[0039] In another aspect, provided herein is a method of treating graft-versus-host disease (GvHD) in a subject. The method comprises administering to the subject a therapeutically effective amount of the composition, wherein the composition is capable of down-regulating an immune response in the subject, thereby alleviating the symptoms of GvHD.

[0040] In another aspect, provided herein is a method of treating an autoimmune disease in a subject. The method comprises administering to the subject a therapeutically effective amount of the composition, wherein the composition is capable of down-regulating an immune response in the subject, thereby suppressing the immune activity of the subject.

[0041] In another aspect, provided herein is a method of treating or preventing cancer in a subject comprising administering to the subject a therapeutically effective amount of the composition comprising a tumor antigen, wherein the composition is capable of potentiating an immune response to the tumor antigen, thereby enhancing the immune response of the subject to cancer.

[0042] In some embodiments, the tumor antigen is selected from the group consisting of: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand.

[0043] In certain embodiments, the tumor antigen is derived from a reference genome sequence. In certain embodiments, the tumor antigen is derived from a genome sequence of a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] **FIGURES 1A and 1B** show a time course of mice injected with radio-labeled exosomes. **FIGURE 1A** shows the intravenous route of administration. **FIGURE 1B** shows the intraperitoneal route of administration.

[0045] **FIGURE 2** is a quantitation of exosome distribution in different mouse tissues after intravenous and intraperitoneal administration of radiolabeled exosomes.

[0046] **FIGURES 3A and 3B** show the effects of B-cell activation in peripheral blood mononuclear cells (PBMCs) from two human donors after incubation with CD40L-expressing exosomes.

[0047] **FIGURES 4A and 4B** show the effects of B-cell activation of purified B-cells from two human donors after incubation with CD40L-expressing exosomes.

[0048] **FIGURE 5A** is a schematic of a CD40 reporter cell line. **FIGURE 5B** shows the concentration-dependent activation of a CD40 reporter cell line treated with an anti-CD40 agonistic antibody or recombinant human CD40L. **FIGURE 5C** shows the effects of CD40L-expressing exosomes on a CD40 reporter cell line.

[0049] **FIGURES 6A and 6B** show the effects of T-cell activation in peripheral blood mononuclear cells (PBMCs) with CD80-expressing exosomes. **FIGURE 6A** shows the effect of CD80-expressing exosomes on the number of $CD8^+$ T-cells. **FIGURE 6B** shows the effect of CD80-expressing exosomes on the number of $CD4^+$ T-cells.

[0050] **FIGURES 7A and 7B** show the effects of CD80-expressing exosomes on IFNγ expression in human PBMCs.

[0051] **FIGURES 8A and 8B** show the effects of CD27L-expressing exosomes on IFNγ expression in human PBMCs from two donors.

[0052] **FIGURES 9A and 9B** show the effects of CD27L-expressing exosomes on IL-2 expression in human PBMCs from two donors.

[0053] **FIGURES 10A and 10B** show the effects of OX40L-expressing exosomes on IFNγ expression in human PBMCs from two donors.

[0054] **FIGURES 11A and 11B** show the effects of OX40L-expressing exosomes on IL-2 expression in human PBMCs from two donors.

[0055] **FIGURE 12A** is a schematic of an OX40 reporter cell line. **FIGURE 12B** shows the concentration-dependent activation of an OX40 reporter cell line treated with an anti-OX40 agonistic antibody or recombinant human OX40L. **FIGURE 12C** shows the effects of OX40L-expressing exosomes on an OX40 reporter cell line.

[0056] **FIGURES 13A and 13B** show the effects of IL-7-expressing exosomes in combination with an anti-CD3 antibody on IFNγ expression in human PBMCs.

[0057] **FIGURE 14A** is a schematic of an IL-7 receptor reporter cell line. **FIGURE 14B** shows the concentration-dependent activation of an IL-7 receptor reporter cell line treated with recombinant human IL-7. **FIGURE 14C** shows the effects of IL-7-expressing exosomes on an IL-7 receptor reporter cell line.

[0058] **FIGURES 15A and 15B** show the effects of IL-7-expressing exosomes on T-cell proliferation in mice *in vivo* as measured by EdU incorporation. **FIGURE 15A** shows the effects of IL-7-expressing exosomes on CD8+ T-cell. **FIGURE 15B** shows the effects of IL-7-expressing exosomes on memory CD8+ T-cell.

[0059] **FIGURES 16A and 16B** show the effects of IL-7-expressing exosomes on T-cell proliferation in mice *in vivo* as measured by CD71 positivity. **FIGURE 16A** shows the effects of IL-7-expressing exosomes on CD8+ T-cell. **FIGURE 16B** shows the effects of IL-7-expressing exosomes on memory CD8+ T-cell.

[0060] **FIGURE 17A** shows a schematic of a PTGFRN/IL-7 fusion protein expressed at high density on the surface of an exosome, and variants of the fusion protein. **FIGURE 17B** is the sequence of the optimized PTGFRN/IL-7 fusion protein.

[0061] **FIGURE 18A** is a Western blot showing the relative expression of different IL-7 fusion proteins on the surface of purified exosomes. **FIGURE 18B** shows the effects of IL-7-expressing exosomes on IL-7 receptor down-regulation as a model of IL-7-mediated T-cell activation.

[0062] **FIGURE 19A** shows the effects of anti-CD3 scFv exosomes on T-cell activation in PBMCs. **FIGURE 19B** shows the effects of anti-CD3 scFv exosomes on B-cell activation in PBMCs.

[0063] **FIGURE 20A** shows the effects of anti-CD3 scFab exosomes on T-cell activation in PBMCs. **FIGURE 20B** shows the effects of anti-CD3 scFab exosomes on B-cell activation in PBMCs.

[0064] **FIGURE 21A** is a histogram showing the extent of T-cell activation after treatment with anti-CD3 scFv exosomes. **FIGURE 21B** is a histogram showing the extent of B-cell activation after treatment with anti-CD3 scFv exosomes.

[0065] **FIGURE 22A** shows the effects of anti-CD3 scFab exosomes on T-cell activation in a plate-coated activation assay compared to soluble anti-CD3 antibody or plate-coated anti-CD3 antibody. **FIGURE 22B** is a bar chart quantitating the results of a separate experiment carried out as in Figure 22A.

[0066] **FIGURE 23A** shows a schematic of a full-length PTGFRN/IL-12 fusion protein. **FIGURE 23B** shows a schematic of a shortened PTGFRN/IL-12 fusion protein.

[0067] **FIGURE 24A** shows the effects of recombinant human IL-12 or exosomes overexpressing either short or full-length PTGFRN-IL-12 inducing IFNγ in human PBMCs. **FIGURE 24B** is a table summarizing the potency of recombinant IL-12 and IL-12-containing exosomes.

[0068] **FIGURE 25** shows the effects of recombinant IL-12 and IL-12-PTGFRN exosomes on reducing tumor growth in a murine model of melanoma.

[0069] **FIGURE 26A** shows the tumor growth curves for each of the tumor-bearing mice shown in Figure 25 treated with PBS. **FIGURE 26B** shows the tumor growth curves for each of the tumor-bearing mice shown in Figure 25 treated with recombinant IL-12. **FIGURE 26C** shows the tumor growth curves for each of the tumor-bearing mice shown in Figure 25 treated with IL-12-PTGFRN exosomes.

[0070] **FIGURE 27** shows images of all B16F10 tumor-bearing mice in the efficacy study shown in Figure 25.

[0071] **FIGURE 28** shows the survival curves of the B16F10 tumor-bearing mice shown in Figure 25.

[0072] **FIGURE 29A** shows the levels of IFNγ gene expression in tumors of mice treated with PBS, rIL-12 or IL-12-PTGFRN exosomes. **FIGURE 29B** shows the levels of CXCL9 gene expression in tumors of mice treated with PBS, rIL-12 or IL-12-PTGFRN exosomes. **FIGURE 29C** shows the levels of CXCL10 gene expression in tumors of mice treated with PBS, rIL-12 or IL-12-PTGFRN exosomes. **FIGURE 29D** shows the levels of TGFβ gene expression in tumors of mice treated with PBS, rIL-12 or IL-12-PTGFRN exosomes.

[0073] **FIGURE 30** shows the percent of IFNγ-positive CD8+ splenic T-cells in tumor-bearing mice treated with PBS, rIL-12 or IL-12-PTGFRN exosomes.

[0074] **FIGURE 31A** shows a schematic of a full-length PTGFRN fused to an IFNγ monomer. **FIGURE 31B** shows a schematic of a full-length PTGFRN fused to an IFNγ tandem dimer.

[0075] **FIGURE 32** shows the PAGE analysis results of purified human and mouse monomeric (m) and tandem dimer (td) PTGFRN IFNγ exosomes.

[0076] **FIGURE 33** shows the monocyte PD-L1 expression after addition of native exosomes (WT), monomeric IFNγ PTGFRN exosomes (m-IFNγ-PTGFRN), and tandem dimer IFNγ PTGFRN exosomes (td-IFNγ-PTGFRN) respectively. LPS-induced PD-L1 activation was used as positive control.

[0077] **FIGURE 34** shows the schematics of 15/IL-15Rα fusion proteins fused to the transmembrane domain of PDGFR.

[0078] **FIGURE 35** shows the NK cell activation measured by the percentage of CD69 positive NK cells after the addition of pDisplay IL-15 exosomes.

[0079] **FIGURE 36A** shows the schematics of IL-15 fused to full-length PTGFRN and IL-15 N72D fused to full-length PTGFRN. **FIGURE 36B** shows the Western blotting of IL-15 fused to full-length PTGFRN and IL-15 N72D fused to full-length PTGFRN.

[0080] **FIGURE 37** shows NK cell activation measured by the percentage of CD69 positive NK cells after the addition of IL-15 fused to full-length PTGFRN and IL-15 N72D fused to full-length PTGFRN.

[0081] **FIGURE 38** shows the schematics of anti-CD3 antibody fragment fused to the PDGFR transmembrane region (exoCD3-PD), a full-length PTGFRN (exoCD3-long), and a PTGFRN fragment (exoCD3-short) respectively.

[0082] **FIGURE 39** shows the results of bio-layer interferometry (BLI) after addition of native exosomes (WT), exosomes with anti-CD3 antibody fragment fused to the PDGFR transmembrane region (pDisplay), exosomes with anti-CD3 antibody fragment fused to a full-length PTGFRN (FL PTGFRN), and exosomes with anti-CD3 antibody fragment fused to a PTGFRN fragment (Short PTGFRN), respectively.

[0083] **FIGURE 40A** shows CD4+ T cell activation measured by the percentage of CD69 positive CD4+ T cells after the addition of anti-CD3 antibody fragment. **FIGURE 40B** shows CD4+ T cell activation measured by the percentage of CD69 positive CD4+ T cells

after the addition of native exosomes (exoNative) and exosomes with anti-CD3 antibody fragment fused to a PTGFRN fragment (exoCD3-Short), respectively.

[0084] **FIGURE 41** shows the schematics of CD40L-GFP PTGFRN fusion proteins and the EC_{50} for each construct in the B-cell activation assay measured by CD69 positivity on B-cells.

[0085] **FIGURE 42A** shows B cell activation measured by the percentage of CD69 positive B cells after the addition of native exosomes, exosomes with trimeric CD40L-PTGFRN constructs pCB-527, and exosomes with trimeric CD40L-PTGFRN constructs pCB-766, respectively. **FIGURE 42B** shows B cell activation measured by the percentage of CD69 positive B cells after the addition of exosomes with trimeric CD40L-PTGFRN constructs pCB-527 and pCB-766 respectively compared to concentration-matched CD40L.

[0086] **FIGURE 43A** shows B cell activation in Donor 1 measured by the percentage of CD69 positive B cells after the addition of exosomes with trimeric CD40L-PTGFRN constructs pCB-527. **FIGURE 43B** shows B cell activation in Donor 2 measured by the percentage of CD69 positive B cells after the addition of exosomes with trimeric CD40L-PTGFRN constructs pCB-527.

[0087] **FIGURE 44A** shows the FACS analysis of native exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibodies against IL-12 and CD40L. **FIGURE 44B** shows the FACS analysis of native exosomes isolated with anti-CD40L-decorated beads and labeled fluorescent antibodies with against CD81 and CD40L.

[0088] **FIGURE 45A** shows the FACS analysis of PTGFRN-CD40L/IL-12 double engineered exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibody against CD81. **FIGURE 45B** shows the FACS analysis of PTGFRN-CD40L/IL-12 double engineered exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibodies against IL-12 and CD40L.

[0089] **FIGURE 46A** shows the FACS analysis of PTGFRN-CD40L/IL-12 double engineered exosomes isolated with anti-IL-12-decorated beads and labeled with fluorescent antibodies against IL-12 and CD40L. **FIGURE 46B** shows the FACS analysis of PTGFRN-CD40L/IL-12 double engineered exosomes isolated with anti-IL-12-decorated beads and labeled with fluorescent antibody against CD81.

[0090] **FIGURE 47A** shows the IFNγ response in Donor 1 human PBMCs after addition of recombinant IL-12, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-IL-12 exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively. **FIGURE 47B** shows the IFNγ response in Donor 2 human PBMCs after addition of recombinant IL-12, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-IL-12 exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively.

[0091] **FIGURE 48** shows EC_{50} of the IFNγ response in Donor 1 and Donor 2 human PBMCs after addition of recombinant IL-12, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-IL-12 exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively.

[0092] **FIGURE 49A** shows the B cell activation in Donor 1 human PBMCs after addition of recombinant CD40L, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-CD40L exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively. **FIGURE 49B** shows the B cell activation in Donor 2 human PBMCs after addition of recombinant CD40L, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-CD40L exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively.

[0093] **FIGURE 50** shows EC_{50} of the IFNγ response in Donor 1 and Donor 2 human PBMCs after addition of recombinant CD40L, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-CD40L exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, and a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes, respectively.

[0094] **FIGURE 51A** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-IL-12-decorated beads and labeled with fluorescent antibodies against IL-12 and CD40L. **FIGURE 51B** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-IL-12-decorated beads and labeled with fluorescent antibodies against IL-12 and FLT3L. **FIGURE 51C** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-IL-12-decorated beads and labeled with fluorescent antibodies against CD40L and FLT3L.

[0095] **FIGURE 52A** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibodies against IL-12 and CD40L. **FIGURE 52B** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibodies against IL-12 and FLT3L. **FIGURE 52C** shows the FACS analysis of PTGFRN-CD40L/IL-12/FLT3L triple engineered exosomes isolated with anti-CD40L-decorated beads and labeled with fluorescent antibodies against CD40L and FLT3L.

DETAILED DESCRIPTION

[0096] Disclosed herein are extracellular vesicles capable of modulating human immune system. Also provided are methods for producing the extracellular vesicles, and methods of using these extracellular vesicles to treat cancer and other immune system related diseases.

[0097] Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0098] 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 can 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.

[0099] 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, representative illustrative methods and materials are now described.

[00100] All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[00101] It is noted that, as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims can be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a negative limitation.

[00102] As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which can be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[00103] In further describing the subject invention, subject systems for use in practicing the subject methods will be discussed in greater detail, followed by a review of associated methods.

[00104] As used herein, the term "**extracellular vesicle**" refers to a cell-derived vesicle comprising a membrane that encloses an internal space. Extracellular vesicles comprise all membrane-bound vesicles that have a smaller diameter than the cell from which they are derived. Generally extracellular vesicles range in diameter from 20 nm to 1000 nm, and can comprise various macromolecular cargo either within the internal space, displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. The cargo can comprise nucleic acids, proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived from cells by direct or indirect manipulation (*e.g.*, by serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (*e.g.*, by direct plasma membrane budding or fusion of the late

endosome with the plasma membrane). Extracellular vesicles can be derived from a living or dead organism, explanted tissues or organs, and/or cultured cells.

[00105] As used herein the term "**exosome**" refers to a cell-derived small (between 20-300 nm in diameter, more preferably 40-200 nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane. The exosome is a species of extracellular vesicle. The exosome comprises lipid or fatty acid and polypeptide and optionally comprises a payload (*e.g.*, a therapeutic agent), a receiver (*e.g.*, a targeting moiety), a polynucleotide (*e.g.*, a nucleic acid, RNA, or DNA), a sugar (*e.g.*, a simple sugar, polysaccharide, or glycan) or other molecules. The exosome can be derived from a producer cell, and isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof.

[00106] As used herein, the term "**nanovesicle**" refers to a cell-derived small (between 20-250 nm in diameter, more preferably 30-150nm in diameter) vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct or indirect manipulation such that the nanovesicle would not be produced by the producer cell without the manipulation. Appropriate manipulations of the producer cell include but are not limited to serial extrusion, treatment with alkaline solutions, sonication, or combinations thereof. The production of nanovesicles can, in some instances, result in the destruction of the producer cell. Preferably, populations of nanovesicles are substantially free of vesicles that are derived from producer cells by way of direct budding from the plasma membrane or fusion of the late endosome with the plasma membrane. The nanovesicle is a species of extracellular vesicle. The nanovesicle comprises lipid or fatty acid and polypeptide, and optionally comprises a payload (*e.g.*, a therapeutic agent), a receiver (*e.g.*, a targeting moiety), a polynucleotide (*e.g.*, a nucleic acid, RNA, or DNA), a sugar (*e.g.*, a simple sugar, polysaccharide, or glycan) or other molecules. The nanovesicle, once it is derived from a producer cell according to the manipulation, can be isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof.

[00107] The term "**extracellular vesicle delivery**" or "**delivery of extracellular vesicles**" refers to the administration and localization of extracellular vesicles to target tissues, cells, and/or organs of the subject. In some embodiments, the immunomodulating component can be delivered to the cytoplasm of a target cell. In other embodiments, the immunomodulating

component is delivered to the membrane of the target cell. In some embodiments, the membrane of the extracellular vesicle fuses with a membrane of a target cell.

[00108] As used herein, the term "**producer cell**" refers to any cell from which an extracellular vesicle can be isolated. A producer cell is a cell which serves as a source for the extracellular vesicle. A producer cell can share a protein, lipid, sugar, or nucleic acid component with the extracellular vesicle. In some embodiments, the producer cell is a modified or synthetic cell. In some embodiments, the producer cell is a cultured or isolated cell. In certain embodiments, the producer cell is a cell line. In certain other embodiments, the producer cell is a primary cell. In some particular embodiments, the producer cell is an immune cell.

[00109] "**Membrane**" as used herein is a boundary layer that separates an interior space from an exterior space comprising one or more biological compounds, typically lipids, and optionally polypeptides and/or carbohydrates. In some embodiments, the membrane comprises lipids and fatty acids. In some embodiments, the membrane comprises phospholipids, glycolipids, fatty acids, sphingolipids, phosphoglycerides, sterols, cholesterols, and phosphatidylserines. In some of these embodiments, the membrane further comprises one or more polypeptide and/or one or more polysaccharide, such as glycan. The extracellular vesicle comprises a membrane as defined herein.

[00110] As used herein, the term "**immunomodulating component**" refers to a therapeutic agent that acts on a target (*e.g.*, a target cell) that is contacted with the extracellular vesicle, and regulates the immune system. The immunomodulating component that can be introduced into an extracellular vesicle and/or a producer cell include therapeutic agents such as, modulators of checkpoint inhibitors or ligands of checkpoint inhibitors, surface antigens and derivatives thereof, cytokines and derivatives thereof. The immunomodulating component can also include an agonist, an antagonist, an antibody, and an antigen-binding fragment, or a polynucleotide, such as siRNA, miRNA, lncRNA, and DNA.

[00111] The term "**receiver**" refers to a molecule that directs the extracellular vesicle to a target and/or promotes the interaction of extracellular vesicle with the target in the subject. In some embodiments, the receiver is a polypeptide. In some embodiments, the receiver is capable of increasing the concentration of the immunomodulating component in the tissue of the subject. Examples of receivers include, but are not limited to, examples listed in Table 3.

[00112] The term "**target**" refers to, a cell, a pathogen, a metabolite, a polypeptide complex or any molecule or structure that resides in a tissue or circulates in the circulatory system or lymphatic system of the subject, such as an immune cell or a cancer cell. Examples of targets include, but are not limited to, examples listed in Table 4.

[00113] A "**therapeutic agent**" or "**therapeutic molecule**" includes a compound or molecule that, when present in an effective amount, produces a desired therapeutic effect, pharmacologic and/or physiologic effect on a subject in need thereof. It includes any compound, *e.g.*, a small molecule drug, or a biologic (*e.g.*, a polypeptide drug or a nucleic acid drug) that when administered to a subject has a measurable or conveyable effect on the subject, *e.g.*, it alleviates or decreases a symptom of a disease, disorder or condition.

[00114] As used herein, the term "**antibody**" encompasses an immunoglobulin whether natural or partly or wholly synthetically produced, and fragments thereof. The term also covers any protein having a binding domain that is homologous to an immunoglobulin binding domain. "Antibody" further includes a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. Use of the term antibody is meant to include whole antibodies, polyclonal, monoclonal and recombinant antibodies, fragments thereof, and further includes single-chain antibodies, humanized antibodies, murine antibodies, chimeric, mouse-human, mouse-primate, primate-human monoclonal antibodies, anti-idiotype antibodies, antibody fragments, such as, *e.g.*, scFv, $(scFv)_2$, Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, and Fd fragments, diabodies, and antibody-related polypeptides. Antibody includes bispecific antibodies and multispecific antibodies so long as they exhibit the desired biological activity or function.

[00115] The term "**antigen-binding fragment**" used herein refers to fragments of an intact immunoglobulin, and any part of a polypeptide including antigen binding regions having the ability to specifically bind to the antigen. For example, the antigen-binding fragment can be a $F(ab')_2$ fragment, a Fab' fragment, a Fab fragment, a Fv fragment, or a scFv fragment, but is not limited thereto. A Fab fragment has one antigen binding site and contains the variable regions of a light chain and a heavy chain, the constant region of the light chain, and the first constant region CH1 of the heavy chain. A Fab' fragment differs from a Fab fragment in that the Fab' fragment additionally includes the hinge region of the heavy chain, including at least one cysteine residue at the C-terminal of the heavy chain CH1 region. The $F(ab')_2$ fragment is produced whereby cysteine residues of the Fab' fragment are joined by a disulfide bond at the

hinge region. An Fv fragment is the minimal antibody fragment having only heavy chain variable regions and light chain variable regions, and a recombinant technique for producing the Fv fragment is well-known in the art. Two-chain Fv fragments can have a structure in which heavy chain variable regions are linked to light chain variable regions by a non-covalent bond. Single-chain Fv (scFv) fragments generally can have a dimer structure as in the two-chain Fv fragments in which heavy chain variable regions are covalently bound to light chain variable regions *via* a peptide linker or heavy and light chain variable regions are directly linked to each other at the C-terminal thereof. The antigen-binding fragment can be obtained using a protease (for example, a whole antibody is digested with papain to obtain Fab fragments, and is digested with pepsin to obtain $F(ab')_2$ fragments), and can be prepared by a genetic recombinant technique. A dAb fragment consists of a VH domain. Single-chain antibody molecules can comprise a polymer with a number of individual molecules, for example, dimer, trimer or other polymers.

[00116] The phrase "**nucleic acid molecule**" refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases. It includes chromosomal DNA and self-replicating plasmids, vectors, mRNA, tRNA, siRNA, miRNA, *etc.* The nucleic acid molecule can be recombinant and exogenous polypeptides can be expressed when the nucleic acid is introduced into a cell.

[00117] The term "**agonist**" refers to a molecule that binds to a receptor and activates the receptor to produce a biological response. Receptors can be activated by either an endogenous or an exogenous agonist. Non-limiting examples of endogenous agonist include hormones and neurotransmitters. Non-limiting examples of exogenous agonist include drugs. The agonist can be a full, partial, or inverse agonist.

[00118] The term "**antagonist**" refers to a molecule that blocks or dampens an agonist mediated response rather than provoking a biological response itself upon bind to a receptor. Many antagonists achieve their potency by competing with endogenous ligands or substrates at structurally defined binding sites on the receptors. Non-limiting examples of antagonists include alpha blockers, beta-blocker, and calcium channel blockers. The antagonist can be a competitive, non-competitive, or uncompetitive antagonist.

[00119] As used herein the term "**a fragment**" of a protein refers to a protein that is N- and/or C-terminally deleted in comparison to the naturally occurring protein. Preferably, a fragment of PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, or ATP

transporter retains the ability to be specifically targeted to exosomes. Such a fragment is also referred to as "**functional fragment**". Whether a fragment is a functional fragment in that sense can be assessed by any art known methods to determine the protein content of exosomes including Western Blots, FACS analysis and fusions of the fragments with autofluorescent proteins like, *e.g.* GFP. In a particular embodiment the fragment of PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, ATP transporter retains at least 50%, 60%, 70%, 80%, 90% or 100% of the ability of the naturally occurring PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, or ATP transporter to be specifically targeted to exosomes.

[00120] As used herein the term "**variant**" of a protein refers to a protein that shares a certain amino acid sequence identity with another protein upon alignment by a method known in the art. A variant of a protein can include a substitution, insertion, deletion, frameshift or rearrangement in another protein. In a particular embodiment, the variant is a variant having at least 70% identity to PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, ATP transporter or a fragment of PTGFRN, BSG, IGSF2, IGSF3, IGSF8, ITGB1, ITGA4, SLC3A2, or ATP transporter. In some embodiments variants or variants of fragments of PTGFRN share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with PTGFRN according to SEQ ID NO: 1 or with a functional fragment thereof. In some embodiments variants or variants of fragments of BSG share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with BSG according to SEQ ID NO: 9 or with a functional fragment thereof. In some embodiments variants or variants of fragments of IGSF2 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with IGSF2 according to SEQ ID NO: 34 or with a functional fragment thereof. In some embodiments variants or variants of fragments of IGSF3 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with IGSF3 according to SEQ ID NO: 20 or with a functional fragment thereof. In some embodiments variants or variants of fragments of IGSF8 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with IGSF8 according to SEQ ID NO: 14 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ITGB1 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ITGB1 according to SEQ ID NO: 21 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ITGA4 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ITGA4 according to SEQ ID NO: 22 or with a functional fragment thereof. In some embodiments variants or variants of fragments of SLC3A2 share at least 70%, 80%, 85%,

90%, 95% or 99% sequence identity with SLC3A2 according to SEQ ID NO: 23 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP1A1 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP1A1 according to SEQ ID NO: 24 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP1A2 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP1A2 according to SEQ ID NO: 25 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP1A3 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP1A3 according to SEQ ID NO: 26 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP1A4 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP1A4 according to SEQ ID NO: 27 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP1B3 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP1B3 according to SEQ ID NO: 28 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP2B1 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP2B1 according to SEQ ID NO: 29 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP2B2 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP2B2 according to SEQ ID NO: 30 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP2B3 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP2B3 according to SEQ ID NO: 31 or with a functional fragment thereof. In some embodiments variants or variants of fragments of ATP2B4 share at least 70%, 80%, 85%, 90%, 95% or 99% sequence identity with ATP2B4 according to SEQ ID NO: 32 or with a functional fragment thereof. In each of above cases, it is preferred that the variant or variant of a fragment retains the ability to be specifically targeted to exosomes.

[00121] Methods of alignment of sequences for comparison are well-known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, Adv. Appl. Math. 2: 482 (1981); Needleman and Wunsch, J. Mol. Bio. 48: 443 (1970); Pearson and Lipman, Methods in Mol. Biol. 24: 307-31 (1988); Higgins and Sharp, Gene 73: 15 237-44 (1988); Higgins and Sharp, CABIOS 5: 151-3 (1989) Corpet et al., Nuc. Acids Res. 16: 10881-90 (1988); Huang et al., Comp. Appl. BioSci. 8: 155-65 (1992); and Pearson et al., Meth. Mol. Biol. 24: 307-31 (1994). The NCBI Basic Local Alignment Search Tool (BLAST) [Altschul 20 et al., J. Mol. Biol. 215: 403-10 (1990) J is available from several

sources, including the National Center for Biological Information (NBCl, Bethesda, Md.) and on the Internet, for use in connection with the sequence analysis programs blastp, blasm, blastx, tblastn and tblastx. BLAST and a description of how to determine sequence identify using the program can be accessed at the official website of NCBI (National Center for Biotechnology Information) under NIH (National Institute of Health).

[00122] Recitation of any protein provided herein encompasses a functional variant of the protein. The term "**functional variant**" of a protein refers to a variant of the protein that retains the ability to be specifically targeted to exosomes.

[00123] As used herein, the term "**pharmaceutical composition**" refers to one or more of the compounds described herein, such as, *e.g.*, an extracellular vesicle mixed or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically-acceptable carriers and excipients. One purpose of a pharmaceutical composition is to facilitate administration of preparations of extracellular vesicles to a subject. The term "**pharmaceutically-acceptable**" and grammatical variations thereof, refers to compositions, carriers, diluents and reagents capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that prohibits administration of the composition. The term "excipient" or "carrier" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. The term "**pharmaceutically-acceptable carrier**" or "**pharmaceutically-acceptable excipient**" encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans, as well as any carrier or diluent that does not cause significant irritation to a subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[00124] As used herein, the terms "**isolate**," "**isolated**," and "**isolating**" or "**purify**," "**purified**," and "**purifying**" as well as "**extracted**" and "**extracting**" are used interchangeably and refer to the state of a preparation (*e.g.*, a plurality of known or unknown amount and/or concentration) of desired extracellular vesicles, that have undergone one or more processes of purification, *e.g.*, a selection or an enrichment of the desired extracellular vesicle preparation. In some embodiments, isolating or purifying as used herein is the process of removing, partially removing (*e.g.* a fraction) of the extracellular vesicles from a sample

containing producer cells. In some embodiments, an isolated extracellular vesicle composition has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In other embodiments, an isolated extracellular vesicle composition has an amount and/or concentration of desired extracellular vesicles at or above an acceptable amount and/or concentration. In other embodiments, the isolated extracellular vesicle composition is enriched as compared to the starting material (*e.g.* producer cell preparations) from which the composition is obtained. This enrichment can be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material. In some embodiments, isolated extracellular vesicle preparations are substantially free of residual biological products. In some embodiments, the isolated extracellular vesicle preparations are 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contaminating biological matter. Residual biological products can include abiotic materials (including chemicals) or unwanted nucleic acids, proteins, lipids, or metabolites. Substantially free of residual biological products can also mean that the extracellular vesicle composition contains no detectable producer cells and that only extracellular vesicles are detectable.

[00125] The terms "**administration**," "**administering**" and variants thereof refer to introducing a composition, such as an extracellular vesicle, or agent into a subject and includes concurrent and sequential introduction of a composition or agent. The introduction of a composition or agent into a subject is by any suitable route, including orally, pulmonarily, intranasally, parenterally (intravenously, intra-arterially, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, intrathecally, intratumorally, periocularly or topically. Administration includes self-administration and the administration by another. A suitable route of administration allows the composition or the agent to perform its intended function. For example, if a suitable route is intravenous, the composition is administered by introducing the composition or agent into a vein of the subject.

[00126] As used herein, the term "**modulate**," "**modulating**", "**modify**," and/or "**modulator**" generally refers to the ability to alter, by increase or decrease, *e.g.*, directly or indirectly promoting/stimulating/up-regulating or interfering with/inhibiting/down-regulating a specific concentration, level, expression, function or behavior, such as, *e.g.*, to act as an

antagonist or agonist. In some instances a modulator can increase and/or decrease a certain concentration, level, activity or function relative to a control, or relative to the average level of activity that would generally be expected or relative to a control level of activity.

[00127] The term "**sufficient amount**" means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to modulate a condition in the subject.

[00128] The term "**therapeutically effective amount**" is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

[00129] As used herein, the term "**substantially**" or "**substantial**" refers, *e.g.*, to the presence, level, or concentration of an entity in a particular space, the effect of one entity on another entity, or the effect of a treatment. For example, an activity, level or concentration of an entity is substantially increased if the increase is 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold, or 1000-fold relative to a baseline. An activity, level or concentration of an entity is also substantially increased if the increase is 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or 500% relative to a baseline.

[00130] The term "***in vivo***" refers to processes that occur in a living organism.

[00131] The term "**mammal**" as used herein includes both humans and non-human mammals.

[00132] Abbreviations used in this application include the following: "**mRNA**" refers to messenger RNA, "**miRNA**" refers to microRNA, "**siRNA**" refers to small interfering RNA, "**antisense RNA**" refers to single stranded RNA that is complementary to an mRNA, "**shRNA**" refers to small or short hairpin RNA, "**lncRNA**" refers to long non-coding RNA, and "**dsDNA**" refers to double stranded DNA.

Compositions

[00133] Aspects of the subject disclosure include a composition capable of regulating the immune system. The composition comprises an extracellular vesicle comprising a cell membrane, and an immunomodulating component associated with the cell membrane or enclosed within the membrane-bound enclosed volume.

The Extracellular Vesicle

[00134] In various embodiments, the composition comprises an extracellular vesicle. In certain embodiments, the extracellular vesicle is a cell-derived vesicle comprising a membrane that encloses an internal space.

[00135] In various embodiments, the extracellular vesicle can be a membrane-bound vesicle that has a smaller diameter than the cell from which it is derived. In some embodiments, the extracellular vesicle has a longest dimension between about 20-1000 nm, such as between about 20-100 nm, 20-200 nm, 20-300 nm, 20-400 nm, 20-500 nm, 20-600 nm, 20-700 nm, 20-800 nm, 20-900 nm, 30-100 nm, 30-200 nm, 30-300 nm, 30-400 nm, 30-500 nm, 30-600 nm, 30-700 nm, 30-800 nm, 30-900 nm, 40-100 nm, 40-200 nm, 40-300 nm, 40-400 nm, 40-500 nm, 40-600 nm, 40-700 nm, 40-800 nm, 40-900 nm, 50-150 nm, 50-500 nm, 50-750 nm, 100-200 nm, 100-500 nm, or 500-1000 nm.

[00136] In certain embodiments, the extracellular vesicle is an exosome. In certain embodiments, the extracellular vesicle is a nanovesicle. In certain embodiments, the extracellular vesicle is an apoptotic body. In certain embodiments, the extracellular vesicle is a fragment of cell. In certain embodiments, the extracellular vesicle is a vesicle derived from cell by direct or indirect manipulation. In certain embodiments, the extracellular vesicle is a vesiculated organelle. In various embodiments, the extracellular vesicle is a vesicle produced by living cells.

[00137] In some embodiments, the extracellular vesicle is derived from a living organism. In some embodiments, the extracellular vesicle is derived from a dead organism. In some embodiments, the extracellular vesicle is derived from an explanted tissue. In some embodiments, the extracellular vesicle is derived from an explanted organ. In some embodiments, the extracellular vesicle is derived from cultured cells. In some of these embodiments, when the extracellular vesicle is generated in a cell culture system, the extracellular vesicle is further isolated (*e.g.*, by separating the extracellular vesicle from the cultured cells). Separation can be achieved by sedimentation. For example, the extracellular vesicle can have a specific density between 0.5-2.0, 0.6-1.0, 0.7-1.0, 0.8-1.0, 0.9-1.0, 1.0-1.1, 1.1-1.2, 1.2-1.3, 1.4-1.5, 1.0-1.5, 1.5-2.0, and 1.0-2.0 kg/m^3. Separation can also be achieved by affinity purification. For example, the extracellular vesicle can be purified by binding a population comprising extracellular vesicles to a resin, said resin comprising a plurality of ligands that have specific affinity for one or more target proteins on the surface of the

extracellular vesicle. The target proteins may be a tetraspanin (*e.g.*, CD63, CD81, CD9), an EWI protein/immunoglobulin superfamily member (*e.g.*, PTGFRN, IGSF8, IGSF3), an integrin (*e.g.*, ITGB1, ITGA4), an ATP transporter protein (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4), SLC3A2, BSG, or CD98hc. The target protein may additionally be the immunomodulating component that is displayed on the surface of the exosomes.

[00138] In various embodiments, the extracellular vesicle comprises lipids or fatty acids and polypeptides. In certain embodiments, the extracellular vesicle further comprises a sugar. In certain embodiments, the extracellular vesicle further comprises a polynucleotide.

[00139] In various embodiments, the extracellular vesicle membrane comprises an interior surface and an exterior surface and encloses an internal space. In some embodiments, the extracellular vesicle further comprises a payload. In certain embodiments, the payload is enclosed within the internal space. In certain embodiments, the payload is displayed on the external surface of the extracellular vesicle. In certain embodiments, the payload is spanning the membrane of the extracellular vesicle. In various embodiments, the payload comprises nucleic acids, proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. In some embodiments, the extracellular vesicle further comprises a receiver.

The exosome

[00140] In various embodiments, the extracellular vesicle is an exosome. In certain embodiments, the exosome is a small membrane-bound vesicle secreted by producer cells.

[00141] In some embodiments, the exosome from the producer cell has a longest dimension between about 20-300 nm, such as between about 20-290 nm, 20-280 nm, 20-270 nm, 20-260 nm, 20-250 nm, 20-240 nm, 20-230 nm, 20-220 nm, 20-210 nm, 20-200 nm, 20-190 nm, 20-180 nm, 20-170 nm, 20-160 nm, 20-150 nm, 20-140 nm, 20-130 nm, 20-120 nm, 20-110 nm, 20-100 nm, 20-90 nm, 20-80 nm, 20-70 nm, 20-60 nm, 20-50 nm, 20-40 nm, 20-30 nm, 30-300 nm, 30-290 nm, 30-280 nm, 30-270 nm, 30-260 nm, 30-250 nm, 30-240 nm, 30-230 nm, 30-220 nm, 30-210 nm, 30-200 nm, 30-190 nm, 30-180 nm, 30-170 nm, 30-160 nm, 30-150 nm, 30-140 nm, 30-130 nm, 30-120 nm, 30-110 nm, 30-100 nm, 30-90 nm, 30-80 nm, 30-70 nm, 30-60 nm, 30-50 nm, 30-40 nm, 40-300 nm, 40-290 nm, 40-280 nm, 40-270 nm, 40-260 nm, 40-250 nm, 40-240 nm, 40-230 nm, 40-220 nm, 40-210 nm, 40-200 nm, 40-190 nm, 40-180 nm, 40-170 nm, 40-160 nm, 40-150 nm, 40-140 nm, 40-130 nm, 40-120 nm, 40-110 nm, 40-100 nm, 40-90 nm, 40-80 nm, 40-70 nm, 40-60 nm, 40-50 nm, 50-300 nm, 50-290 nm,

50-280 nm, 50-270 nm, 50-260 nm, 50-250 nm, 50-240 nm, 50-230 nm, 50-220 nm, 50-210 nm, 50-200 nm, 50-190 nm, 50-180 nm, 50-170 nm, 50-160 nm, 50-150 nm, 50-140 nm, 50-130 nm, 50-120 nm, 50-110 nm, 50-100 nm, 50-90 nm, 50-80 nm, 50-70 nm, 50-60 nm, 60-300 nm, 60-290 nm, 60-280 nm, 60-270 nm, 60-260 nm, 60-250 nm, 60-240 nm, 60-230 nm, 60-220 nm, 60-210 nm, 60-200 nm, 60-190 nm, 60-180 nm, 60-170 nm, 60-160 nm, 60-150 nm, 60-140 nm, 60-130 nm, 60-120 nm, 60-110 nm, 60-100 nm, 60-90 nm, 60-80 nm, 60-70 nm, 70-300 nm, 70-290 nm, 70-280 nm, 70-270 nm, 70-260 nm, 70-250 nm, 70-240 nm, 70-230 nm, 70-220 nm, 70-210 nm, 70-200 nm, 70-190 nm, 70-180 nm, 70-170 nm, 70-160 nm, 70-150 nm, 70-140 nm, 70-130 nm, 70-120 nm, 70-110 nm, 70-100 nm, 70-90 nm, 70-80 nm, 80-300 nm, 80-290 nm, 80-280 nm, 80-270 nm, 80-260 nm, 80-250 nm, 80-240 nm, 80-230 nm, 80-220 nm, 80-210 nm, 80-200 nm, 80-190 nm, 80-180 nm, 80-170 nm, 80-160 nm, 80-150 nm, 80-140 nm, 80-130 nm, 80-120 nm, 80-110 nm, 80-100 nm, 80-90 nm, 90-300 nm, 90-290 nm, 90-280 nm, 90-270 nm, 90-260 nm, 90-250 nm, 90-240 nm, 90-230 nm, 90-220 nm, 90-210 nm, 90-200 nm, 90-190 nm, 90-180 nm, 90-170 nm, 90-160 nm, 90-150 nm, 90-140 nm, 90-130 nm, 90-120 nm, 90-110 nm, 90-100 nm, 100-300 nm, 110-290 nm, 120-280 nm, 130-270 nm, 140-260 nm, 150-250 nm, 160-240 nm, 170-230 nm, 180-220 nm, or 190-210 nm.

[00142] In particularly preferred embodiments, the exosome from the producer cell described herein has a longest dimension between about 30-100 nm. In another preferred embodiment, the exosome from the producer cell has a longest dimension between about 20-300 nm. In another preferred embodiment, the exosome from the producer cell has a longest dimension between about 40-200 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 90% of the exosomes have a longest dimension 20-300 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 95% of the exosomes have a longest dimension 20-300 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 99% of the exosomes have a longest dimension 20-300 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 90% of the exosomes have a longest dimension 40-200 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 95% of the exosomes have a longest dimension 40-200 nm. In another embodiment, a population of the exosomes described herein comprise a population wherein 99% of the exosomes have a

longest dimension 40-200 nm. In other preferred embodiments, the size of the exosome or population of exosomes described herein is measured according to methods described, *infra*.

[00143] In some embodiments, the exosome is generated by a producer cell. In some embodiments, the membrane of the exosome comprises one or more molecules derived from the producer cell. In some embodiments, the exosome is generated in a cell culture system and isolated (*e.g.*, by separating the exosome from the producer cell). Separation can be achieved by sedimentation. For example, the exosome can have a specific density between 0.5-2.0, 0.6-1.0, 0.7-1.0, 0.8-1.0, 0.9-1.0, 1.0-1.1, 1.1-1.2, 1.2-1.3, 1.4-1.5, 1.0-1.5, 1.5-2.0, and 1.0-2.0 kg/m^3. Separation can also be achieved by affinity purification. For example, the extracellular vesicle can be purified by binding a population comprising extracellular vesicles to a resin, said resin comprising a plurality of ligands that have specific affinity for one or more target proteins on the surface of the extracellular vesicle. The one or more target protein may be a tetraspanin (*e.g.*, CD63, CD81 and/or CD9), an EWI protein/immunoglobulin superfamily member (*e.g.*, PTGFRN, IGSF8 and/or IGSF3), an integrin (*e.g.*, ITGB1 and/or ITGA4), an ATP transporter protein (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3 and/or ATP2B4), SLC3A2, BSG, or CD98hc. The target protein may additionally be the immunomodulating component that is displayed on the surface of the exosomes.

[00144] In some embodiments, the exosome membrane comprises an interior surface and an exterior surface. In certain embodiments, the interior surface faces the inner core of the exosome. In certain embodiments, the exterior surface can be in contact with the endosome, the multivesicular bodies, or the membrane/cytoplasm of a producer cell or a target cell.

[00145] In some embodiments, the exosome membrane comprises lipids and fatty acids. In some embodiments, the exosome membrane comprises phospholipids, glycolipids, fatty acids, sphingolipids, phosphoglycerides, sterols, cholesterols, and phosphatidylserines. In some embodiments, the lipid and fatty acid can be one or more of those listed in Table 1.

[00146] In certain embodiments, the exosome comprises a lipid bilayer composed of an inner leaflet and an outer leaflet. The composition of the inner and outer leaflet can be determined by transbilayer distribution assays known in the art, see *e.g.*, Kuypers *et al.* Biohim Biophys Acta 1985 819:170. In some embodiments, the composition of the outer leaflet is between approximately 70-90% choline phospholipids, between approximately 0-15% acidic phospholipids, and between approximately 5-30% phosphatidylethanolamine. In

some embodiments, the composition of the inner leaflet is between approximately 15-40% choline phospholipids, between approximately 10-50% acidic phospholipids, and between approximately 30-60% phosphatidylethanolamine.

[00147] In some embodiments, the exosome membrane further comprises one or more polypeptide. In certain embodiments, the exosome comprises one or more polypeptide selected from the following list, including but not limited to, spectrin, myosin-like polypeptide, band 3, SLC4A1, actin, actin-like polypeptide, glyceraldehyde 3-P dehydrogenase (G3PD), tetraspanins (*e.g.*, CD63, CD81 and/or CD9), Alix and TSG101, integrins (*e.g.*, ITGB1 and/or ITGA4), selectins, CR1, TNFRI, proteolytic enzymes, glycosylphosphatidylinositol (GPI)-linked proteins or histones, EWI protein/immunoglobulin superfamily members (*e.g.*, PTGFRN, IGSF8 and/or IGSF3), ATP transporter proteins (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3 and/or ATP2B4), SLC3A2, BSG, or CD98hc. In some embodiments, the exosome comprises at least one polypeptide selected from Table 2.

[00148] In some embodiments, the exosome comprises polypeptides on its surface. In some embodiments, the exosome is modified to contain the one or more polypeptides. In some embodiments, the producer cell is modified to contain the one or more polypeptides. In some embodiments, the producer cell naturally contains the one or more polypeptides and exosomes derived therefrom also contain the polypeptides. The levels of any desired surface marker can be modified directly on the exosome (*e.g.*, by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the complex). Alternatively or in addition, the levels of any desired surface marker can be modified directly on the producer cell (*e.g.,* by contacting the complex with recombinantly produced polypeptides to bring about insertion in or conjugation to the membrane of the cell). Alternatively, the producer cell can be modified by transducing an exogenous nucleic acid into the producer cell to express a desired surface marker. The surface marker can already be naturally present on the producer cell, in which case the exogenous construct can lead to overexpression of the marker and increased concentration of the marker in or on the producer cell. Alternatively, a naturally expressed surface marker can be removed from the producer cell (*e.g.*, by inducing gene silencing in the producer cell). The polypeptides can confer different functionalities to the exosome (*e.g.*, specific targeting capabilities, delivery functions (*e.g.*, fusion molecules), enzymatic functions, increased or

decreased half-life *in vivo*, *etc.*). In some embodiments, the polypeptides include, but are not limited to CD47, CD55, CD49, CD40, CD133, CD59, glypican-1, CD9, CD63, CD81, integrins, selectins, lectins, and cadherins.

[00149] In specific embodiments, the exosomes comprise one or more polypeptides on their surface, wherein said polypeptides are selected from a group of proteins that was recently identified to be enriched on the surface of exosomes (described in detail in U.S. Patent Application 62/550,543, which is incorporated herein by reference in its entirety). This group of polypeptides includes prostaglandin F2 receptor negative regulator (PTGFRN); basigin (BSG); immunoglobulin superfamily member 3 (IGSF3); immunoglobulin superfamily member 8 (IGSF8); integrin beta-1 (ITGB1); integrin alpha-4 (ITGA4); 4F2 cell-surface antigen heavy chain (SLC3A2); and a class of ATP transporter proteins (ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4)).

[00150] In some embodiments, the exosome membrane further comprises one or more polysaccharide, such as glycan.

[00151] In some embodiments, the exosome delivers the payload (therapeutic agent) to a target. The payload is a therapeutic agent that acts on a target (*e.g.,* a target cell) that is contacted with the exosome. Contacting can occur *in vitro* or in a subject. Payloads that can be introduced into an exosome and/or a producer cell include therapeutic agents such as, nucleotides (*e.g.,* nucleotides comprising a detectable moiety or a toxin or that disrupt transcription), nucleic acids (*e.g.,* DNA or mRNA molecules that encode a polypeptide such as an enzyme, or RNA molecules that have regulatory function such as miRNA, dsDNA, lncRNA, or siRNA), amino acids (*e.g.,* amino acids comprising a detectable moiety or a toxin that disrupt translation), polypeptides (*e.g.,* enzymes), lipids, carbohydrates, and small molecules (*e.g.,* small molecule drugs and toxins).

[00152] The exosome can interact with the target cell *via* membrane fusion and deliver payloads (*e.g.,* therapeutic agents) in an exosome composition to the surface or cytoplasm of a target cell. In some embodiments, membrane fusion occurs between the exosome and the plasma membrane of a target cell. In other embodiments, membrane fusion occurs between the exosome and an endosomal membrane of a target cell.

[00153] In some embodiments, the exosome comprises a receiver polypeptide. The receiver polypeptide can be synthetic. In some embodiments, the receiver polypeptide is introduced into the producer cell (*e.g.*, an exogenous nucleic acid that encodes the receiver polypeptide

is introduced into the producer cell) or a recombinant receiver polypeptide that is made outside the producer cell (*e.g.*, synthesized by a protein expression system). In some embodiments, the receiver polypeptide (*e.g.*, a recombinantly produced polypeptide) is introduced into the exosome directly (*e.g.*, after the exosome is isolated from the producer cell). In some embodiments, the receiver polypeptide can be on the surface of the exosomes. In some embodiments, the receiver polypeptide is capable of targeting the exosome to a specific target (*e.g.*, a target such as a pathogen, a metabolite, a polypeptide complex or a cell such as non-functional cell or cancer cell) that circulates in the circulatory system of the subject, such as the blood, or a target that resides in a tissue (such as a diseased tissue).

[00154] In some embodiments, the exosome is synthetic. For example, the exosome can comprise a payload, such as, *e.g.*, a therapeutic polypeptide, nucleic acid (such as DNA or RNA) or other polynucleotide, polysaccharide or glycan, lipid or fatty acid, large biologic, small molecule or toxin such that the exosome is not naturally occurring. In some embodiments, the exosome is modified (*e.g.*, by introducing a payload or otherwise modifying the content of the complex, such as by changing the protein, lipid or glycan content of the membrane). For example, exosomes are first isolated from a producer cell and then modified as desired, thereby generating synthetic exosomes. In some embodiments, the producer cell is modified. For example, an exogenous nucleic acid, an exogenous polypeptide or small molecule or toxin can be introduced into the producer cell. Alternatively or in addition, the producer cell can otherwise be modified (*e.g.*, by modifying the cellular or membrane content, such as by changing the lipid or glycan content of the cell membrane). Exosomes generated from the modified producer cells comprise one or more of the modifications of the producer cell. The process produces synthetic exosomes. In some embodiments, both the producer cell and the exosome isolated from the producer cell are modified as described herein.

Nanovesicle

[00155] In various embodiments, the extracellular vesicle is a nanovesicle. In certain embodiments, the nanovesicle is a cell-derived small vesicle comprising a membrane that encloses an internal space, and which is generated from the cell by direct or indirect manipulation such that the nanovesicle would not be produced by the cell without the manipulation. Appropriate manipulations of the cell include but are not limited to serial

extrusion, treatment with alkaline solutions, sonication, or combinations thereof and can, in some instances, result in the destruction of the producer cell.

[00156] In various embodiments, the nanovesicle has a longest dimension between about 20-250 nm, such as between about 20-100 nm, 20-150 nm, 20-200 nm, 30-100 nm, 30-150 nm, 30-200 nm, 30-250 nm, 40-100 nm, 40-150 nm, 40-200 nm, 40-250 nm, 50-100 nm, 50-150 nm, 50-200 nm, 50-250 nm, 100-200 nm, or 150-250 nm.

[00157] In various embodiments, the nanovesicle is derived from a producer cell. In certain embodiments, the nanovesicle is generated from a producer cell by direct or indirect manipulation. Appropriate manipulations include but are not limited to serial extrusion, treatment with alkaline solutions, sonication, or combinations thereof. In some of these embodiments, the manipulation can result in the destruction of the producer cell. In some preferred embodiments, the population of the nanovesicle is substantially free of vesicles that are derived from producer cells by way of direct budding from the plasma membrane or fusion of the late endosome with the plasma membrane.

[00158] In some embodiments, the nanovesicle is isolated from the producer cell based on its size, density, biochemical parameters, or a combination thereof. In certain embodiments, the isolation can be achieved by sedimentation. For example, the nanovesicle can have a specific density between 0.5-2.0, 0.6-1.0, 0.7-1.0, 0.8-1.0, 0.9-1.0, 1.0-1.1, 1.1-1.2, 1.2-1.3, 1.4-1.5, 1.0-1.5, 1.5-2.0, and 1.0-2.0 kg/m^3.

[00159] In various embodiments, the nanovesicle comprises lipids or fatty acids and polypeptides. In certain embodiments, the nanovesicle further comprises a sugar. In certain embodiments, the nanovesicle further comprises a polynucleotide. In some embodiments, the nanovesicle further comprises a receiver. In some embodiments, the nanovesicle further comprises a payload. In some of these embodiments, the payload comprises nucleic acids, proteins, carbohydrates, lipids, small molecules, and/or combinations thereof.

The Immunomodulating Component

[00160] In various embodiments, the composition further comprises an immunomodulating component.

[00161] In some embodiments, the immunomodulating compound is a protein that is expressed as a translational fusion protein to an exosome surface protein, such that said protein is retained on the surface of the exosome. In certain embodiments, the

immunomodulating compound is a membrane protein. In certain embodiments, the immunomodulating compound is a soluble protein. In some embodiments, the exosome surface protein is a tetraspanin (*e.g.*, CD63, CD81, CD9), an EWI protein/immunoglobulin superfamily member (*e.g.*, PTGFRN, IGSF8, IGSF3), an integrin (*e.g.*, ITGB1, ITGA4), an ATP transporter protein (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4), SLC3A2, BSG, or CD98hc or a fragment or variant thereof.

[00162] In some embodiments, the immunomodulating compound is a soluble protein that is expressed as a translational fusion protein to an exosome surface protein, such that said soluble protein is retained on the surface of the exosome. In some embodiments, the exosome surface protein is a tetraspanin (*e.g.*, CD63, CD81, CD9), an EWI protein/immunoglobulin superfamily member (*e.g.*, PTGFRN, IGSF8, IGSF3), an integrin (*e.g.*, ITGB1, ITGA4), an ATP transporter protein (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4), SLC3A2, BSG, or CD98hc or a fragment or variant thereof.

[00163] In certain embodiments, the immunomodulating component has anti-tumor activity. In some embodiments, the immunomodulating component regulates the innate immune response. In some of these embodiments, the immunomodulating component targets the natural killer cells. In some other embodiments, the immunomodulating component regulates the adaptive immune response. In some of these embodiments, the immunomodulating component targets the cytotoxic T cells.

[00164] In some embodiments, the immunomodulating component is expressed in the producer cell in its full-length form. In other embodiments, the immunomodulating component is expressed as a translational fusion protein to an exosome surface protein, which results in a higher level of expression of the biologically active portion of the immunomodulating compound on the surface of the exosome. In some embodiments, the immunomodulating compound is a soluble protein that is expressed as a translational fusion protein to an exosome surface protein, such that said soluble protein is retained on the surface of the exosome. In some embodiments, the exosome surface protein is a tetraspanin (*e.g.*, CD63, CD81, CD9), an EWI protein/immunoglobulin superfamily member (*e.g.*, PTGFRN, IGSF8, IGSF3), an integrin (*e.g.*, ITGB1, ITGA4), an ATP transporter protein (*e.g.*, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B3, ATP2B1, ATP2B2, ATP2B3, ATP2B4), SLC3A2, BSG, or CD98hc or a fragment or variant thereof.

[00165] In some embodiments, the immunomodulating component is an inhibitor for a negative checkpoint regulator. In some embodiments, the immunomodulating component is an inhibitor for a binding partner of a negative checkpoint regulator.

[00166] In certain embodiments, the immunomodulating component is an inhibitor of cytotoxic T-lymphocyte-associate protein 4 (CTLA-4). In some of these embodiments, the CTLA-4 inhibitor is a monoclonal antibody of CTLA-4. In certain embodiments, the inhibitor is a fragment of a monoclonal antibody of CTLA-4. In certain embodiments, the antibody fragment is a scFv, $(scFv)_2$, Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, or Fd of a monoclonal antibody of CTLA-4. In certain embodiments, the inhibitor is a nanobody, a bispecific antibody, or a multispecific antibody against CTLA-4. In some specific embodiments, the monoclonal antibody is ipilimumab. In some specific embodiments, the monoclonal antibody is tremelimumab.

[00167] In certain embodiments, the immunomodulating component is an inhibitor of programmed cell death protein 1 (PD-1). In certain embodiments, the immunomodulating component is an inhibitor of programmed death-ligand 1 (PD-L1). In certain embodiments, the immunomodulating component is an inhibitor of programmed death-ligand 2 (PD-L2). In some embodiments, the inhibitor of PD-1, PD-L1, or PD-L2 is a monoclonal antibody of PD-1, PD-L1, or PD-L2. In certain embodiments, the inhibitor is a fragment of a monoclonal antibody of PD-1, PD-L1, or PD-L2. In certain embodiments, the antibody fragment is a scFv, $(scFv)_2$, Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, or Fd of a monoclonal antibody of PD-1, PD-L1, or PD-L2. In certain embodiments, the inhibitor is a nanobody, a bispecific antibody, or a multispecific antibody against PD-1, PD-L1, or PD-L2. In some specific embodiments, the monoclonal antibody is nivolumab. In some specific embodiments, the monoclonal antibody is pembrolizumab. In some specific embodiments, the monoclonal antibody is pidilizumab. In some specific embodiments, the monoclonal antibody is atezolizumab. In some specific embodiments, the monoclonal antibody is avelumab.

[00168] In certain embodiments, the immunomodulating component is an inhibitor of lymphocyte-activated gene 3 (LAG3). In some of these embodiments, the inhibitor of LAG3 is a monoclonal antibody of LAG3.

[00169] In certain embodiments, the immunomodulating component is an inhibitor of T-cell immunoglobulin mucin-containing protein 3 (TIM-3). In certain embodiments, the immunomodulating component is an inhibitor of B and T lymphocyte attenuator (BTLA). In

certain embodiments, the immunomodulating component is an inhibitor of T cell immunoreceptor with Ig and ITIM domains (TIGIT). In certain embodiments, the immunomodulating component is an inhibitor of V-domain Ig suppressor of T cell activation (VISTA). In certain embodiments, the immunomodulating component is an inhibitor of adenosine A2a receptor (A2aR). In certain embodiments, the immunomodulating component is an inhibitor of killer cell immunoglobulin like receptor (KIR). In certain embodiments, the immunomodulating component is an inhibitor of indoleamine 2,3-dioxygenase (IDO). In certain embodiments, the immunomodulating component is an inhibitor of CD20, CD39, or CD73.

[00170] In some embodiments, the immunomodulating component is an activator for a positive co-stimulatory molecule. In some embodiments, the immunomodulating component is an activator for a binding partner of a positive co-stimulatory molecule.

[00171] In some embodiments, the immunomodulating component is an activator of a TNF receptor superfamily member. In certain embodiments, the TNF receptor superfamily member is selected from the group consisting of: CD120a, CD120b, CD18, OX40, CD40, Fas receptor, M68, CD27, CD30, 4-1BB, TRAILR1, TRAILR2, TRAILR3, TRAILR4, RANK, OCIF, TWEAK receptor, TACI, BAFF receptor, ATAR, CD271, CD269, GITR, TROY, CD358, TRAMP, and XEDAR. In some embodiments, the immunomodulating component is a TNF superfamily member. In certain embodiments, the TNF superfamily member is selected from the group consisting of: TNFα, TNF-C, OX40L, CD40L, FasL, LIGHT, TL1A, CD27L, Siva, CD153, 4-1BB ligand, TRAIL, RANKL, TWEAK, APRIL, BAFF, CAMLG, NGF, BDNF, NT-3, NT-4, GITR ligand, and EDA-2.

[00172] In some embodiments, the activator of a TNF receptor superfamily member is expressed as a monomeric protein. In some embodiments, the activator of a TNF receptor superfamily member is expressed as trimeric proteins. In some embodiments, the TNF receptor superfamily member is expressed as a monomeric protein. In some embodiments, the TNF receptor superfamily member is expressed as trimeric proteins.

[00173] In certain embodiments, the immunomodulating component is an activator of TNF Receptor Superfamily Member 4 (OX40). In some of these embodiments, the activator of OX40 is an agonist antibody of OX40. In some other of these embodiments, the activator of OX40 is OX40 ligand (OX40L).

[00174] In certain embodiments, the immunomodulating component is an activator of CD27. In some of these embodiments, the activator of CD27 is an agonist antibody of CD27. In some other of these embodiments, the activator of CD27 is CD27 ligand (CD27L).

[00175] In certain embodiments, the immunomodulating component is an activator of CD40. In some of these embodiments, the activator of CD40 is an agonist antibody of CD40. In some other of these embodiments, the activator of CD40 is CD40 ligand (CD40L). In some embodiments, the CD40L is monomeric CD40L. In some embodiments, the CD40L is trimeric CD40L.

[00176] In some embodiments, trimeric CD40L is fused to PTGFRN or a fragment thereof. In some embodiments, trimeric CD40L is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments, trimeric CD40L is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 19 or SEQ ID NO: 20.

[00177] In certain embodiments, the immunomodulating component is an activator of glucocorticoid-induced TNFR-related protein (GITR). In some of these embodiments, the activator of GITR is an agonist antibody of GITR. In some other of these embodiments, the activator of GITR is a natural ligand of GITR.

[00178] In certain embodiments, the immunomodulating component is an activator of 4-1BB. In some of these embodiments, the activator of 4-1BB is an agonist antibody of 4-1BB. In some other of these embodiments, the activator of 4-1BB is a natural ligand of 4-1BB.

[00179] In some embodiments, the immunomodulating component is Fas receptor (Fas). In some of these embodiments, the Fas receptor is displayed on the surface of the extracellular vesicle. In some other embodiments, the immunomodulating component is Fas ligand (FasL). In some of these embodiments, the Fas ligand is displayed on the surface of the extracellular vesicle. In certain embodiments, the immunomodulating component is an antibody of Fas receptor. In certain embodiments, the immunomodulating component is an antibody of Fas ligand.

[00180] In some embodiments, the immunomodulating component is an activator of a CD28-superfamily co-stimulatory molecule. In certain embodiments, the CD28-superfamily co-stimulatory molecule is ICOS or CD28. In certain embodiments, the immunomodulating component is ICOSL, CD80, or CD86.

[00181] In certain embodiments, the immunomodulating component is an activator of inducible T cell co-stimulator (ICOS). In some of these embodiments, the activator of ICOS is an agonist antibody of ICOS. In some other of these embodiments, the activator of ICOS is ICOS ligand (ICOSL).

[00182] In certain embodiments, the immunomodulating component is an activator of CD28. In some of these embodiments, the activator of CD28 is an agonist antibody of CD28. In some other of these embodiments, the activator of CD28 is a natural ligand of CD28. In certain embodiments, the ligand of CD28 is CD80.

[00183] In certain embodiments, the composition comprises an inhibitor for a negative checkpoint regulator or an inhibitor for a binding partner of a negative checkpoint regulator and an activator for a positive co-stimulatory molecule or an activator for a binding partner of a positive co-stimulatory molecule.

[00184] In certain embodiments, the immunomodulating component is a cytokine. In some embodiments, the cytokine is a soluble cytokine that has been translationally fused to an exosome surface protein or fragment thereof. In some embodiments, the cytokine is interleukin 2 (IL-2). In some embodiments, the cytokine is interleukin 7 (IL-7). In some embodiments, the cytokine is interleukin 12 (IL-12). In some embodiments, the cytokine is interleukin 15 (IL-15).

[00185] In certain embodiments, the cytokine is fused to PTGFRN or a fragment thereof. In some embodiments, IL-7 is fused to PTGFRN or a fragment thereof. In some embodiments, IL-7 is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments, IL-7 is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

[00186] In certain embodiments, the cytokine is fused to PTGFRN or a fragment thereof. In some embodiments, IL-12 is fused to PTGFRN or a fragment thereof. In some embodiments, IL-12 is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments, IL-12 is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6.

[00187] In certain embodiments, the cytokine is fused to PTGFRN or a fragment thereof. In some embodiments, IL-15 is fused to PTGFRN or a fragment thereof. In some embodiments, IL-15 is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments,

IL-15 is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 15 or SEQ ID NO: 16.

[00188] In some embodiments, the cytokine is an interferon (IFN). In certain embodiments, the interferon is fused to PTGFRN or a fragment thereof. In certain embodiments, the interferon is interferon γ (IFNγ). In some embodiments, IFNγ is fused to PTGFRN or a fragment thereof. In some embodiments, IFNγ is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments, IFNγ is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 7 or SEQ ID NO: 8.

[00189] In some embodiments, the immunomodulating component is a T-cell receptor (TCR) or a derivative thereof. In certain embodiments, the immunomodulating component is a TCR α-chain or a derivative thereof. In certain embodiments, the immunomodulating component is a TCR β-chain or a derivative thereof. In some embodiments, the immunomodulating component is a co-receptor of the T-cell or a derivative thereof.

[00190] In some embodiments, the immunomodulating component is a tumor antigen. In certain embodiments, the tumor antigen is selected from the group consisting of: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand.

[00191] In certain embodiments, the tumor antigen is a carcinoembryonic antigen (CEA). In certain embodiments, the tumor antigen is an epithelial tumor antigen (ETA).

[00192] In certain embodiments, the tumor antigen is a mucin. In some of these embodiments, the mucin is a secreted mucin. In some other of these embodiments, the mucin is a transmembrane mucin. In specific embodiments, the tumor antigen is mucin 1 (MUC1). In specific embodiments, the tumor antigen is Tn-MUC1. In specific embodiments, the tumor antigen is mucin 16 (MUC16).

[00193] In certain embodiments, the tumor antigen is a melanoma-associated antigen (MAGE). In some of these embodiments, the MAGE is a type-I MAGE. In some other of

these embodiments, the MAGE is a type-II MAGE. In specific embodiments, the type-I MAGE is MAGE-A2. In specific embodiments, the type-I MAGE is MAGE-A4.

[00194] In certain embodiments, the tumor antigen is alpha-fetoprotein (AFP). In certain embodiments, the tumor antigen is tumor protein p53 (p53). In certain embodiments, the tumor antigen is tyrosinase. In certain embodiments, the tumor antigen is a tyrosinase-related protein (TRP). In some embodiments, the tumor antigen is programmed death ligand 1 (PD-L1) or programmed death ligand 2 (PD-L2). In various embodiments, the tumor antigen is selected from the group consisting of CD4, CD8, CD45, CD80, and CD86.

[00195] In some embodiments, the immunomodulating component is a chimeric antigen receptor (CAR) or a derivative thereof. In some embodiments, the CAR binds to one or more of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand.

[00196] In some embodiments, the immunomodulating component is an activator of a T-cell receptor or co-receptor. In certain embodiments, the immunomodulating component is an activator of CD3. In certain embodiments, the activator is a fragment of a monoclonal antibody of CD3. In certain embodiments, the antibody fragment is a scFv, $(scFv)_2$, Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, or Fd of a monoclonal antibody against CD3. In certain embodiments, the activator is a nanobody, a bispecific antibody, or a multispecific antibody against CD3. In some embodiments, the anti-CD3 antibody fragment is fused to PTGFRN or a fragment thereof. In some embodiments, the anti-CD3 antibody fragment is fused to the N-terminus of PTGFRN or a fragment thereof. In some embodiments, the anti-CD3 antibody fragment is expressed as a fusion protein to PTGFRN, wherein the polypeptide has the sequence of SEQ ID NO: 18 or SEQ ID NO: 21. In certain embodiments, the immunomodulating component is an activator of CD28. In certain embodiments, the inhibitor is a fragment of a monoclonal antibody of CD28. In certain embodiments, the antibody fragment is a scFv, $(scFv)_2$, Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, or Fd of a monoclonal antibody of CD28. In certain embodiments, the inhibitor is a nanobody, a bispecific antibody, or a multispecific antibody against CD28.

[00197] In some embodiments, the immunomodulating component is a major histocompatibility complex (MHC) or a derivative thereof. In some of these embodiments, the immunomodulating component is an MHC class I or a derivative thereof. In some of these embodiments, the immunomodulating component is an MHC class II or a derivative thereof. In some of these embodiments, the immunomodulating component is an MHC class III or a derivative thereof.

[00198] In some embodiments, the immunomodulating component is a human leukocyte antigen (HLA) or a derivative thereof. In some of these embodiments, the immunomodulating component is an HLA-A, HLA-B, HLA-C, or derivative thereof. In some of these embodiments, the immunomodulating component is an HLA-E, HLA-F, HLA-G, or a derivative thereof. In some of these embodiments, the immunomodulating component is an HLA-DP, HLA-DQ, HLA-DR, or a derivative thereof.

[00199] In various embodiments, the immunomodulating component can be a polypeptide, a polynucleotide, a polysaccharide, a lipid, a small molecule, or a toxin.

[00200] In some embodiments, the immunomodulating component can be a protein, a peptide, a glycolipid, or a glycoprotein.

[00201] In certain embodiments, the immunomodulating component is an agonist. In some of these embodiments, the agonist is an endogenous agonist, such as a hormone, or a neurotransmitter. In some other of these embodiments, the agonist is an exogenous agonist, such as a drug. In some embodiments, the agonist is a physical agonist, which can create an agonist response without binding to the receptor. In some embodiments, the agonist is a superagonist, which can produce a greater maximal response than the endogenous agonist. In certain embodiments, the agonist is a full agonist with full efficacy at the receptor. In certain other embodiments, the agonist is a partial agonist having only partial efficacy at the receptor relative to a full agonist. In some embodiments, the agonist is an inverse agonist that can inhibit the constitutive activity of the receptor. In some embodiments, the agonist is a co-agonist that works with other co-agonists to produce an effect on the receptor. In certain embodiments, the agonist is an irreversible agonist that binds permanently to a receptor through formation of covalent bond. In certain embodiments, the agonist is selective agonist for a specific type of receptor.

[00202] In certain embodiments, the immunomodulating component is an antagonist. In some of these embodiments, the antagonist is a competitive antagonist, which reversibly

binds to the receptor at the same binding site as the endogenous ligand or agonist without activating the receptor. Competitive antagonist can affect the amount of agonist necessary to achieve a maximal response. In some other of these embodiments, the antagonist is a non-competitive antagonist, which binds to an active site of the receptor or an allosteric site of the receptor. Non-competitive antagonist can reduce the magnitude of the maximum response that can be attained by any amount of agonist. In some other embodiments, the antagonist is an uncompetitive antagonist, which requires receptor activation by an agonist before its binding to a separate allosteric binding site.

[00203] In various embodiments, the immunomodulating component comprises an antibody or an antigen-binding fragment. The immunomodulating component can be a full length protein or a fragment thereof. The antibody or antigen-binding fragment can be derived from natural sources, or partly or wholly synthetically produced. In some embodiments, the antibody is a monoclonal antibody. In some of these embodiments, the monoclonal antibody is an IgG antibody. In certain embodiments, the monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4. In some other embodiments, the antibody is a polyclonal antibody. In certain embodiments, the antigen-binding fragment is selected from Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, and Fd fragments. In certain embodiments, the antigen-binding fragment is an scFv or $(scFv)_2$ fragment. In certain other embodiments, the antibody or antigen-binding fragment is a Nanobody® (single-domain antibody). In some embodiments, the antibody or antigen-binding fragment is a bispecific or multispecific antibody.

[00204] In various embodiments, the antibody or antigen-binding fragment is fully human. In some embodiments, the antibody or antigen-binding fragment is humanized. In some embodiments, the antibody or antigen-binding fragment is chimeric. In some of these embodiments, the chimeric antibody has non-human V region domains and human C region domains. In some embodiments, the antibody or antigen-binding fragment is non-human, such as murine or veterinary.

[00205] In certain embodiments, the immunomodulating component is a polynucleotide. In some of these embodiments, the polynucleotide includes, but is not limited to, an mRNA, a miRNA, an siRNA, an antisense RNA, an shRNA, a lncRNA, and a dsDNA. In some embodiments, the polynucleotide is an RNA (*e.g.*, an mRNA, a miRNA, an siRNA, an antisense RNA, an shRNA, or an lncRNA). In some of these embodiments, when the polynucleotide is an mRNA, it can be translated into a desired polypeptide. In some

embodiments, the polynucleotide is a microRNA (miRNA) or pre-miRNA molecule. In some of these embodiments, the miRNA is delivered to the cytoplasm of the target cell, such that the miRNA molecule can silence a native mRNA in the target cell. In some embodiments, the polynucleotide is a small interfering RNA (siRNA) or a short hairpin RNA (shRNA) capable of interfering with the expression of an oncogene or other dysregulating polypeptides. In some of these embodiments, the siRNA is delivered to the cytoplasm of the target cell, such that the siRNA molecule can silence a native mRNA in the target cell. In some embodiments, the polynucleotide is an antisense RNA that is complementary to an mRNA. In some embodiments, the polynucleotide is a long non-coding RNA (lncRNA) capable of regulating gene expression and modulating diseases. In some embodiments, the polynucleotide is a DNA that can be transcribed into an RNA. In some of these embodiments, the transcribed RNA can be translated into a desired polypeptide.

[00206] In some embodiments, the immunomodulating component is a protein, a peptide, a glycolipid, or a glycoprotein.

[00207] In various embodiments, the composition comprises two or more above mentioned immunomodulating components, including mixtures, fusions, combinations and conjugates, of atoms, molecules, *etc.* In some embodiments, the composition comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve different immunomodulating components associated with the membrane or enclosed within the enclosed volume of said extracellular vesicle. In certain embodiments, the composition comprises a nucleic acid combined with a polypeptide. In certain embodiments, the composition comprises two or more polypeptides conjugated to each other. In certain embodiments, the composition comprises a protein conjugated to a biologically active molecule. In some of these embodiments, the biologically active molecule is a prodrug.

[00208] In some embodiments, the composition comprises two different immunomodulating components associated with the membrane or enclosed within the enclosed volume of said extracellular vesicle. In certain embodiments, the two different immunomodulating components are IL-12 and CD40L. In some embodiments, the CD40L and IL-12 are fused to PTGFRN or a fragment thereof respectively. In some embodiments, the CD40L and IL-12 are fused to the N-terminus of PTGFRN or a fragment thereof respectively. In some embodiments, the CD40L and IL-12 are expressed as fusion proteins to

PTGFRN, wherein the polypeptides have the sequences of SEQ ID NO: 20 and SEQ ID NO: 3 respectively.

[00209] In some embodiments, the composition comprises three different immunomodulating components associated with the membrane or enclosed within the enclosed volume of said extracellular vesicle. In certain embodiments, the two different immunomodulating components are IL-12, CD40L, and FMS-like tyrosine kinase 3 ligand (FLT3L). In some embodiments, the CD40L, IL-12, and FLT3L are fused to PTGFRN or a fragment thereof respectively. In some embodiments, the CD40L, IL-12, and FLT3L are fused to the N-terminus of PTGFRN or a fragment thereof respectively. In some embodiments, the CD40L, IL-12, and FLT3L are expressed as fusion proteins to PTGFRN, wherein the polypeptides have the sequences of SEQ ID NO: 20, SEQ ID NO: 3, and SEQ ID NO: 22 respectively.

The Pharmaceutical Composition

[00210] The pharmaceutical compositions generally comprise a plurality of extracellular vesicles and a pharmaceutically-acceptable excipient or carrier in a form suitable for administration to a subject. Pharmaceutically-acceptable excipients or carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions comprising a plurality of extracellular vesicles. (*See*, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. 21st ed. (2005)). The pharmaceutical compositions are generally formulated sterile and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[00211] In some embodiments, the pharmaceutical composition comprises one or more therapeutic agents and the extracellular vesicle described herein. In some embodiments, the extracellular vesicles are co-administered with of one or more separate therapeutic agents, wherein co-administration includes administration of the separate therapeutic agent before, after or concurrent with administration of the extracellular vesicles.

[00212] Pharmaceutically-acceptable excipients include excipients that are generally safe, non-toxic, and desirable, including excipients that are acceptable for veterinary use as well as for human pharmaceutical use.

[00213] Examples of carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the extracellular vesicles described herein, use thereof in the compositions is contemplated. Supplementary therapeutic agents can also be incorporated into the compositions. Typically, a pharmaceutical composition is formulated to be compatible with its intended route of administration. The extracellular vesicles can be administered by parenteral, topical, intravenous, oral, subcutaneous, intra-arterial, intradermal, transdermal, rectal, intracranial, intraperitoneal, intranasal, intratumoral, intramuscular route or as inhalants. In certain embodiments, the pharmaceutical composition comprising extracellular vesicles is administered intravenously, *e.g.* by injection. The extracellular vesicles can optionally be administered in combination with other therapeutic agents that are at least partly effective in treating the disease, disorder or condition for which the extracellular vesicles are intended.

[00214] Solutions or suspensions can include the following components: a sterile diluent such as water, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00215] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (if water soluble) or dispersions and sterile powders. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). The composition is generally sterile and fluid to the extent that easy syringeability exists. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of

surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. If desired, isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride can be added to the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[00216] Sterile injectable solutions can be prepared by incorporating the extracellular vesicles in an effective amount and in an appropriate solvent with one or a combination of ingredients enumerated herein, as desired. Generally, dispersions are prepared by incorporating the extracellular vesicles into a sterile vehicle that contains a basic dispersion medium and any desired other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The extracellular vesicles can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner to permit a sustained or pulsatile release of the extracellular vesicles.

[00217] Systemic administration of compositions comprising extracellular vesicles can also be by transmucosal means. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of, *e.g.*, nasal sprays.

[00218] In certain embodiments the pharmaceutical composition comprising extracellular vesicles is administered intravenously into a subject that would benefit from the pharmaceutical composition. In certain other embodiments, the composition is administered to the lymphatic system, *e.g.*, by intralymphatic injection or by intranodal injection (*see e.g.*, Senti *et al.*, PNAS 105(46): 17908 (2008)), or by intramuscular injection, by subcutaneous administration, by intratumoral injection, by direct injection into the thymus, or into the liver.

[00219] In certain embodiments, the pharmaceutical composition comprising extracellular vesicles is administered as a liquid suspension. In certain embodiments, the pharmaceutical composition is administered as a formulation that is capable of forming a depot following

administration. In certain preferred embodiments, the depot slowly releases the extracellular vesicles into circulation, or remains in depot form.

[00220] Typically, pharmaceutically-acceptable compositions are highly purified to be free of contaminants, are biocompatible and not toxic, and are suited to administration to a subject. If water is a constituent of the carrier, the water is highly purified and processed to be free of contaminants, *e.g.*, endotoxins.

[00221] The pharmaceutically-acceptable carrier can be lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginates, gelatin, calcium silicate, micro-crystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and/or mineral oil, but is not limited thereto. The pharmaceutical composition can further include a lubricant, a wetting agent, a sweetener, a flavor enhancer, an emulsifying agent, a suspension agent, and/or a preservative.

[00222] The pharmaceutical compositions described herein comprise the extracellular vesicles described herein and optionally a pharmaceutically active or therapeutic agent. The therapeutic agent can be a biological agent, a small molecule agent, or a nucleic acid agent.

[00223] Dosage forms are provided that comprise a pharmaceutical composition comprising the extracellular vesicles described herein. In some embodiments, the dosage form is formulated as a liquid suspension for intravenous injection. In some embodiments, the dosage form is formulated as a liquid suspension for intratumoral injection.

[00224] In certain embodiments, the preparation of extracellular vesicles is subjected to radiation, *e.g.*, X rays, gamma rays, beta particles, alpha particles, neutrons, protons, elemental nuclei, UV rays in order to damage residual replication-competent nucleic acids.

[00225] In certain embodiments, the preparation of extracellular vesicles is subjected to gamma irradiation using an irradiation dose of more than 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more than 100 kGy.

[00226] In certain embodiments, the preparation of extracellular vesicles is subjected to X-ray irradiation using an irradiation dose of more than 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10000, or greater than 10000 mSv.

Methods

[00227] Aspects of the subject disclosure also include methods of producing the composition comprising the extracellular vesicle and the immunomodulating component. In some embodiments, the method comprises: obtaining the extracellular vesicle from the producer cell, wherein the producer cell naturally contains the immunomodulating component; and optionally isolating the obtained extracellular vesicle. In some embodiments, the method comprises: modifying a producer cell with the immunomodulating component; obtaining the extracellular vesicle from the modified producer cell; and optionally isolating the obtained extracellular vesicles. In some other embodiments, the method comprises: obtaining the extracellular vesicle from a producer cell; isolating the obtained extracellular vesicles; and modifying the isolated extracellular vesicle with the immunomodulating component. In certain embodiments, the method further comprises formulating the isolated extracellular vesicles into a pharmaceutical composition.

Methods of Producing the extracellular vesicles

Methods of Modifying the Producer Cell with the Immunomodulating Component

[00228] In various embodiments, the method comprises modifying a producer cell with the immunomodulating component.

[00229] The producer cell can be a mammalian cell line, a plant cell line, an insect cell line, a fungi cell line, or a prokaryotic cell line. In certain embodiments, the producer cell is a mammalian cell line. The mammalian cell lines include but are not limited to a human embryonic kidney (HEK) cell line, a Chinese hamster ovary (CHO) cell line, an HT-1080 cell line, a HeLa cell line, a PERC-6 cell line, a CEVEC cell line, a fibroblast cell line, an amniocyte cell line, an epithelial cell line, and a mesenchymal stem cell (MSC) cell line. In some preferred embodiments, the mammalian cell line can be HEK-293 cells, BJ human foreskin fibroblast cells, fHDF fibroblast cells, AGE.HN® neuronal precursor cells, CAP® amniocyte cells, adipose mesenchymal stem cells, or RPTEC/TERT1 cells. The producer cell can also be a primary cell. In various embodiments, the primary cell can be a primary mammalian cell, a primary plant cell, a primary insect cell, a primary fungi cell, or a primary prokaryotic cell.

[00230] In certain preferred embodiments, the producer cell is an immune cell, such as a dendritic cell, a T cell, a B cell, a natural killer cell (NK cell), an antigen presenting cell, a macrophage, a T helper cell, or a regulatory T cell (Treg cell).

[00231] In various embodiments, the immunomodulating component can be expressed in a producer cell from a transgene or mRNA introduced into the producer cell by transfection, viral transduction, electroporation, extrusion, sonication, cell fusion, or other methods that are known to the skilled in the art.

[00232] In certain embodiments, the immunomodulating component is introduced to the producer cell by transfection. In some embodiments, the immunomodulating component can be introduced into suitable producer cells using synthetic macromolecules such as cationic lipids and polymers (Papapetrou *et al.*, Gene Therapy 12: S118-S130 (2005)). In some embodiments, the cationic lipids form complexes with the immunomodulating component through charge interactions. In some of these embodiments, the positively charged complexes bind to the negatively charged cell surface and are taken up by the cell by endocytosis. In some other embodiments, a cationic polymer can be used to transfect producer cells. In some of these embodiments, the cationic polymer is polyethylenimine (PEI). In certain embodiments, chemicals such as calcium phosphate, cyclodextrin, or polybrene, can be used to introduce the immunomodulating component to the producer cells. The immunomodulating component can also be introduced into a producer cell using a physical method such as particle-mediated transfection, "gene gun", biolistics, or particle bombardment technology (Papapetrou *et al.*, Gene Therapy 12: S118-S130 (2005)). A reporter gene such as, for example, beta-galactosidase, chloramphenicol acetyltransferase, luciferase, or green fluorescent protein can be used to assess the transfection efficiency of the producer cell.

[00233] In certain embodiments, the immunomodulating component is introduced to the producer cell by viral transduction. A number of viruses can be used as gene transfer vehicles, including moloney murine leukemia virus (MMLV), adenovirus, adeno-associated virus (AAV), herpes simplex virus (HSV), lentiviruses, and spumaviruses. The viral mediated gene transfer vehicles comprise vectors based on DNA viruses, such as adenovirus, adeno-associated virus and herpes virus, as well as retroviral based vectors.

[00234] In certain embodiments, the immunomodulating component is introduced to the producer cell by electroporation. Electroporation creates transient pores in the cell

membrane, allowing for the introduction of various molecules into the cell. In some embodiments, DNA and RNA as well as polypeptides and non-polypeptide therapeutic agents can be introduced into the producer cell by electroporation.

[00235] In certain embodiments, the immunomodulating component is introduced to the producer cell by microinjection. In some embodiments, a glass micropipette can be used to inject the immunomodulating component into the producer cell at the microscopic level.

[00236] In certain embodiments, the immunomodulating component is introduced to the producer cell by extrusion.

[00237] In certain embodiments, the immunomodulating component is introduced to the producer cell by sonication. In some embodiments, the producer cell is exposed to high intensity sound waves, causing transient disruption of the cell membrane allowing loading of an immunomodulating component.

[00238] In certain embodiments, the immunomodulating component is introduced to the producer cell by cell fusion. In some embodiments, the immunomodulating component is introduced by electrical cell fusion. In some other embodiments, polyethylene glycol (PEG) is used to fuse the producer cells. In some other embodiments, sendai virus is used to fuse the producer cells.

[00239] In some embodiments, the immunomodulating component is introduced to the producer cell by hypotonic lysis. In some of these embodiments, the producer cell is exposed to low ionic strength buffer causing them to burst allowing loading of an immunomodulating component. In some alternative embodiments, controlled dialysis against a hypotonic solution is used to swell the producer cell and to create pores in the producer cell membrane. The producer cell is subsequently exposed to conditions that allow resealing of the membrane.

[00240] In some embodiments, the immunomodulating component is introduced to the producer cell by detergent treatment. In certain embodiments, producer cell is treated with a mild detergent which transiently compromises the producer cell membrane by creating pores allowing loading of an immunomodulating component. After producer cells are loaded, the detergent is washed away thereby resealing the membrane.

[00241] In some embodiments, the immunomodulating component is introduced to the producer cell by receptor mediated endocytosis. In certain embodiments, producer cells have

a surface receptor which upon binding of the immunomodulating component induces internalization of the receptor and the associated immunomodulating component.

[00242] In some embodiments, the immunomodulating component is introduced to the producer cell by filtration. In certain embodiments, the producer cells and the immunomodulating component can be forced through a filter of pore size smaller than the producer cell causing transient disruption of the producer cell membrane and allowing the immunomodulating component to enter the producer cell.

[00243] In some embodiments, the producer cell is subjected to several freeze thaw cycles, resulting in cell membrane disruption allowing loading of an immunomodulating component.

Methods of Modifying the Extracellular Vesicle with the Immunomodulating Component

[00244] In various alternative embodiments, the immunomodulating component is introduced directly to the extracellular vesicles after the isolation of the extracellular vesicles.

[00245] In certain embodiments, the immunomodulating component is introduced to the extracellular vesicle by transfection. In some embodiments, the immunomodulating component can be introduced into the extracellular vesicles using synthetic macromolecules such as cationic lipids and polymers (Papapetrou *et al.*, Gene Therapy 12: S118-S130 (2005)). In certain embodiments, chemicals such as calcium phosphate, cyclodextrin, or polybrene, can be used to introduce the immunomodulating component to the extracellular vesicles.

[00246] In certain embodiments, the immunomodulating component is introduced to the extracellular vesicle by electroporation. In some embodiments, extracellular vesicles are exposed to an electrical field which causes transient holes in the extracellular vesicle membrane, allowing loading of an immunomodulating component.

[00247] In certain embodiments, the immunomodulating component is introduced to the extracellular vesicle by microinjection. In some embodiments, a glass micropipette can be used to inject the immunomodulating component directly into the extracellular vesicle at the microscopic level.

[00248] In certain embodiments, the immunomodulating component is introduced to the extracellular vesicle by extrusion.

[00249] In certain embodiments, the immunomodulating component is introduced to the extracellular vesicle by sonication. In some embodiments, extracellular vesicles are exposed to high intensity sound waves, causing transient disruption of the extracellular vesicle membrane allowing loading of an immunomodulating component.

[00250] In some embodiments, the immunomodulating component can be conjugated to the surface of the extracellular vesicle. Conjugation can be achieved chemically or enzymatically, by methods known in the art.

[00251] In some embodiments, the extracellular vesicle comprises an immunomodulating component that is chemically conjugated. Chemical conjugation can be accomplished by covalent bonding of the immunomodulating component to another molecule, with or without use of a linker. The formation of such conjugates is within the skill of artisans and various techniques are known for accomplishing the conjugation, with the choice of the particular technique being guided by the materials to be conjugated. In certain embodiments, polypeptides are conjugated to the extracellular vesicle. In certain other embodiments, non-polypeptides, such as lipids, carbohydrates, nucleic acids, and small molecules, are conjugated to the extracellular vesicle.

[00252] In some embodiments, the immunomodulating component is introduced to the extracellular vesicle by hypotonic lysis. In some of these embodiments, the extracellular vesicles are exposed to low ionic strength buffer causing them to burst allowing loading of an immunomodulating component. In some alternative embodiments, controlled dialysis against a hypotonic solution is used to swell the extracellular vesicle and to create pores in the extracellular vesicle membrane. The extracellular vesicle is subsequently exposed to conditions that allow resealing of the membrane.

[00253] In some embodiments, the immunomodulating component is introduced to the extracellular vesicle by detergent treatment. In certain embodiments, extracellular vesicles are treated with a mild detergent which transiently compromises the extracellular vesicle membrane by creating pores allowing loading of an immunomodulating component. After extracellular vesicles are loaded, the detergent is washed away thereby resealing the membrane.

[00254] In some embodiments, the immunomodulating component is introduced to the extracellular vesicle by receptor mediated endocytosis. In certain embodiments, extracellular

vesicles have a surface receptor which upon binding of the immunomodulating component induces internalization of the receptor and the associated immunomodulating component.

[00255] In some embodiments, the immunomodulating component is introduced to the extracellular vesicle by mechanical firing. In certain embodiments, extracellular vesicles can be bombarded with an immunomodulating component attached to a heavy or charged particle such as gold microcarriers. In some of these embodiments, the particle can be mechanically or electrically accelerated such that it traverses the extracellular vesicle membrane.

[00256] In some embodiments, the immunomodulating component is introduced to the extracellular vesicle by filtration. In certain embodiments, the extracellular vesicles and the immunomodulating component can be forced through a filter of pore size smaller than the extracellular vesicle causing transient disruption of the extracellular vesicle membrane and allowing the immunomodulating component to enter the extracellular vesicle.

[00257] In some embodiments, extracellular vesicles are subjected to several freeze thaw cycles, resulting in extracellular vesicle membrane disruption allowing loading of an immunomodulating component.

Methods of Isolating the Extracellular Vesicles

[00258] The extracellular vesicles can be isolated from the producer cells. In certain embodiments, the extracellular vesicle is released by the producer cell into the cell culture medium. It is contemplated that all known manners of isolation of extracellular vesicles are deemed suitable for use herein. For example, physical properties of extracellular vesicles can be employed to separate them from a medium or other source material, including separation on the basis of electrical charge (*e.g.*, electrophoretic separation), size (*e.g.*, filtration, molecular sieving, *etc.*), density (*e.g.*, regular or gradient centrifugation), Svedberg constant (*e.g.*, sedimentation with or without external force, *etc.*). Alternatively, or additionally, isolation can be based on one or more biological properties, and include methods that can employ surface markers (*e.g.*, for precipitation, reversible binding to solid phase, FACS separation, specific ligand binding, non-specific ligand binding, affinity purification *etc.*).

[00259] Isolation and enrichment can be done in a general and non-selective manner, typically including serial centrifugation. Alternatively, isolation and enrichment can be done in a more specific and selective manner, such as using extracellular vesicle or producer cell-specific surface markers. For example, specific surface markers can be used in

immunoprecipitation, FACS sorting, affinity purification, and magnetic separation with bead-bound ligands.

[00260] In some embodiments, size exclusion chromatography can be utilized to isolate the extracellular vesicles. Size exclusion chromatography techniques are known in the art. Exemplary, non-limiting techniques are provided herein. In some embodiments, a void volume fraction is isolated and comprises the extracellular vesicles of interest. Further, in some embodiments, the extracellular vesicles can be further isolated after chromatographic separation by centrifugation techniques (of one or more chromatography fractions), as is generally known in the art. In some embodiments, for example, density gradient centrifugation can be utilized to further isolate the extracellular vesicles. In certain embodiments, it can be desirable to further separate the producer cell-derived extracellular vesicles from extracellular vesicles of other origin. For example, the producer cell-derived extracellular vesicles can be separated from non-producer cell-derived extracellular vesicles by immunosorbent capture using an antigen antibody specific for the producer cell.

[00261] In some embodiments, the isolation of extracellular vesicles can involve combinations of methods that include, but are not limited to, differential centrifugation, size-based membrane filtration, immunoprecipitation, FACS sorting, and magnetic separation.

Methods of Measuring the Size of Extracellular Vesicles

[00262] In some embodiments, the methods described herein comprise measuring the size of extracellular vesicles and/or populations of extracellular vesicles. Generally, extracellular vesicle size is measured as the longest measurable dimension. Generally, the longest measurable dimension of an extracellular vesicle is also referred to as its diameter.

[00263] Extracellular vesicle size can be measured using dynamic light scattering (DLS) and/or multiangle light scattering (MALS). Methods of using DLS and/or MALS to measure the size of extracellular vesicles are known to those of skill in the art, and include the nanoparticle tracking assay (NTA, *e.g.*, using a Malvern NanoSight NS300 nanoparticle tracking device). In a specific embodiment, the extracellular vesicle size is determined using a Malvern NanoSight NS300. In some embodiments, the extracellular vesicles described herein have a longest dimension of about 20-300 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicles described herein have a longest dimension of about 40-200 nm as measured by NTA (*e.g.*, using a

Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 90% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 95% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 99% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 90% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 95% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 99% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by NTA (*e.g.*, using a Malvern NanoSight NS300).

[00264] Extracellular vesicle size can be measured using tunable resistive pulse sensing (TRPS). In a specific embodiment, extracellular vesicle size as measured by TRPS is determined using an iZON qNANO Gold. In some embodiments, the extracellular vesicles described herein have a longest dimension of about 20-300 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicles described herein have a longest dimension of about 40-200 nm as measured by TRPS (*e.g.*, an iZON qNano Gold). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 90% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 95% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 99% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicle

populations described herein comprise a population, wherein 90% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 95% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 99% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by TRPS (*e.g.*, using an iZON qNano Gold).

[00265] Extracellular vesicles size can be measured using electron microscopy. In some embodiments, the method of electron microscopy used to measure extracellular vesicle size is transmission electron microscopy. In a specific embodiment, the transmission electron microscope used to measure extracellular vesicle size is a Tecnai™ G^2 Spirit BioTWIN. Methods of measuring extracellular vesicle size using an electron microscope are well-known to those of skill in the art, and any such method can be appropriate for measuring extracellular vesicle size. In some embodiments, the extracellular vesicles described herein have a longest dimension of about 20-300 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicles described herein have a longest dimension of about 40-200 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 90% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 95% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicle populations described herein comprise a population, wherein 99% of the extracellular vesicles have a longest dimension of about 20-300 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicle populations described herein comprise a population wherein 90% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments,

the extracellular vesicle populations described herein comprise a population wherein 95% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope). In other embodiments, the extracellular vesicle populations described herein comprise a population wherein 99% of the extracellular vesicles have a longest dimension of about 40-200 nm as measured by a scanning electron microscope (*e.g.*, a Tecnai™ G^2 Spirit BioTWIN scanning electron microscope).

Methods of Treating Cancer, GvHD, and Autoimmune Disease

[00266] Also, provided herein are methods of treating cancer, graft-versus-host-disease (GvHD) and autoimmune disease in a subject.

[00267] In various embodiments, the composition is administered to a subject with cancer. In some of these embodiments, the composition can up-regulate an immune response and enhance the tumor targeting of the subject's immune system. In some embodiments, the cancer being treated is characterized by infiltration of leukocytes (T-cells, B-cells, macrophages, dendritic cells, monocytes) into the tumor microenvironment, or so-called "hot tumors" or "inflammatory tumors". In some embodiments, the cancer being treated is characterized by low levels or undetectable levels of leukocyte infiltration into the tumor microenvironment, or so-called "cold tumors" or "non-inflammatory tumors". In some embodiments, the composition is administered in an amount and for a time sufficient to convert a "cold tumor" into a "hot tumor", *i.e.*, said administering results in the infiltration of leukocytes (such as T-cells) into the tumor microenvironment.

[00268] In some embodiments, the composition comprising an extracellular vesicle and an immunomodulating component is administered to a subject as a cancer vaccine. In some of these embodiments, the composition is administered to a subject as a personalized cancer vaccine. In some embodiments, the immunomodulating component is a tumor antigen or a peptide derived from a tumor antigen. Examples of suitable tumor antigens include: alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), epithelial tumor antigen (ETA), mucin 1 (MUC1), Tn-MUC1, mucin 16 (MUC16), tyrosinase, melanoma-associated antigen (MAGE), tumor protein p53 (p53), CD4, CD8, CD45, CD80, CD86, programmed death ligand 1 (PD-L1), programmed death ligand 2 (PD-L2), NY-ESO-1, PSMA, TAG-72, HER2, GD2, cMET, EGFR, Mesothelin, VEGFR, alpha-folate receptor, CE7R, IL-3, Cancer-testis antigen, MART-1 gp100, and TNF-related apoptosis-inducing ligand. In certain

embodiments, the tumor antigen is derived from a reference genome sequence. In certain embodiments, the tumor antigen is derived a genome sequence of the subject receiving the composition.

[00269] The cancers that can be treated with the composition include but are not limited to the cancers listed in Table 5.

[00270] In certain embodiments, the composition is administered to a subject with graft-versus-host disease (GvHD). In some of these embodiments, the composition can down-regulate an immune response and alleviate the symptoms of GvHD. In some specific embodiments, the composition alleviates the symptoms of GvHD through activation of apoptotic signaling. In certain embodiments, the composition for treating GvHD comprises Fas ligand (FasL). In some of these embodiments, the FasL is expressed on the surface of the extracellular vesicle.

[00271] In various embodiments, the composition is administered to a subject with an autoimmune disease. In some of these embodiments, the composition can down-regulate an immune response and suppress the immune activity of the subject.

[00272] The autoimmune diseases include but are not limited to multiple sclerosis, peripheral neuritis, Sjogren's syndrome, rheumatoid arthritis, alopecia, autoimmune pancreatitis, Behcet's disease, Bullous pemphigoid, Celiac disease, Devic's disease (neuromyelitis optica), Glomerulonephritis, IgA nephropathy, assorted vasculitides, scleroderma, diabetes, arteritis, vitiligo, ulcerative colitis, irritable bowel syndrome, psoriasis, uveitis, and systemic lupus erythematosus.

[00273] In some embodiments, the composition is administered intravenously to the circulatory system of the subject. In some embodiments, the composition is infused in suitable liquid and administered into a vein of the subject.

[00274] In some embodiments, the composition is administered intra-arterialy to the circulatory system of the subject. In some embodiments, the composition is infused in suitable liquid and administered into an artery of the subject.

[00275] In some embodiments, the composition is administered to the subject by intrathecal administration. In some embodiments, the composition is administered *via* an injection into the spinal canal, or into the subarachnoid space so that it reaches the cerebrospinal fluid (CSF).

[00276] In some embodiments, the composition is administered intratumorally into one or more tumors of the subject.

[00277] In some embodiments, the composition is administered to the subject by intranasal administration. In some embodiments, the composition can be insufflated through the nose in a form of either topical administration or systemic administration. In certain embodiments, the composition is administered as nasal spray.

[00278] In some embodiments, the composition is administered to the subject by intraperitoneal administration. In some embodiments, the composition is infused in suitable liquid and injected into the peritoneum of the subject. In some embodiments, said intraperitoneal administration results in distribution of the composition (*e.g.*, the extracellular vesicles in the composition) to the lymphatics. In some embodiments, said intraperitoneal administration results in distribution of the composition (*e.g.*, the extracellular vesicles in the composition) to the thymus, spleen, and/or bone marrow. In some embodiments, said intraperitoneal administration results in distribution of the composition (*e.g.*, the extracellular vesicles in the composition) to one or more lymph nodes. In some embodiments, said intraperitoneal administration results in distribution of the composition (*e.g.*, the extracellular vesicles in the composition) to one or more of the cervical lymph node, the inguinal lymph node, the mediastinal lymph node, or the sternal lymph node. In some embodiments, said intraperitoneal administration results in distribution of the composition (*e.g.*, the extracellular vesicles in the composition) to the pancreas.

[00279] In some embodiments, the composition is administered to the subject by periocular administration. In some embodiments, the composition is injected into the periocular tissues. Periocular drug administration includes the routes of subconjunctival, anterior sub-Tenon's, posterior sub-Tenon's, and retrobulbar administration.

[00280] In some embodiments, the composition is administered into the same subject by multiple routes of administration. In some embodiments, said multiple routes of administration comprise intravenous administration, intra-arterial administration, intrathecal administration, intranasal administration, intratumoral administration, intraperitoneal administration, and/or periocular administration. In a preferred embodiment, said multiple routes of administration comprise intravenous administration and intraperitoneal administration.

[00281] In certain embodiments, the dosage of the extracellular vesicles is between 1ng to 10 ng, 10 ng to 100 ng, 100 ng to 1 μg, 1 μg to 5 μg, 5 μg to 10 μg, 10 μg to 50 μg, 50 μg to 75 μg, 75 μg to 100 μg, 100 μg to 150 μg, 150 μg to 200 μg, 200 μg to 300 μg, 300 μg to 500 μg, 500 μg to 1 mg, or 1mg to 10 mg.

[00282] The compositions can be administered once to the subject. Alternatively, multiple administrations can be performed over a period of time. For example, two, three, four, five, or more administrations can be given to the subject. In some embodiments, administrations can be given as needed, *e.g.*, for as long as symptoms associated with the disease, disorder or condition persists. In some embodiments, repeated administrations can be indicated for the remainder of the subject's life. Treatment periods can vary and can be, *e.g.*, no longer than a year, six months, three months, two months, one month, two weeks, one week, three days, two days, or no longer than one day.

[00283] In certain embodiments, doses of extracellular vesicles are administered at intervals such as once daily, every other day, once weekly, twice weekly, once monthly or twice monthly.

[00284] In some embodiments, the pharmaceutical composition is administered at a frequency sufficient to effectively increase the concentration of the immunomodulating component in the target cell or tissue above a level that is associated with a symptom of the disease, disorder or condition.

[00285] In some embodiments, the compositions are administered at least twice over a treatment period such that the disease, disorder or condition is treated, or a symptom thereof is ameliorated. In some embodiments, the compositions are administered at least twice over a treatment period such that the disease, disorder or condition is treated or a symptom thereof is prevented. In some embodiments, the pharmaceutical composition is administered a sufficient number of times over a treatment period such that a sufficient amount of immunomodulating component is delivered to the target cell or tissue during the treatment period. In some embodiments, the pharmaceutical composition is administered a sufficient number of times over a treatment period such that a sufficient amount of immunomodulating component is delivered to the target cell or tissue during the treatment period such that one or more symptoms of the disease, disorder or condition is prevented, decreased, ameliorated or delayed. In some embodiments, increasing the immunomodulating component concentration in the target cell or tissue includes increasing the peak concentration, while in others it

includes increasing the average concentration. In some embodiments, a substantial increase during the treatment period can be determined by comparing a pretreatment or post-treatment period in the subject, or by comparing measurements made in a population undergoing treatment with a matched, untreated control population.

[00286] In some embodiments, the pharmaceutical composition is administered a sufficient number of times per treatment period such that the concentration of immunomodulating component in the target cell or tissue is increased for at least about one week, two weeks, three weeks, four weeks, one month, two months, three months, four months, five months, six months or greater than six months. In some embodiments, the pharmaceutical composition is administered a sufficient number of times per treatment period such that the concentration of immunomodulating component in the target cell or tissue is increased for a period of time at least as long as the treatment period.

[00287] In some embodiments, the time interval between repeated administrations within a treatment period is no longer than the period in which the number of extracellular vesicles in circulation is reduced to less than about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the number of extracellular vesicles present in the administered pharmaceutical composition.

[00288] In some embodiments, the methods further comprise one or multiple doses of non-therapeutic extracellular vesicles prior to the injection of a suitable therapeutic dose of extracellular vesicles harboring a therapeutic agent. In certain embodiments, the non-therapeutic extracellular vesicle is administered separately to and at a different dosage than the therapeutic extracellular vesicles. In certain embodiments, the dosage of the non-therapeutic extracellular vesicle is greater than the dosage of the therapeutic extracellular vesicle. In certain other embodiments, the dosage of the non-therapeutic extracellular vesicle is smaller than the dosage of the therapeutic extracellular vesicle. In certain embodiments, the dosage of the non-therapeutic extracellular vesicle is the same as the therapeutic extracellular vesicle. In various embodiments, the methods of non-therapeutic extracellular vesicles prior to injection of a suitable dose of therapeutic extracellular vesicles reduce the update of the therapeutic extracellular vesicles in the liver, lung, and/or spleen.

[00289] An effective amount of the composition is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the extracellular vesicle (*e.g.*, size, and in some cases the extent of molecules to be delivered)

and other determinants. In general, an effective amount of the composition provides efficient cellular response of the target cell. Increased efficiency can be demonstrated by increased cell transfection (*i.e.*, the percentage of cells transfected with the extracellular vesicle constituents), increased cellular response or reduced innate immune response of the host subject.

[00290] The dosing and frequency of the administration of the extracellular vesicles and pharmaceutical compositions thereof can be determined, *e.g.*, by the attending physician based on various factors such as the severity of disease, the patient's age, sex and diet, the severity of any inflammation, time of administration and other clinical factors. In an example, an intravenous administration is initiated at a dose which is minimally effective, and the dose is increased over a pre-selected time course until a positive effect is observed. Subsequently, incremental increases in dosage are made limiting to levels that produce a corresponding increase in effect while taking into account any adverse effects that can appear.

EXAMPLES

[00291] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations can be used, *e.g.*, bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); nt, nucleotide(s); and the like.

[00292] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. *See, e.g.*, T.E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's

Pharmaceutical Sciences, 21th Edition (Easton, Pennsylvania: Mack Publishing Company, 2005); Carey and Sundberg Advanced Organic Chemistry 3rd Ed. (Plenum Press) Vols A and B(1992).

Methods

Exosome Purification

[00293] Conditioned culture media was collected and centrifuged at 300 – 800 x g for 5 minutes at room temperature to remove cells and large debris. Media supernatant was then supplemented with 1000 U/L Benzonase® and incubated at 37 °C for 1 hour in a water bath. Supernatant was collected and centrifuged at 16,000 x g for 30 minutes at 4 °C to remove residual cell debris and other large contaminants. Supernatant was then ultracentrifuged at 133,900 x g for 3 hours at 4 °C to pellet the exosomes. Supernatant was discarded and any residual media was aspirated from the bottom of the tube. The pellet was resuspended in 200 – 1000 µL PBS (-Ca -Mg).

[00294] To further enrich exosome populations, the pellet was processed *via* density gradient purification (sucrose or Optiprep™). For sucrose gradient purification, the exosome pellet was layered on top of a sucrose gradient as defined in Table 6 below:

Table 6, Sucrose Density Gradient:

Working Percentage (%)	65% Stock Vol. (mL)	Milli-Q Vol. (mL)
50	3.85	1.15
40	3.08	1.92
25	1.92	3.08
10	0.46	2.54

[00295] The gradient was spun at 200,000 x g for 16 hours at 4 °C in a 12 mL Ultra-Clear (344059) tube placed in a SW 41 Ti rotor to separate the exosome fraction.

[00296] The exosome layer was gently removed from the top layer and diluted in ~32.5 mL PBS in a 38.5 mL Ultra-Clear (344058) tube and ultracentrifuged again at 133,900 x g for 3 hours at 4 °C to pellet the purified exosomes. The resulting pellet was resuspended in a minimal volume of PBS (~200 µL) and stored at 4 °C.

[00297] For Optiprep™ gradient, a 3-tier sterile gradient was prepared with equal volumes of 10%, 30%, and 45% Optiprep in a 12 mL Ultra-Clear (344059) tube for a SW 41 Ti rotor. The pellet was added to the Optiprep™ gradient and ultracentrifuged at 200,000 x g for 16

hours at 4 °C to separate the exosome fraction. The exosome layer was then gently collected from the top ~3 mL of the tube.

[00298] The exosome fraction was diluted in ~32 mL PBS in a 38.5 mL Ultra-Clear (344058) tube and ultracentrifuged at 133,900 x g for 3 hours at 4 °C to pellet the purified exosomes. The pelleted exosomes were then resuspended in a minimal volume of PBS (~200 μL) and store at 4 °C.

Example 1: Engineering Exosomes to Display an Immune Checkpoint Regulator Antibody

[00299] A human embryonic kidney (HEK) cell line is grown to high density, and the resulting exosomes are isolated from culture medium according to methods known to those of skill in the art (*e.g.*, the methods described herein). Exosomes engineered with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody are prepared by chemical conjugation according to the techniques known in the art. The exosomes modified with CTLA4 antibody are selected by flow cytometry. At the same time, unmodified exosomes are isolated according to the same standard methods.

[00300] The two exosome populations are labeled with a radioactive tracer, and 150 μg of each preparation is injected into live mice (*e.g.* mouse model of melanoma). The mice receiving either the exosomes displaying the CTLA-4 antibody or the unmodified exosomes are monitored continuously for 30 minutes, and again at four hour intervals by whole-animal PET/CT. Whole-animal imaging allows for real-time, high resolution tracking of labeled exosomes to various tissues.

[00301] 150 μg of each exosome population are injected into two mouse cohorts intravenously without first labeling with a radioactive tracer. The mice are euthanized five weeks post-administration. The tumor samples are collected and analyzed by immunohistochemistry and real-time PCR.

Example 2: Engineering Exosomes to Display Fas Ligand

[00302] Human antigen-presenting cells are transfected with a plasmid encoding a puromycin-resistant selectable marker and Fas ligand. Transfected cells are treated with puromycin, and resistant colonies are selected and assayed for surface expression of Fas ligand by flow cytometry. Stable Fas ligand-expressing cells are grown to high concentration, and the resulting exosomes are isolated from culture medium according to methods known to

those of skill in the art (*e.g.*, the methods described herein). At the same time, untransfected producer cells are cultured and the resulting exosomes are isolated according to the same standard methods.

[00303] The two exosome populations are labeled with a radioactive tracer, and 150 μg of each preparation is injected into live mice (*e.g.* mouse model of GvHD). The mice receiving either the exosomes derived from unmodified cells or the exosomes derived from Fas ligand-expressing cells are monitored continuously for 30 minutes, and again at four hour intervals by whole-animal PET/CT. Whole-animal imaging allows for real-time, high resolution tracking of labeled exosomes to various tissues.

[00304] Purified exosome populations from unmodified producer cells and producer cells engineered to express Fas ligand are purified according to the methods described herein. 150 μg of each exosome population are injected into two mouse cohorts without first labeling with a radioactive tracer. Animals of both cohorts are euthanized three to five weeks post-administration for immunohistochemical analysis and real-time PCR.

Example 3: Lymphatic Uptake of Exosomes after Intraperitoneal Administration

[00305] To determine the biodistribution of purified exosomes *in vivo*, the following experiment was performed:

[00306] Conditioned culture media from 293T cells was collected and centrifuged at 300 – 800 x g for 5 minutes at room temperature to remove cells and large debris. Media supernatant was then supplemented with 1000 U/L Benzonase® and incubated at 37 °C for 1 hour in a water bath. Supernatant was collected and centrifuged at 16,000 x g for 30 minutes at 4 °C to remove residual cell debris and other large contaminants. Supernatant was then ultracentrifuged at 133,900 x g for 3 hours at 4 °C to pellet the exosomes. Supernatant was discarded and residual media was aspirated from the bottom of the tube. The pellet was then resuspended in 200 – 1000 μL PBS (-Ca -Mg).

[00307] To further enrich exosome populations, the pellet was processed via sucrose density gradient purification as defined in Table 6.

[00308] The gradient was spun at 200,000 x g for 16 hours at 4 °C in a 12 mL Ultra-Clear (344059) tube placed in a SW 41 Ti rotor to separate the exosome fraction.

[00309] The exosome layer was gently removed from the top layer and diluted in ~32.5 mL PBS in a 38.5 mL Ultra-Clear (344058) tube and ultracentrifuged again at 133,900 x g for 3

hours at 4 °C to pellet the purified exosomes. The resulting pellet was resuspended in a minimal volume of PBS (~200 µL) and stored at 4 °C.

[00310] To radiolabel the purified exosomes for *in vivo* imaging, $1x10^{11}$ purified exosomes in 100 µL were diluted with HEPES (200 µL, 0.1M, pH 8.5) and conjugated to p-SCN-Bn-DFO (5 µg) for one hour at 37°C followed by overnight incubation at 4°C, separately. DFO-exosomes were incubated with 89Zr (7.5mCi) diluted in HEPES (100 µL, 1M, pH 7.3) for one hour at 37°C and purified on a qEv column. This resulted in a total yield (0.4 mCi of 89Zr-DFO-exosomes in up to 0.8 mL PBS) at 100 µCi/$1x10^{10}$ exosomes. Quality control (HPLC) was performed prior to release to ensure >95% RCP.

In vitro stability

[00311] Exosomes (20 µCi/$2x10^{10}$) were incubated at room temperature in:

a. Formulation buffer
b. Mouse serum (10% v/v exosome solution in serum, if possible)

[00312] 2 hours after initiation of incubation solutions were injected into HPLC to determine stability of tracer.

***In vivo* imaging**

[00313] Mice (SKH-1, n=8, age 5-8 weeks) were randomized into two groups, weighed and injected (with the second group injected immediately after the first group's dynamic scan is over) with $1x10^{10}$/g exosomes to give a minimum radioactive dose of 100 µCi/mouse. Group 1 was injected intravenously (IV) while group 2 was injected intraperitoneally (IP).

[00314] Mice receive a whole-body PET/CT scan in a 4-mouse hotel using the following schedule: 1h dynamic (5x60, 5x180, 8x300 seconds) and static imaging at 4h (20 min), 24h (Thursday, 20min) and 48h (Friday, 30min). Each imaging time point was followed by CT for anatomical reference.

[00315] After the last imaging time point, mice were euthanized and the following organs were collected, weighed and counted in the gamma counter: blood, lung (one), liver (lobe), spleen, pancreas, kidney (one), liver, colon and additional organs of high uptake.

[00316] Organs were allowed to decay for 2-3 days if counts were extremely high and counted again.

Table 7

Group (mouse	Tracer	Injection route	Imaging	Imaging time

# and type)				points
1 (n=4, SKH-1)	^{89}Zr-DFO-exosomes (100 μCi, <200 μL)	IV	Whole body PET/CT using a 4 mouse hotel	1h dynamic followed by static at 4h and 24h (20min) 48h (30min)
2 (n=4, SKH-1)	^{89}Zr-DFO-exosomes (100 μCi, <200 μL)	IP	Whole body PET/CT using a 4 mouse hotel	1h dynamic followed by static at 4h and 24h (20min) 48h (30min)

Results

[00317] The two cohorts of treated mice were imaged 4 hours, 24 hours, and 48 hours after treatment. Whole body PET/CT imaging revealed robust delivery to the livers of all mice in group 1 treated IV (Figure 1A), and a distinct non-overlapping distribution for mice in group 2 treated IP (Figure 1B). Organs were dissected and analyzed by radiographic gamma counter, which revealed significant liver and spleen uptake in mice treated IV (Figure 2). In contrast, for mice treated IP, uptake was primarily observed in the pancreas, spleen, thymus, and lymph nodes, with additional uptake in the liver and ovaries. These results demonstrate that different routes of administration result in substantially different biodistribution profiles. Importantly, IP administration led to significant uptake in the lymphatics, suggesting that IP administration can be a suitable route of administration to reach immune cells.

Example 4: B-Cell Activation by Engineered CD40L Exosomes

[00318] CD40L is a member of the tumor necrosis factor (TNF) superfamily primarily expressed on T-cells. The CD40L receptor, CD40, is expressed on antigen presenting cells including macrophages, dendritic cells and B-cells. Signaling through CD40 activates B-cells and induces an antigen-specific response. Activating the CD40 pathway therefore has implications in the development of anti-tumor immunity in a broad array of tumor types. To determine whether engineered exosomes could be generated to induce a specific immunological effect, exosomes were generated from HEK293SF cells transfected with a plasmid containing full-length human CD40L. Transfected cells were put under puromycin selection and resistant cell populations were grown to high density. The resulting exosomes were collected from the conditioned culture medium and purified over an Optiprep™ gradient as described above. Exosomes from unmodified HEK293SF cells were also isolated to be used as a control. Human peripheral blood mononuclear cells (PBMCs) were plated at

150,000 cells per well of a 96-well plate, and incubated with purified CD40L exosomes or native exosomes overnight at 37 ºC. One sample of PBMCs was incubated with 1 μg/mL of soluble recombinant CD40L-Fc as a positive control. As shown in Figures 3A and 3B, CD40L exosomes activated B-cells in a dose-dependent manner, as measured by CD69 expression in two different donor samples. Native exosomes failed to induce B-cell activation. Importantly, the level of B-cell activation by CD40L exosomes was comparable to the activation caused by the CD40L-Fc.

[00319] To determine whether the observed exosome-mediated B-cell activation was due to direct activation of B-cells or through trans-acting immune cells, a similar experiment was carried out using purified human B-cells. 50,000 purified human B-cells were plated in a 96-well plate and incubated with either CD40L exosomes, native exosomes, or CD40L-Fc. One sample of high concentration CD40L exosomes was put through a freeze-thaw cycle (CD40L-EVs [F/T]) and tested for B-cell activation as well. As shown in Figures 4A and 4B, CD40L exosomes activated purified B-cells from two donors to a similar extent as CD40L-Fc. Native exosomes failed to activate B-cells, while the CD40L exosome freeze-thaw samples successfully activated B-cells, indicating that the effect of CD40L exosomes is mediated directly through B-cells, and that the presence of CD40L is sufficient for B-cell activation. Additionally, the engineered exosomes remain stable and active for at least one freeze-thaw cycle.

[00320] To further validate the CD40L exosomes, a reporter system was used to measure the activity of the engineered exosomes. Activation of CD40 pathway results in activation of NF-κB. Using a modified U2OS cell line engineered to overexpress CD40 on its surface and contain a luciferase reporter downstream of the NF- κB promoter (Promega Corporation), CD40 activation was confirmed by incubating the cells in the presence of an agonistic anti-CD40 antibody (BioLegend, Inc.) crosslinked with an anti-Fc antibody (Jackson ImmunoResearch, Inc.) or recombinant human CD40L (ACROBiosystems) cross-linked with an anti-IgG antibody (Jackson ImmunoResearch, Inc.) (Figures 5A and 5B). CD40L engineered exosomes were incubated with the engineered cells and resulted in a robust increase in luciferase activity comparable to the effects of anti-CD40 + anti-Fc. Importantly, the engineered exosomes did not require a cross-linking antibody, demonstrating that CD40L on the surface of exosomes can form functional CD40L trimers sufficient to activate CD40.

Example 5: T-Cell Activation by Engineered CD80 Exosomes

[00321] CD80 is expressed on antigen presenting cells and binds to CD28 and CTLA-4 on the surface of T-cells. Stimulation by CD80 (and CD86) through CD28 and CTLA-4 activates T-cells during the initiation of an immune response. To determine whether exosomes could be engineered to activate T-cells, CD80-containing exosomes were generated by transfection and selection of HEK293SF cells as described in Example 4. To validate the activity of CD80 exosomes, human PBMCs were plated at 150,000 cells per well of a 96-well plate, and incubated with (i) purified CD80 exosomes and anti-CD3 antibody, (ii) native exosomes and anti-CD3 antibody, (iii) anti-CD3 antibody alone, or (iv) a combination of anti-CD28 and anti-CD3 antibodies. The samples were incubated at 37 °C for three days and assayed for T-cell counts for both $CD4^+$ T-cells (Figure 6A) and $CD8^+$ T-cells (Figure 6B). CD80 exosomes activated T-cells in a dose-dependent manner and to an extent comparable to the positive control of CD3 and CD28 antibodies. In contrast, the native exosomes had no effect on T-cell proliferation.

[00322] To confirm that CD80 exosomes induce a functional activation of T-cells, IFNγ levels were measured by AlphaLISA in PBMCs incubated with native exosomes and CD80 exosomes with additional anti-CD3 antibody. As shown in Figure 7A, there was a dose-dependent increase in IFNγ levels for the CD80 exosomes but not for the native exosomes. As shown in Figure 7B, the highest concentrations of CD80 exosomes resulted in greater IFNγ levels than any other condition, including the positive control (anti-CD28/anti-CD3). These results demonstrate that exosomes can be engineered with specific activity that results in immune cell activation.

Example 6: Pro-Inflammatory Cytokine Production by Engineered CD27L and OX40L Exosomes

[00323] CD27L (CD70) and OX40L are members of the TNF super-family, and bind to cognate receptors (CD27 and OX40, respectively) on T-cells. CD27L is expressed by certain populations of T- and B-cells, while OX40L is expressed by certain populations of antigen presenting cells. Signaling through CD27 or OX40 therefore have implications in immuno-oncology, specifically as a method of activating anergic T-cells. To determine whether exosomes could be engineered to induce pro-inflammatory cytokine production in PBMCs, CD27L- and OX40L-containing exosomes were generated by transfection and selection of HEK293SF cells as described in Example 4. To validate the activity of CD27L exosomes, human PBMCs were plated in a 96-well plate, and incubated with purified CD27L exosomes

and anti-CD3 antibody, native exosomes and anti-CD3 antibody, anti-CD3 antibody alone, or a combination of anti-CD28 and anti-CD3 antibodies. The samples were incubated at 37C for two days and assayed for Interferon Gamma (IFNγ) production (Figures 8A and 8B) and IL-2 production (Figures 9A and 9B) in two different donors. CD27L exosomes induced IFNγ and IL-2 production in a dose-dependent manner and to an extent comparable to (Donor 1) or significantly more than (Donor 2) the positive control of CD3 and CD28 antibodies. In contrast, the native exosomes had no effect on IFNγ or IL-2 production. Similarly, OX40L exosomes were sufficient to induce IFNγ and IL-2 production in two different donors to a similar or greater extent (Figures 10A and 10B and Figures 11A and 11B).

[00324] To further validate the OX40L exosomes, a report system was used to measure the activity of the engineered exosomes. Activation of the OX40 pathway results in activation of NF-κB. Using a modified Jurkat T-cell line engineered to overexpress OX40 on its surface and contain a luciferase reporter downstream of the NF- κB promoter (Promega Corporation), OX40 activation was confirmed by incubating the cells in the presence of an agonistic anti-OX40 antibody (Biolegend) crosslinked with an anti-Fc antibody (Jackson ImmunoResearch, Inc.) or recombinant human OX40L (ACROBiosystems) cross-linked with an anti-IgG antibody (Jackson Immunoresearch) (Figures 12A and 12B). The anti-OX40L antibody crosslinked with anti-IgG failed to activate the reporter cells, while the recombinant OX40L cross-linked with anti-Fc led to a robust activation of the reporter gene (Figure 12B). Strikingly, the engineered OX40L exosomes induced reporter gene expression to a greater extent than either the anti-OX40 antibody or the recombinant OX40L (Figure 12C). Importantly, the engineered exosomes did not require a cross-linking antibody, demonstrating that OX40L on the surface of exosomes can form functional OX40L trimers sufficient to activate OX40.

Example 7: T-Cell Activation by IL-7 Engineered Exosomes

[00325] IL-7 is a cytokine involved in B-cell and T-cell proliferation and has implications in immunotherapy. Specifically, IL-7 may activate T-cells and induce a tumor antigen response in tumors that are poorly infiltrated by leukocytes or in tumor microenvironments that have induced T-cell anergy. IL-7 signaling through the heterodimeric IL-7 receptor induces Interferon Gamma (IFNγ) signaling, which can enhance tumor-specific antigen response by T-cells. To determine whether exosomes could be engineered to induce T-cell activation, IL-7-containing exosomes were generated by transfection and selection of HEK293SF cells with the pDisplay™ plasmid (ThermoFisher) encoding a fusion of IL-7 and

PDGF Receptor. The engineered exosomes were purified as described in the Methods. To validate the activity of IL-7 exosomes, human PBMCs were plated in a 96-well plate, and incubated with purified IL-7 exosomes and anti-CD3 antibody, native exosomes and anti-CD3 antibody, anti-CD3 antibody alone, or a combination of anti-CD28 and anti-CD3 antibodies. The samples were incubated at 37 °C for two days and assayed for IFNγ (Figures 13A and 13B). IL-7 exosomes in combination with anti-CD3 antibody induced peak IFNγ production to a greater extent than anti-CD3 alone (Figure 13A). Additionally, IL-7 exosomes induced IFNγ in a dose-dependent manner and to an extent comparable to the positive control of CD3 and CD28 antibodies. In contrast, the native exosomes had no effect on IFNγ production (Figure 13B).

[00326] The IL-7 receptor is a heterodimeric complex consisting of IL-7R and IL-2RG, which form a ternary complex in the presence of IL-7 and induces downstream signaling through the JAK/STAT pathway, resulting in cell proliferation. A synthetic cell-based assay was used to measure IL-7 signaling through the IL-7 receptor to assess the functional activity of engineered IL-7 exosomes (DiscoverX Corporation) (Figure 14A). Recombinant human IL-7 (rhIL-7) was sufficient to increase signaling through the IL-7 receptor (Figure 14B), and engineered IL-7 exosomes were able to induce signaling through the IL-7 receptor while native exosomes were not (Figure 14C). These data demonstrate that IL-7-expressing exosomes are sufficient to induce signaling through the IL-7 receptor *in vitro*.

[00327] To determine whether the effects of IL-7 exosomes observed *in vitro* could be recapitulated in an *in vivo* model, the IL-7 exosomes were administered to C57BL/6 mice. A cohort of 20 mice were separated into the following groups: (1) PBS, (2) recombinant human IL-7 (rhIL-7), (3) IL-7 engineered exosomes, and (4) unmodified native exosomes. Five mice in each group were injected intraperitoneally (IP) with 1 mg of EdU and either PBS, $1x10^{11}$ native or IL-7 exosomes, or 10 μg of rhIL-7 once daily for three days. Mice were sacrificed, spleens were isolated, and EdU levels were measured in splenic cells by flow cytometry. As shown in Figure 15A, the percent-positive CD8+ T-cell were significantly increased in the IL-7 exosome mice and the rhIL-7 mice compared to the control cohorts. Although the T-cell counts in IL-7 exosome mice were lower than the rhIL-7 cohort, it is estimated that there was five-fold fewer IL-7 molecules administered in the IL-7 exosome cohort (data not shown). A similar trend was observed for Memory CD8+ T-cells by measuring the levels of the memory marker CD45RO (Figure 15B).

[00328] As an orthogonal approach, the levels of CD71 (Transferrin receptor) were measured in splenic cells isolated from exosome-treated mice. CD71 is required for proliferation, and CD71 levels correlate with T-cell number. As shown in Figures 15A and 15B, CD8+ T-cell and Memory CD-8+ T-cell numbers followed the same trend as observed in Figure 16A and 16B. Together, these data demonstrate that engineered exosomes can induce a specific immune cell effect *in vivo*, and that this activation can be more potent on a per-molecule basis compared to recombinant agonists.

Example 8: IL-7 Fusion to Proprietary Scaffolds Enhances Specific Activity

[00329] To enhance the activity of IL-7 engineered exosomes, the IL-7 sequence was fused to a truncated portion of PTGFRN, a novel exosome transmembrane protein that is highly expressed on the surface of HEK293SF exosomes. IL-7 was expressed as a translational fusion upstream of a short fragment of PTGFRN encompassing the region before the C-terminal-most IgV domain, the transmembrane domain, and the intracellular domain of PTGFRN, as well as a FLAG tag. A series of expression constructs was generated by introducing a series of four amino acid deletions between IL-7 and PTGFRN (Figure 17A). The resulting constructs were numbered pX-1 through pX-4 (pX-4 complete sequence shown in Figure 17B). As shown by Western blot analysis using an anti-IL-7 antibody, constructs pX-3 and pX-4 showed the highest levels of expression. The level of IL-7 expression in the PTGFRN backbone was dramatically higher than pDisplay-IL-7, which was used in Example 7 (Figure 18A). The increased expression of IL-7 suggested that these novel fusion proteins could induce a much greater level of IL-7-mediated T-cell activation. To determine the potency of PTGFRN-IL-7 fusions, an *in vitro* model of T-cell activation was carried out. Upon IL-7-mediated activation of T-cells, IL-7 receptor (IL-7R) levels decrease in a dose-dependent manner within 24 hours (Ghawazi *et al.*, Immunol Cell Biol. 2013 Feb;91(2):149-58). Thus, IL-7R levels were monitored after incubation of PBMCs with various IL-7 engineered exosomes. As shown in Figure 18B, native exosomes failed to reduce IL-7R levels, while pDisplay-IL-7 exosomes (IL-7-pD) reduced IL-7R levels only at high doses. In contrast, PTGFRN-IL-7 exosomes (IL-7-pX3 to pX4) completely reduced IL-7R levels at much lower doses, demonstrating an increased potency of these engineered exosomes. As a measure of IC50, the PTGFRN-IL-7 exosomes were 20- to 76-fold more potent than the IL-7-pD exosomes (Table 2), demonstrating that increased ligand density is sufficient to increase biological potency. Furthermore, these results demonstrate that specific truncations of PTGFRN may be ideal scaffolds for use in engineering therapeutic exosomes.

Table 8

Exosomes	pX1	pX2	pX3	pX4	pD
IC50 (p/ml)	4.2E+09	5.4E+09	1.4E+09	1.5E+09	1.1E+11
Fold increase in potency	25.6	19.8	76.5	71.0	N/A

Example 9: Exosomes Engineered with Anti-CD3 Antibody Fragments

[00330] As shown in the previous examples, exosomes can be engineered to overexpress functional endogenous sequences of immunomodulatory proteins. To determine if synthetic agonists can be engineered on the surface of exosomes, anti-CD-3 antibodies were expressed as fusions to either pDisplay as described in Example 4, or the transmembrane domain of CD80. Human PBMCs were plated in a 96-well plate at 100,000 cells per well and incubated overnight with exosomes engineered to express an anti-CD3 single chain Fv (scFv) (Figures 19A and 19B) or single chain Fab (scFab) (Figures 20A and 20B). As a positive control, PBMCs were incubated with ImmunoCult™ CD3/CD28 Activator (Stem Cell Technologies) according to the manufacturers' protocol. In the presence of anti-CD28 co-stimulation, all engineered exosomes induced T-cell (Figures 19A and 20A) and B-cell (Figures 19B and 20B) activation comparable to the positive control, while the non-engineered exosome controls did not. To measure the effects of anti-CD3 exosomes on immune cell populations, T-cell and B-cells were assayed for CD69 positivity by flow cytometry. As shown in Figure 21A, PBMCs incubated with exosomes expressing anti-CD3 scFv fused to the CD80 transmembrane domain led to activation of ~40% of T-cells. Similar effects were observed for the activation of B-cells (Figure 21B).

[00331] To determine whether anti-CD-3 exosome-mediated T-cell activation was due to direct T-cell activation or through trans-acting immune cells, activation of purified T-cells was measured. 100,000 purified human T-cells were plated in 96-well format in wells that were pre-coated with a non-targeting antibody or anti-CD3 exosomes in the presence or absence of anti-CD28 antibody, or in wells that were incubated with soluble anti-CD3 exosomes in the presence or absence of anti-CD28 antibody. As shown in Figure 22A, both soluble and plate-coated anti-CD3 scFv exosomes activated T-cells in the presence of anti-CD28 antibody as measured by CD69 expression. As shown in Figure 22B, plate-coated anti-CD3 antibody in the presence of anti-CD28 antibody activated T-cells to the same extent as plate-coated anti-CD3 scFv in the presence of anti-CD28 antibody. Strikingly, while soluble anti-CD3 antibody in the presence of anti-CD28 antibody was sufficient to activate ~30% of

T-cells, soluble anti-CD3 scFv exosomes in the presence of anti-CD28 antibody activated a significantly higher proportion of T-cells, demonstrating that exosomes engineered to overexpress an antibody fragment can induce higher levels of T-cell activation compared to soluble antibody. Together, these results demonstrate that exosomes can be engineered to overexpress antibody fragments with functional activity against specific cell types.

Example 10: IL-12-PTGFRN Exosomes Have Potent Immunomodulatory Activity *in vitro* and *in vivo*.

[00332] IL-12 is a potent immunostimulatory cytokine produced by antigen presenting cells in response to infection and other antigenic stimulation. IL-12 production by activated dendritic cells, macrophages, and neutrophils induces IFNγ production by both CD8+ and CD4+ T-cells and induces cytotoxic effects of Natural Killer (NK) cells. The combined impact of IL-12 secretion in the tumor microenvironment results in the secretion of Th1 cytokines including IFNγ, leading to tumor cell killing, reprogramming of myeloid-derived suppressor cells (MDSCs) and anti-angiogenic effects. IL-12-mediated anti-tumor effects result in a durable T-cell response and anti-tumor immunity in numerous animal models. IL-12 has previously been tested as an immunotherapy agent in humans but resulted in significant toxicity in renal cell carcinoma patients despite a detectable induction of a robust IFNγ response (Leonard *et al.*, Blood. 1997 Oct 1;90(7):2541-8). Exosomes therefore may represent an ideal delivery modality for IL-12 due to the high local concentration of the cytokine and presumed tumor-retained pharmacology.

[00333] IL-12 consists of two domains, p35 and p40. The human IL-12 dimer was encoded as a fusion protein to either full-length PTGFRN (Figure 23A, construct 871, SEQ ID NO: 3) or a shortened fragment of PTGFRN that enables high-density surface display (Figure 23B, construct 873, SEQ ID NO: 5), and the constructs were stably expressed in HEK293SF cells. Stable cell lines were grown in chemically defined media and the exosomes from the culture supernatant were purified over an Optiprep™ gradient as described in the Methods. The amount of IL-12 protein on the surface of the exosomes was measured by ELISA and concentration-matched to the rIL-12 for all functional studies. Purified full-length and short hIL-12-PTGFRN exosomes or recombinant hIL-12 (rhIL-12; BioLegend, Catalog No. 573004) were titrated in human PBMCs in the presence of a sub-optimal concentration anti-CD3 antibody to induce IFNγ expression. rhIL-12 resulted in robust IFNγ expression with an EC_{50} of 0.029 ng/ml, which was comparable to full-length IL12-PTGFRN, both of which were ~10x more potent than IL12-short-PTGFRN (Figure 24A-B). These results

suggest that IL-12 displayed on the full-length PTGFRN scaffold may be a more potent immunomodulating reagent than the short PTGFRN construct.

[00334] Mouse and human IL-12 proteins do not cross-react, and the *in vitro* data shown in Figure 24 suggest that mIL-12 fused to full-length PTGFRN would be more a more potent than using the short scaffold of PTGFRN. To determine the potency of mIL-12-PTGFRN exosomes in an *in vivo* model of cancer, C57BL/6 mice were implanted subcutaneously with $1x10^6$ B16F10 murine melanoma cells (n=5 mice per group). On days 5, 6, and 7 after tumor inoculation the animals were injected intratumorally with PBS, 0.2μg of recombinant murine IL-12 (mIL12; BioLegend, Catalog No. 577004), or $1x10^{11}$ exosomes displaying full-length IL-12-PTGFRN (mIL12-Exosomes; SEQ ID NO: 4). Animals were sacrificed once tumor volumes reached 2,000mm^3. As shown in Figures 25-27, tumors in the PBS group grew rapidly while tumors in the rmIL12 and mIL12-Exo groups were dramatically reduced (~65-80% reduction in volume). Importantly, by day 16, tumors in the mIL12-Exo group were smaller than those in the rmIL12 group demonstrating superior efficacy of IL-12 when displayed on the surface of exosomes compared to the soluble cytokine. There was also a survival advantage for the IL-12 treated groups compared to the PBS treated groups (Figure 28).

[00335] To understand the mechanistic advantage of IL-12-PTGFRN-exosomes over rmIL12, Th1 gene expression was profiled in the tumors of the control and treated groups. IFNγ (Figure 29A), the T-cell chemoattractants CXCL9 (Figure 29B) and CXCL10 (Figure 29C), and TGFβ (Figure 29D) were all increased in the IL-12 treated groups compared to the control group. In most cases, the cytokine signals were higher in the animals treated with mIL12-Exo compared to rmIL-12. IFNγ levels in splenic CD8+ T-cells were measured by flow cytometry, and the Exo-mIL-12-treated mice showed significantly greater signal than either the PBS group or the rmIL-12 group (Figure 30). Together, these data demonstrate that IL-12 displayed on the surface of an exosome represents a novel and potent immunomodulatory strategy that promotes robust T-cell activation *in vitro* and can be used to elicit potent anti-tumor effects in an aggressive model of murine melanoma *in vivo*. Mechanistically, the IL-12 exosomes show superiority over rIL-12, and thus represent a novel, differentiated therapeutic modality in cancer immunotherapy.

Example 11: Interferon Gamma-Displaying Exosomes Are Potent Immune Cell Activators

[00336] Interferon gamma (IFNγ) is a cytokine involved in priming innate and adaptive immune responses. It is expressed from a variety of cell types in response to numerous signals including IL-12, and is sufficient to activate NK cells, drive antigen presentation in antigen presenting cells, and promote leukocyte activation and invasion. IFNγ is naturally expressed as a homodimer and is secreted as a soluble factor. IFNγ expressing exosomes were generated by stably transfecting HEK293SF cells with full-length PTGFRN fused to monomeric or dimeric human and mouse IFNγ (Figures 31A and 31B, respectively). Exosomes from suspension cell cultures were purified as described above and analyzed by PAGE. Monomeric (m) and tandem dimer (td) PTGFRN IFNγ exosomes were expressed at the predicted molecular weights (arrow heads) at comparable levels (Figure 32). The purified exosomes were analyzed by ELISA and compared to a standard curve using recombinant IFNγ (Biolegend, Catalog No. 570206) to calculate the number of IFNγ molecules per exosome. The results in Table 9 show the number of IFNγ molecules in each of the four types of purified exosomes. Notably, the tandem dimer IFNγ PTGFRN exosomes contain at least twice as many IFNγ molecules as the monomeric IFNγ PTGFRN exosomes, suggesting that the tandem dimer exosomes are appropriately expressing the dimeric IFNγ constructs.

Table 9

Construct	IFN γ molecules/exosome
h-mIFNγ-PTGFRN	53
h-tdIFNγ-PTGFRN	173
m-mIFNγ-PTGFRN	47
m-tdIFNγ-PTGFRN	113

[00337] Human monomeric and tandem dimer PTGFRN- IFNγ exosomes were incubated with human PBMCs for 24 hours at increasing concentrations. Monocyte activation was measured by PD-L1 expression, a downstream surface protein induced by IFNγ signaling. As shown in Figure 33, native HEK293SF exosomes (WT) failed to induce PD-L1 expression, while both monomeric and tandem dimer IFNγ PTGFRN exosomes induced PD-L1 in a dose-dependent manner, with greater activation by the tandem dimer IFNγ PTGFRN exosomes. Exosome-mediated PD-L1 activation was comparable to LPS-induced activation (Figure 33). These data demonstrate that a soluble cytokine, in either monomeric or dimeric format, can be functionally expressed on the surface of an exosome and induce immune cell activation.

The use of IFNγ expressing exosomes in immuno-oncology may be useful for the induction of NK and T-cell responses against tumor cells.

Example 12: IL-15 Expressing Exosomes Induce NK Cell Activation

[00338] Interleukin 15 (IL-15) is a cytokine produced by mononuclear cells after pathogenic infection. IL-15 can be secreted as a soluble protein or presented as a dimeric membrane-anchored protein bound to IL-15Rα. IL-15 activates NK cells and T-cells and is implicated as a potential therapeutic molecule in immuno-oncology and other immune intervention therapies. IL-15-expressing exosomes were produced by stably transfecting HEK293SF cells with expression plasmids encoding the transmembrane domain of PDGFR (pDisplay) fused to IL-15/IL-15Rα fusion proteins (Figure 34). Exosomes were purified by Optiprep™ density-gradient ultracentrifugation as described in the Methods above. Purified exosomes were incubated with human PBMCs for 24 hours, and NK cell activation was measured as percent positive for CD69 by flow cytometry. None of the pDisplay IL-15 exosomes induced NK cell activation at doses up to 10^5 exosomes per cell of PBMC culture (Figure 35; exosome construct number as in Figure 34). To investigate whether higher density IL-15 display was required to induce NK cell activation, HEK293SF cells were stably transfected with an expression plasmid encoding IL-15 fused to full-length PTGFRN. Additionally, HEK293SF cells were stably transfected with an expression plasmid encoding a more potent IL-15 fused to full-length PTGFRN (IL-15 N72D, as described in *J Immunol.* 2009 Sep 15;183(6):3598-607; Figure 36A). Expression was confirmed by anti-PTGFRN Western blotting (Figure 36B). IL-15 levels were quantified by ELISA (R&D Systems, Catalog No. D1500), normalized to recombinant IL-15 (Biolegend, Catalog No. 570302). The IL-15 PTGFGN exosomes were added to two independent PBMC cultures overnight and compared to concentration-matched recombinant IL-15. All three IL-15 sources induced NK cell activation in PBMCs in a dose-dependent manner as measured by the percentage of NK cells positive for CD69. Furthermore, all constructs were comparable to each other across both donors demonstrating meaningful comparative efficacy (Figure 37; exosome construct number as in Figure 36). These data demonstrate that IL-15 can be actively and potently displayed on the surface of exosomes, but this requires high expression levels such as those bestowed by PTGFRN.

Example 13: Exosomes Displaying Anti-CD-3 Antibody Fragments on a PTGFRN Scaffold Activate T-Cells

[00339] The results in Example 9 demonstrate that exosomes displaying anti-CD3 antibody fragments can activate T-cells. To determine whether the PTGFRN scaffold supports this activity, anti-CD3 antibody fragments (OKT3 variants) were fused to the PDGFR transmembrane region (exoCD3-PD), full-length PTGFRN (exoCD3-long), or a PTGFRN fragment (exoCD3-short) and stably expressed in HEK293SF cells (Figure 38). Exosome binding was confirmed by bio-layer interferometry (BLI) using an Octet® RED96 (Pall). A CD3 fragment was bound to the BLI probe (Figure 39, ii), washed (Figure 39, iii), and the exosome constructs were added (Figure 39, iv). Exosomes from WT HEK293SF cells did not bind the BLI probe, but all engineered constructs did. Both PTGFRN fragments bound to the probe with a greater affinity and remained stably bound (Figure 39, v). Anti-CD3 display exosomes were tested for *in vitro* activity. T-cell activation was measured by CD69 positivity on CD4+ T-cells as measured by flow cytometry. In contrast to the unmodified native exosomes (exoNative), the exosomes with anti-CD3 fused to the PTGFRN fragment (exoCD3-short) were effective in activating CD4+ T-cells *in vitro* (Figure 40).

Example 14: Exosomes Displaying CD40L Are Potent Activators of B-Cells

[00340] CD40 ligand (CD40L) is a ligand of the tumor necrosis superfamily (TNFSF) that binds to the costimulatory receptor CD40, which is highly expressed on B-cells and other antigen presenting cells. TNFSF ligand-mediated cellular activation requires the formation of trimeric ligand complexes that form on the cell surface and bind to cognate receptors. To investigate whether exosomes displaying different conformations of CD40L on their surface were sufficient to activate B-cells, over 40 different CD40L expression constructs were designed and individually transfected in HEK293SF cells. CD40L was expressed as a fusion to the transmembrane domain of PDGFR, full-length PTGFRN, and a short single-domain fragment of PTGFRN (Figure 41A, bottom). CD40L-GFP PTGFRN fusions were expressed as a monomer (pCB-518 to pCB-526) or as a forced trimer (pCB-607 and pCB-527) (Figure 41A, bottom). To promote trimerization of monomeric CD40L, constructs were designed which expressed a fusion to multimerization domains from TRAF2 (pCB-521 to pCB-523) or Collagen XV (pCB-524 to pCB-526). Among the monomeric CD40L constructs, pCB-518/521/524 contained full-length N-terminal stem sequences from endogenous CD40L; pCB-519/522/525 contained a truncated N-terminal stem sequence from endogenous CD40L; and pCB-520/523/526 contained only the soluble portion of CD40L. Each of the engineered

exosome populations was incubated with purified B-cells, isolated from human peripheral blood by using RosetteSep™ Human B Cell Enrichment Cocktail (Stemcell Technologies#15064) and B-cell activation was measured by CD69 positivity on B-cells by flow cytometry. The EC_{50} for each of the constructs was calculated as a function of particles concentration of cell culture and is plotted in the graph shown in Figure 41, top. Interestingly, all of the monomeric CD40L constructs had modest potency, while the trimeric constructs were at least ten-fold more potent than the monomers (Figure 41, top). These results demonstrate that monomeric CD40L is a poor activator of B-cells when presented on the surface of exosomes, but that forced trimeric CD40L can induce robust B-cell activation. Furthermore, PTGFRN has been shown to form dimeric structures (PCT/US2018/048026), suggesting that higher order multimeric structures may be forming on the exosome surface to further promote target engagement and immune cell activation.

[00341] The results shown in Figure 41 all employed exosomes containing luminal GFP fused to the C-terminus of PTGFRN. With the goal of generating a tag-less CD40L exosome, the same trimeric CD40L-PTGFRN construct as the lead construct pCB-527 but lacking the C-terminal GFP was stably expressed in HEK293SF cells (pCB-766). The absolute concentration of CD40L on the surface of the engineered exosomes was quantified using ELISA (R&D Systems, Catalog No., DCDL40), as shown in Table 10, below.

Table 10

EC50	pCB-0766	pCB-0527	rhCD40L
particles/mL	6.63E+08	4.53E+08	N/A
ng/mL	1.68	1.89	28.51

[00342] The purified CD40L-PTGFRN exosomes were tested in B-cell activation assays as described above, compared to concentration-matched recombinant human CD40L (Biolegend, Catalog No. 591702). The GFP-containing and the tag-less CD40L exosomes were comparable B-cell activators when measured as a function of particle number or CD40L concentration (Figure 42A), and both exosome preparations were more potent than concentration-matched CD40L (Figure 42B). Native, non-engineered exosomes from HEK293SF cells failed to activate B-cells, demonstrating that the engineered CD40L trimeric constructs on the exosome surface were sufficient to potently activate B-cells.

[00343] An alternative modality to agonize CD40 and activate B-cells is to use an agonistic antibody cross-linked with a secondary antibody. To compare the potency of

trimeric CD40L-expressing exosomes to an agonistic CD40L antibody, PBMC cultures were incubated with 2μg/ml anti-CD40L antibody (Biolegend®; Clone 5C3) with a secondary cross-linking antibody (JacksonImmuno Research, Catalog No. 115-006-071). Maximal B-cell activation is shown as the dotted line in Figures 43A and 43B. pCB-527 exosomes (PTGFRN-trimeric CD40L-GFP) induced a greater maximal B-cell activation than the cross-linked agonistic antibody in two independent donor PBMC pools (Figures 43A and 43B) demonstrating superiority of trimeric CD40L exosomes in activating immune cells.

Example 15: Simultaneous Display of Multiple Immuno-oncology Molecules on Individual Exosomes

[00344] The previous examples demonstrate that individual immune-modulating proteins can be displayed on the surface of an exosome and induce functional changes in one or more immune cell types. In certain applications, the use of combinatorially engineered exosomes may be required, *i.e.*, an exosome containing more than one molecule on the exosome surface, each of which is capable of signaling a distinct immune cell pathway. HEK293SF cells were stably transfected with a plasmid expressing both PTGFRN-IL-12 and PTGFRN-CD40L fusion proteins. Exosomes were isolated and purified as described above. Exosomes from unmodified HEK293SF cells were used as negative controls.

[00345] To demonstrate simultaneous loading of different ligands, a pull-down co-stain assay was developed:

REAGENTS:

Dynabeads (Thermofisher Exosome-Streptavidin Isolation/Detection Reagent, Catalog No. 10608D): $1x10^7$beads/mL, 50% slurry

Isolation buffer: 0.5% BSA/PBS (1:4 from 2% BSA)

Block buffer: 2% BSA/PBS (1gr/50mL, filter)

- Wash 0.5 ml beads with 0.5 ml isolation buffer and resuspend in 0.5mL isolation buffer
- Add 1μg biotinylated capture antibody (2.2 ul of 0.5ug/ul stock)
- 1hr rotation, RT
- Wash 500μl isolation buffer
- Resuspend in 500μl block buffer, 10 min rotation RT
- Incubate in 500μl isolation buffer ($1x10^7$beads/mL, 50% slurry)
- Store at 4C

A. Exosome capture and flow

$1x10^5$ beads per sample (10 µl beads, 20µl slurry)

50,000 exosomes per bead; $5x10^9$ exosomes per sample ($1.2x10^9$ exosomes/µL stock)

5µl of each fluorescently labeled detection antibody for flow

Mix $5x10^9$ exosomes + 20µl Dynabeads slurry + 0.7 ml 0.1% BSA/PBS

PROCEDURE:

1. 120 µl slurry beads, remove sup, add 0.7 ml block buffer, mix, rotate 10 min RT, remove sup
2. Suspend beads in 0.7ml isolation buffer + 25.2 µl exosomes, rotate ON @ 4C
3. Next day: quick spin exosomes and beads, 5 sec
4. Place tube on magnet, remove sup
5. Block in 700µl, 10 min rotate RT
6. Place tube on magnet, remove sup
7. Resuspend in 600µl isolation buffer: 6 x 100µl per tube
8. Add 1µl labeled detection antibody, mix, incubate 30 min @ 4C in dark
9. Spin 2 min @ 500g, remove sup
10. Wash 2x isolation buffer
11. Resuspend in 200µl isolation buffer, run flow.

[00346] Native exosomes were isolated with anti-CD40L-decorated beads and labeled fluorescent antibodies against IL-12 and CD40L (Figure 44A) or CD81, an exosome marker present on native and engineered exosomes, and CD40L (Figure 44B). The CD40L beads did not pull down any of the native exosomes, since no fluorescent signal was detected for IL-12, CD40L or CD81. In contrast, PTGFRN-CD40L/IL-12 double engineered exosomes were incubated with anti-CD40L beads and isolated as above. Staining for CD81 (Figure 45A), IL-12 or CD40L (Figure 45B) were all detected with the engineered exosomes (greater than 97% of counted beads), indicating that CD40L-mediated isolation could also isolate IL-12 exosomes. Similarly, anti-IL-12-decorated beads were incubated with the IL-12/CD40L engineered exosomes and stained for IL-12, CD40L, and CD81. Greater than 98% of all beads were positive for both CD40L and IL-12 or for CD81 (Figures 46A and 46B), demonstrating that the exosomes contained both IL-12 and CD40L on their surface.

[00347] IL-12 and CD40L concentration was quantified by ELISA (Abcam Catalog No. ab119517) for testing the engineered exosomes for potency *in vitro*. Equal concentrations of

recombinant IL-12, recombinant IL-12 mixed with recombinant CD40L, PTGFRN-IL-12 exosomes, double-positive PTGFRN-CD40L/IL-12 exosomes, or a mixture of PTGFRN-IL-12 exosomes and PTGFRN-CD40L exosomes were added to human PBMCs at increasing concentrations (rhIL-12 - BioLegend, Catalog No. 573004; rhCD40L – Biolegend, Catalog No. 591702). The cells were co-stimulated with anti-CD3 antibody, and IFNγ production was measured by (PerkinElmer, Catalog No. AL217C). As shown in Figures 47A and 47B, all IL-12-containing exosome preparations elicited an IFNγ response comparable to the recombinant cytokines. Calculation of the EC50 for the various conditions revealed that exosome-associated IL-12 was more potent than concentration-matched IL-12, whether expressed singly or combinatorially on the exosome surface (Figure 48). Similar results were achieved with recombinant CD40L and singly or doubly engineered CD40L exosomes in the context of B-cell activation (Figure 49A and B). Again, the CD40L engineered exosomes were more potent than the soluble recombinant cytokine, and in this case the doubly engineered exosomes were the most potent construct tested in the assay (Figure 50).

[00348] To further explore the possibility of combinatorial surface display exosomes, HEK293SF cells were stably transfected with three independent constructs expressing either PTGFRN-IL-12, PTGFRN-CD40L, or PTGFRN-FLT3L fusion proteins. Exosomes were purified and isolated by the affinity bead methods as described above, but were also interrogated for the presence of surface FLT3L using an anti-FLT3L-PE conjugated antibody. Exosomes isolated with anti-IL-12 beads were doubly positive for IL-12 and CD40L (Figure 51A), IL-12 and FLT3L (Figure 51B), and CD40L and FLT3L (Figure 51C). Exosomes isolated with anti-CD40L beads were doubly positive for IL-12 and CD40L (Figure 52A), IL-12 and FLT3L (Figure 52B), and CD40L and FLT3L (Figure 52C), confirming that individual exosomes expressed each of the three immunomodulatory ligands. These results demonstrate that multiply engineered immuno-modulatory exosomes are a feasible therapeutic modality, and that they are comparable or more potent than soluble cytokines in immune cell activation.

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[00349] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this disclosure that certain

changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

[00350] Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, *i.e.*, any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

SEQUENCE LISTING

>SEQ ID NO: 1
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QVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSAGG
GGSDYKDDDDKGGGGSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDDMAFDVSWF
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>SEQ ID NO: 2
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KAPVLLSSLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSP
TGSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVGLSTVIGLLSCLIGYCSSHWCCKKEVQ
ETRRERRRLMSMEMD

>hIL-12-PTGFRN; 871 (SEQ ID NO: 3)
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DTPEEDGITW TLDQSSEVLG SGKTLTIQVK EFGDAGQYTC HKGGEVLSHS
LLLLHKKEDG IWSTDILKDQ KEPKNKTFLR CEAKNYSGRF TCWWLTTIST
DLTFSVKSSR GSSDPQGVTC GAATLSAERV RGDNKEYEYS VECQEDSACP
AAEESLPIEV MVDAVHKLKY ENYTSSFFIR DIIKPDPPKN LQLKPLKNSR
QVEVSWEYPD TWSTPHSYFS LTFCVQVQGK SKREKKDRVF TDKTSATVIC
RKNASISVRA QDRYYSSSWS EWASVPCSGG SGGGSGGGGS GGGGSGGGSG
GRNLPVATPD PGMFPCLHHS QNLLRAVSNM LQKARQTLEF YPCTSEEIDH
EDITKDKTST VEACLPLELT KNESCLNSRE TSFITNGSCL ASRKTSFMMA
LCLSSIYEDL KMYQVEFKTM NAKLLMDPKR QIFLDQNMLA VIDELMQALN
FNSETVPQKS SLEEPDFYKT KIKLCILLHA FRIRAVTIDR VMSYLNASSA
GGGGSGGGGS RVVRVPTATL VRVVGTELVI PCNVSDYDGP SEQNFDWSFS
SLGSSFVELA STWEVGFPAQ LYQERLQRGE ILLRRTANDA VELHIKNVQP
SDQGHYKCST PSTDATVQGN YEDTVQVKVL ADSLHVGPSA RPPPSLSLRE
GEPFELRCTA ASASPLHTHL ALLWEVHRGP ARRSVLALTH EGRFHPGLGY
EQRYHSGDVR LDTVGSDAYR LSVSRALSAD QGSYRCIVSE WIAEQGNWQE
IQEKAVEVAT VVIQPSVLRA AVPKNVSVAE GKELDLTCNI TTDRADDVRP
EVTWSFSRMP DSTLPGSRVL ARLDRDSLVH SSPHVALSHV DARSYHLLVR
DVSKENSGYY YCHVSLWAPG HNRSWHKVAE AVSSPAGVGV TWLEPDYQVY
LNASKVPGFA DDPTELACRV VDTKSGEANV RFTVSWYYRM NRRSDNVVTS
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NYYCVVSAWT KQRNNSWVKS KDVFSKPVNI FWALEDSVLV VKARQPKPFF
AAGNTFEMTC KVSSKNIKSP RYSVLIMAEK PVGDLSSPNE TKYIISLDQD
SVVKLENWTD ASRVDGVVLE KVQEDEFRYR MYQTQVSDAG LYRCMVTAWS
PVRGSLWREA ATSLSNPIEI DFQTSGPIFN ASVHSDTPSV IRGDLIKLFC
IITVEGAALD PDDMAFDVSW FAVHSFGLDK APVLLSSLDR KGIVTTSRRD
WKSDLSLERV SVLEFLLQVH GSEDQDFGNY YCSVTPWVKS PTGSWQKEAE
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EVQETRRERR RLMSMEMD*

>mIL-12-PTGFRN; 872 (SEQ ID NO: 4)
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DTPEEDDITW TSDQRHGVIG SGKTLTITVK EFLDAGQYTC HKGGETLSHS
HLLLHKKENG IWSTEILKNF KNKTFLKCEA PNYSGRFTCS WLVQRNMDLK
FNIKSSSSSP DSRAVTCGMA SLSAEKVTLD QRDYEKYSVS CQEDVTCPTA
EETLPIELAL EARQQNKYEN YSTSFFIRDI IKPDPPKNLQ MKPLKNSQVE
VSWEYPDSWS TPHSYFSLKF FVRIQRKKEK MKETEEGCNQ KGAFLVEKTS
TEVQCKGGNV CVQAQDRYYN SSCSKWACVP CRVRSGGSGG GSGGGGSGGG
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MMTLCLGSIY EDLKMYQTEF QAINAALQNH NHQQIILDKG MLVAIDELMQ
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SFSSLGSSFV ELASTWEVGF PAQLYQERLQ RGEILLRRTA NDAVELHIKN
VQPSDQGHYK CSTPSTDATV QGNYEDTVQV KVLADSLHVG PSARPPPSLS
LREGEPFELR CTAASASPLH THLALLWEVH RGPARRSVLA LTHEGRFHPG
LGYEQRYHSG DVRLDTVGSD AYRLSVSRAL SADQGSYRCI VSEWIAEQGN
WQEIQEKAVE VATVVIQPSV LRAAVPKNVS VAEGKELDLT CNITTDRADD
VRPEVTWSFS RMPDSTLPGS RVLARLDRDS LVHSSPHVAL SHVDARSYHL
LVRDVSKENS GYYYCHVSLW APGHNRSWHK VAEAVSSPAG VGVTWLEPDY
QVYLNASKVP GFADDPTELA CRVVDTKSGE ANVRFTVSWY YRMNRRSDNV
VTSELLAVMD GDWTLKYGER SKQRAQDGDF IFSKEHTDTF NFRIQRTTEE
DRGNYYCVVS AWTKQRNNSW VKSKDVFSKP VNIFWALEDS VLVVKARQPK
PFFAAGNTFE MTCKVSSKNI KSPRYSVLIM AEKPVGDLSS PNETKYIISL
DQDSVVKLEN WTDASRVDGV VLEKVQEDEF RYRMYQTQVS DAGLYRCMVT
AWSPVRGSLW REAATSLSNP IEIDFQTSGP IFNASVHSDT PSVIRGDLIK
LFCIITVEGA ALDPDDMAFD VSWFAVHSFG LDKAPVLLSS LDRKGIVTTS
RRDWKSDLSL ERVSVLEFLL QVHGSEDQDF GNYYCSVTPW VKSPTGSWQK
EAEIHSKPVF ITVKMDVLNA FKYPLLIGVG LSTVIGLLSC LIGYCSSHWC
CKKEVQETRR ERRRLMSMEM D*

>hIL-12-short PTGFRN; 873 (SEQ ID NO: 5)
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LLLLHKKEDG IWSTDILKDQ KEPKNKTFLR CEAKNYSGRF TCWWLTTIST
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QVEVSWEYPD TWSTPHSYFS LTFCVQVQGK SKREKKDRVF TDKTSATVIC
RKNASISVRA QDRYYSSSWS EWASVPCSGG SGGGSGGGGS GGGGSGGGSG
GRNLPVATPD PGMFPCLHHS QNLLRAVSNM LQKARQTLEF YPCTSEEIDH
EDITKDKTST VEACLPLELT KNESCLNSRE TSFITNGSCL ASRKTSFMMA
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FNSETVPQKS SLEEPDFYKT KIKLCILLHA FRIRAVTIDR VMSYLNASSA
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LLQVHGSEDQ DFGNYYCSVT PWVKSPTGSW QKEAEIHSKP VFITVKMDVL
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EMD*

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FNIKSSSSSP DSRAVTCGMA SLSAEKVTLD QRDYEKYSVS CQEDVTCPTA

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VSWEYPDSWS TPHSYFSLKF FVRIQRKKEK MKETEEGCNQ KGAFLVEKTS
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IDHEDITRDQ TSTLKTCLPL ELHKNESCLA TRETSSTTRG SCLPPQKTSL
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ASAGGGGSGG GGSGPIFNAS VHSDTPSVIR GDLIKLFCII TVEGAALDPD
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MSMEMD*

SEQ ID NO: 7 PTGFRN_IFN_gamma monomer

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SLHVGPSARPPPSLSLREGEPFELRCTAASASPLHTHLALLWEVHRGPARRSVLALTH
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KLFCIITVEGAALDPDDMAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKS
DLSLERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITV
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D

SEQ ID NO: 8 PTGFRN_IFN_gamma dimer

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SEQ ID NO: 9 PTGFRN_IFN_gamma mouse monomer
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GAALDPDDMAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLERVS
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SEQ ID NO: 10 PTGFRN_IFN_gamma mouse dimer
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ITTFFSNSKAKKDAFMSIAKFEVNNPQVQRQAFNELIRVVHQLLPESSLRSAGGGGSG
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PRYSVLIMAEKPVGDLSSPNETKYIISLDQDSVVKLENWTDASRVDGVVLEKVQEDE
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GIVTTSRRDWKSDLSLERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPTGSWQK
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RRERRRLMSMEMD

SEQ ID NO: 11 IL-15 441
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DTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSSADYK
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SEQ ID NO: 12 IL-15 442
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SSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTGGSGGGSGGGGSTLDPRSFLL
RNPNDKYEPFWEDEEKNESGGGGSGGGSGGSNWVNVISDLKKIEDLIQSMHIDATLY
TESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGC
KECEELEEKNIKEFLQSFVHIVQMFINTSSADYKDDDDKFEGGGGSGGGGSAVGQDT
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SEQ ID NO: 13 IL-15 443
METDTLLLWVLLLWVPGSTGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTSGGSGGGSGGGGSGGGGSGGGSGGSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQ
RPAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTG
TTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTSADYKDDDDKFEG
GGGSGGGGSAVGQDTQEVIVVPHSLPFKVVVISAILALVVLTIISLIILIMLWQKKPRS
GLLTGRT

SEQ ID NO: 14 IL-15 444
METDTLLLWVLLLWVPGSTGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTSDYKDDDDKGGSGGGSGGGGSTLDPRSFLLRNPNDKYEPF
WEDEEKNESGGGGSGGGSGGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRK
AGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSP
SGKEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNW

ELTASASHQPPGVYPQGHSDTTSAFEGGGGSGGGGSAVGQDTQEVIVVPHSLPFKVV
VISAILALVVLTIISLIILIMLWQKKPRSGLLTGRTHHHHHH

SEQ ID NO: 15 IL-15 1009
METDTLLLWVLLLWVPGSTGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTSGGSSGSGSGSTGTSSSGTGTSAGTTGTSASTSGSGSGGGGG
SGGGGSAGGTATAGASSGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG
TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSG
KEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWEL
TASASHQPPGVYPQGHSDTTSAGGGGSGGGGSRVVRVPTATLVRVVGTELVIPCNVS
DYDGPSEQNFDWSFSSLGSSFVELASTWEVGFPAQLYQERLQRGEILLRRTANDAVE
LHIKNVQPSDQGHYKCSTPSTDATVQGNYEDTVQVKVLADSLHVGPSARPPPSLSLR
EGEPFELRCTAASASPLHTHLALLWEVHRGPARRSVLALTHEGRFHPGLGYEQRYHS
GDVRLDTVGSDAYRLSVSRALSADQGSYRCIVSEWIAEQGNWQEIQEKAVEVATVVI
QPSVLRAAVPKNVSVAEGKELDLTCNITTDRADDVRPEVTWSFSRMPDSTLPGSRVL
ARLDRDSLVHSSPHVALSHVDARSYHLLVRDVSKENSGYYYCHVSLWAPGHNRSW
HKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGFADDPTELACRVVDTKSGEANV
RFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLKYGERSKQRAQDGDFIFSKEHTD
TFNFRIQRTTEEDRGNYYCVVSAWTKQRNNSWVKSKDVFSKPVNIFWALEDSVLVV
KARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGDLSSPNETKYIISLDQ
DSVVKLENWTDASRVDGVVLEKVQEDEFRYRMYQTQVSDAGLYRCMVTAWSPVR
GSLWREAATSLSNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDD
MAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVH
GSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVG
LSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLMSMEMD

SEQ ID NO: 16 IL-15 1010
METDTLLLWVLLLWVPGSTGNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKV
TAMKCFLLELQVISLESGDASIHDTVENLIILANDSLSSNGNVTESGCKECEELEEKNI
KEFLQSFVHIVQMFINTSGGSSGSGSGSTGTSSSGTGTSAGTTGTSASTSGSGSGGGGG
SGGGGSAGGTATAGASSGSITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAG
TSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSG

KEPAASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWEL
TASASHQPPGVYPQGHSDTTSAGGGGSGGGGSRVVRVPTATLVRVVGTELVIPCNVS
DYDGPSEQNFDWSFSSLGSSFVELASTWEVGFPAQLYQERLQRGEILLRRTANDAVE
LHIKNVQPSDQGHYKCSTPSTDATVQGNYEDTVQVKVLADSLHVGPSARPPPSLSLR
EGEPFELRCTAASASPLHTHLALLWEVHRGPARRSVLALTHEGRFHPGLGYEQRYHS
GDVRLDTVGSDAYRLSVSRALSADQGSYRCIVSEWIAEQGNWQEIQEKAVEVATVVI
QPSVLRAAVPKNVSVAEGKELDLTCNITTDRADDVRPEVTWSFSRMPDSTLPGSRVL
ARLDRDSLVHSSPHVALSHVDARSYHLLVRDVSKENSGYYYCHVSLWAPGHNRSW
HKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGFADDPTELACRVVDTKSGEANV
RFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLKYGERSKQRAQDGDFIFSKEHTD
TFNFRIQRTTEEDRGNYYCVVSAWTKQRNNSWVKSKDVFSKPVNIFWALEDSVLVV
KARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGDLSSPNETKYIISLDQ
DSVVKLENWTDASRVDGVVLEKVQEDEFRYRMYQTQVSDAGLYRCMVTAWSPVR
GSLWREAATSLSNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDD
MAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVH
GSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVG
LSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLMSMEMD

SEQ ID NO: 17 pDisplay-anti-CD3
MKIICLALVALLLTAQPAMAEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ
KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPL
TFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGECGGSSGSGSGSTGTSSSGTGTSAGTTGTSASTSGSGSGGGGSGGGGSAGGTAT
AGASSGSQVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWV
AVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARQMGYW
HFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTGGSGGGSGGGGSGGGGSGGGSGGSAVGQDTQEVIVVPHSLPFKVVVIS
AILALVVLTIISLIILIMLWQKKPRDYKDDDDK

SEQ ID NO: 18 PTGFRN-anti-CD3

MKIICLALVALLLTAQPAMAEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ
KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPL
TFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGECGGSSGSGSGSTGTSSSGTGTSAGTTGTSASTSGSGSGGGGSGGGGSAGGTAT
AGASSGSQVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWV
AVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARQMGYW
HFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTGGSGGGSGGGGSGGGGSGGGSGGSRVVRVPTATLVRVVGTELVIPCNV
SDYDGPSEQNFDWSFSSLGSSFVELASTWEVGFPAQLYQERLQRGEILLRRTANDAV
ELHIKNVQPSDQGHYKCSTPSTDATVQGNYEDTVQVKVLADSLHVGPSARPPPSLSL
REGEPFELRCTAASASPLHTHLALLWEVHRGPARRSVLALTHEGRFHPGLGYEQRYH
SGDVRLDTVGSDAYRLSVSRALSADQGSYRCIVSEWIAEQGNWQEIQEKAVEVATV
VIQPSVLRAAVPKNVSVAEGKELDLTCNITTDRADDVRPEVTWSFSRMPDSTLPGSR
VLARLDRDSLVHSSPHVALSHVDARSYHLLVRDVSKENSGYYYCHVSLWAPGHNRS
WHKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGFADDPTELACRVVDTKSGEAN
VRFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLKYGERSKQRAQDGDFIFSKEHT
DTFNFRIQRTTEEDRGNYYCVVSAWTKQRNNSWVKSKDVFSKPVNIFWALEDSVLV
VKARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGDLSSPNETKYIISLD
QDSVVKLENWTDASRVDGVVLEKVQEDEFRYRMYQTQVSDAGLYRCMVTAWSPV
RGSLWREAATSLSNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDD
MAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVH
GSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVG
LSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLMSMEMDTGGSGGSVSKGEELFT
GVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTY
GVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNR
IELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA
DHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFVTAAGITLGMDEL
YKDYKDDDDK

SEQ ID NO: 19 PTGFRN_CD40L trimer mouse

METDTLLLWVLLLWVPGSTGMQRGDEDPQIAAHVVSEANSNAASVLQWAKKGYYT
MKSNLVMLENGKQLTVKREGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERI
LLKAANTHSSSQLCEQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSSFGLLKL
GSGGSGGSGGSGMQRGDEDPQIAAHVVSEANSNAASVLQWAKKGYYTMKSNLVM
LENGKQLTVKREGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERILLKAANTH
SSSQLCEQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSSFGLLKLGSGGSGGS
GGSGMQRGDEDPQIAAHVVSEANSNAASVLQWAKKGYYTMKSNLVMLENGKQLT
VKREGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERILLKAANTHSSSQLCEQ
QSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSSFGLLKLSAGGGGSGGGGSRVV
RVPTATLVRVVGTELVIPCNVSDYDGPSEQNFDWSFSSLGSSFVELASTWEVGFPAQL
YQERLQRGEILLRRTANDAVELHIKNVQPSDQGHYKCSTPSTDATVQGNYEDTVQV
KVLADSLHVGPSARPPPSLSLREGEPFELRCTAASASPLHTHLALLWEVHRGPARRSV
LALTHEGRFHPGLGYEQRYHSGDVRLDTVGSDAYRLSVSRALSADQGSYRCIVSEWI
AEQGNWQEIQEKAVEVATVVIQPSVLRAAVPKNVSVAEGKELDLTCNITTDRADDV
RPEVTWSFSRMPDSTLPGSRVLARLDRDSLVHSSPHVALSHVDARSYHLLVRDVSKE
NSGYYYCHVSLWAPGHNRSWHKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGF
ADDPTELACRVVDTKSGEANVRFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLK
YGERSKQRAQDGDFIFSKEHTDTFNFRIQRTTEEDRGNYYCVVSAWTKQRNNSWVK
SKDVFSKPVNIFWALEDSVLVVKARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIM
AEKPVGDLSSPNETKYIISLDQDSVVKLENWTDASRVDGVVLEKVQEDEFRYRMYQ
TQVSDAGLYRCMVTAWSPVRGSLWREAATSLSNPIEIDFQTSGPIFNASVHSDTPSVI
RGDLIKLFCIITVEGAALDPDDMAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRR
DWKSDLSLERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKP
VFITVKMDVLNAFKYPLLIGVGLSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLM
SMEMD

SEQ ID NO: 20 PTGFRN_CD40L trimer human
METDTLLLWVLLLWVPGSTGMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYT
MSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERIL
LRAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKLGS
GGSGGSGGSGMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENG
KQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKP
CGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKLGSGGSGGSGGSGM

QKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTVKRQGLY
YIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGV
FELQPGASVFVNVTDPSQVSHGTGFTSFGLLKLSAGGGGSGGGGSRVVRVPTATLVR
VVGTELVIPCNVSDYDGPSEQNFDWSFSSLGSSFVELASTWEVGFPAQLYQERLQRG
EILLRRTANDAVELHIKNVQPSDQGHYKCSTPSTDATVQGNYEDTVQVKVLADSLH
VGPSARPPPSLSLREGEPFELRCTAASASPLHTHLALLWEVHRGPARRSVLALTHEGR
FHPGLGYEQRYHSGDVRLDTVGSDAYRLSVSRALSADQGSYRCIVSEWIAEQGNWQ
EIQEKAVEVATVVIQPSVLRAAVPKNVSVAEGKELDLTCNITTDRADDVRPEVTWSF
SRMPDSTLPGSRVLARLDRDSLVHSSPHVALSHVDARSYHLLVRDVSKENSGYYYC
HVSLWAPGHNRSWHKVAEAVSSPAGVGVTWLEPDYQVYLNASKVPGFADDPTELA
CRVVDTKSGEANVRFTVSWYYRMNRRSDNVVTSELLAVMDGDWTLKYGERSKQR
AQDGDFIFSKEHTDTFNFRIQRTTEEDRGNYYCVVSAWTKQRNNSWVKSKDVFSKP
VNIFWALEDSVLVVKARQPKPFFAAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGD
LSSPNETKYIISLDQDSVVKLENWTDASRVDGVVLEKVQEDEFRYRMYQTQVSDAG
LYRCMVTAWSPVRGSLWREAATSLSNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLF
CIITVEGAALDPDDMAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLS
LERVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMD
VLNAFKYPLLIGVGLSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLMSMEMD

SEQ ID NO: 21 PTGFRN_short-anti-CD3
MKIICLALVALLLTAQPAMAEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQ
KPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPL
TFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA
LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGECGGSSGSGSGSTGTSSSGTGTSAGTTGTSASTSGSGSGGGGSGGGGSAGGTAT
AGASSGSQVQLVESGGGVVQPGRSLRLSCAASGFKFSGYGMHWVRQAPGKGLEWV
AVIWYDGSKKYYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARQMGYW
HFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
KSCDKTHTGGSGGGSGGGGSGGGGSGGGSGGSGPIFNASVHSDTPSVIRGDLIKLFCII
TVEGAALDPDDMAFDVSWFAVHSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLE
RVSVLEFLLQVHGSEDQDFGNYYCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMDVL
NAFKYPLLIGVGLSTVIGLLSCLIGYCSSHWCCKKEVQETRRERRRLMSMEMDTGGS

GGSVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLP
VPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA
EVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKI
RHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSKLSKDPNEKRDHMVLLEFV
TAAGITLGMDELYKDYKDDDDK

SEQ ID NO: 22 FLT3L-PTGFRN
MTVLAPAWSPTTYLLLLLLLSSGLSGTQDCSFQHSPISSDFAVKIRELSDYLLQDYPVT
VASNLQDEELCGGLWRLVLAQRWMERLKTVAGSKMQGLLERVNTEIHFVTKCAFQ
PPPSCLRFVQTNISRLLQETSEQLVALKPWITRQNFSRCLELQCQPDSSTLPPPWSPRPL
EATAPTAPQPPSAGGGGSGGGGSRVVRVPTATLVRVVGTELVIPCNVSDYDGPSEQN
FDWSFSSLGSSFVELASTWEVGFPAQLYQERLQRGEILLRRTANDAVELHIKNVQPSD
QGHYKCSTPSTDATVQGNYEDTVQVKVLADSLHVGPSARPPPSLSLREGEPFELRCT
AASASPLHTHLALLWEVHRGPARRSVLALTHEGRFHPGLGYEQRYHSGDVRLDTVG
SDAYRLSVSRALSADQGSYRCIVSEWIAEQGNWQEIQEKAVEVATVVIQPSVLRAAV
PKNVSVAEGKELDLTCNITTDRADDVRPEVTWSFSRMPDSTLPGSRVLARLDRDSLV
HSSPHVALSHVDARSYHLLVRDVSKENSGYYYCHVSLWAPGHNRSWHKVAEAVSS
PAGVGVTWLEPDYQVYLNASKVPGFADDPTELACRVVDTKSGEANVRFTVSWYYR
MNRRSDNVVTSELLAVMDGDWTLKYGERSKQRAQDGDFIFSKEHTDTFNFRIQRTT
EEDRGNYYCVVSAWTKQRNNSWVKSKDVFSKPVNIFWALEDSVLVVKARQPKPFF
AAGNTFEMTCKVSSKNIKSPRYSVLIMAEKPVGDLSSPNETKYIISLDQDSVVKLENW
TDASRVDGVVLEKVQEDEFRYRMYQTQVSDAGLYRCMVTAWSPVRGSLWREAATS
LSNPIEIDFQTSGPIFNASVHSDTPSVIRGDLIKLFCIITVEGAALDPDDMAFDVSWFAV
HSFGLDKAPVLLSSLDRKGIVTTSRRDWKSDLSLERVSVLEFLLQVHGSEDQDFGNY
YCSVTPWVKSPTGSWQKEAEIHSKPVFITVKMDVLNAFKYPLLIGVGLSTVIGLLSCL
IGYCSSHWCCKKEVQETRRERRRLMSMEMD

TABLES

Table 1. Exosome lipids	
Lysobisphosphatidic acid	Ganglioside GM3 24:1
Sphingomyelin (SM)	Ganglioside GM3 16:0
Ganglioside GM3	PE40:5
Phosphatidylserine (PS)	PE40:6
Phosphatidylinositol (PI)	PE38:3
Phosphatidylcholine (PC)	PE38:4
Phosphatidylethanolamine (PE)	PE36:1
Lysophosphatidylcholine (LPC)	PE36:2
Cholesterol (Chol)	PE34:1
Diacylglycerol (DG)	PE34:2
PI18:0/20:3	PE-ether38:5
PI18:0/20:4	PE-ether38:6
PI18:0/18:1	PE-ether34:1
PI18:1/18:1	PE-ether34:2
PI18:0/16:0	PC34:1
PA18:0/18:1	PC36:4
PS18:0/18:1	PC34:3
BMP18:0/18:1	PC32:0
BMP18:1/18:1	PC30:0
BMP18:1/16:0	SM24:1
CL(18:1)3/16:1	SM16:0
CL(18:1)2/(16:1)2	Dihydrosphingomyelin16:0

Table 2. Exosome polypeptides			
ACLY	TCP1	ACTR1A	LY75
ACTB	PRDX2	THOC4	ABCC1
ACTG1	TSPAN6	INADL	MYO1E
ALB	CCT3	CTDSPL	NACA
ALDOA	TSTA3	ZMPSTE24	NAP1L4
ALDOB	TUBA3C	DNAJA2	NCL
AKR1B1	HIST1H2AK	NDRG1	NEDD8
AMBP	HIST1H2AJ	RAPGEF3	YBX1
ANPEP	HIST1H2AB	SPON2	PA2G4
ANXA2	HIST2H2AC	UBAC1	PECAM1
ANXA3	IFITM1	N4BP2L2	PFAS
ANXA4	PDXK	CAP1	SERPINB9
ANXA5	LIN7A	VAT1	PI4KA
ANXA6	BUB3	NEBL	PLAT
ANXA7	MAP4K4	DCTN2	PLCG2
ANXA11	EDIL3	ARPC1A	PPA1
	ATP6AP2	C6orf108	PPP2CA
CAPZB	PSME3	SMC2	PRKCB
CD63	TUBB3	AHSA1	PSMA6
CD81	IFITM3	STAMBP	PSMA7

CKB	ACAA2	PMVK	PSMB8
CLU	CCT7	GIPC1	PSMB9
CLIC1	CCT4	HBS1L	PSMD7
TPP1	IFITM2	NCKAP1	PSME1
CLTC	GNA13	ALDH1L1	PTPRA
CNP	RUVBL2	FTCD	RAC2
COL6A1	PRSS23	FGL2	RPL3
CR1	ACOT7	CFHR3	RPL4
CTNND1	CCT5	MMP24	RPL5
ACE	DIP2C	COPS8	RPL11
DDT	ASCC3L1	CKAP4	RPL22
DEFA1	TNIK	C10orf116	RPL24
DEFA3	NEDD4L	SLC27A2	RPL27
DNAH8	NCSTN	MID2	RPL30
DPEP1	TSPAN15	KIF3A	RPL28
DPP4	PLXNB2	NUDT5	RPL31
EEF1A1	SDCBP2	TREH	RPL34
EEF2	IGKV1-5	CEP250	RPL35A
EGF	IGHV4-31	PDCD10	RPL37A
EIF5A	IGKV3-20	PADI2	RPS2
ENO1	IGKV2-24	PACSIN2	RPS3A
ENO3	MINK1	CHP	RPS5
ENPEP	IGKα	SNF8	RPS9
STOM	VPS36	DDX19B	RPS19
EPS8	DERA	SCN11A	RPS25
FABP3	GOLGA7	LYPLA2	RPS26
FGA	KRT76	PARK7	RPS28
MLANA	EIF3EIP	COBLL1	RPS29
FN1	LSR	CNKSR2	RSU1
FTL	TUBA8	ENPP4	SARS
FUS	RAB4B	RAB3GAP1	SLAMF1
GAA	SETD4	AKR7A3	SLC1A4
GAPDH	TOLLIP	SPEN	SLC2A3
GDI2	PLEKHB2	GANAB	SNRPD2
GGT1	VPS37C	MGRN1	SPINK1
GLB1	LIN7C	CUX2	SPN
GLG1	H2AFJ	DNAJC13	STK10
GNA11	CAND1	ZCCHC11	STXBP3
GNAI1	PLSCR3	PHF15	TALDO1
GNAI2	KIAA1199	KIAA0841	TNFAIP3
GNAI3	GNB4	ARHGEF12	TPM3
GNAS	MYH14	COTL1	TPM4
GNB1	TSPAN14	ANGPTL2	TYK2
GNB2	NCALD	DDAH2	VIM
GNG7	REG4	HEBP2	WARS
SFN	VPS25	CD2AP	WAS
GPI	TUBB6	PLD3	LAT2
GSTA1	TUBA1C	TMEM2	HIST1H2BL

GSTA2	TNKS1BP1	SH3BP4	STX7
GSTA3	FAM125B	BHMT2	CPNE1
GSTM3	LRSAM1	GCA	RPL14
GSTP1	HIST3H2A	MXRA5	PDCD5
GUSB	TUBA3E	AHCTF1	SYNGR2
HIST1H2AD	TUBA3D	PTPN23	RPL23
HLA-A	DCD	DAK	RAB9A
HLA-B	HIST4H4	ACOT11	IGSF2
HLA-DQB1	ALDH16A1	APPL1	EEF1E1
HLA-DRA	RPS4Y2	PHGDH	SCAMP2
HLA-DRB1	MYL6B	TIAM2	SCAMP3
HLA-DRB5	BRI3BP	KCNG2	DPP3
HPGD	AGR3	CYFIP2	ARPC1B
HRAS	EEF1AL3	GHITM	PDIA6
HSPA1A	KRT28	C11orf54	WASF2
HSPA1B	KRT24	DBNL	ANP32B
HSPA8	RPLP0-like	ATAD2	PAICS
HSP90AA1	RPSAP15	PHPT1	AHCYL1
	RANP1	C16orf80	VAMP5
KRT1	PCSK9	OLA1	41891
KRT9	METRNL	ZDHHC1	HSPH1
KRT10	LOC284889	SNX12	SUB1
LDHA	KRT6C	PSAT1	CDC37
LDHB	KRT79	NT5C	CORO1A
TACSTD1	RAB43	EHD2	CD300A
MCAM	KRT27	TAX1BP3	TMC6
MDH1	ACTBL2	CRNN	RFTN1
MEP1A	RP11-631M21.2	NOX3	SCRIB
MSN	TUBB2B	ATP6V0A4	SERBP1
2-Sep	KRT77	ITSN2	TTLL3
PGAM1	AGRN	GEMIN4	CACYBP
PGK1	RAB15	LAP3	SIT1
PKM2	LOC388524	CRYL1	SLC43A3
PPP1CA	LOC388720	MYO15A	PILRA
	HSP90AB2P	ATP6V1D	RPL26L1
PTPRC	ACTBL3	SNX9	MPP6
RAN	LOC442497	PCYOX1	GNG2
RDX	A26C1A	ANKFY1	TMED9
SDCBP	HIST2H4B	UFC1	DOCK10
STX3	hCG_1757335	FAM49B	C3orf10
STXBP1	HLA-A29.1	CUTA	MYO1G
STXBP2	LOC653269	ATP6V1H	FLJ21438
TPI1	A26C1B	VPS24	SLC38A1
EZR	LOC100128936	CMPK1	FERMT3
YWHAE	LOC100130553	UPB1	ITFG3
TUBA1A	LOC100133382	CLIC5	HIST1H2AH
WDR1	LOC100133739	MUPCDH	SLAMF6
PDCD6IP	AP2A2	CLIC6	TMC8

GPA33	ALDH3B1	SIAE	LOC153364
TUBA1B	FASLG	CPVL	SVIP
TUBB2C	ATP4A	RHOF	TMEM189-UBE2V1
CAPN7	CAPS	ARL15	hCG_16001
DDAH1	COL12A1	ZNHIT6	FABP5L7
PGLS	DMBT1	GIPC2	Del(X)1Brd
SAMM50	DSP	PCDH24	ABP1
CLIC4	EGFR	VPS13C	ACTN3
CHMP2B	EPHA5	CC2D1A	AFM
ULK3	EPHB1	EPS8L1	AKT1
RNF11	FAT	C10orf18	ALDH3A2
VPS4A	HSD17B4	CHCHD3	ALOX12P2
ARFIP1	L1CAM	C2orf18	ANXA2P1
CHMP2A	LAMA5	C17orf80	KRT33B
SMPDL3B	MUC4	EPN3	MYOC
PACSIN3	NOTCH1	UACA	SERPINE1
EHD4	PPP2R1B	VPS13D	PIK3CA
EHD3	PTPRF	APPL2	NRP1
HEBP1	SORT1	ARL8B	SPRY1
VPS28	SERPINB3	DDX19A	EMILIN1
DCXR	SELP	NAGK	LRG1
RHCG	FSCN1	ITLN1	AZGP1P1
CHMP5	TGFB1	CCDC132	LOC728533
VTA1	CLTCL1	OTUB1	ALDH7A1
RAB14	CHST1	CDK5RAP2	AXL
GPRC5B	EIF3I	MBD5	CFB
CAB39	TNFSF10	SLC22A11	C1S
RAB8B	MAP7	SUSD2	CAT
TM7SF3	COPB2	SUCNR1	CD47
MXRA8	HEPH	BDH2	CD151
C11orf59		NIT2	CDH13
MOBKL1B	CIB1	RPL23AP13	CFTR
UEVLD	SLC34A2	FAM20C	CEACAM8
TSNAXIP1	SLC6A14	SLC12A9	AP1S1
GPRC5C	DIP2A	RAB25	CLTA
GNG12	TNPO3	SMURF1	CNGB1
BAIAP2L1	FER1L3	TMEM27	COL1A1
MUC13	CNTLN	RAB22A	COL1A2
CHMP1B	TUBB4Q	NDRG3	COL2A1
SLC44A2	KIF15	ERMN	COL3A1
CPNE5	SERINC1	TAOK1	COL4A1
TMBIM1	PDIA2	KIAA1529	COL4A2
EPS8L3	EPS8L2	RNF213	COL4A3
MMRN2	PLVAP	WIZ	COL5A1
TTYH3	MYADM	ACE2	COL5A2
SLC44A4	MUC16	PLEKHA1	COL7A1
RAB1B	KRT25	SCPEP1	COMP

RAB33B	SERINC5	AASDHPPT	CPS1
RBP5	LOC440264	FIGNL1	CSF1
C5orf32	AGT	PBLD	VCAN
ABHD14B	ALPP	KIF9	SLC25A10
MOBKL1A	APOA2	LEPRE1	CTBP2
ARRDC1	APOB	RAB17	CTNNA2
	APOE	IKZF5	DCTN1
FAM125A	SERPING1	MMP25	DECR1
SNX18	C1QB	MPP5	DNASE1L1
CHMP4B	C1R	TEKT3	ENG
MITD1	C4A	ALDH8A1	STX2
S100A16	C4B	SLC13A3	ETFB
CPNE8	C4BPA	DUSP26	F2R
C1orf58	C4BPB	GGCT	F8
GLIPR2	CD5L	TMEM38A	ACSL1
TUBB	FCN1	C1orf116	FAP
ATP6V1C2	FCN2	GDPD3	FBLN1
FTLL1	FGB	OR2A4	FBN1
PEF1	FGG	FAM65A	FBN2
SERPINA3	GRIN1	NARG1L	FEN1
ACP2	MSH6	CHMP6	FLT1
ACPP	HBA1	DYNC2H1	FUCA2
ACTA2	HBA2	PRKRIP1	GAS6
ACTC1	ITGA2B	GSTCD	GDI1
ACTG2	PPARG	PIP4K2C	GLDC
ACY1	PDLIM7	CYBRD1	GNAL
APCS	CD274	FUZ	GRM2
APOD	A1BG	ARMC9	GRM3
APRT	ACAT1	NAT13	GRM7
AQP1	ACO1	COASY	GSTM1
AQP2	ADCY1	UBXN6	GSTM5
ARF1	ADFP	COL18A1	H2AFX
ARF3	ADH5	BHLHB9	HBE1
ARF4	ADH6	WNT5B	HMGCS2
ARF5	PARP4	CAB39L	TNC
ARF6	AHSG	ITM2C	IDH3B
RHOA	AK1	LOC81691	IFRD1
ARL3	ALAD	AMN	ITGA5
ASAH1	ALCAM	SH3BGRL3	ITGB5
ASS1	ALDH2	C9orf58	ITPR2
FXYD2	ALDH9A1	BCL2L12	KRT84
BHMT	ALDOC	RAB34	LAMB1
BST2	ALK	TBC1D10A	LCN1
C3	ALOX12	GPR98	LGALS8
CA2	ALPL	HDHD2	LMNA
CA4	ANXA13	ARL6	LOXL2
CALB1	AOX1	IQCG	LTBP2
CALR	APAF1	C2orf16	MAP1A

CD9	APOA4	PARD6B	MAT1A
CD59	SHROOM2	TXNDC17	MC1R
HSPA5	RHOB	ABCC11	MCC
HSPA6	ARHGAP1	FAM40A	ME1
HSP90AB1	ARHGDIB	SCIN	MECP2
HSPD1	ARSE	SCRN2	MAP3K1
IDH1	ARSF	ZNF486	MFAP4
KNG1	ASL	ACY3	SCGB2A1
KRAS	ASNA1	C11orf52	ALDH6A1
LAMP1	ATIC	CRB3	MOS
LGALS3BP	ATP6V1A	C20orf114	CITED1
LRP2	ATP6V1B1	NAPRT1	NEFH
MAN1A1	ATP6V1B2	RG9MTD2	OPRM1
RAB8A	ATP6V0C	SAT2	OTC
MIF	ATP6V1C1	KIF12	OXTR
MME	ATP6V1E1	MAL2	PAPPA
MUC1	ATP6V0A1	OSBPL1A	PC
MYH9	ATP6AP1	VASN	PCOLCE
NAGLU	AZU1	SLC22A12	PDGFRB
NONO	BCR	ACSM1	PFKFB3
NPM1	BGN	TTC18	PGAM2
NRAS	BLMH	GSTO2	SERPINE2
P2RX4	BLVRA	CLRN3	PLP2
P4HB	BLVRB	LRRK2	PPP1CC
PEBP1	BPI	C12orf59	SRGN
SERPINA5	BTG1	LOC124220	MAP2K6
PFN1	BTN1A1	SLC5A10	PSMB7
PFN2	TSPO	CCDC105	PSMB10
ABCB1	C1QC	C1orf93	PTK7
SERPINA1	CAPN5	ARL8A	PTPRK
PIGR	C5	LOC128192	PZP
PIK3C2B	C9	GALM	RAD21
PKD1	PTTG1IP	LRRC15	RASA1
PLSCR1	CACNA2D1	LOC131691	RDH5
PODXL	CALML3	H1FOO	RPL18
CTSA	CAMK4	ENPP6	RPL29
PPIA	CAMP	CMBL	RPS10
PSAP	CAPG	MUM1L1	RPS24
PSMB3	CAPN1	C20orf117	S100A13
PTBP1	CAPN2	SIRPA	SAA4
PTPRJ	CAPZA2	PLEKHA7	ATXN1
RAB1A	CD14	A2ML1	CLEC11A
RAB2A	CD80	C16orf89	SDC2
RAB3B	CD36	TOM1L2	SMARCA4
RAB5A	SCARB2	KIF18B	SPOCK1
RAB5B	CD40	C19orf18	STAT1
RAB13	CDC2	PM20D1	STC1
RAB27B	CEL	PROM2	SURF4

RAB5C	CETP	GPR155	SYT1
RAC1	CTSC	SLC36A2	TAGLN
RALB	AP2M1	VPS37D	TCN1
RAP1B	CSN1S1	SLC5A12	TERF1
RBM3	CSN2	SLC5A8	TGFB2
RNASE2	CSN3	EML5	TSPAN4
S100A6	ACSL3	TBC1D21	TSN
S100A11	FOLR1	ZNF114	TSNAX
S100P	B4GALT1	ANO6	COL14A1
SLC1A1	GNAQ	SLC5A9	WNT5A
SLC2A5	HBB	CRTC2	ZNF134
SLC12A1	HBD	C20orf106	PXDN
SLC12A3	CFH	TMEM192	SMC1A
SNCG	HLA-G	ARMC3	OFD1
SNRPD1	HP	NAPEPLD	COPS3
SOD1	HPR	C10orf30	STC2
SRI	IGHA1	ATP6V0D2	ADAM9
TF	IGJ	STXBP4	CREG1
THBS1	IGLC1	C17orf61	CDK5R2
THY1	IGLC2	TXNDC8	TNFSF18
TMPRSS2	IGLC3	LRRC57	MPZL1
TSG101	LAMC1	HSPA12A	SEMA5A
TUBB2A	LPA	MAGI3	CLDN1
UBE2N	LPL	C11orf47	RGN
UMOD	LRP1	SLC39A5	SLC16A3
UPK2	LTF	C12orf51	ARHGEF1
VTN	TACSTD2	SLC46A3	LRRFIP2
EIF4H	MBL2	VMO1	TAAR2
YWHAB	MYH8	SLC26A11	CRIPT
YWHAG	NEB	LOC284422	ENTPD4
YWHAZ	PON1	CRB2	IFT140
NPHS2	PKN2	HIST2H2AB	RNF40
RAB7A	PROS1	FAM151A	RB1CC1
PSCA	MASP1	SLC6A19	PSMD6
CUBN	RELN	PKD1L3	MRC2
BBOX1	PTX3	LOC342897	HDAC5
RAB11A	RARS	EGFL11	RASA4
NAPA	SILV	SERINC2	SLC25A13
PROM1	THBS2	PDDC1	PSMD14
FCGBP	TLR2	SLCO4C1	TFG
CPNE3	TTN	SFT2D2	CDIPT
MGAM	TTR	C9orf169	CRTAP
GPRC5A	TYRP1	LOC377711	UNC13B
RAB11B	VWF	OR11L1	ARL6IP5
VAMP3	CLIP2	RAB19	TGOLN2
SLC9A3R1	XDH	LOC440335	POSTN
ITM2B	APOL1	HIST2H2BF	CLPX
NAPSA	FCN3	LOC441241	TSPAN9

VPS4B	SELENBP1	KPRP	TMED10
RAB3D	SMC3	HSP90AB6P	SLC38A3
PRDX6	DDX21	LOC643751	IL1RAPL1
KIAA0174	CCPG1	LOC651536	GALNT5
PDCD6	ABCG2	LOC652968	PRR4
ARPC4	SFI1	AEBP1	ITGA11
TSPAN1	MVP	AMY1A	CLASP2
PDZK1IP1	AKAP9	AMY1B	EPB41L3
NUTF2	PRG4	AMY1C	KIAA0467
FLOT1	AKR1A1	AMY2A	DULLARD
HRSP12	ABCA7	ANGPT1	NOMO1
A2M	COLEC10	APLP2	KIAA0146
ACP1	GNB5	APP	SLC39A14
ACTA1	MMRN1	AQP5	DNPEP
ACTN4	CLASP1	AZGP1	CASP14
ACTN1	SYNE1	CEACAM1	STX12
ACTN2	NIPBL	BMP3	BRMS1
ADAM10	CHRDL2	CA6	ABI3BP
AHCY	HSPB8	DDR1	PLEKHG3
ALDH1A1	ANGPTL4	CAPNS1	FBXW8
SLC25A4	NIN	COL6A2	GAPDHS
SLC25A5	ZNF571	COPA	GREM1
SLC25A6	LRP1B	CPD	DKK3
ANXA1	CNDP2	DLD	SRPX2
ANXA2P2	DNAH7	ETFA	IGHV3-11
APOA1	HCN3	GLUD1	IGHV3-7
ARHGDIA	EXOC4	HSD17B10	IGLV4-3
ARVCF	SNX25	IMPDH2	IGLV3-21
	TC2N	HTATIP2	IGLV1-40
	HAPLN3	MARVELD2	ST6GALNAC6
ATP1B1	CD163L1	CST4	COPS4
ATP5A1	HRNR	CST5	HERC5
ATP5B	P704P	CTSB	NUSAP1
ATP5I	CD24	DAG1	PLUNC
ATP5O	COL6A3	DSG2	PPME1
B2M	COL15A1	TOR1A	MBD3
CALM1	COMT	ECM1	SLC38A2
CALM2	CP	EIF4G1	FAM64A
CALM3	CPN2	EXT2	GTPBP2
CANX	CRABP2	FAT2	DIRAS2
CAPZA1	CRK	GPC4	DCHS2
CD2	CRYAB	FOLH1	QPCTL
CD247	CRYM	FUT2	PARP16
CD86	CSE1L	FUT3	TMEM51
CD37	CSK	FUT6	MCM10
CD44	CSTB	FUT8	CHST12
CD53	CTH	GLRX	LYAR
CDC42	CTNS	GPC1	ODZ3

CDH1	CTSD	GPX3	WDR52
CFL1	CTSG	IGHA2	ASH1L
CFL2	DDB1	IGHVα	UNC45A
COX4I1	DDC	IGLα	SLC7A10
COX5B	DDX3X	IVL	PNO1
CLDN3	DDX5	KRT12	CD248
CSPG4	CFD	LAMA4	AHRR
CSRP1	DNM2	LAMB2	ZBTB4
CST3	DPYS	LGALS7	SPTBN4
CTNNA1	DSC2	LMAN1	LGR6
CTNNB1	DSG3	LPO	RNF123
NQO1	ECE1	LTBP3	PRDM16
DYNC1H1	MEGF8	DNAJB9	PARVG
EEF1A2	ELA2	MEST	RMND5A
EFNB1	SERPINB1	MGAT1	FAT4
CTTN	EPHX2	MGP	FLJ13197
EPHB4	FBL	MUC5AC	TREML2
ERBB2	EVPL	MUC7	SVEP1
F5	F11	NEU1	OBFC1
FASN	FABP1	NUCB1	ZNF614
FKBP1A	ACSL4	NUCB2	FLJ22184
FLNA	FAH	FURIN	DBF4B
FLNB	EFEMP1	PAM	CD276
G6PD	FBP1	PLG	CMIP
GCNT2	FKBP4	FXYD3	ADAMTS12
PDIA3	FKBP5	PLOD2	SPACA1
GSN	FRK	PLTP	VANGL1
HADHA	FTH1	PON3	SPRY4
HLA-DMB	FUCA1	PPP1CB	HYI
HLA-E	GABRB2	PRELP	FAM108A1
HNRNPA2B1	GALK1	DNAJC3	TMEM47
HNRNPH2	GBE1	HTRA1	MYCBPAP
HSPA1L	GDF2	RARRES1	RAB6C
HSPA2	GFRA1	SAA1	FAM71F1
HSPA4	GK2	SAA2	ZNF503
HSPA7	GLO1	SEPP1	PARP10
HSPA9	GLUL	SFRP1	SHANK3
HSP90AA4P	GM2A	ST3GAL1	LACRT
HSP90AA2	GNG5	SLC5A5	TRIM41
HSP90AB3P	GOT1	SLC9A1	OXNAD1
HSPE1	GPD1	SLC20A2	LDHAL6B
HSPG2	GPM6A	SLPI	LOC92755
ICAM1	GPT	SRPR	CACNA2D4
ITGA6	GPX4	STAU1	ARHGAP18
ITGA2	GRB2	HSPA13	AHNAK2
ITGAV	GRID1	TGFBI	RPLP0P2
	GSR	TGM1	PGLYRP2
ITGB2	GSS	TGM3	RAB39B

ITGB4	GSTM2	YES1	GYLTL1B
JUP	HGD	HIST2H2AA3	KRT74
CD82	HINT1	HIST2H2BE	SLAIN1
KPNB1	HNMT	GALNT4	LOC122589
KRT2	HNRNPL	B4GALT3	NLRP8
KRT5	HPD	TNFSF13	PODN
KRT8	HPX	TNFSF12	C5orf24
KRT13	HRG	ANGPTL1	CD109
KRT14	DNAJA1	GCNT3	TRIM40
KRT15	HSPB1	TM9SF2	GPR112
KRT16	DNAJB1	DDX23	KRT72
KRT18	CFI	ADAMTS3	VTI1A
KRT19	IGF2R	GPR64	SYT9
LAMP2	IGFALS	LHFPL2	KRT80
LGALS4	IL1RN	ST3GAL6	CCDC64B
LYZ	IRF6	PRDX4	ATP8B3
	ITGA1	MAN1A2	C1orf84
MFGE8	EIF6	OS9	LOC149501
MMP7	ITGB8	MGAT4A	LOC150786
MYH10	ITIH4	TWF2	WDR49
MYL6	KHK	CLCA4	NEK10
MYO1C	KIFC3	TXNDC4	STOML3
MYO1D	KLK1	PLCB1	SASS6
NME1	LBP	CES3	DCLK2
NME2	LCN2	B3GAT3	FREM3
PRDX1	LCP1	TOR1B	C9orf91
PCBP1	LTA4H	IGHV3OR16-13	TREML2P
CHMP1A	BCAM	IGLV2-11	CCDC129
SERPINF1	MAN2A1	IGLV1-44	PAN3
PHB	MDH2	IGKV3D-15	MAMDC2
PPIB	MFI2	IGKV4-1	RCOR2
PRKAR2A	MLLT3	C1GALT1C1	LOC283412
PRKDC	MLLT4	RACGAP1	LOC283523
PSMA2	MNDA	EFEMP2	NOMO2
QSOX1	MPO	DUOX2	SEC14L4
PYGB	MPST	SDF4	LCN1L1
RAB6A	MYO1B	CYB5R1	LOC286444
RALA	MSRA	ERAP1	TAS2R60
RAP1A	MTAP	NUDT9	KRT18P19
RPL6	MTHFD1	FAM3B	LOC343184
RPL8	MYH3	FAM20A	LOC345041
RPLP1	MYO5B	FAM55D	GNAT3
RPLP2	MYO6	ANO1	POLN
RPN1	NID1	LRRC16A	LOC376693
RPS3	NKX6-1	TTC17	ARMS2
RPS7	NQO2	PDGFC	LOC387867
RPS13	NP	PCDHGB5	LOC388339
RPS14	NPC1	CCL28	FLG2

RPS15A	NPHS1	UGCGL1	LOC388707
RPS18	NRF1	SEMA3G	LOC389141
RPS20	NT5E	CORO1B	LOC390183
RPS21	PAFAH1B1	NDRG2	KRT8P9
RPS27A	PAFAH1B2	KIAA1324	LOC391777
RRAS	PCBD1	TXNDC16	LOC391833
S100A10	PCK1	ARHGAP23	LOC399942
SDC1	PDCD2	MUTED	LOC400389
SDC4	PDE8A	TINAGL1	LOC400578
SLC1A5	ENPP3	TOR3A	LOC400750
SLC2A1	SLC26A4	VWA1	LOC400963
	PDZK1	CHID1	FLJ21767
SLC12A2	PEPD	TMEM109	LOC401817
SLC16A1	PFKL	GAL3ST4	NOMO3
SPTBN1	PGD	THSD4	LOC439953
SSBP1	PGM1	UXS1	RPL12P6
SSR4	SLC25A3	TXNDC5	LOC440589
TBCA	SERPINA4	CRISPLD1	LOC440917
TCEB1	SERPINB6	LOXL4	LOC440991
TFRC	SERPINB13	GNPTG	LOC441876
TKT	PIK3C2A	SCGB3A1	LOC442308
TSPAN8	PIP	CHST14	DIPAS
TPM1	PKD2	C1QTNF1	LOC643300
HSP90B1	PKLR	C1QTNF3	LOC643358
TUBA4A	PKHD1	SLC26A9	LOC643531
TUFM	PLCD1	FAM129A	RPSAP8
TXN	PLOD1	HIST2H3C	LOC644464
UBA52	PLS1	TPRG1L	LOC644745
UBB	UBL3	TMPRSS11B	LOC645018
UBC	PPL	C20orf70	LOC645548
UBA1	PPP1R7	PPM1L	LOC646127
UBE2V2	PRCP	GBP6	LOC646316
UGDH	PRKCA	KRT78	LOC646359
UQCRC2	PRKCD	SLC37A2	LOC646785
VCP	PRKCH	NPNT	LOC646875
VIL1	PRKCI	KRT73	LOC646949
YWHAH	PRKCZ	HIST2H3A	LOC647000
CXCR4	PRNP	VWA2	LOC647285
SLC7A5	PRSS8	GSTK1	LOC650405
HIST1H4I	PRTN3	SBSN	LOC650901
HIST1H4A	PSMA1	C5orf46	LOC652493
HIST1H4D	PSMA3	LRRC26	LOC652797
HIST1H4F	PSMA4	C4orf40	LOC653162
HIST1H4K	PSMA5	LOC440786	PPIAL3
HIST1H4J	PSMB1	SCFV	LOC653232
HIST1H4C	PSMB2	LGALS7B	HSPBL2
HIST1H4H	PSMB5	HIST2H3D	LOC728002
HIST1H4B	PSMB6	ACAT2	LOC728088

HIST1H4E	PSMC5	ACTL6A	LOC728576
HIST1H4L	PSMD12	ADK	LOC728590
HIST2H4A	PSME2	ANXA8L2	LOC728791
TAGLN2	PTPN6		LOC728979
RUVBL1	PTPN13		ANG
VAMP8	PTPRO		BDNF
SNAP23	QDPR	CAV1	CALU
IQGAP1	RAB27A	CD70	CCR4
KRT75	RAP1GDS1	CS	CCR5
TJP2	RBL2	DARS	CSF2
ROCK2	RBP4	DHX9	CSF3
ARPC3	RENBP	DPYSL2	DCN
ACTR3	RFC1	EEF1D	EPO
LRPPRC	RHEB	EPRS	F3
TRAP1	RNH1	FDPS	GPC5
TUBB4	RNPEP	FLNC	GDF1
GNB2L1	ROBO2	XRCC6	GDF9
BAIAP2	RP2	GFPT1	GFRA3
HYOU1	RPS11	HIST1H1B	GRN
AGR2	RREB1	HIST1H2BB	CXCL2
OLFM4	RYR1	H3F3A	GZMA
CCT2	S100A4	H3F3B	HIST1H2BD
ATP5L	S100A8	HNRNPF	HGF
CCT8	S100A9	HNRNPK	IFNG
SLC12A7	SERPINB4	IARS	IGFBP3
MASP2	SCN10A	LAMA3	IGFBP4
IQGAP2	SEC13	LAMB3	IGFBP6
RAB10	SECTM1	LAMC2	IGFBP7
PRDX3	SH3BGRL	LGALS1	IL1RAP
EHD1	SHMT1	NBR1	IL3
TMED2	SHMT2	MARS	IL5
LMAN2	SLC3A1	MX1	IL6ST
YWHAQ	SLC4A1	PFKP	IL7
GCN1L1	SLC5A1	PLAU	IL8
RAB35	SLC5A2	PSMB4	IL10
DSTN	SLC6A13	PSMC2	IL11
UPK1A	SLC9A3	PSMC4	IL13
PHB2	SLC15A2	PSMD2	IL15RA
RRAS2	SLC25A1	PSMD13	INHBA
SEC31A	SLC22A2	PYGL	INHBB
CLSTN1	SLC22A5	RPL10	IPO5
PTGR1	SMO	RPL15	LIF
RAB21	SORD	STX4	LRP6
CYFIP1	SORL1	TARS	LTBP1
SLC44A1	SPAST	CLDN5	MMP1
CORO1C	SPR	TPBG	MMP2
MTCH2	SPRR3	XPO1	MMP3
QPCT	SRC	XRCC5	MMP10

PRDX5	ST13	BAT1	NBL1
SND1	STK11	HIST1H2BG	TNFRSF11B
F11R	VAMP7	HIST1H2BF	OSM
LIMA1	SYPL1	HIST1H2BE	PDGFA
RAB6B	SERPINA7	HIST1H2BI	PRKCSH
KRT20	TECTA	HIST1H2BC	CCL2
VPS35	TGM4	HIST1H4G	CCL7
TOMM22	TGFBR3	EIF3A	CCL20
AKR1B10	TGM2	EIF3B	SFRP4
S100A14	TLN1	EIF3C	SOD3
DIP2B	DNAJC7	SLC5A6	SPARC
RAP2C	UBE2G1	HIST2H2AA4	TIMP1
FAM129B	UPK1B	LOC728358	TIMP2
	UGP2	LOC730839	TIMP3
AHNAK	UPK3A	LOC100126583	ICAM5
VPS37B	UTRN	AARS	TNFRSF1A
TUBA4B	VASP	AK2	VEGFC
ARPC5L	VCL	APEH	GDF5
EPPK1	VDAC1	FAS	HIST3H3
ADSL	VDAC3	BAX	HIST1H2AI
AP2A1	XPNPEP2	FMNL1	HIST1H2AL
RHOC	BTG2	CASP9	HIST1H2AC
RHOG	GCS1	CD19	HIST1H2AM
ASNS	BAT2	MS4A1	HIST1H2BN
	PTP4A2	CD22	HIST1H2BM
CAD	DYSF	TNFRSF8	HIST1H2BH
CBR1	EEA1	SCARB1	HIST1H2BO
CBR3	STK24	ENTPD1	HIST1H3A
CCT6A	CUL4B	CD48	HIST1H3D
CDH17	CUL3	CD58	HIST1H3C
CEACAM5	ATRN	CD74	HIST1H3E
COPB1	CDC42BPA	CD79B	HIST1H3I
CLDN4	PPFIA2	CD97	HIST1H3G
CLDN7	AKR7A2	41889	HIST1H3J
CRYZ	PPAP2A	CR2	HIST1H3H
CD55	ABCB11	CSNK2B	HIST1H3B
EEF1G	MAP2K1IP1	DBI	FADD
EPHA2	EIF3H	DHCR7	IL1RL2
EIF4A1	SLC4A4	DLG1	FGF18
EIF4A2	SNX3	DOCK2	FGF16
ENO2	MYH13	DUT	HIST1H3F
SLC29A1	NAPG	ECH1	HIST1H2AG
EPHB2	FBP2	VAPA	HIST1H2BJ
EPHB3	SCEL	H2AFY	NRG2
ESD	SUCLA2	PDIA4	GDF3
F7	GGH	EIF4A3	FGF19
FLOT2	PROZ	ACTR1B	GDF11
GARS	SQSTM1	OPTN	FST

GMDS	AP1M1	NAMPT	LASS1
GNB3	RAB7L1	MPZL2	HPSE
HIST1H2AE	WASL	STIP1	ESM1
HLA-C	PLOD3	PKP3	DKK1
HLA-H	PGLYRP1	POFUT2	IL17B
HPCAL1	KALRN	QPRT	IL19
	CLIC3	WBP2	TNFRSF12A
IGHα	BAZ1B	ERO1L	IL23A
IGHG1	SPAG9	H2AFY2	FGFRL1
IGHG2	SLC13A2	RCC2	TREM1
IGHG3	ATP6V0D1	RTN4	IL1F9
IGHG4	HGS	GLT25D1	CXCL16
IGHM	AP4M1	RNASE7	IL22RA1
IGKC	ATP6V1F	FCRLA	HIST1H2BK
ITGA3	PTER	H2AFV	HIST3H2BB
KRT3	TRIP10	MRLC2	LOC440093
KRT4	SLC9A3R2	PAGE2	PGAM4
KRT6A	SLIT2	HIST1H2BA	PC-3
KRT6B	SLC22A6	SNX33	LOC729500
KRT7	KL	PTRF	KRT18P26
KRT17	KIF3B	HIST2H2BC	S100A11P
RPSA	SLC22A8	ANXA8	LOC729679
LFNG	GRHPR	NME1-NME2	KRT17P3
LGALS3	SLC22A13	EIF2S1	RCTPI1
LRP4	TMPRSS11D	EIF2S3	LOC729903
CD46	GSTO1	EIF4E	RP11-556K13.1
MICA	NPEPPS	EPB41L2	LOC100129982
MYH11	TMEM59	EVI2B	LOC100130100
NARS	ATP6V1G1	FCER2	LOC100130446
NEDD4	CDC42BPB	FGR	LOC100130562
RPL10A	CREB5	FH	LOC100130624
PCNA	CROCC	GART	LOC100130711
PLEC1	DHX34	GOT2	LOC100130819
PLXNA1	TMEM63A	NCKAP1L	LOC100131713
PPP2R1A	SLK	HLA-DPB1	LOC100131863
PSMC6	RUSC2	HLA-DQA1	LOC100132795
PSMD3	OXSR1	HNRNPA1	LOC100133211
PSMD11	SLC23A1	HNRNPC	LOC100133690
RAC3	DOPEY2	HPRT1	SET
RAP2A	ABI1	ICAM3	CCT6B
RAP2B	GNPDA1	INSR	ACTR3B
RPL12	TOM1	EIF3E	PSMA8
RPLP0	ABCB6	ITGAL	ARP11
RPS4X	ABCC9	ITGB3	BCHE
RPS4Y1	HUWE1	ITGB7	H2AFZ
RPS8	ARPC5	ITIH2	SNRPE
RPS16	ACTR2	STMN1	TFPI
SPTAN1	TSPAN3	LCK	ADAMTS1

VAMP1	ARPC2	LSP1	GDF15

Table 3. Polypeptide payloads and receivers			
General Classes			
Ankyrin repeat proteins		**Fibronectins**	**Lyases**
Antibodies	Complement receptors	GPI-linked polypeptides	Nanobodies
Aptamers	Cyclic peptides	HEAT repeat proteins	Nucleic Acids
ARM repeat proteins	DARPins	Hydrolases	Polypeptides
Carbohydrates	DNAses	Kinases	Single-chain variable fragments (scFv)
Cell surface receptors	Enzymes	Lipoproteins	Tetratricopeptide repeat proteins
Complement			
C1 inhibitor	C4 binding protein	CR3	Factor I
C3 Beta chain Receptor	CD59	CR4	Homologous restriction factor
C3aR	CR1	Decay-accelerating factor (DAF)	Membrane cofactor protein (MCP)
C3eR	CR2	Factor H	PRELP
Enzymes			
triacylglycerol lipase	bile-acid-CoA hydrolase	feruloyl esterase	phosphatidate phosphatase
(S)-methylmalonyl-CoA hydrolase	bis(2-ethylhexyl)phthalate esterase	formyl-CoA hydrolase	phosphatidylglycero phosphatase
[acyl-carrier-protein] phosphodiesterase	bisphosphoglycerate phosphatase	fructose-bisphosphatase	phosphatidylinositol deacylase
[phosphorylase] phosphatase	Carboxylic-Ester Hydrolases	fumarylacetoacetase	phosphodiesterase I
1,4-lactonase	carboxymethylenebuten olidase	fusarinine-C ornithinesterase	phosphoglycerate phosphatase
11-cis-retinyl-palmitate hydrolase	cellulose-polysulfatase	galactolipase	phosphoglycolate phosphatase
1-alkyl-2-acetylglycerophosphocholine esterase	cephalosporin-C deacetylase	gluconolactonase	phosphoinositide phospholipase C
2'-hydroxybiphenyl-2-sulfinate desulfinase	cerebroside-sulfatase	glucose-1-phosphatase	phospholipase A1
2-pyrone-4,6-dicarboxylate lactonase	cetraxate benzylesterase	glucose-6-phosphatase	phospholipase A2

3', 5'-bisphosphate nucleotidase	chlorogenate hydrolase	glutathione thiolesterase	phospholipase C
3-hydroxyisobutyryl-CoA hydrolase	chlorophyllase	glycerol-1-phosphatase	phospholipase D
3'-nucleotidase	cholinesterase	glycerol-2-phosphatase	phosphonoacetaldehyde hydrolase
3-oxoadipate enol-lactonase	choline-sulfatase	glycerophosphocholine phosphodiesterase	phosphonoacetate hydrolase
3-phytase	choloyl-CoA hydrolase	Glycosidases, *i.e.* enzymes that hydrolyse O- and S-glycosyl compounds	phosphonopyruvate hydrolase
4-hydroxybenzoyl-CoA thioesterase	chondro-4-sulfatase	glycosulfatase	phosphoprotein phosphatase
4-methyloxaloacetate esterase	chondro-6-sulfatase	Glycosylases	Phosphoric-diester hydrolases
4-phytase	citrate-lyase deacetylase	histidinol-phosphatase	Phosphoric-monoester hydrolases
4-pyridoxolactonase	cocaine esterase	hormone-sensitive lipase	Phosphoric-triester hydrolases
5'-nucleotidase	cutinase	Hydrolysing N-glycosyl compounds	phosphoserine phosphatase
6-acetylglucose deacetylase	cyclamate sulfohydrolase	Hydrolysing S-glycosyl compounds	poly(3-hydroxybutyrate) depolymerase
6-phosphogluconolactonase	Cysteine endopeptidases	hydroxyacylglutathione hydrolase	poly(3-hydroxyoctanoate) depolymerase
a-amino-acid esterase	Cysteine-type carboxypeptidases	hydroxybutyrate-dimer hydrolase	polyneuridine-aldehyde esterase
a-Amino-acyl-peptide hydrolases	D-arabinonolactonase	hydroxymethylglutaryl-CoA hydrolase	protein-glutamate methylesterase
acetoacetyl-CoA hydrolase	deoxylimonate A-ring-lactonase	iduronate-2-sulfatase	quorum-quenching N-acyl-homoserine lactonase
acetoxybutynylbithiophene deacetylase	dGTPase	inositol-phosphate phosphatase	retinyl-palmitate esterase
acetylajmaline esterase	dihydrocoumarin hydrolase	juvenile-hormone esterase	Serine dehyrdatase or serine hydroxymethyl transferase
acetylalkylglycerol acetylhydrolase	Dipeptidases	kynureninase	Serine endopeptidases
acetylcholinesterase	Dipeptide hydrolases	L-arabinonolactonase	serine-ethanolaminephosph

			ate phosphodiesterase
acetyl-CoA hydrolase	Dipeptidyl-peptidases and tripeptidyl-peptidases	limonin-D-ring-lactonase	Serine-type carboxypeptidases
acetylesterase	Diphosphoric-monoester hydrolases	lipoprotein lipase	S-formylglutathione hydrolase
acetylpyruvate hydrolase	disulfoglucosamine-6-sulfatase	L-rhamnono-1,4-lactonase	sialate O-acetylesterase
acetylsalicylate deacetylase	dodecanoyl-[acyl-carrier-protein] hydrolase	lysophospholipase	sinapine esterase
acetylxylan esterase	Endodeoxyribonucleases producing 3'-phosphomonoesters	mannitol-1-phosphatase	Site specific endodeoxyribonucleases: cleavage is not sequence specific
acid phosphatase	Endodeoxyribonucleases producing 5'-phosphomonoesters	Metallocarboxypeptidases	Site-specific endodeoxyribonucleases that are specific for altered bases .
Acting on acid anhydrides to catalyse transmembrane movement of substances	Endopeptidases of unknown catalytic mechanism	Metalloendopeptidases .	Site-specific endodeoxyribonucleases: cleavage is sequence specific
Acting on acid anhydrides to facilitate cellular and subcellular movement	Endoribonucleases producing 3'-phosphomonoesters	methylphosphothioglycerate phosphatase	sphingomyelin phosphodiesterase
Acting on GTP to facilitate cellular and subcellular movement	Endoribonucleases producing 5'-phosphomonoesters	methylumbelliferyl-acetate deacetylase	S-succinylglutathione hydrolase
Acting on phosphorus-nitrogen bonds	Endoribonucleases that are active with either ribo- or deoxyribonucleic acids and produce 3'-phosphomonoesters	monoterpene e-lactone hydrolase	steroid-lactonase
Acting on sulfur-nitrogen bonds	Endoribonucleases that are active with either ribo- or deoxyribonucleic acids and produce 5'-phosphomonoesters	N-acetylgalactosamine-4-sulfatase	sterol esterase
actinomycin lactonase	Enzymes acting on acid anhydrides	N-acetylgalactosamine-	steryl-sulfatase

		6-sulfatase	
acylcarnitine hydrolase	Enzymes Acting on carbon-carbon bonds	N-acetylgalactosaminoglycan deacetylase	succinyl-CoA hydrolase
acyl-CoA hydrolase	Enzymes acting on carbon-nitrogen bonds, other than peptide bonds	N-acetylglucosamine-6-sulfatase	sucrose-phosphate phosphatase
acylglycerol lipase	Enzymes acting on carbon-phosphorus bonds	N-sulfoglucosamine sulfohydrolase	sugar-phosphatase
acyloxyacyl hydrolase	Enzymes acting on carbon-sulfur bonds	oleoyl-[acyl-carrier-protein] hydrolase	Sulfuric-ester hydrolases
acylpyruvate hydrolase	Enzymes Acting on ether bonds	Omega peptidases	tannase
ADAMTS13	Enzymes acting on halide bonds	orsellinate-depside hydrolase	Thioester hydrolases
Adenosine deaminase	Enzymes acting on peptide bonds (peptidases)	oxaloacetase	Thioether and trialkylsulfonium hydrolases
adenylyl-[glutamate—ammonia ligase] hydrolase	Enzymes acting on phosphorus-nitrogen bonds	palmitoyl[protein] hydrolase	Threonine endopeptidases
ADP-dependent medium-chain-acyl-CoA hydrolase	Enzymes acting on sulfur-nitrogen bonds	palmitoyl-CoA hydrolase	thymidine phosphorylase
ADP-dependent short-chain-acyl-CoA hydrolase	Enzymes acting on sulfur-sulfur bonds	pectinesterase	trehalose-phosphatase
ADP-phosphoglycerate phosphatase	Ether hydrolases .	Peptidyl peptide hydrolases	triacetate-lactonase
alkaline phosphatase	Exodeoxyribonucleases producing 5'-phosphomonoesters	Peptidyl-amino-acid hydrolases	Triphosphoric-monoester hydrolases
all-trans-retinyl-palmitate hydrolase	Exonucleases that are active with either ribo- or deoxyribonucleic acids and produce 3'-phosphomonoesters	Peptidylamino-acid hydrolases or acylamino-acid hydrolases	trithionate hydrolase
aminoacyl-tRNA hydrolase	Exonucleases that are active with either ribo- or deoxyribonucleic acids and produce 5'-phosphomonoesters	Peptidyl-dipeptidases	tropinesterase
Aminopeptidases	Exoribonucleases producing 3'-phosphomonoesters	phenylacetyl-CoA hydrolase	ubiquitin thiolesterase

arylesterase	Exoribonucleases producing 5'-phosphomonoesters .	Phenylalanine ammonia lyase	UDP-sulfoquinovose synthase
arylsulfatase	Factor IX	Phenylalanine hydroxylase	uricase
Asparaginase	Factor VIII	pheophorbidase	uronolactonase
Aspartic endopeptidases	fatty-acyl-ethyl-ester synthase	phloretin hydrolase	wax-ester hydrolase
b-diketone hydrolase		phorbol-diester hydrolase	xylono-1,4-lactonase

Table 4. Targets			
General Classes of Targets			
Microbes	Polypeptides	DNA	Amino Acids
Fungi	Toxins	RNA	Prions
Bacteria	Lipids	Parasites	Cytokines
Virus	Cells	Cellular Debris	
Infectious Disease-Related Targets			
Lipopolysaccharides	Cell invasion protein	Intermedilysin	Secreted effector protein sptP
Zona occludens toxin	Cholera enterotoxin	Invasion protein sipA	Seeligeriolysin
Actin polymerization protein RickA	Cysteine protease	Iota toxin component Ia	Serine protease
Actin polymerization protein RickA	Cytolethal distending toxin	Ivanolysin	Shiga toxin
Adenosine monophosphate-protein transferase vopS	Cytolysin	LepB	Sphingomyelinase
adenylate cyclase	Cytotoxic necrotizing factor	Lethal factor	Staphylokinase
Adenylate cyclase ExoY	Cytotoxin	Leukotoxin	Streptokinase
ADP-ribosyltransferase enzymatic component	Dermonecrotic toxin	Listeriolysin	Streptolysin
Aerolysin	Deubiquitinase	Microbial collagenase	Streptopain
Alpha-toxin	Diphtheria toxin	Outer membrane protein IcsA autotransporter	Suilysin
Alveolysin	Enterohemolysin	Panton-Valentine Leucocidin F	Superantigen
Alveolysin	Enterotoxin	Perfringolysin	T3SS secreted

			effector EspF
Anthrolysin O	Epidermal cell differentiation inhibitor	Pertussis toxin	Tetanus toxin
Arp2/3 complex-activating protein rickA	Exoenzyme	Phospholipase	Tir
Binary ADP-ribosyltransferase CDT toxin	Exotoxin	Plasminogen activator	TolC
Botulinum neurotoxin	G-nucleotide exchange factor	Pneumolysin	Toxic shock syndrome toxin
C2 toxin, component II	Guanine nucleotide exchange factor sopE	Protective antigen	Zink-carboxypeptidase
CagA	Heat stable enterotoxin	Protein kinase	Zink-carboxypeptidase
Calmodulin-sensitive adenylate cyclase	IgA-specific serine endopeptidase autotransporter	Pyolysin	Zn-dependent peptidase
Cell cycle inhibiting factor	Inositol phosphate phosphatase sopB	RTX toxin	
Lipid & Cell Targets			
Circulating tumor cells	very low density lipid (VLDL)	triglycerides	Fatty acids
Metastases	high density lipoprotein	chylomicrons	Cholesterol
Eukaryotic cells	low density lipoprotein	apolipoproteins	

Table 5. Cancers			
Acute lymphoblastic leukaemia (ALL)	Colorectal cancer	Macroglobulinemia, Waldenström	Pleuropulmonary Blastoma, Childhood
Acute myeloid leukaemia (AML)	Craniopharyngioma, Childhood	Male Breast Cancer	Pregnancy and Breast Cancer
Adrenocortical Carcinoma	Cutaneous T-Cell Lymphoma	Malignant Fibrous Histiocytoma of Bone and Osteosarcoma	Primary Central Nervous System (CNS) Lymphoma
AIDS-Related Kaposi Sarcoma	Ductal Carcinoma In Situ (DCIS)	Melanoma	Prostate Cancer
AIDS-Related lymphoma	Embryonal Tumors, Childhood	Merkel Cell Carcinoma	Rare cancers
Anal Cancer	Endometrial Cancer	Mesothelioma	Rectal Cancer
Appendix Cancer	Ependymoma, Childhood	Metastatic Squamous Neck Cancer with Occult Primary	Renal cell carcinoma
Astrocytomas, Childhood	Epithelial cancer	Midline Tract Carcinoma Involving NUT Gene	Renal Pelvis and Ureter, Transitional Cell Cancer
Atypical Teratoid/Rhabdoid	Esophageal Cancer	Molar pregnancy	Retinoblastoma

Tumor, Childhood			
Basal Cell Carcinoma	Esthesioneuroblastoma, Childhood	Mouth and oropharyngeal cancer	Rhabdomyosarcoma
Bile duct cancer	Ewing sarcoma	Multiple Endocrine Neoplasia Syndromes, Childhood	Salivary Gland Cancer
Bladder cancer	Extragonadal Germ Cell Tumor	Multiple Myeloma/Plasma Cell Neoplasm	Sarcoma
Bone cancer	Extrahepatic Bile Duct Cancer	Mycosis Fungoides	Secondary cancers
Bowel cancer	Eye Cancer	Myelodysplastic Syndromes	Sézary Syndrome
Brain Stem Glioma, Childhood	Gallbladder Cancer	Myelodysplastic/Myeloproliferative Neoplasms	Skin Cancer
Brain tumours	Gastric cancer	Myeloproliferative Disorders, Chronic	Skin cancer (non melanoma)
Breast cancer	Gastrointestinal Carcinoid Tumor	Nasal Cavity and Paranasal Sinus Cancer	Small Cell Lung Cancer
Bronchial Tumors, Childhood	Germ Cell Tumor	Nasopharyngeal cancer	Small Intestine Cancer
Burkitt Lymphoma	Gestational trophoblastic tumours (GTT)	Neuroblastoma	Soft Tissue Sarcoma
Cancer of unknown primary	Glioma	Non-Hodgkin Lymphoma	Squamous Cell Carcinoma
Cancer spread to bone	Hairy cell leukaemia	Non-Small Cell Lung Cancer	Squamous Neck Cancer with Occult Primary, Metastatic
Cancer spread to brain	Head and neck cancer	Oesophageal cancer	Stomach (Gastric) Cancer
Cancer spread to liver	Heart Cancer, Childhood	Oral Cancer	Stomach cancer
Cancer spread to lung	Hepatocellular (Liver) Cancer	Oral Cavity Cancer	T-Cell Lymphoma, Cutaneous - see Mycosis Fungoides and Sézary Syndrome
Carcinoid Tumor	Histiocytosis, Langerhans Cell	Oropharyngeal Cancer	Testicular cancer
Carcinoma of Unknown Primary	Hodgkin Lymphoma	Osteosarcoma (Bone Cancer)	Throat Cancer
Cardiac (Heart) Tumors, Childhood	Hypopharyngeal Cancer	Osteosarcoma and Malignant Fibrous Histiocytoma	Thymoma and Thymic Carcinoma
Central Nervous System Atypical Teratoid/Rhabdoid	Intraocular Melanoma	Ovarian Cancer	Thyroid Cancer

Tumor, Childhood			
Central Nervous System Embryonal Tumors, Childhood	Islet Cell Tumors, Pancreatic Neuroendocrine Tumors	Pancreatic Cancer	Transitional Cell Cancer of the Renal Pelvis and Ureter
Central Nervous System, Childhood	Kidney cancer	Pancreatic Neuroendocrine Tumors (Islet Cell Tumors)	Unknown primary cancer
Cervical cancer	Langerhans Cell Histiocytosis	Papillomatosis, Childhood	Ureter and Renal Pelvis, Transitional Cell Cancer
Chordoma, Childhood	Laryngeal Cancer	Paraganglioma	Urethral Cancer
Choriocarcinoma	Leukemia	Parathyroid Cancer	Uterine Cancer, Endometrial
Chronic Lymphocytic Leukemia (CLL)	Lip and Oral Cavity Cancer	Penile Cancer	Uterine Sarcoma
Chronic myeloid leukaemia (CML)	Liver cancer	Pharyngeal Cancer	Vaginal cancer
Chronic Myeloproliferative Disorders	Lobular Carcinoma In Situ (LCIS)	Pheochromocytoma	Vulvar Cancer
Colon cancer	Low Malignant Potential Tumor	Pituitary Tumor	Waldenström Macroglobulinemia
Lymphoma	Lung Cancer	Plasma Cell Neoplasm/Multiple Myeloma	Wilms Tumor