disclosure are able to prevent activation of allogeneic CD8 T cells or CD4 T cells including without triggering killing by allogeneic NK cells. In such cases the cells are NKT cells that are engineered to express little or no HLA class I molecules, and these cells, surprisingly, remain resistant to killing by the host NK cells. The NKT cells as used herein may include iNKT, UTNKT, or natural killer T cells, for example. In some cases donor cells are able to be utilized for immunotherapy of HLA unmatched subject, including those with cancer. In specific cases, RNA interference is used with B2M-specific shRNAs, and a range of B2M and HLA class I expression can be found in NKT cells that would prevent activation of allogeneic CD8 T cells without triggering killing by allogeneic NK cells. In specific cases, RNA interference is used with Ii-specific shRNAs, and downregulation of Ii and HLA class II expression can be found in NKT cells that would prevent activation of allogeneic CD4 T cells. In some specific cases, B2M-specific shRNA, Ii-specific shRNA, or both and a tumor-specific CAR can be engineered to be expressed within a single retroviral construct for a one-hit generation of tumor-specific and universally tolerated healthy donor-derived NKT cells for immunotherapy of HLA unmatched cancer patients. In particular examples, the cells are not derived from induced pluripotent stem cells. The cells are resistant to elimination by NK cells, in specific examples.

[0011] In one example of the disclosure, there is an isolated NKT cell or a plurality of cells thereof, having reduced expression of endogenous beta-2-microglobulin (B2M) and/or MHC class II-associated invariant chain (Ii), or both. The cells have reduced expression of B2M and/or Ii because of manipulation by the hand of man and are not located in nature or similar to cells in nature. The reduced expression may be further defined as a knockout or a knockdown in the cells. In specific examples, the cell or cells comprise one or more agents that target the B2M gene and/or Ii gene, such as one or more synthetic DNA or RNA that targets the B2M gene or Ii gene, for example the 3′ end of the B2M or Ii gene. In certain cases, the synthetic RNA is a shRNA or a CRISPR guide RNA.

[0011a] In one aspect, the present disclosure refers to an isolated CD1d-restricted human natural killer T (NKT) cell or a plurality of CD1d-restricted human NKT cells thereof, wherein the CD1d-restricted human NKT cell or cells comprise one or more agents that target one or more genes, wherein the one or more agents is ~~a DNA vector,~~ morpholinos, siRNA, S-DNA, TALEN, ZFNs, shRNA or a CRISPR guide RNA, wherein the CD1d-restricted human NKT cell or cells have reduced or no detectable expression of the one or more genes, wherein the reduction of expression is in comparison with CD1d-restricted human NKT cell or cells not comprising the one or more agents, and wherein the one or more genes comprise: (a) endogenous beta-2-microglobulin (B2M); (b) endogenous major histocompatibility complex (MHC) class II-associated invariant chain (Ii); or (c) both.

[0012] In particular examples of the disclosure, a cell or cells that have reduced expression of endogenous B2M and/or Ii comprise one or more engineered receptors, such as a chimeric antigen receptor or a T cell receptor. In some cases, the NKT cell or cells recombinantly express one or more cytokines and/or one or more cytokine receptors, such as IL-15, IL-15Ra, IL-7, IL-12, IL-18, IL-21, IL-27, IL-33, or a combination thereof. In some cases, the NKT cell or cells have reduced expression of a second endogenous gene. In particular cases the cell or cells are autologous or allogeneic in reference to a subject.

[0013] In one example, there is a method of generating the NKT cell or cells as encompassed herein by exposing NKT cells to one or more agents that reduce expression of endogenous B2M and/or MHC class II-associated invariant chain (Ii) in the cell or cells. The agent may be of any kind, including without being limited to a DNA vector, morpholinos, an antisense RNA, antigomer RNA, siRNA, S-DNA, TALEN, Zinc finger nuclease (ZFN) or a CRISPR guide RNA. Said DNA vector encodes an agent that inactivates or reduces expression of one or more target gene. In some cases, the NKT cells are manipulated to express one or more entities other than manipulation to have reduced expression of endogenous B2M, such as manipulated to have one or more engineered receptors and/or one or more cytokines. In some cases cells other than NKT cells are employed.

[0013a] In another aspect, the present disclosure refers to a method of generating the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells as disclosed herein, the method comprising exposing CD1d-restricted human NKT cell or cells to: (a) said one or more agents that reduce expression of endogenous B2M in the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells; (b) said one or more agents that reduce expression of endogenous Ii in the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells; or (c) both.

[0014] In some examples, there are a cell or cells as encompassed herein for use in the treatment of a medical condition in a subject, such as cancer. In some cases, the cancer is of the brain, lung, breast, prostate, pancreas, skin, kidney, liver, testes, ovary, gall bladder, spleen, endometrium, cervix, esophagus, thyroid, pituitary gland, stomach, colon, anus, blood, bone, bladder, bile duct, head and neck, oral cavity, salivary gland, small intestine, and/or urethra, or premalignant conditions, such as myelodysplastic syndrome (MDS).

[0015] In specific examples there is a composition comprising an allogeneic cell or allogeneic cells as encompassed herein for use in the treatment of a medical condition, preferably cancer or a premalignant condition, in a subject.

[0016] In some examples, there is a method of treating a medical condition in a subject by providing to the subject an effective amount of one or more cells as encompassed herein. The medical condition may or may not be cancer. In some cases the cells are allogeneic in reference to the subject, although in alternative cases they are autologous to the subject. In particular examples they are NKT cells.

[0016a] In another aspect, the present disclosure refers to use of a therapeutically amount of CD1d-restricted human NKT cell or cells as disclosed herein in the manufacture of a medicament for treating one or more medical conditions in a subject in need thereof, wherein the one or more medical conditions is cancer or a premalignant condition.

[0017] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description of the disclosure that follows may be better understood. Additional features and advantages of the disclosure will be described hereinafter which form the subject of the claims of the disclosure. It should be appreciated by those skilled in the art that the conception and specific example disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present disclosure. It should also be realized by those skilled in the art that such equivalent

CLAIMS

What is claimed is:

1. An isolated CD1d-restricted human natural killer T (NKT) cell or a plurality of CD1d-restricted human NKT cells thereof, wherein the CD1d-restricted human NKT cell or cells comprise one or more agents that target one or more genes, wherein the one or more agents is ~~a DNA vector,~~ morpholinos, siRNA, S-DNA, TALEN, ZFNs, shRNA or a CRISPR guide RNA, wherein the CD1d-restricted human NKT cell or cells have reduced or no detectable expression of the one or more genes, wherein the reduction of expression is in comparison with CD1d-restricted human NKT cell or cells not comprising the one or more agents, and wherein the one or more genes comprise:

(a) endogenous beta-2-microglobulin (B2M);

(b) endogenous major histocompatibility complex (MHC) class II-associated invariant chain (Ii); or

(c) both.

2. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of claim 1, wherein the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells comprise synthetic DNA or RNA that target the B2M gene or Ii gene.

3. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of claim 2, wherein the synthetic DNA or RNA targets the 3' end of the B2M gene or Ii gene.

4. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of claim 2 or 3, wherein the synthetic RNA is a shRNA or a CRISPR guide RNA.

5. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-4, wherein the CD1d-restricted human NKT cell or cells comprise~~s~~ one or more recombinantly engineered receptors.

6. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of claim 5, wherein the receptor is a chimeric antigen receptor, chimeric cytokine receptor, or a T cell receptor.

7. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-6, wherein the CD1d-restricted cell or cells recombinantly express~~es~~ one or more cytokines.

8. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of claim 7, wherein the cytokine is IL-15, IL-7, IL-12, IL-18, IL-21, IL-27, IL-33, or a combination thereof.

9. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-8, further having reduced expression of another endogenous gene besides B2M and/or Ii.

10. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-9, wherein the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells are CD1d-restricted NKT cells that are CD62L-positive and/or CD4-positive and/or PD1-negative/low cells.

11. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-10, wherein the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells are autologous in reference to a subject.

12. The CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-10, wherein the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells are allogeneic in reference to a subject.

13. A method of generating the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells of any one of claims 1-12, the method comprising exposing CD1d-restricted human NKT cell or cells to:

(a) said one or more agents that reduce expression of endogenous B2M in the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells;

(b) said one or more agents that reduce expression of endogenous Ii in the CD1d-restricted human NKT cell or ~~CD1d-restricted~~ cells; or

(c) both.

14. The method of claim 13, wherein the CD1d-restricted human NKT cell or cells are manipulated to express a recombinantly engineered receptor and/or one or more cytokines.

15. Use of a therapeutically amount of CD1d-restricted human NKT cell or cells of any one of claims 1-12 in the manufacture of a medicament for treating one or more medical conditions in a subject in need thereof, wherein the one or more medical conditions is cancer or a premalignant condition.

16. The use of claim 15, wherein the cancer is neuroblastoma, breast cancer, cervical cancer, ovary cancer, endometrial cancer, melanoma, bladder cancer, lung cancer, pancreatic cancer, colon cancer, prostate cancer, hematopoietic tumors of lymphoid lineage, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, B-cell lymphoma, Burkitt's lymphoma, multiple myeloma, Hodgkin's lymphoma, Non-Hodgkin's lymphoma, myeloid leukemia, acute myelogenous leukemia (AML), chronic myelogenous leukemia, thyroid cancer, thyroid follicular cancer, tumors of mesenchymal origin, fibrosarcoma, rhabdomyosarcomas, melanoma, uveal melanoma, teratocarcinoma, neuroblas-

toma, glioma, glioblastoma, benign tumor of the skin, renal cancer, anaplastic large-cell lymphoma, esophageal squamous cells carcinoma, hepatocellular carcinoma, follicular dendritic cell carcinoma, intestinal cancer, muscle-invasive cancer, seminal vesicle tumor, epidermal carcinoma, spleen cancer, bladder cancer, head and neck cancer, stomach cancer, liver cancer, bone cancer, brain cancer, cancer of the retina, biliary cancer, small bowel cancer, salivary gland cancer, cancer of uterus, cancer of testicles, cancer of connective tissue, prostatic hypertrophy, myelodysplasia, Waldenstrom's macroglobinaemia, nasopharyngeal, neuroendocrine cancer myelodysplastic syndrome, mesothelioma, angiosarcoma, Kaposi's sarcoma, carcinoid, oesophagogastric, fallopian tube cancer, peritoneal cancer, papillary serous mullerian cancer, malignant ascites, gastrointestinal stromal tumor (GIST), or a hereditary cancer syndrome selected from Li-Fraumeni syndrome and Von Hippel-Lindau syndrome (VHL), or wherein the premalignant condition is myelodysplastic syndrome (MDS).

17. The use of claim 16, wherein the subject has cancer and is to be provided an additional cancer therapy.

18. The use of claim 17, wherein the additional cancer therapy is surgery, radiation, chemotherapy, immunotherapy, proton therapy, hormone therapy, or a combination thereof.