(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 June 2019 (27.06.2019)

(10) International Publication Number
WO 2019/126799 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 9/22* (2006.01)
C07K 14/705 (2006.01)

(21) International Application Number:
PCT/US2018/067380

(22) International Filing Date:
21 December 2018 (21.12.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/609,589 22 December 2017 (22.12.2017) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

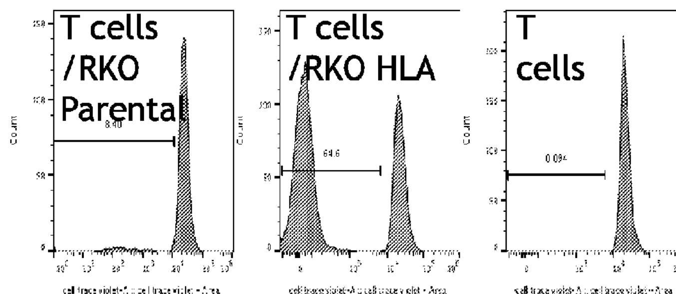
Published:
— *with international search report (Art. 21(3))*

WO 2019/126799 A1

(54) **Title:** MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) COMPOSITIONS AND METHODS OF USE THEREOF

Violet Trace Cell proliferation

FIG. 8A.



(57) **Abstract:** Immunotherapeutic compositions including class I MHC component, non-classical MHC class I component, or class II MHC components and methods of use thereof are described. The class I MHC, non-classical class I MHC, class II MHC components can be non-naturally occurring MHC component. Additionally, immunotherapeutic compositions comprising a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a gRNA targeting methylated regions of genetic elements controlling expression of MHC genes and method of use thereof are described. The compositions and methods described herein can further comprise administration of the immunotherapeutic composition with an immune checkpoint inhibitor.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/609,589, filed December 22, 2017. This provisional application is hereby incorporated herein in its entirety for all purposes.

BACKGROUND OF THE DISCLOSURE

[0002] Major histocompatibility complex (MHC) molecules are important in the immune response of the body as they bind to antigens derived from pathogens or tumors, displaying them on the cell surface for recognition by T-cells. Genes in the MHC, often referred to in humans as human leukocyte antigen (HLA) genes, include class I, class II MHC, non-classical MHC I, and non-classical MHC II genes. Class I MHC molecules are ubiquitously expressed on the surfaces of adult somatic cells and usually present peptides of cytosolic origin, although through mechanisms of cross-presentation they can present extracellular antigens. Non-classical MHC I molecules can be recognized by natural killer (NK) cells and $CD8^+$ T cells. Class II MHC molecules bind to peptides derived from proteins degraded in the endocytic pathway and are usually restricted to professional antigen presenting cells (APCs), such as dendritic cells, macrophages, and B cells, however, expression of MHC class II molecules can be induced in other types of cells, such as tumor cells. Non-classical MHC II molecules are generally not exposed on cell surface, but exposed on internal membranes in lysosomes.

[0003] One way tumor cells avoid recognition by T-cells is to express immune checkpoints, masking their identity as cancerous cells and evading immune system attack. Immune checkpoint inhibitors have been used to block this method of action and allow T-cells to recognize these cells as cancerous. However, these therapies have proven ineffective in some cancers.

[0004] Immune checkpoint inhibitors can only be effective if the T-cell is first able recognize a tumor cell. Some cancers have been shown to lack or significantly reduce expression of MHC molecules which can interfere with this tumor recognition, and could be a way in which tumor cells avoid detection. Therefore, it is desirable to develop methods of increasing the expression of MHC in cancer cells, as this could increase not only the innate immune response of the body in absence of any additional therapies but may also serve as a way to enhance the effectiveness of therapeutic agents, such as immune checkpoint inhibitors, in previously unresponsive cancers.

SUMMARY OF THE DISCLOSURE

[0005] Provided herein are immunotherapeutic compositions, comprising a nucleic acid molecule encoding a MHC component or a fragment thereof. The MHC component can be formulated with at least one, two, three, four or more different excipients for delivery to a subject or an individual. The MHC component can be a naturally occurring MHC component, or alternatively the MHC component can be non-naturally occurring. In some embodiments, the MHC component is non-naturally occurring and shows enhanced recognition by a T cell relative to a naturally occurring MHC component. In some embodiments, the MHC component is naturally occurring, and a cell expressing the heterologous MHC component has an enhanced recognition by a T cell relative to a similar cell not modified to express the heterologous MHC component. In some instances the modified cell is a cancer cell. Such cancer cell can be a solid tumor cancer cell. Such cancer cell can be a breast cancer cell, a prostate cancer cell, a lung cancer cell, a pancreatic cancer cell, an ovarian cancer cell, a liver cancer cell, a colon cancer cell, or any other cancer cell.

[0006] In some embodiments, a nucleic acid molecule of the disclosure encodes a non-naturally occurring MHC component. A non-naturally occurring MHC component can be an engineered MHC component having a high sequence homology to a naturally occurring MHC component.

[0007] In some instances, a composition herein comprises a non-naturally occurring homolog of a naturally occurring MHC component. Such homolog can comprise at least one variant compared to a nucleic acid molecule encoding a naturally occurring MHC component. In some embodiments, the variant is a mutation, an insertion, a deletion, or a duplication. An MHC homolog herein preferably has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% amino acid sequence homology to a naturally occurring MHC component. In some embodiments, a nucleic acid molecule is at least 80%, 90%, 95%, 98%, or 99% similar or has at least 80%, 90%, 95%, 98%, or 99% sequence homology to the nucleic acid sequence encoding the naturally occurring MHC component. In some embodiments, a nucleic acid molecule encodes an MHC component that is at least 80%, 90%, 95%, 98%, or 99% similar or has at least 80%, 90%, 95%, 98%, or 99% sequence homology to an MHC component that is naturally occurring. In some embodiments, the nucleic acid molecule is at least 80%, 90%, 95%, 98%, or 99% similar to the nucleic acid sequence encoding the naturally occurring MHC component. In some embodiments, the nucleic acid encodes an MHC component that is at least 80%, 90%, 95%, 98%, or 99% similar to a naturally occurring MHC component.

[0008] In some embodiments, the MHC component is a gene selected from the list consisting of: HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, HLA-F, HLA-DRA, HLA-DRB1,

HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1. The MHC component can be a class I MHC component. In some embodiments, the class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof.

[0009] In some embodiments, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class I MHC component or functional (e.g., antigenic) fragment thereof. In some embodiments, the second class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof. In some embodiments, the second class I MHC component is a naturally occurring or a non-naturally occurring MHC component. In some embodiments, a naturally occurring or a non-naturally occurring MHC component is a class II MHC component. In some embodiments, the class II MHC component comprises an alpha (α) chain, a beta (β) chain, or a combination thereof. In some embodiments, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class II MHC component or a functional fragment thereof. In some embodiments, the second class II MHC component comprises an alpha (α) chain, a beta (β) chain or a combination thereof. In some embodiments, the second class II MHC component is a naturally occurring or a non-naturally occurring MHC component. In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a vesicle such as a liposome, exosome, lipid nanoparticle, or a biomaterial. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer cell. In some embodiments, the method further comprises at least one pharmaceutically acceptable excipient, diluent, or carrier. In some embodiments, the method further comprises a unit dose of between about 0.01 μg to about 100 μg of the nucleic acid disclosed herein. In other embodiments, the method further comprises a unit does of between about 0.01 μg to about 100 μg of the MHC molecules encoded by the nucleic acid disclosed herein.

[0010] Also provided herein are methods for treating a cancer in an individual, comprising administering to the individual a nucleic acid molecule encoding a MHC component or a functional fragment thereof. In some embodiments, the MHC component can be non-naturally occurring. In other embodiments, the MHC component is naturally occurring. In some

embodiments, the non-naturally occurring MHC component shows enhanced recognition by a T cell relative to a naturally occurring MHC component. In some embodiments, the cancer is ovarian cancer, pancreatic cancer, or colon cancer. In some embodiments, the cancer has reduced MHC expression. In some embodiments, the method further comprises determining the sequence of a native MHC component of the individual. In some embodiments, the method further comprises diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced relative to a control. In some embodiments, the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof. In some embodiments, the method further comprises administering an additional therapeutic compound to the individual. In some embodiments, the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy. In some embodiments, the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof. In some embodiments, the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof. In some embodiments, the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor. In some embodiments, the cytokine is INFα, INFβ, IFNγ, or TNF. In some embodiments, the cellular therapy is an adoptive T cell transfer (ACT) therapy. Additionally or alternatively, the cellular therapy can be chimeric antigen receptor (CAR) T-cell therapy or T-cell antigen coupler (TAC) T-cell therapy.

[0011] In some embodiments, administration of the nucleic acid molecule to the individual results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound. In some embodiments, the nucleic acid molecule is a non-naturally occurring MHC component that comprises at least one variant compared to a nucleic acid molecule encoding a naturally occurring MHC component. In some embodiments, the variant is a mutation, an insertion, a deletion, or a duplication. In some embodiments, the MHC component is a gene selected from the list consisting of: HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-E, HLA-G, HLA-F, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1. In some embodiments, the nucleic acid molecule is at least 95% similar to the nucleic acid sequence

encoding the naturally occurring MHC component. In some embodiments, the nucleic acid molecule is at least 80% similar to the nucleic acid sequence encoding the naturally occurring MHC component. In some embodiments, the MHC component is a class I MHC component. In some embodiments, the class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof. In some embodiments, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class I MHC component or fragment thereof. In some embodiments, the second class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof. In some embodiments, the second class I MHC component is a naturally occurring or a non-naturally occurring MHC component. In some embodiments, the MHC component is a class II MHC component. In some embodiments, the class II MHC component comprises an alpha (α) chain, a beta (β) chain, or a combination thereof. In some embodiments, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class II MHC component or a fragment thereof. In some embodiments, the second class II MHC component comprises an alpha (α) chain, a beta (β) chain or a combination thereof. In some embodiments, the second class II MHC component is a naturally occurring or a non-naturally occurring MHC component. In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a liposome. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer cell.

[0012] Also provided herein are immunotherapeutic compositions, comprising: a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to an enzyme that modifies a nucleic acid molecule (e.g., a TET enzyme) and a guide RNA (gRNA) with a region complementary to a transcription factor or a promoter of an MHC gene. In some embodiments, the MHC gene is HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, HLA-F, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1. In some embodiments, the deactivated CRISPR-associated nuclease is deactivated Cas9 (dCas9). In some embodiments, the TET enzyme is TET1, TET2, TET3, or a catalytic domain thereof. In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some

embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a liposome. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer cell. In some embodiments, the composition further comprises at least one pharmaceutically acceptable excipient, diluent, or carrier.

[0013] Also provided herein are methods for increasing expression of an MHC gene in a cancer in an individual, comprising administering to the individual an immunotherapeutic composition comprising: a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a guide RNA (gRNA) with a region complementary to a transcription factor or a promoter of the MHC gene. In some embodiments, the MHC gene is HLA-A, HLA-B, HLA-C, HLA-E, HLA-G, HLA-F,HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1. In some embodiments, the cancer is ovarian cancer, pancreatic cancer, or colon cancer. In some embodiments, the cancer has reduced MHC expression. In some embodiments, the method further comprises diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced. In some embodiments, the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof. In some embodiments, the method further comprises administering an additional therapeutic compound to the individual. In some embodiments, the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy. In some embodiments, the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof. In some embodiments, the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof. In some embodiments, the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor. In some

embodiments, the cytokine is INFα, INFβ, IFNγ, or TNF. In some embodiments, the cellular therapy is an adoptive T cell transfer (ACT) therapy. Additionally or alternatively, the cellular therapy can be chimeric antigen receptor (CAR) T-cell therapy or T-cell antigen coupler (TAC) T-cell therapy.

[0014] In some embodiments, expression of the nucleic acid molecule by the cancer results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound. In some embodiments, the deactivated CRISPR-associated nuclease is deactivated Cas9 (dCas9). In some embodiments, the TET enzyme is TET1, TET2, TET3, or a catalytic domain thereof. In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a liposome. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer.

[0015] Further, provided herein are immunotherapeutic compositions, comprising a nucleic acid molecule encoding a regulator of an MHC molecule. In some embodiments, the regulator of the MHC molecule is selected from the group consisting of: transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, and any combination thereof. In some embodiments, the transactivator is selected from the group consisting of: class II, major histocompatibility complex, transactivator (CIITA) and NOD-like receptor family CARD domain containing 5 (NLRC5). In some embodiments, the transcription factor is selected from the group consisting of: a nuclear transcription factor Y (NF-Y), cAMP response element-binding protein (CREB), a regulatory factor X (RFX), an interferon regulatory factor (IRF), a signal transducer and activator of transcription (STAT), a ubiquitous transcription factor (USF), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). In some embodiments, wherein the NF-Y is selected from the group consisting of: NF-Ya, NF-Yb, and NF-Yc. In some embodiments, the RFX is selected from the group consisting of: RFXANK/RFXB, RFX5, and RFXAP. In some embodiments, the IRF is selected form the group consisting of: IRF-1, IRF-2, IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8, and IRF-9. In some embodiments, the STAT is selected from the group consisting of: STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, and STAT-6. In some embodiments, the USF is selected from the group consisting of: USF-1 and USF-2. In some embodiments, the acetyltransferase is selected from

the group consisting of: CREB-binding protein (CBP), p300, and p300/CBP-associated factor (pCAF). In some embodiments, the methyltransferase is Enhancer of Zeste Homolog 2 (EZH2), protein arginine N-methyltransferase 1 (PRMT1), and coactivator-associated arginine methyltransferase 1 (CARM1). In some embodiments, the elongation factor is positive transcriptional elongation factor ($pTEF_b$). In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a liposome. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer cell. In some embodiments, the immunotherapeutic compositions further comprise at least one pharmaceutically acceptable excipient, diluent, or carrier.

[0016] Moreover, provided herein are methods for treating a cancer in an individual, comprising administering to the individual a nucleic acid molecule encoding a regulator of an MHC molecule. In some embodiments, the cancer is ovarian cancer, pancreatic cancer, or colon cancer. In some embodiments, the cancer has reduced MHC expression. In some embodiments, the methods further comprise diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced relative to a control. In some embodiments, the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof. In some embodiments, the methods further comprise administering an additional therapeutic compound to the individual. In some embodiments, the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy. In some embodiments, the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof. In some embodiments, the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof. In some embodiments, the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-

dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor. In some embodiments, the cytokine is INFα, INFβ, IFNγ, or TNF. In some embodiments, the cellular therapy is an adoptive T cell transfer (ACT) therapy. Additionally or alternatively, the cellular therapy can be chimeric antigen receptor (CAR) T-cell therapy or T-cell antigen coupler (TAC) T-cell therapy.

[0017] In some embodiments, administration of the nucleic acid molecule to the individual results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound. In some embodiments, the regulator of the MHC molecule is selected from the group consisting of: transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, and any combination thereof. In some embodiments, the transactivator is selected from the group consisting of: class II, major histocompatibility complex, transactivator (CIITA) and NOD-like receptor family CARD domain containing 5 (NLRC5). In some embodiments, the transcription factor is selected from the group consisting of: a nuclear transcription factor Y (NF-Y), cAMP response element-binding protein (CREB), a regulatory factor X (RFX), an interferon regulatory factor (IRF), a signal transducer and activator of transcription (STAT), a ubiquitous transcription factor (USF), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). In some embodiments, the NF-Y is selected from the group consisting of: NF-Ya, NF-Yb, and NF-Yc. In some embodiments, the RFX is selected from the group consisting of: RFXANK/RFXB, RFX5, and RFXAP. In some embodiments, the IRF is selected form the group consisting of: IRF-1, IRF-2, IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8, and IRF-9. In some embodiments, the STAT is selected from the group consisting of: STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, and STAT-6. In some embodiments, the USF is selected from the group consisting of: USF-1 and USF-2. In some embodiments, the acetyltransferase is selected from the group consisting of: CREB-binding protein (CBP), p300, and p300/CBP-associated factor (pCAF). In some embodiments, the methyltransferase is Enhancer of Zeste Homolog 2 (EZH2), protein arginine N-methyltransferase 1 (PRMT1), and coactivator-associated arginine methyltransferase 1 (CARM1). In some embodiments, the elongation factor is positive transcriptional elongation factor ($pTEF_b$). In some embodiments, the nucleic acid molecule is DNA or RNA. In some embodiments, the nucleic acid is a plasmid. In some embodiments, the nucleic acid is a viral vector. In some embodiments, the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). In some embodiments, the nucleic acid is formulated for targeted delivery to a tumor cell. In some embodiments, the nucleic acid is formulated in a liposome. In some embodiments, the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an

aptamer, an antibody, or a combination thereof. In some embodiments, the liposome is formulated for targeted delivery to a cancer cell.

INCORPORATION BY REFERENCE

[0018] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] Features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0020] **FIGS. 1A-1D** illustrate transfection of HLA-DR alleles in an RKO colonic carcinoma cell line. FIG. 1A shows no surface expression of any HLA receptor in parental RKO. FIG. 1B shows that in RKO transfected with HLADR A alone, there is no detected HLA-DR expression on the cell surface. However, intracellular expression for the Myc-DKK tag (data not shown) indicated successful transfection. FIG. 1C shows no HLA-DR surface expression in an RKO cell line transfected with HLADR B1 alone. However, GFP expression indicated successful transfection. FIG. 1D shows high and medium GFP expression with surface expression of both alpha and beta chains in HLA-DR A and B co-transfected cells.

[0021] **FIGS. 2A-2C** illustrate transfection of HLA-DR alleles in RKO colonic carcinoma and SKOV3 cell lines. FIG. 2A is a flow cytometry analysis of parental RKO cells. FIG. 2B is a flow cytometry analysis of GFP HLA-DRAB1*15 RKO cells. FIG. 2C shows punctate GFP in co-transfected RKO cells v. green fluorescent cytoplasm when only HLA-DR B was transfected.

[0022] **FIGS. 3A-3D** illustrate fluorescent pictures of stably co-transfected RKO and SKOV3 cells listed as: RKO HLA-DR AB1 (FIG. 3A); SKOV3 HLA-DR AB1 (FIG. 3B); RKO HLA-DR AB3 (FIG. 3C); and SKOV3 HLA-DR AB3 (FIG. 3D).

[0023] **FIG. 4A** illustrates a vector structure of HLA-DR B3.

[0024] **FIG. 4B** illustrates a vector structure of HLA-DR B4.

[0025] **FIG. 4C** illustrates a vector structure of HLA-DR B5.

[0026] **FIG. 4D** illustrates a vector structure of HLA-DR alpha a.

[0027] **FIG. 4E** illustrates a vector structure of HLA-DR B1*15.

[0028] **FIG. 5A** illustrates two representative dendritic cells prepared from two different donors expressing high levels of HLA-DR and PD-L1.

[0029] **FIG. 5B** illustrates primary T-cells prepared for the mixed lymphocute reaction (MLR) assays from two different donors genotyped as HLA-DR1.

[0030] **FIG. 5C** illustrate RKO cells expressing high levels of PD-L1.

[0031] **FIGS. 6A-6F** illustrate T cell proliferation when cultured with HLA-DR transfected RKO cells together with anti-PD-1 antibodies.

[0032] **FIG. 6G** illustrates that T cells were not proliferated when cultured with RKO parental cells.

[0033] **FIG. 6H** illustrates that T cells were not proliferated without any treatment.

[0034] **FIG. 7A** illustrates T cell proliferation when cultured with parental RKO cells together with anti-PD-1 antibodies.

[0035] **FIG. 7B** illustrates T cell proliferation when cultured with HLA-DR transfected RKO cells together with anti-PD-1 antibodies.

[0036] **FIG. 7C** illustrates T cell proliferation when cultured with HLA-DR transfected RKO cells.

[0037] **FIG. 7D** illustrates T cell proliferation when cultured with HLA-DR transfected RKO cells together with anti-PD-1 antibodies.

[0038] **FIG. 8A-8C** illustrate HLA-DR transfected RKO cells increased T cell proliferations and inflammatory cytokine secretion.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0039] Disclosed herein are immunotherapeutic compositions and methods of using the same to treat or prevent a condition such as cancer. An immunotherapeutic composition herein can comprise a nucleic acid molecule encoding an MHC component or a functional fragment thereof or a regulator of the nucleic acid molecule encoding the MHC component or functional fragment thereof. Further disclosed herein are immunotherapeutic compositions comprising a an MHC component polypeptide or a functional fragment thereof or a regulator of the nucleic acid molecule encoding the MHC component or functional fragment thereof.

MHC components

[0040] As used herein, “MHC component” or “MHC molecule” refers to a nucleic acid encoding an MHC gene, a polypeptide encoded by an MHC gene, a gene or gene product associated with an MHC, or a regulator of an MHC or a regulator of nucleic acids encoding an MHC component, or a functional fragment thereof. Thus, unless a sentence is limiting, the term MHC molecule should encompass both the nucleic acid sequences encoding an MHC protein as well as the proteins. Moreover, functional fragments refer to those fragments of the proteins and nucleic acid molecules that result in substantially the same function as the full sequence. So, in

some embodiments, a functional fragment is the extracellular portion of a molecule described herein or the nucleic acid sequences encoding the extracellular portion of the protein. In other instances, a function fragment comprises both the extracellular domain and the transmembrane domain of a molecule (or nucleic acids encoding the same).

[0041] The MHC components herein can be mammalian MHC components, or more specifically a human MHC component, which can alternatively be referred to as a human leukocyte antigen (HLA). For example, HLA genes that are MHC components include HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, HLA-J, HLA-K, HLA-N, HLA-P, HLA-S, HLA-T, HLA-U, HLA-V, HLA-W, HLA-X, HLA-Y, HLA-Z, HLA-DRA, HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DRB6, HLA-DRB7, HLA-DRB8, HLA-DRB9, HLA-DQA1, HLA-DQB1, HLA-DQA2, HLA-DQB2, HLA-DQB3, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB HLA-DPA1, HLA-DPB1, HLA-DPA2, HLA-DPB2, and HLA-DPA3. A gene or gene product associated with the MHC component can be β2 microglobulin (B2M). MHC component can be used to describe an entire MHC molecule or a portion or functional fragment thereof. An MHC molecule herein can be a MHC class I molecule, a non-classical MHC molecule, or a MHC class II molecule, or a homolog or functional fragment of any of the above.

[0042] Class I MHC molecules can present peptides derived from cytosolic proteins to cytotoxic T-cells to trigger an immune response. Class I MHC molecules can also present exogenous peptides through cross-presentation. The class I MHC molecule can comprise two domains: a heavy (α) chain and a light chain (β_2 microglobulin), wherein the heavy chain and the light chain are linked non-covalently. The heavy (α) chain can further comprise three extracellular domains: an α1 domain, an α2 domain, and an α3 domain, with the α2 domain and the α3 domain forming the groove to which the peptide that the class I MHC molecule presents is bound. Non-classical MHC I molecules of the disclosure can be recognized by natural killer (NK) cells and $CD8^+$ T cells. HLA-E, HLA-F, and HLA-G are non-classical MHC I molecules encoded in the MHC I locus with low levels of heterogeneity compared to classical MHC I molecules. HLA-E molecule expression is IFN-γ-inducible and HLA-G expression can be induced by interferon-inducible transcription factors, such as IRF-1 and other stimuli.

[0043] The MHC components herein can be a class I MHC component or a functional fragment thereof. Examples of functional fragments include any of the above domains but not the entire MHC gene. For example, in one instance, an MHC component comprises the heavy (α) chain without a light chain (β_2 microglobulin). In other instances, an MHC component comprises a light chain (β_2 microglobulin) without the heavy (α) chain. In other instances, a class I MHC component can comprise a heavy (α) chain, a light chain (β_2 microglobulin), or a

combination thereof. In some instances, an MHC component includes one or two of: an $\alpha1$ domain, an $\alpha2$ domain, and an $\alpha3$ domain, but not all three domains.

[0044] A class I MHC component can be a human HLA-A gene, an HLA-B gene, an HLA-C gene or a polypeptide product thereof, or a homolog thereof, or functional fragment thereof. The class I MHC component can be a molecule encoded by any suitable HLA-A allele from a human genome. The class I MHC component can be a molecule encoded by any suitable HLA-B allele from a human genome. The class I MHC component can be a molecule encoded by any suitable HLA-C allele from a human genome. The class I MHC component can be a molecule encoded by any suitable β_2 microglobulin allele from a human genome. In some instances, the class I MHC component is a fragment of a class I MHC component. For example, a class I MHC component can be an exon or specific domain of a class I MHC component, such as the $\alpha2$ domain and the $\alpha3$ domain of the heavy chain. In some instances, the class I MHC component is a polypeptide encoded by a class I MHC gene. Thus, the present disclosure contemplates both the MHV and HLA polypeptide products and fragments (domains) described herein as well as nucleic acid molecules encoding the same.

[0045] The heavy chain of a class I MHC component can be functionally variable, wherein a plurality of different gene products can be produced by a single gene. The functionally variable products of a class I MHC gene can be referred to as a class I MHC serotypes. There can be at least 25 serotypes of HLA-A, at least 50 serotypes of HLA-B, and at least 12 serotypes of HLA-C. The class I MHC component can be any suitable class I MHC serotype. The class I MHC serotype can be HLA-A2, HLA-A3, or HLA-B8. The alleles representing these different serotypes can be selected from Table 3 attached herein. In some embodiments, a composition herein comprises nucleic acids encoding one or more, 2 or more, 3 or more, 4 or more, 5 or more, or 6 or more, 7 or more, 8 or more, 9 or more, or 10 or more different MHC components, HLA alleles, or HLA alleles described in Table 3, or functional fragments thereof.

[0046] A nucleic acid encoding a class I MHC component can comprise a nucleic acid encoding class I MHC component polypeptide. In one example, a nucleic acid encoding a class I MHC component comprises a nucleic acid that encodes an allele of HLA-A2, HLA-A3, or HLA-B8.

[0047] In some instances, the nucleic acid sequence encoding an MHC component is identical to a naturally occurring class I MHC nucleic acid sequence. In other instances, the nucleic acid sequence encoding an MHC component has been codon optimized or engineered for more efficient transfection or expression in a target cell. For example, in one instance, all intronic sequences are removed. In some instances, the nucleic acid molecule encoding an MHC component is non-naturally occurring, but the MHC component encoded by it has an amino acid

sequence that is naturally occurring. This is true for all of the MHC components described herein. In some instances, the nucleic acid sequence is different from a naturally occurring class I MHC nucleic acid sequence but encodes a polypeptide identical to a class I MHC polypeptide owing to codon degeneracy. For example, a class I MHC nucleic acid sequence can be a codon optimized class I MHC nucleic acid sequence. In some instances, the nucleic acid encoding the class I MHC component comprises a nucleic acid optimized to improve expression of the class I MHC component. In some instances, the nucleic acid sequence encoding the class I MHC component is different from a naturally occurring class I MHC nucleic acid sequence but encodes a polypeptide identical to a class I MHC polypeptide and shows increased expression relative to the expression of a naturally occurring class I MHC nucleic acid sequence.

[0048] Further, the MHC component can be a non-classical MHC I component or a fragment thereof. Non-classical MHC-I molecules are usually nonpolymorphic and tend to show a more restricted pattern of expression than their MHC class I counterparts. The non-classical MHC I component can be a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof. The non-classical MHC component can be an HLA-E gene, an HLA-G gene, an HLA-F gene or a polypeptide product thereof. The non-classical MHC component can be a molecule encoded by any suitable HLA-E allele from a human genome. The non-classical MHC component can be a molecule encoded by any suitable HLA-G allele from a human genome. The non-classical MHC component can be a molecule encoded by any suitable HLA-F allele from a human genome. The non-classical MHC component can be a molecule encoded by any suitable β_2 microglobulin allele from a human genome. In some instances, the non-classical MHC component is a functional fragment of a non-classical MHC component. For example, the non-classical MHC component can be an exon or specific domain of a non-classical MHC component, such as the α2 domain and the α3 domain of the heavy chain. In some instances, the class I MHC component is a polypeptide encoded by a non-classical MHC gene. Different alleles representing HLA-E, HLA-G, and HLA-F can be selected from Table 3.

[0049] A nucleic acid encoding a non-classical MHC I component can comprise a nucleic acid encoding a non-classical MHC I component. In some instances, the nucleic acid sequence is identical to a naturally occurring non-classical MHC I nucleic acid sequence. In some instances, the nucleic acid sequence is different from a naturally occurring non-classical MHC I nucleic acid sequence but encodes a polypeptide identical to a non-classical MHC I polypeptide owing to codon degeneracy. For example, a non-classical MHC I nucleic acid sequence can be a codon optimized non-classical MHC I nucleic acid sequence. In some instances, the nucleic acid encoding the non-classical MHC I component comprises a nucleic acid optimized to improve expression of the non-classical MHC I component. In some instances, the nucleic acid sequence

encoding the non-classical MHC I component is different from a naturally occurring non-classical MHC I nucleic acid sequence but encodes a polypeptide identical to a non-classical MHC I polypeptide and shows increased expression relative to the expression of a naturally occurring non-classical MHC I nucleic acid sequence.

[0050] Class II MHC molecules can present peptides derived from extracellular proteins. These class II molecules can usually be found on antigen-presenting cells (APC), such as dendritic cells, macrophages, and B cells, although their expression can be induced in non-antigen-presenting cells such as tumor cells. A class II MHC molecule can comprise an alpha (α) chain and a beta (β) chain. The alpha chain can comprise an α1 domain and an α2 domain, while the beta chain can comprise a β1 domain and a β2 domain, with the α1 domain and the β1 domain forming the groove to which the peptide the class II MHC molecule presents is bound. In some instances, an MHC component comprises less than all of the domains of a Class II MHC molecule.

[0051] The MHC component can be a class II MHC component or a fragment thereof. The class II MHC component can be an alpha (α) chain, a beta (β) chain, or a combination thereof. The class II MHC component can be an HLA-DM gene, HLA-DO gene, an HLA-DP, an HLA-DQ gene, an HLA-DR gene, or a polypeptide product thereof. The alpha chains and beta chains for each of the HLA-DM, HLA-DO, HLA-DP, and HLA-DQ are described in Table 1. The class II MHC component can be a molecule encoded by any suitable HLA-DM, HLA-DO, HLA-DP, or HLA-DQ allele from a human genome. In some instances, the class II MHC component is a fragment of a class II MHC component. For example, the class II MHC component can be an exon or specific domain of a class II MHC component, such as the α1 domain of the alpha chain and the β1 domain of the beta chain. In some instances, the class II MHC component is a polypeptide encoded by a class II MHC gene in Table 1. In some instances, the class II MHC component is a polypeptide encoded by HLA-DR4 or HLA-DR15.

Table 1. Genes encoding alpha and beta chains of class II MHC molecules

Class II MHC molecule	**Alpha chain**	**Beta chain**
HLA-DM	HLA-DMA	HLA-DMB
HLA-DO	HLA-DOA	HLA-DOB
HLA-DP	HLA-DPA1, HLA-DPA2, HLA-DPA3	HLA-DPB1, HLA-DPB2
HLA-DQ	HLA-DQA1, HLA-DQA2	HLA-DQB1, HLA-DQB2, HLA-DQB3
HLA-DR	HLA-DRA	HLA-DRB1, HLA-DRB2, HLA-DRB3, HLA-DRB4,

		HLA-DRB5, HLA-DRB6, HLA-DRB7, HLA-DRB8, HLA-DRB9

[0052] A class II MHC component can be class II MHC molecule such as HLA-DM, HLA-DO, HLA-DP, HLA-DQ, or HLA-DR. Each of these class II MHC molecules can comprise an alpha chain and a beta chain encoded by a gene in Table 1. The alpha chain and beta chain genes in Table 1 can be functionally variable, wherein a plurality of different gene products can be produced by a single gene. In one example, different gene products can be produced by a single gene through alternative splicing of exons. The functionally variable products of an alpha chain and beta chain as shown in Table 1 can be referred to as a class II MHC serotypes. There can be at least 21 serotypes of HLA-DR, and at least 8 serotypes of HLA-DQ. The class II MHC component can be any suitable class II MHC serotype. The class II MHC component can be HLA-DR4 or HLA-DR15. The alleles representing these different serotypes can be selected from Table 3 attached herein.

[0053] A nucleic acid encoding a class II MHC component can comprise a nucleic acid encoding a class II MHC component. In some instances, the nucleic acid sequence is identical to a naturally occurring class II MHC nucleic acid sequence. In some instances, the nucleic acid sequence is different from a naturally occurring class II MHC nucleic acid sequence but encodes a polypeptide identical to a class II MHC polypeptide owing to codon degeneracy. For example, a class II MHC nucleic acid sequence can be a codon optimized class II MHC nucleic acid sequence. In some instances, the nucleic acid sequence encoding the class II MHC component is different from a naturally occurring class II MHC nucleic acid sequence but encodes a polypeptide identical to a class II MHC polypeptide and shows increased expression relative to the expression of a naturally occurring class II MHC nucleic acid sequence. In some instances, the nucleic acid encoding the class II MHC component comprises a nucleic acid optimized to improve expression of the class II MHC component. In some instances, the nucleic acid sequence encoding the class II MHC component is different from a naturally occurring class II MHC nucleic acid sequence but encodes a polypeptide identical to a class II MHC polypeptide and shows increased expression relative to the expression of a naturally occurring class II MHC nucleic acid sequence.

[0054] Disclosed herein, in certain embodiments is a non-naturally occurring MHC component or a fragment thereof. In some instances, the non-naturally occurring MHC component is a homolog of any of a class I MHC component or class II MHC component. A

homolog is a non-naturally occurring sequence that has high sequence similarity or sequence identity to a naturally occurring sequence.

[0055] In general, “sequence similarity,” “sequence identity,” or “sequence homology,” which can be used interchangeably, refer to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Typically, techniques for determining sequence identity include determining the nucleotide sequence of a polynucleotide and/or determining the amino acid sequence encoded thereby, and comparing these sequences to a second nucleotide or amino acid sequence. Two or more sequences (polynucleotide or amino acid) can be compared by determining their “percent identity”, also referred to as “percent homology”. The percent identity to a reference sequence (e.g., nucleic acid or amino acid sequences), which may be a sequence within a longer molecule (e.g., polynucleotide or polypeptide), may be calculated as the number of exact matches between two optimally aligned sequences divided by the length of the reference sequence and multiplied by 100. Percent identity may also be determined, for example, by comparing sequence information using the advanced BLAST computer program, including version 2.2.9, available from the National Institutes of Health. The BLAST program is based on the alignment method of Karlin and Altschul, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990) and as discussed in Altschul, et al., J. Mol. Biol. 215:403-410 (1990); Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5877 (1993); and Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Briefly, the BLAST program defines identity as the number of identical aligned symbols (i.e., nucleotides or amino acids), divided by the total number of symbols in the shorter of the two sequences. The program may be used to determine percent identity over the entire length of the sequences being compared. Default parameters are provided to optimize searches with short query sequences, for example, with the blastp program. The program also allows use of an SEG filter to mask-off segments of the query sequences as determined by the SEG program of Wootton and Federhen, Computers and Chemistry 17: 149-163 (1993). A high sequence identity between a disclosed sequence and a claimed sequence contemplates at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%. In some cases, reference to percent sequence identity refers to sequence identity as measured using BLAST (Basic Local Alignment Search Tool). As used herein, percent sequence identity or homology can be determined by any one or more of the conventional methods. Methods for analyzing sequence homology include, but are not limited to, pairwise sequence alignment, which is used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences (protein or nucleic acid); and multiple sequence alignment (MSA), which is an alignment of three or more biological sequences of similar length. Various software and

analytic tools are available for determining sequence homology based on global alignment, local alignment, or genomic alignment. Examples include, but are not limited to, EMBOSS Needle provides an optimal global alignment of two sequences using the Needleman-Wunsch algorithm; EMBOSS Stretcher uses a modified version of the Needleman-Wunsch algorithm that allows larger sequences to be globally aligned; EMBOSS Water uses the Smith-Waterman algorithm to calculate local alignment of two sequences; EMBOSS Matcher provides local similarities between two sequences using a rigorous algorithm based on the LALIGN application; LALIGN identifies internal duplications by calculating non-intersecting local alignments of protein or DNA sequences; Wise2DBA (DNA Block Aligner) aligns two sequences based on the assumption that the sequences share a number of colinear blocks of conservation separated by potentially large and varied lengths of DNA in the two sequences; GeneWise compares a protein sequence to a genomic DNA sequence, allowing for introns and frameshifting errors; PromoterWise compares two DNA sequences allowing for inversions and translocations, ideal for promoters; BLAST provides local search with fast k-tuple heuristic; FASTA provides local search with fast k-tuple heuristic, faster but less sensitive than BLAST; and ClustalW provides local or global progressive alignment. In some cases, ClustalW can be used for multiple sequence alignment. In some cases, Smith-Waterman and/or BLAST can be used to find homologous sequences by searching and comparing a query sequence with sequences in a database. In some cases, Smith–Waterman algorithm is preferably used to determine sequence identity within a domain or for local sequence alignment instead of comparing full-length or entire sequences, as the Smith–Waterman algorithm compares segments of all possible lengths and optimizes the similarity measure. In some cases, the Needleman–Wunsch algorithm is preferably used for aligning entire protein or nucleotide sequences to determine global or overall sequence identity. EMBOSS Needle and Stretcher tools use the Needleman-Wunsch algorithm for global alignment. EMBOSS Water tool uses the Smith-Waterman algorithm for local alignment. In various embodiments disclosed herein, overall or local sequence identity is determined preferably using BLAST.

[0056] The non-naturally occurring MHC component can show expression in a cell that does not normally express a corresponding naturally occurring MHC component. The non-naturally occurring MHC component can show enhanced expression by a cell relative to a naturally occurring MHC component. Expression of the non-naturally occurring MHC component by the cell can result in enhanced recognition by a T-cell relative to a naturally occurring MHC component. Expression of the non-naturally occurring MHC component can result in increased apoptosis of the cell expressing the non-naturally occurring MHC component. The cell can be a tumor cell.

[0057] A nucleic acid encoding a non-naturally occurring MHC component can comprise at least one variant compared to a nucleic acid molecule encoding a naturally occurring MHC component. The variant can be a mutation, an insertion, a deletion, or a duplication. The mutation can result in a substitution, which can further encode a synonymous or non-synonymous mutation, a frameshift mutation, or a nonsense mutation. In some instances, the mutation is in a protein coding portion of a gene encoding the non-naturally occurring MHC component. In some instances, the mutation is in a promoter region of the gene encoding the non-naturally occurring MHC component.

[0058] The nucleic acid molecule of the non-naturally occurring MHC component can be at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% similar to the nucleic acid sequence encoding a corresponding naturally occurring MHC component. In some instances, the nucleic acid molecule is at least 20% similar to the nucleic acid sequence encoding the naturally occurring MHC component. In some instances, the nucleic acid molecule is at least 80% similar to the nucleic acid sequence encoding the naturally occurring MHC component. In some instances, the nucleic acid molecule is at least 95% similar to the nucleic acid sequence encoding the naturally occurring MHC component.

[0059] The polypeptide of the non-naturally occurring MHC component can be at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% similar to the polypeptide of the naturally occurring MHC component. In some instances, the polypeptide is at least 80% similar to the polypeptide of the naturally occurring MHC component. In some instances, the polypeptide is at least 95% similar to the polypeptide of the naturally occurring MHC component.

[0060] Regulators of MHC molecules can be regulators of class I MHC molecules or class II MHC molecules. The regulator can regulate transcription of a nucleic acid encoding the MHC molecule. Regulation of the transcription of the nucleic acid encoding the MHC molecule can comprise an increase in the level of transcription of the MHC molecule. Regulation of the transcription of the nucleic acid encoding the MHC molecule can comprise a decrease in the level of transcription of the MHC molecule. The regulator can be a transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, or any combination thereof.

[0061] The transactivator can be class II, major histocompatibility complex, transactivator (CIITA) or NOD-like receptor family CARD domain containing 5 (NLRC5). In some instances, CIITA is a transactivator for class II MHC molecules. In some instances NLRC5 is a transactivator for class I MHC molecules.

[0062] The transcription factor can be a nuclear transcription factor Y (NF-Y), cAMP response element-binding protein (CREB), a regulatory factor X (RFX), an interferon regulatory factor (IRF), a signal transducer and activator of transcription (STAT), a ubiquitous transcription factor (USF), or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). The NF-Y can be NF-Ya, NF-Yb, or NF-Yc. The RFX can be RFXANK/RFXB, RFX5, or RFXAP. The IRF can be IRF-1, IRF-2, IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8, or IRF-9. The STAT can be STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, or STAT-6. The USF can be USF-1 or USF-2.

[0063] The acetyltransferase can be a histone acetyltransferase (HAT). The HAT can be a CREB-binding protein (CBP), p300, or p300/CBP-associated factor (pCAF). In some embodiments, the regulator is a histone deacetylase inhibitor (DAI).

[0064] The methyltransferase can be a histone methyltransferase (HMTase), a DNA/RNA methyltransferase, or an arginine methyltransferase. The HTMase can be Enhancer of Zeste Homolog 2 (EZH2). The arginine methyltransferase can be protein arginine N-methyltransferase 1 (PRMT1) or coactivator-associated arginine methyltransferase 1 (CARM1). In one example, decreased expression of EZH2 can increase expression of CIITA.

[0065] The elongation factor can be a positive transcriptional elongation factor ($pTEF_b$).

[0066] In some embodiments, regulators of MHC molecules are upregulated by an additional factor. The additional factor upregulating a regulator of an MHC molecule can be IFN-γ, lipopolysaccharide (LPS), or IL-4. In other embodiments, regulators of MHC molecules are downregulated by an additional factor. The additional factor downregulating a regulator of an MHC molecule can be IFN-β, IL-10, nitric oxide (NO), or TGFβ. The regulator of an MHC molecule upregulated or downregulated by an additional factor can be CIITA or NLRC5.

[0067] Regulators of MHC molecules can be a ligand of a costimulatory molecule. The costimulatory molecule can be a molecule required for T-cell activation. A costimulatory molecule can be CD40. The regulator of an MHC molecule can be a ligand of CD40.

Immunotherapeutic compositions

[0068] Disclosed herein, in certain embodiments, are immunotherapeutic compositions comprising a nucleic acid molecule encoding an MHC component or a fragment thereof. In certain embodiments, the immunotherapeutic compositions comprise a polypeptide of an MHC component or a fragment thereof. Further disclosed herein, in certain embodiments, are immunotherapeutic compositions comprising a nucleic acid molecule encoding a regulator of an MHC component or a fragment thereof or a polypeptide of a regulator of an MHC component or

a fragment thereof. The nucleic acid molecule can be DNA or RNA. Any of the MHC components herein can be used as immunotherapeutic compositions.

[0069] The immunotherapeutic composition can comprise a nucleic acid molecule encoding a class I MHC component, such as a class I MHC heavy (α) chain. The nucleic acid molecule can further encode a second class I MHC component, such as a class I MHC light chain (β_2 microglobulin). For example, the immunotherapeutic composition can comprise a nucleic acid molecule encoding a class I MHC heavy (α) chain and a class I MHC light chain (β_2 microglobulin). In some instances, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class I MHC component. For example, the immunotherapeutic composition can comprise a first nucleic acid molecule encoding a class I MHC heavy (α) chain and a second nucleic acid molecule encoding a class I MHC light chain (β_2 microglobulin).

[0070] The immunotherapeutic composition can comprise a nucleic acid molecule encoding a class II MHC component, such as a class II MHC alpha (α) chain. The nucleic acid molecule can further encode a second class II MHC component, such as a class II MHC beta (β) chain. For example, the immunotherapeutic composition can comprise a nucleic acid molecule encoding a class II MHC alpha (α) chain and a class II MHC beta (β) chain. In some instances, the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class II MHC component. For example, the immunotherapeutic composition can comprise a first nucleic acid molecule encoding a class II MHC alpha (α) chain and a second nucleic acid molecule encoding a class II MHC beta (β) chain.

[0071] The immunotherapeutic composition can comprise a nucleic acid encoding a regulator of an MHC component or a fragment thereof. The immunotherapeutic composition can comprise a polypeptide of a regulator of an MHC component or a fragment thereof. The regulator can be a transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, or any combination thereof as previously described herein. The immunotherapeutic composition can comprise an additional factor regulating a regulator of an MHC component or fragment thereof. The additional factor regulating the regulator of the MHC component can be IFN-γ, lipopolysaccharide (LPS), IL-4, IFN-β, IL-10, nitric oxide (NO), or TGFβ. The additional factor can be administered as a polypeptide or as a small molecule (e.g. NO).

[0072] The additional factor can be administered simultaneous with the nucleic acid encoding the regulator of the MHC component or fragment thereof. The additional factor can be administered sequentially following administration of the nucleic acid encoding the regulator of the MHC component or fragment thereof. The nucleic acid encoding the regulator of the MHC

component or fragment thereof can be administered sequentially following administration of the additional factor.

[0073] The immunotherapeutic composition can comprise a ligand of a costimulatory molecule. The costimulatory molecule can be CD40.

[0074] The immunotherapeutic composition can comprise a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme. The nucleic acid encoding a deactivate CRIPSR-associated nuclease fused to a TET enzyme can further encode at least one guide RNA (gRNA). The immunotherapeutic composition comprising a nucleic acid encoding a deactivate CRIPSR-associated nuclease fused to a TET enzyme can further comprise a second nucleic acid encoding the gRNA. The gRNA can comprise a region complementary to a transcription factor, a regulator of an MHC component, or a promoter of an MHC gene. The deactivated CRISPR-associated nuclease can be a deactivated Cas9 (dCas9) or a deactivated Cpf1 (dCfp1). The TET enzyme can be TET1, TET2, TET3, or a catalytic domain thereof. In some instance, the TET enzyme is a TET1 enzyme or a catalytic domain of the TET1 enzyme. Administration of an immunotherapeutic composition comprising a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme can be used to demethylate a promoter, a regulator of an MHC component, or a transcription factor associated with an MHC gene. Demethylating a promoter, a regulator of an MHC component, or transcription factor associated with an MHC gene can result in increased expression of the MHC gene.

[0075] The immunotherapeutic composition can further comprise at least a second nucleic acid encoding a second deactivated CRISPR-associated nuclease fused to a TET enzyme. The second nucleic acid can further encode at least one second guide RNA. In some instances, the immunotherapeutic composition comprises a plurality of nucleic acids encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a plurality of guide RNAs. In some instances, the immunotherapeutic composition comprises a single nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a plurality nucleic acids encoding a plurality of guide RNAs. In some instances, a gRNA is designed to target a single methylated CpG site. In other instances, the gRNA is designed to target at least two methylated CpG sites.

[0076] The immunotherapeutic composition can be formulated as an aqueous solution. The immunotherapeutic composition can be formulated as a powder, for example a dry powder nucleic acid composition comprising a lipid-DNA complex. The powder formulation can further be suspended in an aqueous solution. The immunotherapeutic composition can be lyophilized, sterilized, or a combination thereof.

[0077] The immunotherapeutic composition can further comprise at least pharmaceutically acceptable excipient. The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0078] Any suitable pharmaceutically acceptable excipient can be used. An excipient can be a carrier, a diluent, a detergent, a buffer, a salt, a peptide, a surfactant, an oligosaccharide, an amino acid, a carbohydrate, or an adjuvant. In some instances, a hydrophilic excipient is used, for example a dry powder immunotherapeutic composition comprising nucleic acid dispersed within a hydrophilic excipient. Examples of excipients include, but are not limited to, human serum albumin, collagen, gelatin, hyaluronic acid, glucose, lactose, sucrose, xylose, ribose, trehalose, mannitol, raffinose, stachyose, dextran, maltodextrin, cylcodextrin, cellulose, methylcellulose, glycine, alanine, glutamate, ascorbic acid, ascorbate salts, citric acid, citrate salts, NaCl, $NaHCO_3$, NH_4HCO_3, $MgSO_4$, and Na_2SO_4.

[0079] In some instances, excipients are used to stabilize the immunological composition. The excipient can be salts dissolved in buffered solutions (which also can provide pH control or maintenance), including, but not limited to a phosphate buffered saline solution. In some instances, the excipient increases bulk of the immunological composition. The excipient can increase or decrease the absorption of the immunological composition by the individual.

[0080] The compositions herein can be formulated for oral delivery, or delivery that is intravenous, intramuscular, subcutaneous, subdermal, subcutaneous, sublingual, as well as other routes.

[0081] Solid dosage forms suitable for oral administration in accordance with the present teachings include but are not limited to capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia; (c) humectants such as glycerol; (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (e) solution retarding agents such as paraffin; (f) absorption accelerators such as quaternary ammonium compounds; (g) wetting agents such as, for example, acetyl alcohol and glycerol monostearate; (h) absorbents such as kaolin and bentonite clay, and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and

mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0082] The active compounds may also be in micro-encapsulated form with one or more excipients as noted above. Encapsulation can include the use of liposomes, exosomes, lipid nanoparticles, or a biomaterial.

[0083] Liquid dosage forms for oral administration include but are not limited to pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs.

[0084] Injectable preparations (e.g., sterile injectable aqueous or oleaginous suspensions) may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents.

[0085] Any of the formulations or compositions herein are preferably designed to specifically target cancer cells. For example, in some instances, the MHC component is formulated in an exosome that selectively targets cancer cells. Examples of such exosomes are described in Gomari et al., Onco Targets (2018) 11: 5753-5762 "Targeted cancer therapy using engineered exosome as a natural drug delivery vehicle." In some instances, the MHC component or a vesicle encapsulating the same comprises an aptamer that selectively targets the MHC component or the vesicle encapsulating it to a cancer cell. Examples of aptamers that selectively target cancer cells are described in Cerchia et al, Trends Biotechnol. (2010) Oct 28(10): 517-25 "Targeting cancer cells with nucleic acid aptamers". In another example, the MHC component or vesicle encapsulating it is coupled to a nano-material that selectively targets cancer cells, such as cancer stem cells. Examples of such nano-materials include those described in Qin et al. (2017) Front. Pharmacol. "Nanomaterials in targeting cancer stem cells for cancer therapy". In another example, the MHC component or vesicle encapsulating it is coupled to an antibody that selectively targets cancer stem cells. This can form a drug-antibody conjugate. Or alternatively the antibody can be displayed on the surface of a vesicle that directs an encapsulated MHC component to the cancer cells. Examples of drug antibody conjugation is described in Thomas et al, (2016) Lancet Oncol., June 17(6), "Antibody-drug conjugates for cancer therapy" and Dan et al., (2018) Pharmaceutical (Basel) (2018) June; 11(2):32, "Antibody-drug conjugates for cancer therapy: chemistry to clinical implications."

[0086] A nucleic acid encoding an MHC component, a nucleic acid encoding a regulator of the MHC component, or a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme can be delivered to the cell via a vector. The nucleic acid can be RNA or DNA. The cell can be a tumor cell. The vector can be a viral vector or a non-viral vector. Non-viral vector delivery systems include DNA plasmids, RNA (e.g. a transcript of a vector described

herein), naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a lipid or a liposome.

[0087] A lipid can be a cationic lipid, an anionic lipid, or neutral lipid. The lipid can be a liposome, a small unilamellar vesicle (SUV), a lipidic envelope, a lipidoid, or a lipid nanoparticle (LNP). The lipid can be mixed with the nucleic acid to form a lipoplex (a nucleic acid-liposome complex). The lipid can be conjugated to the nucleic acid. The lipid can be a non-pH sensitive lipid or a pH-sensitive lipid. The lipid can further comprise a polythethylene glycol (PEG).

[0088] The cationic lipid can be a monovalent cationic lipid, such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), [1,2-bis(oleoyloxy)-3-(trimethylammonio)propane] (DOTAP), or 3β[N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol). The cationic lipid can be a multivalent cationic lipid, such as Di-octadecyl-amido-glycyl-spermine (DOGS) or {2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate} (DOSPA).

[0089] The anionic lipid can be a phospholipid or dioleoylphosphatidylglycerol (DOPG). Examples of phospholipids include, but are not limited to, phosphatidic acid, phosphatidylglycerol, or phosphatidylserine. In some instances, the anionic lipid further comprises a divalent cation, such as Ca^2+, Mg^2+, Mn2+, and Ba^2+.

[0090] The cationic lipid or the anionic lipid can further comprise a neutral lipid. The neutral lipid can be dioleoylphosphatidyl ethanolamine (DOPE) or dioleoylphosphatidylcholine (DOPC). In some instances, the use of a helper lipid in combination with a charged lipid yields higher transfection efficiencies.

[0091] The liposome can further comprise a polymer, a lipid, a peptide, a magnetic nanoparticle (MNP), an additional compound, or a combination thereof. The polymer, lipid, or magnetic nanoparticle can be attached to the liposome or integrated into the liposomal membrane. The polymer can be a polyethylene glycol (PEG). The polymer can be N-[2-hydroxypropyl] methacrylamide (HPMA), poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA), or arginine-grafted bioreducible polymers (ABPs). The peptide can be a cell-penetrating peptide, a cell adhesion peptide, or a peptide which binds to a receptor on a cell. The cell can be a tumor cell. Any suitable cell-penetrating peptide can be used. Examples of cell-penetrating peptides include, but are not limited to a polylysine peptide and a polyarginine peptide. The cell adhesion peptide can be an arginylglycylaspartic acid (RGD) peptide. An additional compound can be a compound which binds to a receptor on a cell, such as folic acid.

[0092] The vector can be a viral vector. The viral vector can be a replication-competent viral vector or a replication-incompetent viral vector. The viral vector can be an oncolytic virus.

Examples of viral vectors include, but are not limited to, an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV). The alphavirus can be a Semliki Forest virus (SFV), a Sindbis virus (SIN), or a Venezuelan Equine Encephalitis (VEE). The pox virus can be a vaccinia virus. The herpes virus can be a herpes simplex virus (HSV) or an Epstein-barr virus (EBV). The adeno associated virus can be AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, or AAV8.

[0093] The viral vector can be a modified viral vector. The modified viral vector can show reduced immunogenicity, an increase in the persistence of the vector in the blood stream, or impaired uptake of the vector by macrophages and antigen presenting cells.

[0094] The modified viral vector can further comprise a polymer, a lipid, a peptide, a magnetic nanoparticle (MNP), an additional compound, or a combination thereof. The polymer, lipid, or magnetic nanoparticle can be attached to a capsid of the viral vector. The polymer can be a polyethylene glycol (PEG). The polymer can be N-[2-hydroxypropyl] methacrylamide (HPMA), poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA), or arginine-grafted bioreducible polymers (ABPs). The peptide can be a cell-penetrating peptide, a cell adhesion peptide, or a peptide which binds to a receptor on a cell. The cell can be a tumor cell. Any suitable cell-penetrating peptide can be used. Examples of cell-penetrating peptides include, but are not limited to a polylysine peptide and a polyarginine peptide. The cell adhesion peptide can be an arginylglycylaspartic acid (RGD) peptide. An additional compound can be a compound which binds to a receptor on a cell, such as folic acid.

[0095] The magnetic nanoparticle can be a superparamagnetic nanoparticle. In some instances, binding of an MNP can result a lower viral vector dose for optimal transgene delivery. In some instances, binding of an MNP improves transduction efficiency.

[0096] In some instances, the modified viral vector is a genetically modified vector. The genetically modified vector can have reduced immunogenicity, reduced genotoxicity, increased loading capacity, increased transgene expression, or a combination thereof. In some instances, the genetically modified viral vector is a pseudotyped viral vector. The pseudotyped viral vector can have at least one foreign viral envelope protein. The foreign viral envelope protein can be an envelope protein from a lyssavirus, an arenavirus, a hepadnavirus, a flavivirus, a paramyxovirus, a baculovirus, a filovirus, or an alphavirus. The foreign viral envelope protein can be the glycoprotein G of a vesicular stomatitis virus (VSV). In some instances, the foreign viral envelope protein is a genetically modified viral envelope protein. The genetically modified viral envelope protein can be a non-naturally occurring viral envelope protein.

[0097] In some instances, a capsid of the viral vector is conjugated with a bi-specific antibody. The bi-specific antibody can be targeted to bind to a cell of interest. The cell of interest can be a tumor cell.

[0098] Any of the compositions and immunotherapies herein can further comprise one or more therapeutic moieties. Such therapeutic moieties can include an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

Method of use

[0099] The compositions herein can be used to increase T cell activation and/or cytokine release. This can occur in vivo or in vitro. Such methods can further be used to treat conditions that evade the immune system, such as cancer for example. Thus described herein, in certain embodiments, are methods for activating the immune system and/or enhancing T cell activity and/or increasing cytokine mediated response in a subject. Such cytokine releases may be of interferon-gamma and TNF alpha. Also described herein, in certain embodiments, are methods of treating a cancer in an individual, comprising administering to the individual an immunotherapeutic composition comprising a nucleic acid molecule encoding an MHC component or polypeptide thereof. Further described herein, in certain embodiments, are methods of treating a cancer in an individual, comprising administering to the individual an immunotherapeutic composition comprising a nucleic acid molecule encoding a regulator of an MHC component, or a polypeptide thereof. Further described herein, in certain embodiments, are methods of treating a cancer in an individual, comprising administering to the individual an immunotherapeutic composition comprising a nucleic acid molecule encoding an nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme. In some cases, methods of treating a cancer in an individual comprise administering to the individual an immunotherapeutic composition comprising at least one nucleic acid molecule encoding at least two of the following: an MHC component, a regulator of an MHC component, an additional factor regulating a regulator of an MHC molecule, and a deactivated CRISPR-associated nuclease fused to a TET enzyme. The at least one nucleic acid molecule can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 nucleic acid molecules. Thus, a composition herein can include at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 different nucleic acid molecules either operably linked to one another or in separate plasmids, each of which includes a nucleic acid molecule encoding an MHC component.

[0100] The compositions herein can be used to treat cancer. The cancer can be solid tumor cancer, hematological cancer, acute myeloid leukemia, chronic lymphocytic leukemia, chronic

myeloid leukemia, acute lymphoblastic leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, bladder cancer, pancreatic cancer, cervical cancer, endometrial cancer, lung cancer, bronchus cancer, liver cancer, ovarian cancer, colon and rectal cancer, stomach cancer, gastric cancer, gallbladder cancer, gastrointestinal stromal tumor cancer, thyroid cancer, head and neck cancer, oropharyngeal cancer, esophageal cancer, melanoma, non-melanoma skin cancer, Merkel cell carcinoma, virally induced cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, renal cell cancer, renal pelvis cancer, leukemia, lymphoma, sarcoma, glioma, brain tumor, and carcinoma. In some instances, the cancer is ovarian cancer, pancreatic cancer, or colon cancer. The cancer can be a cancer that does not express an MHC molecule. The cancer can be a cancer that shows reduced expression of the MHC molecule. The MHC molecule can be a class I MHC molecule or a class II MHC molecule. In some instances, the cancer is a cancer that does not respond to an immune checkpoint inhibitor therapy.

[0101] In some instances, the method further comprises diagnosing the cancer with no or reduced MHC molecule expression. Diagnosing the cancer with no or reduced MHC molecule expression can comprise: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC molecule expression in the isolated cancerous cells is reduced or eliminated relative to a control. The control can be a predetermined level, the level of MHC expression in a non-cancerous tissue of the individual, or a level of MHC molecule expression in a non-cancerous tissue of a different subject.

[0102] In some instances, the method further comprises determining the sequence of an MHC component of the individual. The sequence of the MHC component can include exons and introns of an MHC gene as well as a promoter, 5’UTR, and 3’UTR region thereof. The MHC component of the individual can be the sequence of the native or endogenous MHC component of the individual. Sequencing the MHC component of the individual can comprise Sanger or next generation sequencing (NGS). Sequencing the MHC component can further comprise an initial step of treating the nucleic acid of the individual with bisulfite prior to sequencing. Comparing a nucleic acid sequence to a bisulfite treated nucleic acid sequence can be used to identify methylated CpG sites. In some instances, sequencing the MHC component of the individual is informative for the desired sequence of the immunotherapeutic composition. For example, if a promoter of an MHC component from a cancerous cell is hypermethylated compared to the MHC component from a non-cancerous cell, an immunotherapeutic composition can be designed to demethylate at least one methylated CpG site of the promoter. In another example, sequencing the MHC component of the individual allows for a non-naturally MHC component to be designed which will be immunologically compatible with the individual.

[0103] The method can further comprising administering an additional therapeutic compound to the individual. The additional therapeutic compound can be a therapeutic agent which binds to an immune checkpoint gene or a ligand thereof, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof. The therapeutic agent which binds to an immune checkpoint molecule or a ligand thereof can be an immune checkpoint inhibitor or an immune checkpoint agonist. Examples of immune checkpoint molecules include, but are not limited to, CD27, CD28, CD40, CD122, OX40, ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, 4-1BB, and GITR. Examples of immune checkpoint inhibitors include, but are not limited to, Ipilimumab, Tremelimumab, Nivolumab, Pembrolizumab, Atezolizumab, Avelumab, Durvaumab, and Lirilumab. The small molecule therapy can be a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor. The cytokine can be INFα, INFβ, IFNγ, or TNF. The cellular therapy can be an adoptive T cell transfer (ACT) therapy. Additionally or alternatively, the cellular therapy can be chimeric antigen receptors (CARs) T cell therapy or T-cell antigen couplers (TACs) T cell therapy. TAC receptors operate through the native T-cell receptors (TCRs). Further, a TAC comprises (1) an antigen-binding domain, (2) a TCR recruitment domain, and (3) a co-receptor domain (hinge, transmembrane, and cytosolic regions).

[0104] The additional therapeutic compound can be administered simultaneous with administration of the immunotherapeutic compound, or can be administered before or after administration of the immunotherapeutic compound. In some instances, administration of the immunotherapeutic composition results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound.

[0105] In some instances, a immunotherapeutic composition is delivered via a variety of routes. Exemplary delivery routes include oral (including buccal and sub-lingual), rectal, nasal, topical, transdermal patch, pulmonary, vaginal, suppository, or parenteral (including intramuscular, intraarterial, intrathecal, intradermal, intraperitoneal, subcutaneous and intravenous) administration or in a form suitable for administration by aerosolization, inhalation or insufflation. In some instances, the immunotherapeutic composition described herein is administered to muscle, or can be administered via intradermal or subcutaneous injections, or transdermally, such as by iontophoresis. In some cases, epidermal administration of the immunotherapeutic composition is employed.

[0106] The immunotherapeutic composition can be administered to a subject in need thereof, for example, one or more times (e.g., 1-10 times or more) daily, weekly, monthly, biannually, annually, or as medically necessary. Dosages may be provided in either a single or

multiple dosage regimens. The timing between administrations can decrease as the medical condition improves or increase as the health of the patient declines.

[0107] The dosage of the pharmaceutical compositions of the disclosure depends on factors including the route of administration, the disease to be treated, and physical characteristics, e.g., age, weight, general health, of the subject. Typically, the amount of the pharmaceutical composition contained within a single dose can be an amount that effectively prevents, delays, or treats the disease without inducing significant toxicity. The effective amount for use in humans can be determined from animal models. For example, a dose for humans can be formulated to achieve circulating, liver, topical, and/or gastrointestinal concentrations that have been found to be effective in animals. Based on animal data, and other types of similar data, those skilled in the art can determine the effective amounts of a vaccine composition appropriate for humans. The dosage can be adapted by the physician in accordance with conventional factors such as the extent of the disease and different parameters of the subject.

[0108] The immunotherapeutic composition can be administered before, during, or after the onset of a symptom associated with a disease or condition (e.g., a cancer). In some instances, the immunotherapeutic composition is administered for treatment of a cancer. In some cases, the immunotherapeutic composition is administered for prevention, such as a prophylactic treatment of a cancer. In some cases, the immunotherapeutic composition is administered to illicit an immune response from a patient.

[0109] In some aspects, the immunotherapeutic composition and kit described herein are stored at between 2°C and 8°C. In some instances, the immunotherapeutic composition is not stored frozen. In some instances, the immunotherapeutic composition is stored in temperatures of such as at -20°C or -80°C. In some instances, the immunotherapeutic composition is stored away from sunlight.

Kits

[0110] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more methods described herein. The kit can comprise an immunotherapeutic composition described herein formulated in a compatible pharmaceutical excipient and placed in an appropriate container.

[0111] The kit can include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. A container can be formed from a variety of materials such as glass or plastic.

[0112] The kit can include an identifying description, a label, or a package insert. The label or package insert can list contents of kit or the immunological composition, instructions relating to its use in the methods described herein, or a combination thereof. The label can be on or associated with the container. The label can be on a container when letters, numbers, or other characters forming the label are attached, molded or etched into the container itself. The label can be associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In some instances, the label is used to indicate that the contents are to be used for a specific therapeutic application.

[0113] A kit herein can further comprises one or more reagents such as site specific primers or probes to extract, enrich, and/or determine the sequence of the HLA alleles of an individual. The kit may further comprise one or more different HLA alleles. A therapeutic treatment comprises administering to the individual MHC components that have the same HLA alleles as what is found in the individual being treated.

[0114] The kit can further comprise one or more other therapeutic agents such as an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

Certain terminology

[0115] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. The below terms are discussed to illustrate meanings of the terms as used in this specification, in addition to the understanding of these terms by those of skill in the art. 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.

[0116] Certain ranges are presented herein with numerical values being preceded by the term “about.” The term “about” is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating un-recited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. 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 methods and compositions described herein are. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the methods and compositions described herein, 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 methods and compositions described herein.

[0117] The terms “individual,” “patient,” or “subject” are used interchangeably. None of the terms require or are limited to situation characterized by the supervision (e.g. constant or intermittent) of a health care worker (e.g. a doctor, a registered nurse, a nurse practitioner, a physician’s assistant, an orderly, or a hospice worker). Further, these terms refer to human or animal subjects.

[0118] “Treating” or “treatment” refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder, as well as those prone to have the disorder, or those in whom the disorder is to be prevented. For example, a subject or mammal is successfully “treated” for cancer, if, after receiving a therapeutic amount of a subject oligonucleotide conjugate according to the methods of the present disclosure, the subject shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of cancer cells or absence of the cancer cells; reduction in the tumor size; inhibition (i.e., slowing to some extent and preferably stopping) of cancer cell infiltration into peripheral organs, including the spread of cancer into soft tissue and bone; inhibition (i.e., slowing to some extent and preferably stopping) of tumor metastasis; inhibition, to some extent, of tumor growth; and/or relief to some extent of one or more of the symptoms associated with the specific cancer; reduced morbidity and/or mortality, and improvement in quality of life issues.

[0119] 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 the methods and compositions described herein belong. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the methods and compositions described herein, representative illustrative methods and materials are now described.

EXAMPLES

[0120] Example 1:

[0121] Co-transfection of complete alpha and beta HLA-DR chains into cell lines that do not natively express HLA-DR resulted in HLA-DR expression on the cell surface.

[0122] The RKO colonic carcinoma cell line (ATCC CRL-2577), which lack HLA-DR expression, was stably transfected with either HLA-DR A plasmid (alpha, cat#RC209920 (NM_019111)) (FIG. 4D) or HLADRB1*15 plasmid (beta cat#RG218764 (NM_002124)) (FIG. 4F), which were obtained from OriGene Technologies Inc. The RKO cells were also co-transfected with both plasmids. All transfection used electroporation (Using Mirus Bio LLC Kit) according to the manufacture protocol. Transfected cells were subjected to selection pressure using the antibiotic Geneticin® (G418-ThermoFisher) for at least 2 weeks. Transfected cells were tested by FACS using antibodies for HLA-DR A and B (ThermoFisher). For the flow cytometry testing, cells were detached from the flasks and stained with anti HLA-DR alpha (LN3, APC) or HLA-DR beta (UT36, PE) for 30 minutes at 4 degrees C. Further, the transfected cells were washed with FACS buffer twice (PBS with 2% FBS). Cells were then run on a FACS analyzer (CytoFlex S) and data were analyzed using Flowjo software version 10.2.

[0123] Referring to FIG. 1A, the parental RKO had no surface expression for any HLA receptor. FIG. 1B shows that successful transfection of RKO cells with HLA-DR A (as evidenced by intracellular expression of the Myc-DKK tag; data not shown). However, no HLA-DR was detected expression on the cell surface. Moreover, FIG. 1C shows that in an RKO cell line transfected with HLADR B1 alone, no HLA-DR surface expression was detected even though GFP expression indicated successful transfection. Additionally, FIG. 1D shows surface expression of both alpha and beta chains in cells co-transfected with HLA-DR A and B (transfection confirmed by high and medium GFP expression). This data supports the conclusion that the HLA-DR gene is silent in RKO cells, and surface expression of HLA-DR occurs only when both the A and B1 chains are expressed concurrently.

[0124] Further, according to the left column of the FACS plots in FIG. 1A-1D, the large square indicates the GFP positive gated population (i.e. GFP expression) and the small squares indicate the cells expressing medium (dark green overlay displayed in a circle in FIGS.1C and 1D) and high (light green overlay displayed in a square in FIGS. 1C and 1D) GFP expression. The middle column of the FACS plots indicates surface expression of alpha chain (X axis) and beta chain (Y axis). In the co-transfected cells, both medium and high expression GFP populations present surface expression of HLA DR A and B as seen in the right column of the FACS plots. Overlay and dark green indicated the medium GFP expression population that expresses medium intensity of HLA-DR A and B, and the light green indicated the high GFP expression population with the high HLA-DR A and B expression.

[0125] Referring to FIGS. 2A and 2B, the high GFP HLA-DRAB1*15 RKO transfected cell line was sorted (Using Sony Sorter, Sony Biotech) and re-evaluated using flow cytometry analysis (FIG. 2B) in comparison to the parental RKO cell line (FIG. 2A). FIG.2C shows

representative fluorescent pictures (Magnification 20×) of co-transfected GFP HLA-DRAB1*15 RKO cells displayed in the left column (GFP/Bright field) versus GFP HLA-DR B1*15 only in the right column. Co-transfected cells with both alpha and beta units show punctate GFP versus green fluorescent proteins scattered in cytoplasm when only HLA-DR B was transfected. This indicates the association of the alpha and beta chain and migration to the surface of the cells.

[0126] FIGS. 3A-3D show representative fluorescent pictures of stably co-transfected RKO and SKOV3 cells listed as: RKO HLA-DR AB1, SKOV3 HLA-DR AB1, RKO HLA-DR AB3, and SKOV3 HLA-DR AB3. The RKO parental cell line was also co-transfected with HLA-DR A in combination with B3 (RG210732, NM_022555), or B4 (RG202743, NM_021983) or B5 (RG203646, NM_002125), which are all obtained from Origene. Data were confirmed using flow cytometry as described above (data not shown). SKOV3 is an ovarian adenocarcinoma cell line (HTB-7, ATCC), a second cell line that lacks HLA DR expression due to lack of A and B chains expression and was co-transfected with HLA-DR A B1, HLA-DR A B3, HLA-DR A B4 and HLA-DR A B5. The transfected SKOV3 cells were sorted. GFP and HLA-DR expression in RKO cell line and pancreatic adenocarcinoma BxPC3 cell line (CRL-1687, ATCC) was not shown. Plasmids of the different Beta chains are presented in FIG. 4A-4C. Fluorescent pictures of RKO HLA-AB1 and RKO HLA-AB3 are shown in FIGS. 6A and 6C, and fluorescent pictures of SKOV3 HLA-AB1 and SKOV3 HLA-AB3 are shown in FIGS. 6B and 6D. White errors indicate punctuated vesicle expression of GFPs, which indicate the migration of MHC molecules to cell surface.

[0127] **Example 2: Proliferation of T Cells is Dependent upon HLA Expression**

[0128] Functional mixed lymphocyte reaction, T-cell proliferation, and cytokine release assays are used to test the effect of tumor cells lines expressing HLA-DR in activating T cells compared to non- expressing tumor cells.

[0129] Human Mixed Lymphocyte Reaction Assay

[0130] To test whether the co-transfected HLA-DR RKO cells can activate T cells with similar and different HLA-DRs, dendritic cells (DCs) were used as a positive control for the MLR assay. To generate these DCs, the protocol described below was followed:
Human buffy coat was purchased from Stanford Blood Center (Stanford, CA), diluted with PBS, and layered over Ficoll for the isolation of human PBMCs. The human PBMCs were washed 4 times with PBS and cluster of differentiation 14 (CD14+) monocytes were isolated using a human specific CD14+ cell isolation kit with positive selection, as described in the manufacturer's protocol (Miltenyi Biotec, San Diego, CA). CD14+ cells were then seeded at 5 x 10^5 cells/mL in complete Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% fetal bovine serum (FBS) for 7 days. Cultures were supplemented with recombinant

human (rh-) IL-4 (1000 U/mL) (R&D Systems, Minneapolis, MN) and with rh granulocyte-macrophage colony-stimulating factor (GM-CSF) (rh-GMCSF) (500 U/mL) (R&D Systems, Minneapolis, MN) at Days 0, 2 and 5. Immature DCs were harvested, washed, and counted on Day 7.

[0131] These DCs expressed high HLA-DR and PD-L1 as shown in FIG. 5A of two representative DCs prepared from two different donors (D1 and D2). The sample of each preparation was tested for PD-L1 expression using r-phycoerythrin (RPE) labeled anti-hu-PD-L1 (eBioscience/Affymatrix, Santa Clara, CA) by flow cytometry using a Cytoflex analyzer (Beckman Culture). Further, HLA- DR alpha (APC) and HLA DR beta PE (eBioscience/Affymatrix, Santa Clara, CA) expressions were also evaluated in cells isolated from D1 and D2.

[0132] Referring to FIG. 5B, human T Lymphocytes were isolated from buffy coats (Stanford blood Center, CA), diluted with phosphate buffered saline (PBS), and layered over Ficoll for the isolation of PBMCs. The human-PBMCs were washed 4 times with PBS and T lymphocytes and were isolated using a human-specific Pan T-cell isolation kit with negative selection as described in the manufacturer's protocol (Miltenyi Biotec, San Diego, CA). As shown in FIG. 5B, DCs expressed minimal levels of co-inhibitory receptors, such as LAG 3 and PD-1, as expected from rested T cells. Further, referring to FIG. 5C, the parental RKO cell line and HLA-DR AB1*15 co-transfected cells were grown with Eagle's Minimum Essential Medium (MEM) (Corning, Fisher Scientific) with 10% FBS. For stably transfected RKO cells, the G418 was added as a selection antibiotic. Both parental and HLA-DR AB transfected RKO cells expressed high level of PD-L1.

[0133] T-Cell Proliferation Assay & Cytokine Release Assay

[0134] The MLR protocol was adapted from Kruisbeek et al, 2004, with some modifications. Primary human-DCs differentiated, HLA-DR AB1 transfected RKO cells, and RKO parental were harvested on the day of experiment for optimal antigen presenting cells status and verified by flow cytometry for high levels of PD-L1 expression and co-stimulatory markers, such as CD80 and CD86, necessary for T-cell activation (data not shown). Cells were counted and treated with a low dose of 50ug/mL mitomycin C (sigma Aldrich, Saint Louis, MO) to prevent cells from secreting cytokines but functioning only as antigen presentation support to the T cells. Thus, the outcome of the assay was only induced by T cells.

[0135] Freshly isolated human-T cells from allogenic donors were harvested following the same protocol described above. T cells were plated with irradiated DCs at a ratio of 10:1 (T: DCs or RKO- for optimal assay conditions) in the presence of different concentrations of anti-PD-1 antibodies (Nivolumab and Pembrolizumab), anti LAG3 antibodies, negative and positive

control antibodies, or media alone (to evaluate the baseline reaction). All conditions were plated in 96-well flat bottom tissue culture treated plates (Fisher Scientific Pittsburg, PA). Cells were cultured using serum free X-vivo15 media (Lonza, Walkersville, MD) to prevent human serum variability between experiments. Cultures were incubated at 37°C with 5% CO_2 for 5-8 days dependent on different donors. The generation of T cells clumps was monitored under light microscope to catch any indication of T cell proliferation (examples in FIGS. 6A-6F, 7A-7D, and 8A). On the day of harvest, supernatants were collected, and cytokine concentrations were measured using Meso Scale Discovery (MSD LLC., Maryland, MD) kits for IFN- gamma and TNF alpha according to the manufacturer's protocol. For T cells proliferation measurement from MLR assay, T cells were treated with Violet CellTrace™ Violet Cell Proliferation Kit (ThermoFisher, San Diego, CA). On harvest day, cells were stained with anti CD3 antibody PE (ThermoFisher, San Diego, CA). Dead cells (Stained with Lived and dead stain eFlour510, ThermoFisher, San Diego, CA) and GFP positive cells were gated out. CD3 positive cells were gated and analyzed for Violet trace staining.

[0136] As shown in the middle histogram of FIG. 8A, RKO transfected cells were able to activate the T cells to cause proliferation. T cells that proliferated lost the dye due to equal division of the dye in each proliferation cycle and appeared as negative. When T cells did not proliferate as shown in both the left and right panels of the histograms in FIG. 8A, T cells maintained the dye. DCs showed similar results compared to RKO HLA-DR AB (data not shown). Since RKO and DCs express high level of PD-L1, proliferation in T cells was inhibited due to the expression of PD-L1. As shown in FIG. 8B, addition of anti PD-L1 antibodies increased the proliferation of T cells. Data were acquired using flow cytometry (CytofLEX S analyzer, Beckman Coulter) and data analysis was performed using Flowjo Software Version 10.2.

[0137] FIGS. 6A-6F and FIGS. 7A-7D showed representative pictures of proliferating T cells obtained from donor 1 and 2 with different magnifications. FIG. 7A demonstrates that donor 1 (D1) T cells were not proliferated when cultured together with RKO parental cells. FIG. 6A shows D1 T cells proliferated after treating with anti PD-1 antibodies and FIG. 6E shows donor 2 (D2) T cells proliferated after treating with anti PD-1 antibodies, respectively. FIGS. 6B and 6C shows D1 T cells proliferated when cultured together with HLA-DR (with both alpha and beta units) transfected RKO cells. Similarly, FIGS. 6D and 6F shows D2 T cells proliferated when cultured together with HLA-DR (with both alpha and beta units) transfected RKO cells. As a contrastF, T cells were not proliferated when T cells were cultured with RKO parental cells (FIG. 6G) or without any treatment (FIG. 6H).

[0138] Further, referring to FIGS. 7B-7D, T cells proliferated when cultured together with HLA-DR A+B (with both alpha and beta units) transfected RKO cells. T cell blasts and clusters are shown in circles with solid lines and RKO cells are circled in dashed lines.

[0139] Cytokines were measured from the supernatants of the above described cultures using MSD U-Plex Kits (Meso Scale Discovery LLC (Maryland MD). Results were run on MSD MESO QuickPlex SQ 120 analyzer and analyzed using MSD software and GraphPad Prism. For statistical analysis, 2 Way Anova was used. Levels of IFN-gamma, TNF-alpha IL-1beta, and IL-6 were measured. Referring to FIG. 8C, IFN-gamma and TNF- alpha were increased from T cells incubated with RKO HLA-DR cells or DCs (not shown, positive control used as positive control only) when compared to RKO parental line, treatment with checkpoint inhibitors increased the cytokine secretion in these cultures. IL-1beta and IL-6 were not detected or detected at low level indicating that cytokines were secreted due to T cell activation and not from innate cells like DCs or the tumor RKO cells. Data presented in duplicates with SEM. Data from RKO or RKO HLA-DR1 are shown in FIG. 8C and table below.

Table 2. Cytokine secretion from T cells activated in MLR with Parental RKO cell Line versus HLA-DR AB Co-transfected RKO Cell Line.

IFN gamma

	aPD-1 mouse IgG1			antiPD-1 huIgG4			antiPD-1 huIgG4 + anti CD 28		
	Mean pg/mL	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**
RKO HLADR αβ	89406.7111	41050.7769	2	46922.14235	16630.99135	2	91365.31965	31971.20435	2
RKO parental	344.0823453	252.1879897	2	304.7829054	241.6331026	2	5071.261095	3863.607395	2

	antiPD-1 huIgG4 + anti LAG3			anti LAG3			Isotype hIgG4		
	Mean pg/mL	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**
RKO HLADR αβ	93004.7066	20627.1864	2	30500.31218	22379.25672	2	14967.45875	2260.14585	2
RKO parental	616.772007	198.844166	2	126.2591885	115.3029116	2	247.4901336	243.5212534	2

TNF alpha

	aPD-1 mouse IgG1			antiPD-1 huIgG4			antiPD-1 huIgG4 + anti CD 28		
	Mean pg/mL	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**
RKO HLADR αβ	89406.7111	41050.7769	2	46922.14235	16630.99135	2	91365.31965	31971.20435	2
RKO parental	344.0823453	252.1879897	2	304.7829054	241.6331026	2	5071.261095	3863.607395	2

	antiPD-1 huIgG4 + anti LAG3			anti LAG3			Isotype hIgG4		
	Mean pg/mL	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**	**Mean pg/mL**	**SEM**	**N**
RKO HLADR αβ	93004.7066	20627.1864	2	30500.31218	22379.25672	2	14967.45875	2260.14585	2
RKO parental	616.772007	198.844166	2	126.2591885	115.3029116	2	247.4901336	243.5212534	2

[0140] Example 3: Administration of a non-naturally occurring class I MHC component

[0141] An individual suffering from ovarian cancer is determined to show reduced HLA-A expression in the ovarian cancer relative to baseline HLA-A expression levels in ovarian tissue. The patient is administered an adenoviral vector comprising a non-naturally occurring HLA-A

gene modified for enhanced expression in ovarian tissue. Expression of the non-naturally occurring HLA-A gene in the individual is restored.

[0142] Example 4: Targeted demethylation of hypermethylated HLA promoter regions in a colon cancer

[0143] An individual suffering from colon cancer previously shown to be unresponsive to immune checkpoint inhibitor therapy has a tumor biopsy. First, expression of each class I HLA and class II HLA gene is determined. Each of the class I HLA genes is shown to have severely reduced expression relative to normal class I HLA expression. DNA from the tumor is extracted as well as DNA from non-cancerous tissue of the same individual. An aliquot of each DNA sample is sequenced for each of HLA-A, HLA-B, and HLA-C genes. The remaining DNA samples are treated with bisulfite and the same genes are subsequently sequenced. Comparison of the non-bisulfite treated sequence with the bisulfite treated DNA reveals that the promoters of each of the three HLA class I genes are methylated with respect to the non-cancerous HLA class I genes at two different CpG sites per promoter.

[0144] An immunotherapeutic composition comprising seven different nucleic acid molecules is created, one nucleic acid molecule encodes a deactivated CRISPR-associated nuclease fused to a TET enzyme (a demethylation enzyme) and the remaining six nucleic acid molecules encode guide RNA (gRNA), each gRNA targeted one of the six methylated CpG sites identified in the promoters. The composition is administered to the individual. Expression of class I HLA molecules in the individual is assessed one day later and shown to have risen. An immune checkpoint inhibitor therapy is then administered to the individual.

[0145] Example 5: Administration of a class II MHC component

[0146] An individual suffering from pancreatic cancer is administered a liposome comprising a plasmid encoding the HLA-DQA1 and HLA-DQB1 genes.

[0147] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Table 3. HLA alleles

1	A*01	A*02	A*03	A*11	A*23	A*24	A*25	A*26	A*29	A*30
2	A*31	A*32	A*33	A*34	A*36	A*43	A*66	A*68	A*69	A*74
3	A*80	B*07	B*08	B*13	B*14	B*15	B*18	B*27	B*35	B*37
4	B*38	B*39	B*40	B*41	B*42	B*44	B*45	B*46	B*47	B*48
5	B*49	B*50	B*51	B*52	B*53	B*54	B*55	B*56	B*57	B*58
6	B*59	B*67	B*73	B*78	B*81	B*82	B*83	C*01	C*02	C*03
7	C*04	C*05	C*06	C*07	C*08	C*12	C*14	C*15	C*16	C*17
8	C*18	E*01	F*01	G*01	H*01	H*02	H*03	J*01	J*02	K*01
9	L*01	N*01	P*01	P*02	S*01	T*01	T*02	T*03	U*01	V*01
10	W*01	W*02	W*03	W*04	W*05	Y*01	Y*02	Y*03	DRA*01	DQA1*01
11	DQA1*02	DQA1*03	DQA1*04	DQA1*05	DQA1*06	DQB1*02	DQB1*03	DQB1*04	DQB1*05	DQB1*06
12	DPA1*01	DPA1*02	DPA1*03	DPA1*04	DPA2*01	DPA2*02	DPB1*01	DPB1*02	DPB1*03	DPB1*04
13	DPB1*05	DPB1*06	DPB1*08	DPB1*09	DPB1*10	DPB1*100	DPB1*101	DPB1*102	DPB1*103	DPB1*104
14	DPB1*105	DPB1*106	DPB1*107	DPB1*108	DPB1*109	DPB1*11	DPB1*110	DPB1*111	DPB1*112	DPB1*113
15	DPB1*114	DPB1*115	DPB1*116	DPB1*117	DPB1*118	DPB1*119	DPB1*120	DPB1*121	DPB1*122	DPB1*123
16	DPB1*124	DPB1*125	DPB1*126	DPB1*127	DPB1*128	DPB1*129	DPB1*13	DPB1*130	DPB1*131	DPB1*132
17	DPB1*133	DPB1*134	DPB1*135	DPB1*136	DPB1*137	DPB1*138	DPB1*139	DPB1*14	DPB1*140	DPB1*141
18	DPB1*142	DPB1*143	DPB1*144	DPB1*145	DPB1*146	DPB1*147	DPB1*148	DPB1*149	DPB1*15	DPB1*150
19	DPB1*151	DPB1*152	DPB1*153	DPB1*154	DPB1*155	DPB1*156	DPB1*157	DPB1*158	DPB1*159	DPB1*16
20	DPB1*160	DPB1*161	DPB1*162	DPB1*163	DPB1*164	DPB1*165	DPB1*166	DPB1*167	DPB1*168	DPB1*169
21	DPB1*17	DPB1*170	DPB1*171	DPB1*172	DPB1*173	DPB1*174	DPB1*175	DPB1*176	DPB1*177	DPB1*178
22	DPB1*179	DPB1*18	DPB1*180	DPB1*181	DPB1*182	DPB1*183	DPB1*184	DPB1*185	DPB1*186	DPB1*187
23	DPB1*188	DPB1*189	DPB1*19	DPB1*190	DPB1*191	DPB1*192	DPB1*193	DPB1*194	DPB1*195	DPB1*196
24	DPB1*197	DPB1*198	DPB1*199	DPB1*20	DPB1*200	DPB1*201	DPB1*202	DPB1*203	DPB1*204	DPB1*205
25	DPB1*206	DPB1*207	DPB1*208	DPB1*209	DPB1*21	DPB1*210	DPB1*211	DPB1*212	DPB1*213	DPB1*214
26	DPB1*215	DPB1*216	DPB1*217	DPB1*218	DPB1*219	DPB1*22	DPB1*220	DPB1*221	DPB1*222	DPB1*223
27	DPB1*224	DPB1*225	DPB1*226	DPB1*227	DPB1*228	DPB1*229	DPB1*23	DPB1*230	DPB1*231	DPB1*232
28	DPB1*233	DPB1*234	DPB1*235	DPB1*236	DPB1*237	DPB1*238	DPB1*239	DPB1*24	DPB1*240	DPB1*241
29	DPB1*242	DPB1*243	DPB1*244	DPB1*245	DPB1*246	DPB1*247	DPB1*248	DPB1*249	DPB1*25	DPB1*250
30	DPB1*251	DPB1*252	DPB1*253	DPB1*254	DPB1*255	DPB1*256	DPB1*257	DPB1*258	DPB1*259	DPB1*26
31	DPB1*260	DPB1*261	DPB1*262	DPB1*263	DPB1*264	DPB1*265	DPB1*266	DPB1*267	DPB1*268	DPB1*269
32	DPB1*27	DPB1*270	DPB1*271	DPB1*272	DPB1*273	DPB1*274	DPB1*275	DPB1*276	DPB1*277	DPB1*278
33	DPB1*279	DPB1*28	DPB1*280	DPB1*281	DPB1*282	DPB1*283	DPB1*284	DPB1*285	DPB1*286	DPB1*287
34	DPB1*288	DPB1*289	DPB1*29	DPB1*290	DPB1*291	DPB1*292	DPB1*293	DPB1*294	DPB1*295	DPB1*296
35	DPB1*297	DPB1*298	DPB1*299	DPB1*30	DPB1*300	DPB1*301	DPB1*302	DPB1*303	DPB1*304	DPB1*305
36	DPB1*306	DPB1*307	DPB1*308	DPB1*309	DPB1*31	DPB1*310	DPB1*311	DPB1*312	DPB1*313	DPB1*314
37	DPB1*315	DPB1*316	DPB1*317	DPB1*318	DPB1*319	DPB1*32	DPB1*320	DPB1*321	DPB1*322	DPB1*323
38	DPB1*324	DPB1*325	DPB1*326	DPB1*327	DPB1*328	DPB1*329	DPB1*33	DPB1*330	DPB1*331	DPB1*332
39	DPB1*333	DPB1*334	DPB1*335	DPB1*336	DPB1*337	DPB1*338	DPB1*339	DPB1*34	DPB1*340	DPB1*341
40	DPB1*342	DPB1*343	DPB1*344	DPB1*345	DPB1*346	DPB1*347	DPB1*348	DPB1*349	DPB1*35	DPB1*350
41	DPB1*351	DPB1*352	DPB1*353	DPB1*354	DPB1*355	DPB1*356	DPB1*357	DPB1*358	DPB1*359	DPB1*36
42	DPB1*360	DPB1*361	DPB1*362	DPB1*363	DPB1*364	DPB1*365	DPB1*366	DPB1*367	DPB1*368	DPB1*369
43	DPB1*37	DPB1*370	DPB1*371	DPB1*372	DPB1*373	DPB1*374	DPB1*375	DPB1*376	DPB1*377	DPB1*378
44	DPB1*379	DPB1*38	DPB1*380	DPB1*381	DPB1*382	DPB1*383	DPB1*384	DPB1*385	DPB1*386	DPB1*387

45	DPB1*388	DPB1*389	DPB1*39	DPB1*390	DPB1*391	DPB1*392	DPB1*393	DPB1*394	DPB1*395	DPB1*396
46	DPB1*397	DPB1*398	DPB1*399	DPB1*40	DPB1*400	DPB1*401	DPB1*402	DPB1*403	DPB1*404	DPB1*405
47	DPB1*406	DPB1*407	DPB1*408	DPB1*409	DPB1*41	DPB1*410	DPB1*411	DPB1*412	DPB1*413	DPB1*414
48	DPB1*415	DPB1*416	DPB1*417	DPB1*418	DPB1*419	DPB1*420	DPB1*421	DPB1*422	DPB1*423	DPB1*424
49	DPB1*425	DPB1*426	DPB1*427	DPB1*428	DPB1*429	DPB1*430	DPB1*431	DPB1*432	DPB1*433	DPB1*434
50	DPB1*435	DPB1*436	DPB1*437	DPB1*438	DPB1*439	DPB1*44	DPB1*440	DPB1*441	DPB1*442	DPB1*443
51	DPB1*444	DPB1*445	DPB1*446	DPB1*447	DPB1*448	DPB1*449	DPB1*45	DPB1*450	DPB1*451	DPB1*452
52	DPB1*453	DPB1*454	DPB1*455	DPB1*456	DPB1*457	DPB1*458	DPB1*459	DPB1*46	DPB1*460	DPB1*461
53	DPB1*462	DPB1*463	DPB1*464	DPB1*465	DPB1*466	DPB1*467	DPB1*468	DPB1*469	DPB1*47	DPB1*470
54	DPB1*471	DPB1*472	DPB1*473	DPB1*474	DPB1*475	DPB1*476	DPB1*477	DPB1*478	DPB1*479	DPB1*48
55	DPB1*480	DPB1*481	DPB1*482	DPB1*483	DPB1*484	DPB1*485	DPB1*486	DPB1*487	DPB1*488	DPB1*489
56	DPB1*49	DPB1*490	DPB1*491	DPB1*492	DPB1*493	DPB1*494	DPB1*495	DPB1*496	DPB1*497	DPB1*498
57	DPB1*499	DPB1*50	DPB1*500	DPB1*501	DPB1*502	DPB1*503	DPB1*504	DPB1*505	DPB1*506	DPB1*507
58	DPB1*508	DPB1*509	DPB1*51	DPB1*510	DPB1*511	DPB1*512	DPB1*513	DPB1*514	DPB1*515	DPB1*516
59	DPB1*517	DPB1*518	DPB1*519	DPB1*52	DPB1*520	DPB1*521	DPB1*522	DPB1*523	DPB1*524	DPB1*525
60	DPB1*526	DPB1*527	DPB1*528	DPB1*529	DPB1*53	DPB1*530	DPB1*531	DPB1*532	DPB1*533	DPB1*534
61	DPB1*535	DPB1*536	DPB1*537	DPB1*538	DPB1*539	DPB1*54	DPB1*540	DPB1*541	DPB1*542	DPB1*543
62	DPB1*544	DPB1*545	DPB1*546	DPB1*547	DPB1*548	DPB1*549	DPB1*55	DPB1*550	DPB1*551	DPB1*552
63	DPB1*553	DPB1*554	DPB1*555	DPB1*556	DPB1*557	DPB1*558	DPB1*559	DPB1*56	DPB1*560	DPB1*561
64	DPB1*562	DPB1*563	DPB1*564	DPB1*565	DPB1*566	DPB1*567	DPB1*568	DPB1*569	DPB1*57	DPB1*570
65	DPB1*571	DPB1*572	DPB1*573	DPB1*574	DPB1*575	DPB1*576	DPB1*577	DPB1*578	DPB1*579	DPB1*58
66	DPB1*580	DPB1*581	DPB1*582	DPB1*583	DPB1*584	DPB1*585	DPB1*586	DPB1*587	DPB1*588	DPB1*589
67	DPB1*59	DPB1*590	DPB1*591	DPB1*592	DPB1*593	DPB1*594	DPB1*595	DPB1*596	DPB1*597	DPB1*598
68	DPB1*599	DPB1*60	DPB1*600	DPB1*601	DPB1*602	DPB1*603	DPB1*604	DPB1*605	DPB1*606	DPB1*607
69	DPB1*608	DPB1*609	DPB1*61	DPB1*610	DPB1*611	DPB1*612	DPB1*613	DPB1*614	DPB1*615	DPB1*616
70	DPB1*617	DPB1*618	DPB1*619	DPB1*62	DPB1*620	DPB1*621	DPB1*622	DPB1*623	DPB1*624	DPB1*625
71	DPB1*626	DPB1*627	DPB1*628	DPB1*629	DPB1*63	DPB1*630	DPB1*631	DPB1*632	DPB1*633	DPB1*634
72	DPB1*635	DPB1*636	DPB1*637	DPB1*638	DPB1*639	DPB1*64	DPB1*640	DPB1*641	DPB1*642	DPB1*643
73	DPB1*644	DPB1*645	DPB1*646	DPB1*647	DPB1*648	DPB1*649	DPB1*65	DPB1*650	DPB1*651	DPB1*652
74	DPB1*653	DPB1*654	DPB1*655	DPB1*656	DPB1*657	DPB1*658	DPB1*659	DPB1*66	DPB1*660	DPB1*661
75	DPB1*662	DPB1*663	DPB1*664	DPB1*665	DPB1*666	DPB1*667	DPB1*668	DPB1*669	DPB1*67	DPB1*670
76	DPB1*671	DPB1*672	DPB1*673	DPB1*674	DPB1*675	DPB1*676	DPB1*677	DPB1*678	DPB1*679	DPB1*68
77	DPB1*680	DPB1*681	DPB1*682	DPB1*683	DPB1*684	DPB1*685	DPB1*686	DPB1*687	DPB1*688	DPB1*689
78	DPB1*69	DPB1*690	DPB1*691	DPB1*692	DPB1*693	DPB1*694	DPB1*695	DPB1*696	DPB1*697	DPB1*698
79	DPB1*699	DPB1*70	DPB1*700	DPB1*701	DPB1*702	DPB1*703	DPB1*704	DPB1*705	DPB1*706	DPB1*707
80	DPB1*708	DPB1*709	DPB1*71	DPB1*710	DPB1*711	DPB1*712	DPB1*713	DPB1*714	DPB1*715	DPB1*716
81	DPB1*717	DPB1*718	DPB1*719	DPB1*72	DPB1*720	DPB1*721	DPB1*722	DPB1*723	DPB1*724	DPB1*725
82	DPB1*726	DPB1*727	DPB1*728	DPB1*729	DPB1*73	DPB1*730	DPB1*731	DPB1*732	DPB1*733	DPB1*734
83	DPB1*735	DPB1*736	DPB1*737	DPB1*738	DPB1*739	DPB1*74	DPB1*740	DPB1*741	DPB1*742	DPB1*743
84	DPB1*744	DPB1*745	DPB1*746	DPB1*747	DPB1*748	DPB1*749	DPB1*75	DPB1*750	DPB1*751	DPB1*752
85	DPB1*753	DPB1*754	DPB1*755	DPB1*756	DPB1*757	DPB1*758	DPB1*759	DPB1*76	DPB1*760	DPB1*761
86	DPB1*762	DPB1*763	DPB1*77	DPB1*78	DPB1*79	DPB1*80	DPB1*81	DPB1*82	DPB1*83	DPB1*84
87	DPB1*85	DPB1*86	DPB1*87	DPB1*88	DPB1*89	DPB1*90	DPB1*91	DPB1*92	DPB1*93	DPB1*94
88	DPB1*95	DPB1*96	DPB1*97	DPB1*98	DPB1*99	DPB2*01	DPB2*02	DPB2*03	DMA*01	DMB*01
89	DOA*01	DOB*01	DRB1*01	DRB1*03	DRB1*04	DRB1*07	DRB1*08	DRB1*09	DRB1*10	DRB1*11
90	DRB1*12	DRB1*13	DRB1*14	DRB1*15	DRB1*16	DRB2*01	DRB3*01	DRB3*02	DRB3*03	DRB4*01

91	DRB4*02	DRB4*03	DRB5*01	DRB5*02	DRB6*01	DRB6*02	DRB7*01	DRB8*01	DRB9*01	HFE*001
92	MICA*002	MICA*007	MICA*008	MICA*009	MICA*010	MICA*012	MICA*018	MICA*019	MICB*002	MICB*004
93	MICB*005	TAP1*01	TAP1*02	TAP1*03	TAP1*04	TAP1*05	TAP1*06	TAP2*01	TAP2*02	

WHAT IS CLAIMED IS:

1. An immunotherapeutic composition, comprising a nucleic acid molecule encoding a first MHC component or a fragment thereof and at least one pharmaceutically acceptable excipient, diluent, or carrier.

2. The immunotherapy composition of claim 1, wherein the nucleic acid molecule is a non-naturally-occurring nucleic acid molecule and the first MHC component is naturally occurring.

3. The immunotherapy composition of claim 1, wherein the first MHC component is a non-naturally occurring protein or polypeptide.

4. The immunotherapeutic composition of claim 3, wherein the non-naturally occurring MHC component shows enhanced recognition by a T cell relative to a naturally occurring MHC component.

5. The immunotherapeutic composition of claim 1, wherein the first MHC component is HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, HLA-DPB1, or a functional fragment thereof.

6. The immunotherapeutic composition of claim 1, further comprising an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

7. The immunotherapeutic composition of claim 2, wherein the nucleic acid molecule is at least 80% identical to a nucleic acid sequence encoding a naturally occurring MHC component.

8. The immunotherapeutic composition of claim 1, wherein MHC component is a class I MHC component.

9. The immunotherapeutic composition of claim 8, wherein the class I MHC component: (a) is a heavy (α) chain and a light chain (β_2 microglobulin), or (b) comprises an allele represented by Table 3.

10. The immunotherapeutic composition of claim 1, further comprising a second nucleic acid molecule encoding a second class I MHC component or fragment thereof wherein the first MHC component and the second MHC component are different.

11. The immunotherapeutic composition of claim 10, wherein the second class I MHC component is a heavy (α) chain and a light chain (β_2 microglobulin).

12. The immunotherapeutic composition of claim 11, wherein the second class I MHC component is a naturally occurring MHC component.

13. The immunotherapeutic composition of claim 1, wherein the first MHC component is a class II MHC component.

14. The immunotherapeutic composition of claim 13, wherein the class II MHC component comprises an alpha (α) chain, a beta (β) chain, or a combination thereof.

15. The immunotherapeutic composition of claim 13, further comprising a second nucleic acid molecule encoding a second class II MHC component or a fragment thereof.

16. The immunotherapeutic composition of claim 15, wherein the second class II MHC component comprises an alpha (α) chain, a beta (β) chain or a combination thereof.

17. The immunotherapeutic composition of claim 16, wherein the second class II MHC component is a naturally occurring component.

18. The immunotherapeutic composition of claim 2, wherein the nucleic acid encoding the MHC component is DNA or RNA.

19. The immunotherapeutic composition of claim 2, wherein the nucleic acid encoding the MHC component is part of a plasmid.

20. The immunotherapeutic composition of claim 2, wherein the nucleic acid encoding the MHC component is part of a viral vector.

21. The immunotherapeutic composition of claim 20, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

22. The immunotherapeutic composition of claim 2, wherein the nucleic acid encoding the MHC component is formulated for targeted delivery to a tumor cell.

23. The immunotherapeutic composition of claim2, wherein the nucleic acid is formulated in a liposome, exosome, lipid nanoparticle, or a biomaterial.

24. The immunotherapeutic composition of claim 23, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

25. The immunotherapeutic composition of claim 23, wherein the liposome is formulated for targeted delivery to a cancer cell.

26. The immunotherapeutic composition of claim 1, wherein the first MHC component is an HLA with an allele of Table 3.

27. A method for treating a cancer in an individual, comprising administering to the individual a therapeutically effective amount of a nucleic acid molecule encoding a major histocompatibility complex (MHC) component or a functional fragment thereof.

28. The method of claim 26, wherein the non MHC component increases T cell activation or enhanced recognition of a cancer cell by a T cell.

29. The method of claim 26, wherein the cancer is ovarian cancer, pancreatic cancer, or colon cancer.

30. The method of claim 26, wherein the cancer has reduced MHC expression.

31. The method of claim 26, further comprising determining a sequence of a native MHC component of the individual prior to the administering.

32. The method of claim 26, further comprising diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced relative to a control.

33. The method of claim 26, wherein the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

34. The method of claim 26, further comprising administering an additional therapeutic compound to the individual.

35. The method of claim 34, wherein the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy.

36. The method of claim 35, wherein the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof.

37. The method of claim 35, wherein the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof.

38. The method of claim 35, wherein the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor.

39. The method of claim 35, wherein the cytokine is INFα, INFβ, IFNγ, or TNF.

40. The method of claim 35, wherein the cellular therapy is an adoptive T cell transfer (ACT) therapy.

41. The method of claim 40, wherein the ACT therapy utilizes a plurality of chimeric antigen receptor (CAR) T-cells.

42. The method of claim 40, wherein the ACT therapy utilizes a plurality of T-cell antigen coupler (TAC) T-cells.

43. The method of claim 34, wherein administration of the nucleic acid molecule to the individual results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound.

44. The method of claim 26, wherein the nucleic acid molecule encoding the non-naturally occurring MHC component comprises at least one variant compared to a nucleic acid molecule encoding a naturally occurring MHC component.

45. The method of claim 44, wherein the variant is a mutation, an insertion, a deletion, or duplication.

46. The method of claim 44, wherein the MHC component is a gene selected from the list consisting of: HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1.

47. The method of claim 44, wherein the nucleic acid molecule is at least 95% similar to the nucleic acid sequence encoding the naturally occurring MHC component.

48. The method of claim 44, wherein the nucleic acid molecule is at least 80% similar to the nucleic acid sequence encoding the naturally occurring MHC component.

49. The method of claim 26, wherein the non-naturally occurring MHC component is a class I MHC component.

50. The method of claim 49, wherein the class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof.

51. The method of claim 49, wherein the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class I MHC component or fragment thereof.

52. The method of claim 51, wherein the second class I MHC component is a heavy (α) chain, a light chain (β_2 microglobulin), or a combination thereof.

53. The method of claim 52, wherein the second class I MHC component is a naturally occurring or a non-naturally occurring MHC component.

54. The method of claim 26, wherein the non-naturally occurring MHC component is a class II MHC component.

55. The method of claim 54, wherein the class II MHC component comprises an alpha (α) chain, a beta (β) chain, or a combination thereof.

56. The method of claim 54, wherein the immunotherapeutic composition further comprises a second nucleic acid molecule encoding a second class II MHC component or a fragment thereof.

57. The method of claim 56, wherein the second class II MHC component comprises an alpha (α) chain, a beta (β) chain or a combination thereof.

58. The method of claim 57, wherein the second class II MHC component is a naturally occurring or a non-naturally occurring MHC component.

59. The method of claim 26, wherein the nucleic acid molecule is DNA or RNA.

60. The method of claim 26, wherein the nucleic acid is a plasmid.

61. The method of claim 26, wherein the nucleic acid is a viral vector.

62. The method of claim 61, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

63. The method of claim 26, wherein the nucleic acid is formulated for targeted delivery to a tumor cell.

64. The method of claim 26, wherein the nucleic acid is formulated in a liposome, exosome, a lipid nanoparticle or a biomaterial.

65. The method of claim 64, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

66. The method of claim 64, wherein the liposome is formulated for targeted delivery to a cancer cell.

67. An immunotherapeutic composition, comprising: a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a guide RNA (gRNA) with a region complementary to a transcription factor or a promoter of an MHC gene.

68. The immunotherapeutic composition of claim 67, wherein the MHC gene is HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1.

69. The immunotherapeutic composition of claim 67, wherein the deactivated CRISPR-associated nuclease is deactivated Cas9 (dCas9).

70. The immunotherapeutic composition of claim 67, wherein the TET enzyme is TET1, TET2, TET3, or a catalytic domain thereof.

71. The immunotherapeutic composition of claim 67, wherein the nucleic acid molecule is DNA or RNA.

72. The immunotherapeutic composition of claim 67, wherein the nucleic acid is a plasmid.

73. The immunotherapeutic composition of claim 67, wherein the nucleic acid is a viral vector.

74. The immunotherapeutic composition of claim 73, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

75. The immunotherapeutic composition of claim 67, wherein the nucleic acid is formulated for targeted delivery to a tumor cell.

76. The immunotherapeutic composition of claim 67, wherein the nucleic acid is formulated in a liposome.

77. The immunotherapeutic composition of claim 76, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

78. The immunotherapeutic composition of claim 76, wherein the liposome is formulated for targeted delivery to a cancer cell.

79. The immunotherapeutic composition of claim 67, further comprising at least one pharmaceutically acceptable excipient, diluent, or carrier.

80. A method for increasing expression of an MHC gene in a cancer in an individual, comprising administering to the individual an immunotherapeutic composition comprising: a nucleic acid encoding a deactivated CRISPR-associated nuclease fused to a TET enzyme and a guide RNA (gRNA) with a region complementary to a transcription factor or a promoter of the MHC gene.

81. The method of claim 80, wherein the MHC gene is HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DOA, HLA-DOB, HLA-DMA, HLA-DMB, HLA-DPA1, and HLA-DPB1.

82. The method of claim 80, wherein the cancer is ovarian cancer, pancreatic cancer, or colon cancer.

83. The method of claim 80, wherein the cancer has reduced MHC expression.

84. The method of claim 80, further comprising diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced.

85. The method of claim 80, wherein the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

86. The method of claim 80, further comprising administering an additional therapeutic compound to the individual.

87. The method of claim 86, wherein the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy.

88. The method of claim 87, wherein the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof.

89. The method of claim 87, wherein the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof.

90. The method of claim 87, wherein the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor.

91. The method of claim 87, wherein the cytokine is INFα, INFβ, IFNγ, or TNF.

92. The method of claim 87, wherein the cellular therapy is an adoptive T cell transfer (ACT) therapy.

93. The method of claim 92, wherein the ACT therapy utilizes a plurality of chimeric antigen receptor (CAR) T-cells.

94. The method of claim 92, wherein the ACT therapy utilizes a plurality of T-cell antigen coupler (TAC) T-cells.

95. The method of claim 86, wherein expression of the nucleic acid molecule by the cancer results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound.

96. The method of claim 80, wherein the deactivated CRISPR-associated nuclease is deactivated Cas9 (dCas9).

97. The method of claim 80, wherein the TET enzyme is TET1, TET2, TET3, or a catalytic domain thereof.

98. The method of claim 80, wherein the nucleic acid molecule is DNA or RNA.

99. The method of claim 80, wherein the nucleic acid is a plasmid.

100. The method of claim 80 wherein the nucleic acid is a viral vector.

101. The method of claim 100, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

102. The method of claim 80, wherein the nucleic acid is formulated for targeted delivery to a tumor cell.

103. The method of claim 80, wherein the nucleic acid is formulated in a liposome.

104. The method of claim 103, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

105. The method of claim 103, wherein the liposome is formulated for targeted delivery to a cancer.

106. An immunotherapeutic composition, comprising a nucleic acid molecule encoding a regulator of an MHC molecule.

107. The immunotherapeutic composition of claim 106, wherein the regulator of the MHC molecule is selected from the group consisting of: transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, and any combination thereof.

108. The immunotherapeutic composition of claim 107, wherein the transactivator is selected from the group consisting of: class II, major histocompatibility complex, transactivator (CIITA) and NOD-like receptor family CARD domain containing 5 (NLRC5).

109. The immunotherapeutic composition of claim 107, wherein the transcription factor is selected from the group consisting of: a nuclear transcription factor Y (NF-Y), cAMP response element-binding protein (CREB), a regulatory factor X (RFX), an interferon regulatory factor (IRF), a signal transducer and activator of transcription (STAT), a ubiquitous transcription factor (USF), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).

110. The immunotherapeutic composition of claim 109, wherein the NF-Y is selected from the group consisting of: NF-Ya, NF-Yb, and NF-Yc.

111. The immunotherapeutic composition of claim 109, wherein the RFX is selected from the group consisting of: RFXANK/RFXB, RFX5, and RFXAP.

112. The immunotherapeutic composition of claim 109, wherein the IRF is selected form the group consisting of: IRF-1, IRF-2, IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8, and IRF-9.

113. The immunotherapeutic composition of claim 109, wherein the STAT is selected from the group consisting of: STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, and STAT-6.

114. The immunotherapeutic composition of claim 109, wherein the USF is selected from the group consisting of: USF-1 and USF-2.

115. The immunotherapeutic composition of claim 107, wherein the acetyltransferase is selected from the group consisting of: CREB-binding protein (CBP), p300, and p300/CBP-associated factor (pCAF)

116. The immunotherapeutic composition of claim 107, wherein the methyltransferase is Enhancer of Zeste Homolog 2 (EZH2), protein arginine N-methyltransferase 1 (PRMT1), and coactivator-associated arginine methyltransferase 1 (CARM1).

117. The immunotherapeutic composition of claim 107, wherein the elongation factor is positive transcriptional elongation factor ($pTEF_b$).

118. The immunotherapeutic composition of claim 106, wherein the nucleic acid molecule is DNA or RNA.

119. The immunotherapeutic composition of claim 106, wherein the nucleic acid is a plasmid.

120. The immunotherapeutic composition of claim 106, wherein the nucleic acid is a viral vector.

121. The immunotherapeutic composition of claim 120, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

122. The immunotherapeutic composition of claim 106, wherein the nucleic acid is formulated for targeted delivery to a tumor cell.

123. The immunotherapeutic composition of claim 106, wherein the nucleic acid is formulated in a liposome.

124. The immunotherapeutic composition of claim 123, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

125. The immunotherapeutic composition of claim 123, wherein the liposome is formulated for targeted delivery to a cancer cell.

126. The immunotherapeutic composition of claim 106, further comprising at least one pharmaceutically acceptable excipient, diluent, or carrier.

127. A method for treating a cancer in an individual, comprising administering to the individual a nucleic acid molecule encoding a regulator of an MHC molecule.

128. The method of claim 127, wherein the cancer is ovarian cancer, pancreatic cancer, or colon cancer.

129. The method of claim 127, wherein the cancer has reduced MHC expression.

130. The method of claim 127, further comprising diagnosing the cancer with reduced MHC expression comprising: (a) obtaining a biological sample from the individual, (b) isolating cancerous cells from the biological sample; and (c) detecting whether MHC expression in the isolated cancerous cells is reduced relative to a control.

131. The method of claim 127, wherein the individual has previously been administered an additional therapeutic compound selected from the group consisting of: an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, a cellular therapy, or a combination thereof.

132. The method of claim 127, further comprising administering an additional therapeutic compound to the individual.

133. The method of claim 132, wherein the additional therapeutic compound is an immune checkpoint inhibitor, an immune checkpoint stimulator, a cancer vaccine, a small molecule therapy, a monoclonal antibody, a cytokine, or a cellular therapy.

134. The method of claim 133, wherein the immune checkpoint inhibitor is a molecule which binds to A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM-3, VISTA, or a ligand thereof.

135. The method of claim 133, wherein the immune checkpoint stimulator is a molecule which binds to CD27, CD28, CD40, CD122, CD137, OX40, GITR, ICOS, or a ligand thereof.

136. The method of claim 133, wherein the small molecule therapy is a proteasome inhibitor, a tyrosine kinase inhibitor, a cyclin-dependent kinase inhibitor, or a polyADP-ribose polymerase (PARP) inhibitor.

137. The method of claim 133, wherein the cytokine is INFα, INFβ, IFNγ, or TNF.

138. The method of claim 133, wherein the cellular therapy is an adoptive T cell transfer (ACT) therapy.

139. The method of claim 133, wherein the ACT therapy utilizes a plurality of chimeric antigen receptor (CAR) T-cells.

140. The method of claim 133, wherein the ACT therapy utilizes a plurality of T-cell antigen coupler (TAC) T-cells.

141. The method of claim 132, wherein administration of the nucleic acid molecule to the individual results in the cancer showing an increased sensitivity to the at least one additional therapeutic compound.

142. The method of claim 127, wherein the regulator of the MHC molecule is selected from the group consisting of: transactivator, a transcription factor, an acetyltransferase, a methyltransferase, an elongation factor, and any combination thereof.

143. The method of claim 142, wherein the transactivator is selected from the group consisting of: class II, major histocompatibility complex, transactivator (CIITA) and NOD-like receptor family CARD domain containing 5 (NLRC5).

144. The method of claim 142, wherein the transcription factor is selected from the group consisting of: a nuclear transcription factor Y (NF-Y), cAMP response element-binding protein (CREB), a regulatory factor X (RFX), an interferon regulatory factor (IRF), a signal transducer and activator of transcription (STAT), a ubiquitous transcription factor (USF), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).

145. The method of claim 144, wherein the NF-Y is selected from the group consisting of: NF-Ya, NF-Yb, and NF-Yc.

146. The method of claim 144, wherein the RFX is selected from the group consisting of: RFXANK/RFXB, RFX5, and RFXAP.

147. The method of claim 144, wherein the IRF is selected form the group consisting of: IRF-1, IRF-2, IRF-3, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8, and IRF-9.

148. The method of claim 144, wherein the STAT is selected from the group consisting of: STAT-1, STAT-2, STAT-3, STAT-4, STAT-5, and STAT-6.

149. The method of claim 144, wherein the USF is selected from the group consisting of: USF-1 and USF-2.

150. The method of claim 142, wherein the acetyltransferase is selected from the group consisting of: CREB-binding protein (CBP), p300, and p300/CBP-associated factor (pCAF)

151. The method of claim 142, wherein the methyltransferase is Enhancer of Zeste Homolog 2 (EZH2), protein arginine N-methyltransferase 1 (PRMT1), and coactivator-associated arginine methyltransferase 1 (CARM1).

152. The method of claim 142, wherein the elongation factor is positive transcriptional elongation factor ($pTEF_b$).

153. The method of claim 127, wherein the nucleic acid molecule is DNA or RNA.

154. The method of claim 127, wherein the nucleic acid is a plasmid.

155. The method of claim 127, wherein the nucleic acid is a viral vector.

156. The method of claim 155, wherein the viral vector is an alphavirus, a retrovirus, an adenovirus, a herpes virus, poxvirus, lentivirus, oncolytic virus, reovirus, or an adeno associated virus (AAV).

157. The method of claim 127, wherein the nucleic acid is formulated for targeted delivery to a tumor cell.

158. The method of claim 127, wherein the nucleic acid is formulated in a liposome.

159. The method of claim 158, wherein the liposome comprises the additional therapeutic compound, a polyethylene glycol (PEG), a cell-penetrating peptide, a ligand, an aptamer, an antibody, or a combination thereof.

160. The method of claim 158, wherein the liposome is formulated for targeted delivery to a cancer cell.

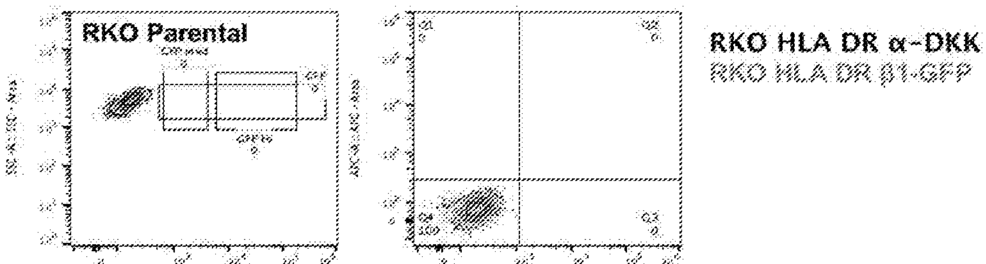


FIG. 1A. Non-Transfected

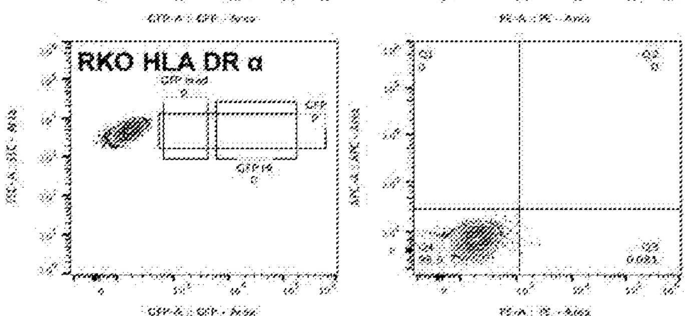


FIG. 1B. HLA-R α Transfected

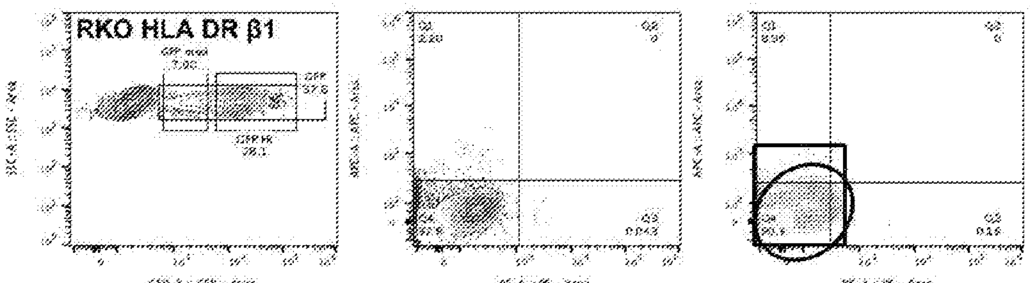


FIG. 1C. HLA-DR β Transfected

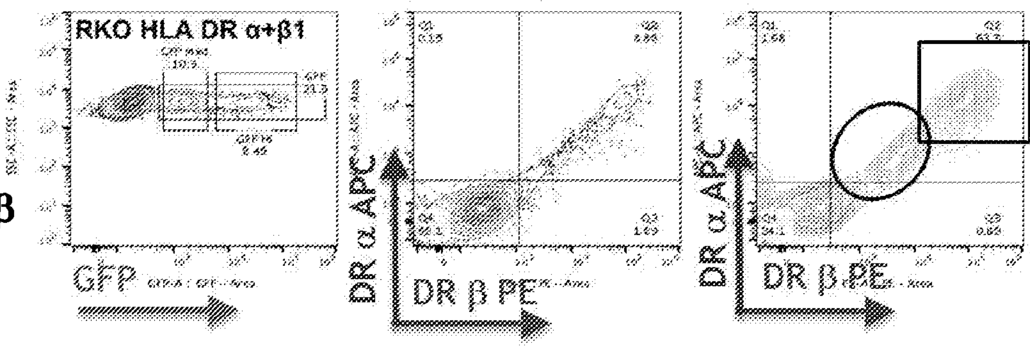


FIG. 1D. HLA-DR α+β co-Transfected

FIG.2A. RKO Parental

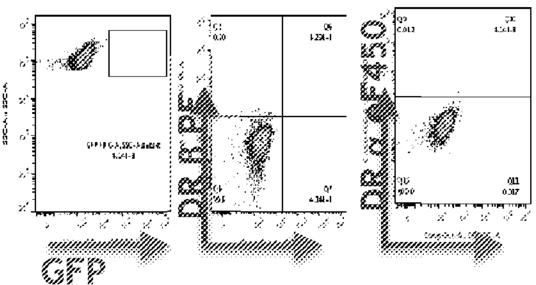


FIG. 2B. RKO HLA DRAB1*15 sorted

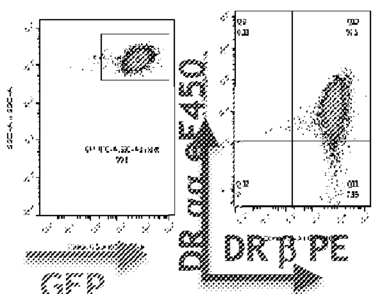
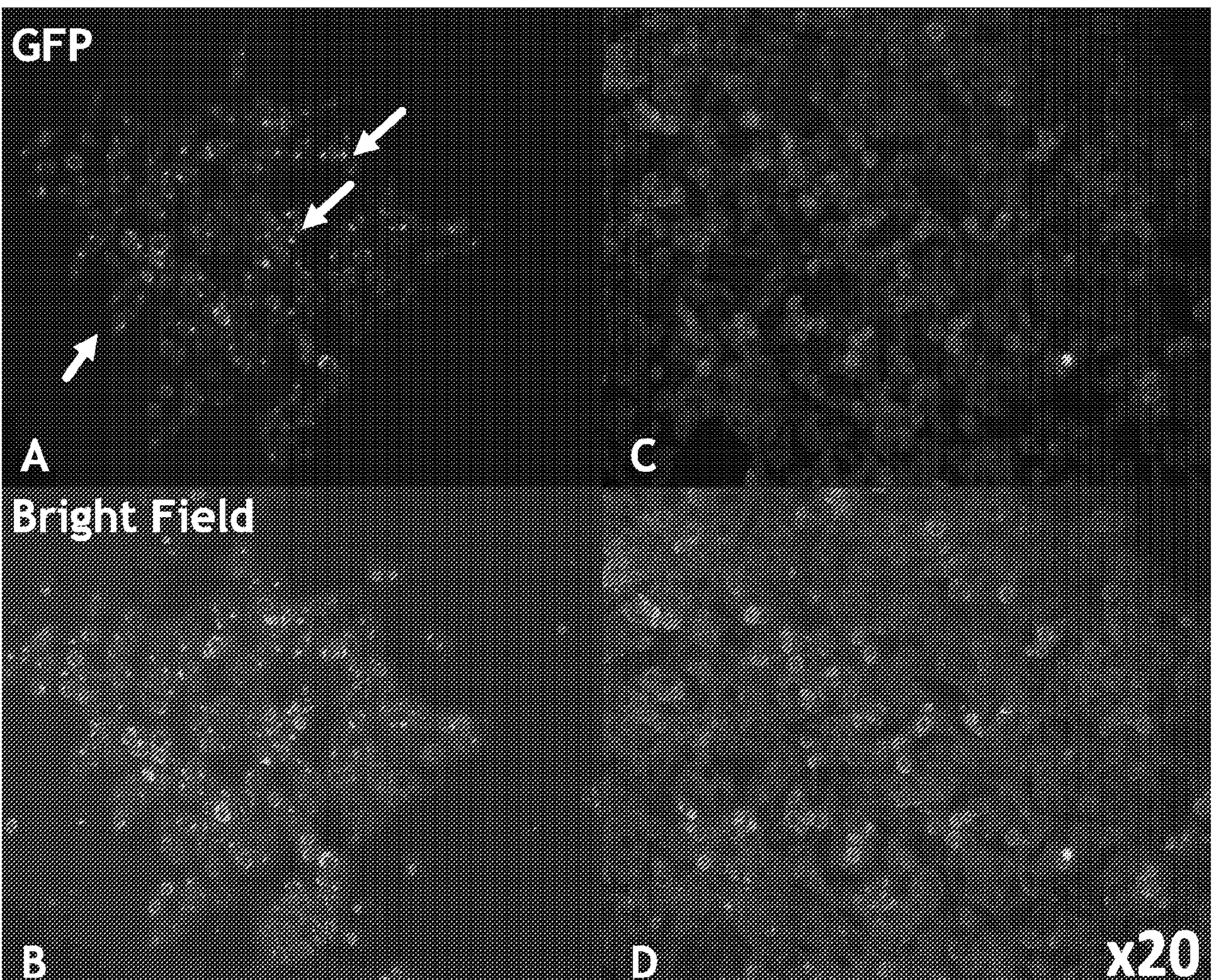


FIG.2C. HLA-DR A/DKK MYC Tag+B1*15 GFP Tag   HLA-DR B1*15 GFP Tag



HLA-DR AB 1*15 Co-Transfected

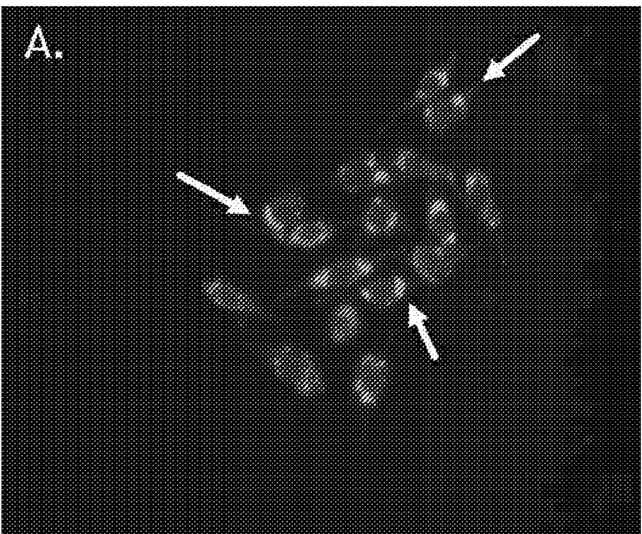


FIG.3A
RKO

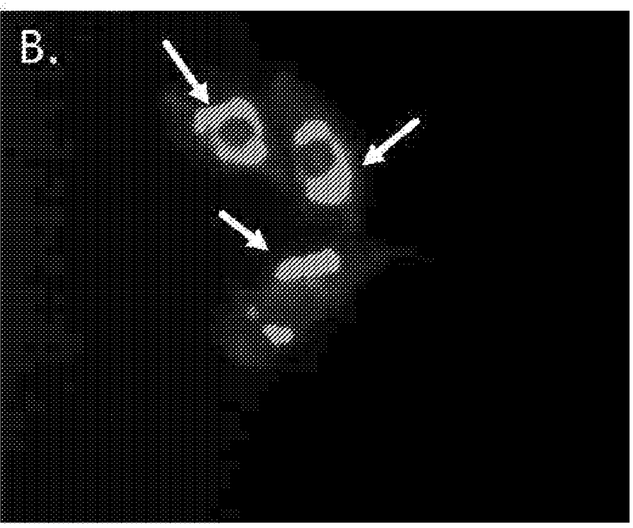


FIG. 3B
SKOV3

HLA-DR AB 3 Co-Transfected

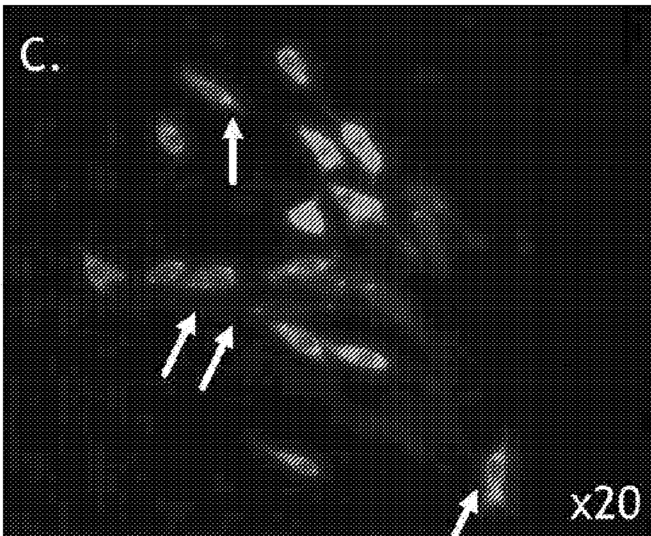


FIG.3C
RKO

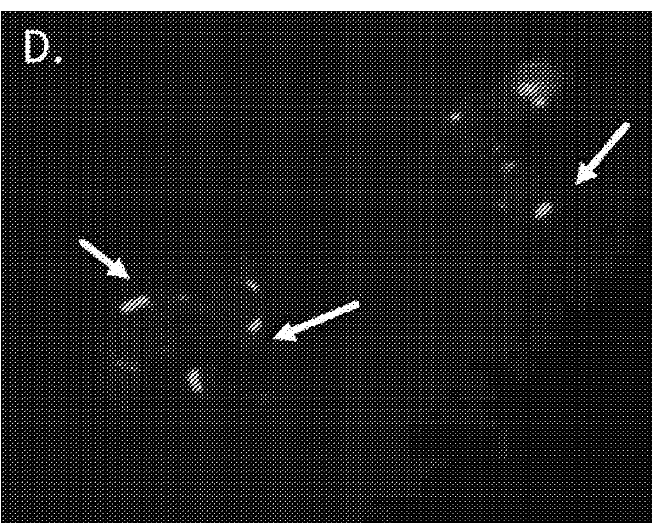


FIG. 3D
SKOV3

FIG.4A

RG210732
(7.3 kb)

FIG.4B

RG202743
(7.3 kb)

FIG.4C

CMV promoter
VP1.5 primer
T7 promoter
SgfI
HLA
MluI
GFP
PolyA signal
SV40 ori
Neo
Col E1
RG203646
(7.3 kb)

FIG.4D

CMV promoter
VP1.5 primer
T7 promoter
SgfI
HLA DR alphaa
HLA
MluI
Myc-DDK
XL39 primer
PolyA signal
Col E1
SV40 ori
f1 ori
RC209920
(5.6 kb)

FIG.4E

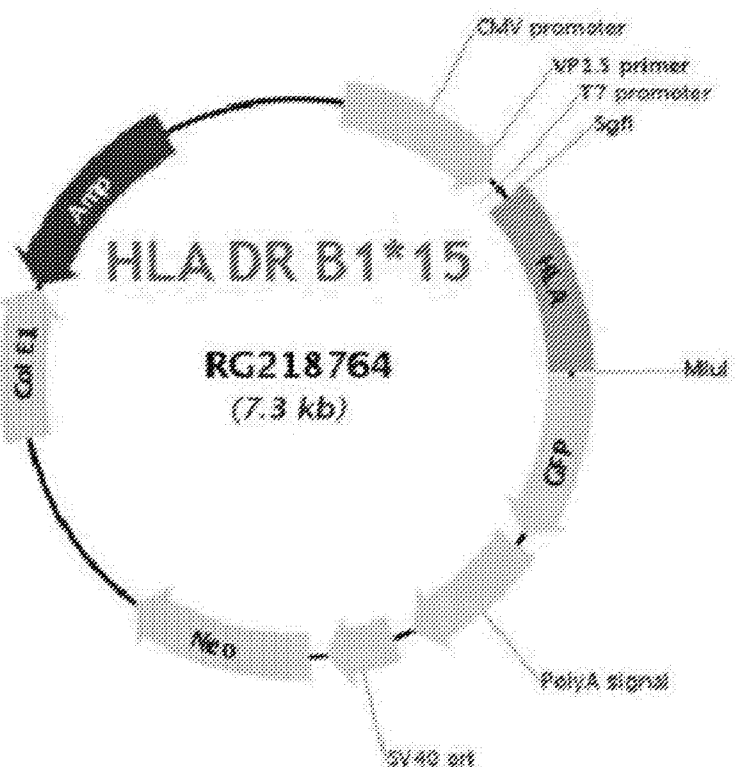


FIG. 5C.

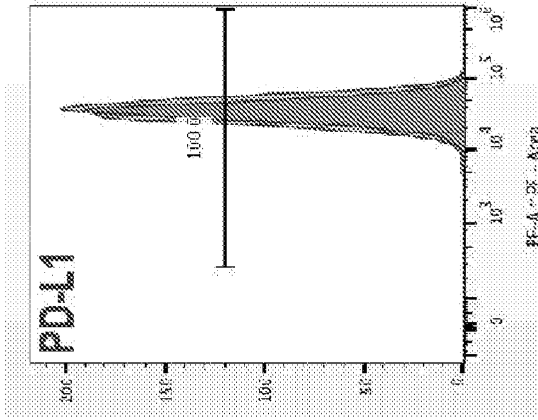


FIG. 5A.

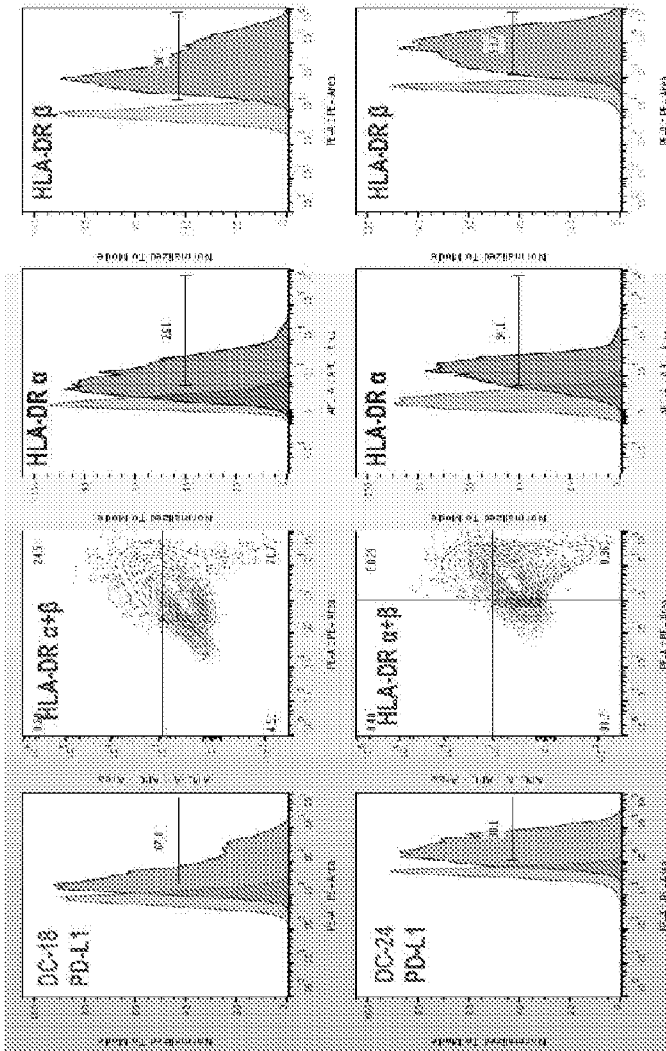


FIG. 5B.

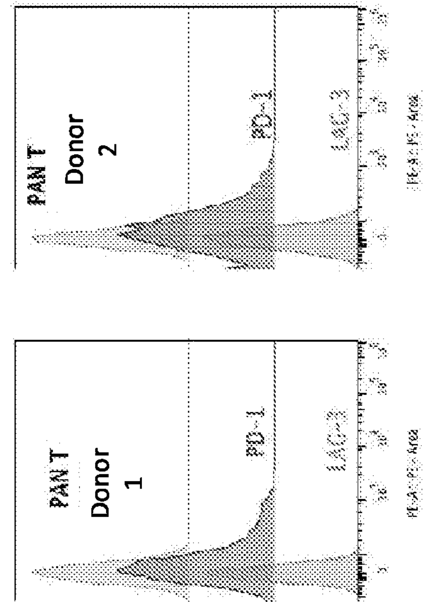
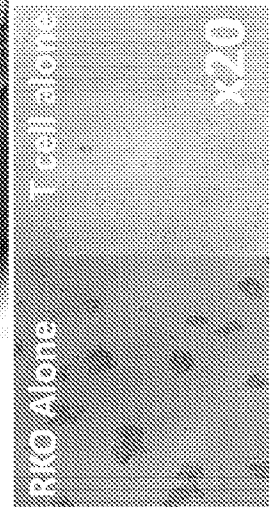


FIG. 6A

FIG. 6B

FIG. 6C

FIG. 6D



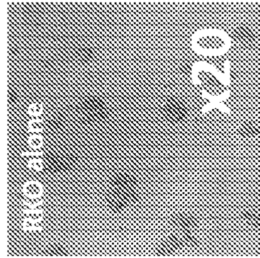


FIG. 6G

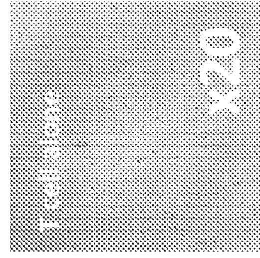


FIG. 6H

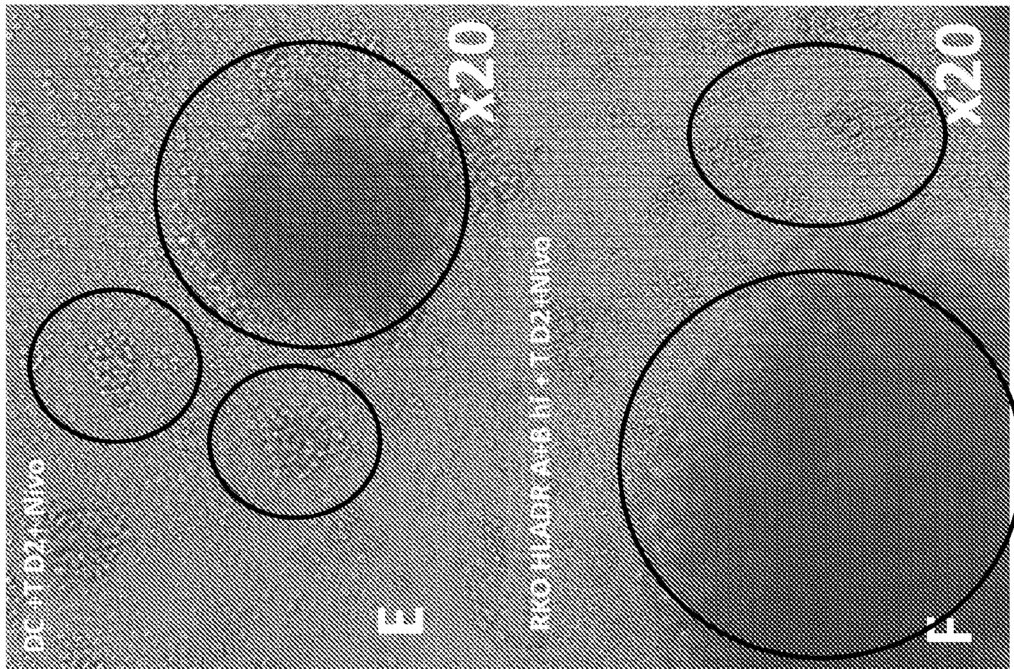


FIG. 6E

FIG. 6F

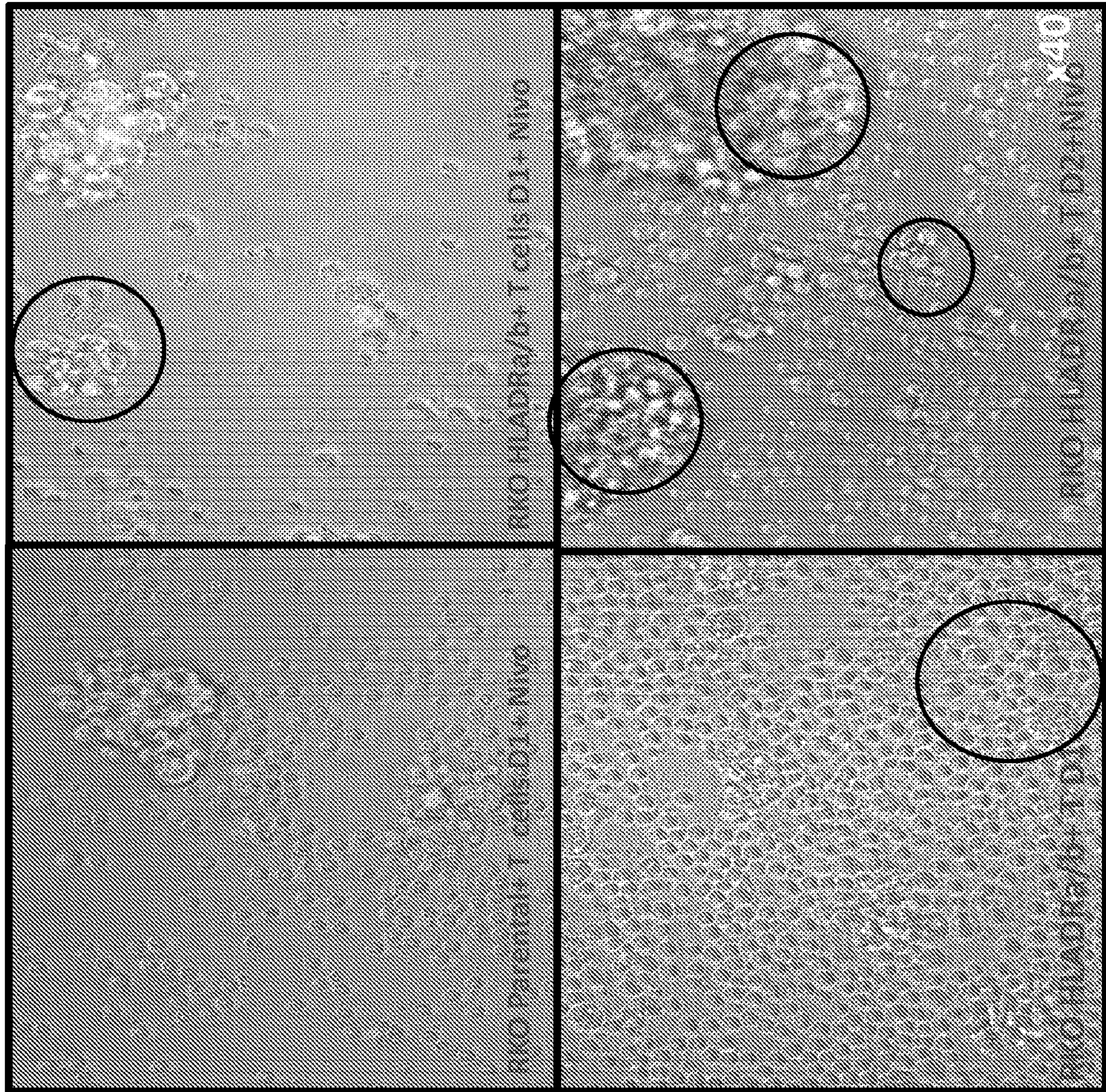


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 7D

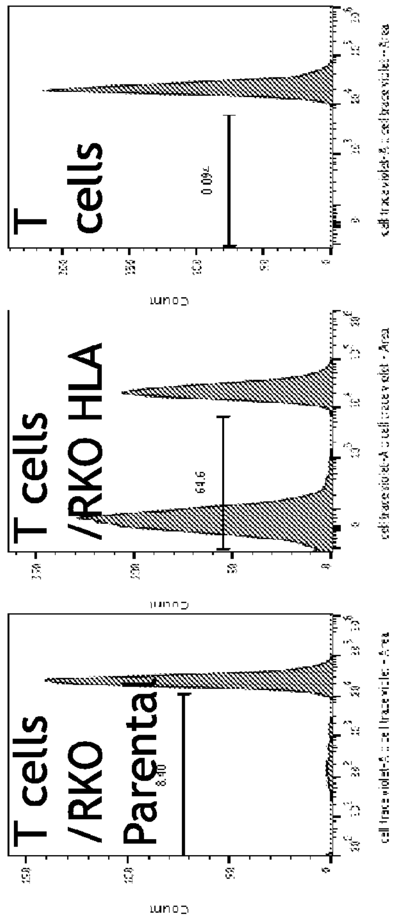


FIG. 8A.

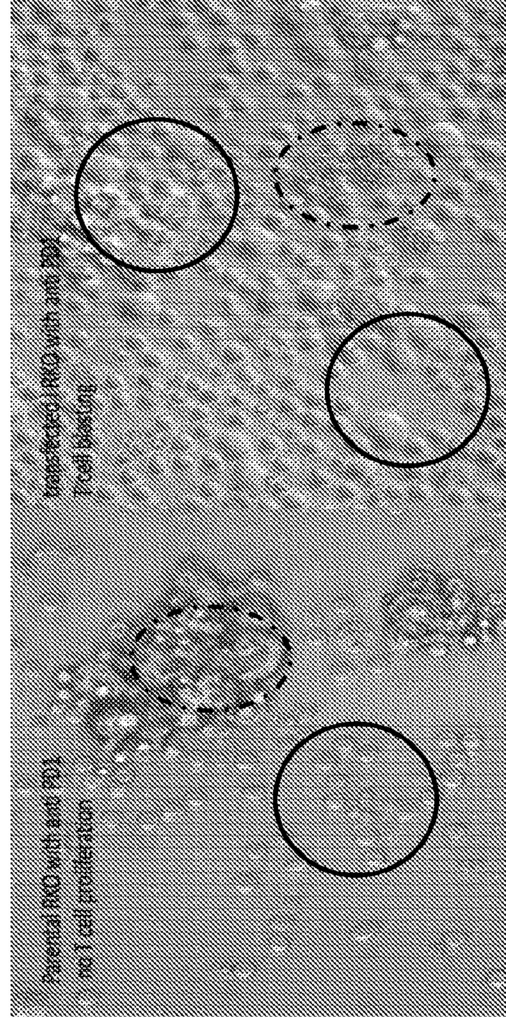
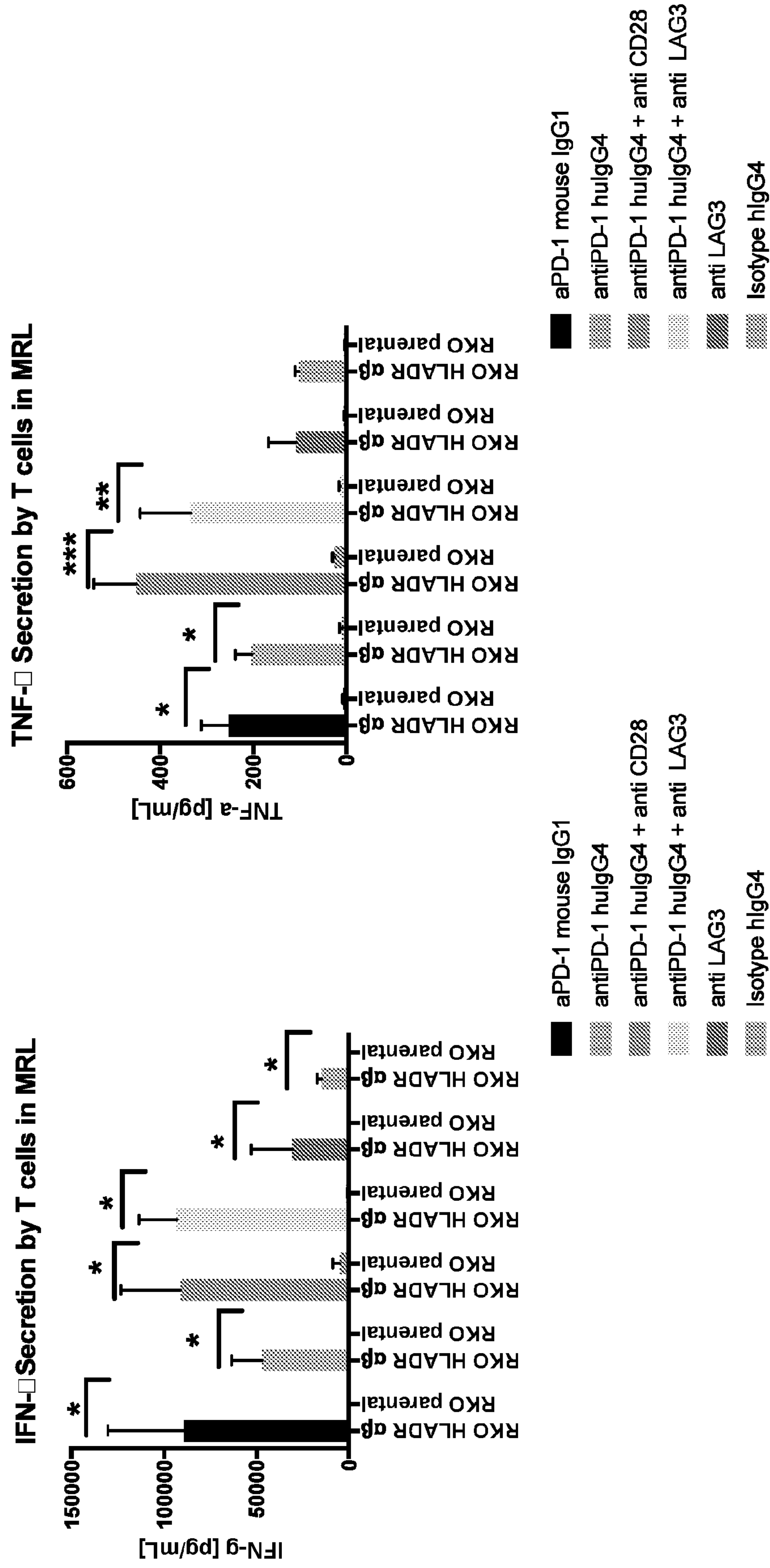


FIG. 8B.

FIG. 8C.



*- P<0.05; **- P <0.01; *** -P<0.001 in 2way Anova

INTERNATIONAL SEARCH REPORT

International application No. PCT/US2018/067380

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 48/00; C07K 14/705; C12N 9/22 (2019.01)
CPC - C07K 14/70539; C12N 15/85; C12N 2310/20 (2019.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/185.1; 435/199; 514/19.3 (keyword delimited)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0112141 A1 (TERMAN) 26 May 2005 (26.05.2005) entire document	1, 2, 6, 8, 13, 18-25, 27-29, 33-37, 39, 40, 49, 54, 59-66 --- 3-5, 7, 9-12, 14-17, 26, 30-32, 38, 41-48, 50-53, 55-58, 74, 89, 101, 121, 135, 156
X --- Y	US 2017/0321285 A1 (THE TEXAS A&M UNIVERSITY SYSTEM) 09 November 2017 (09.11.2017) entire document	106-108, 118-120, 122-129, 131-134, 137-139, 141-143, 153-155, 157-160 --- 30, 41, 43, 67-105, 109-117, 121, 130, 135, 136, 140, 144-152, 156
Y	US 2015/0343055 A1 (OREGON HEALTH & SCIENCE UNIVERSITY et al) 03 December 2015 (03.12.2015) entire document	3-5, 7, 9, 14, 26, 30, 44-48, 50, 55
Y	US 2014/0134195 A1 (RUSSELL et al) 15 May 2014 (15.05.2014) entire document	10-12, 51-53
Y	US 2002/0198144 A1 (WONG et al) 26 December 2002 (26.12.2002) entire document	15-17, 56-58

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
26 February 2019	15 MAR 2019

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/067380

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/122131 A1 (DIXON et al) 20 July 2017 (20.07.2017) entire document	31, 32, 84, 130
Y	ANGELL et al. "MHC Class I Loss is a Frequent Mechanism of Immune Escape in Papillary Thyroid Cancer that is Reversed by Interferon and Selumetinib Treatment in vitro," Clincal Cancer Research, 07 October 2017 (07.10.2014), Vol. 20, No. 23, Pgs. 6034-6044. entire document	38, 90, 136
Y	US 2016/0368964 A1 (MCMASTER UNIVERSITY) 22 December 2016 (22.12.2016) entire document	42, 94, 140
Y	WO 2014/172470 A2 (WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH) 23 October 2014 (23.10.2014) entire document	67-105
Y	US 2002/0156258 A1 (MASTERNAK et al) 24 October 2002 (24.10.2002) entire document	109-114, 144-149
Y	US 2004/0116369 A1 (KROEGER et al) 17 June 2004 (17.06.2004) entire document	112, 147
Y	WO 2017/191274 A2 (CUREVAC) 09 November 2017 (09.11.2017) entire document	113-116, 148-151
Y	KANAZAWA et al. "Combinations of dominant-negative class II transactivator, p300 or CDK9 proteins block the expression of MHC II genes," International Immunology, 01 July 2001 (01.07.2001), Vol. 13, Issue 7, Pgs. 951-958. entire document	117, 152
A	CHATTERJEE-KISHORE et al. "How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene," The EMBO Journal, 01 August 2000 (01.08.2000), Vol. 19, No. 15, Pgs. 4111-4122. entire document	1-160
A	GOBIN et al. "HLA-G Transactivation by cAMP-response Element-binding Protein (CREB)," Journal of Biological Chemistry, 18 October 2002 (18.10.2002), Vol. 277, No. 42, Pgs. 39525-39531. entire document	1-160
A	HOWCROFT et al. "Upstream Stimulatory Factor Regulates Major Histocompatibility Complex Class I Gene Expression: the U2DE4 Splice Variant Abrogates E-Box Activity," Molecular and Cellular Biology, 30 July 1999 (30.07.1999), Vol. 19, No. 7, Pgs. 4788-4797. entire document	1-160
A	TIFFEN et al. "EZH2 as a mediator of treatment resistance in melanoma," Pigment Cell Melanoma Research, 25 May 2016 (25.05.2016), Vol. 29, No. 5, Pgs. 500-507. entire document	1-160