A METHOD OF ENGINEERING NATURAL KILLER CELLS TO TARGET CD70-POSITIVE TUMORS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/958563, filed January 8, 2020, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

[0002] Embodiments of the disclosure include at least the fields of cell biology, molecular biology, immunology, and medicine, including cancer medicine.

BACKGROUND

[0003] Genetic reprogramming of Natural Killer (NK) cells for adoptive cancer immunotherapy has clinically relevant applications and benefits such as 1) innate anti-tumor surveillance without prior need for sensitization; 2) allogeneic efficacy without graft versus host reactivity; and 3) direct cell-mediated cytotoxicity and cytolysis of target tumors. Human NK cell development and acquisition of self-tolerance, alloreactivity, and effector functions is an adaptive process of licensing, calibration, and arming. At the molecular level, specific activating and inhibitory receptors direct NK cellular functions by aggregating, balancing, and integrating extracellular signals into distinct effector functions. The functional activity of NK cells and responsiveness to extrinsic stimuli follow the 'rheostat' model of continuous education and thus amenable to reprogramming. Genetic modification of NK cells to redirect their effector functions is an effective method to harness their cytotoxic capability to kill tumor cells.

[0004] The present disclosure concerns improvements in cell therapy and adoptive cell therapy directed at Cluster of Differentiation (CD70)-positive cancers.

~~BRIEF~~ SUMMARY

[0005] ~~Embodiment~~Examples of the disclosure encompass methods and compositions related to engineered cellular receptors that target CD70 (also known as CD27 ligand, CD27LG, and TNFSF7, for example). In specific ~~embodiment~~examples, the engineered receptors that target CD70 are in the form of polynucleotides, polypeptides, or are comprised on the surface of cells of any kind, including immune cells. In specific cases, the cells are immune cells, and in certain ~~embodiment~~examples the immune cells are NK cells, NK T cells, invariant NKT cells, gamma delta T

cells, regulatory T cells, B cells, macrophages, mesenchymal stromal cells (MSCs), dendritic cells, and so forth from any source. In certain ~~embodiment~~examples, reprogrammed NK cells from cord blood (CB-NK) are encompassed for targeting tumors expressing CD70 molecules.

[0006] CD70 is utilized as a target antigen for methods and compositions because it is expressed on many cancers, including acute myeloid leukemia (AML), lymphoma, lung cancer, melanoma, breast cancer, glioblastoma, mesothelioma, head and neck cancer, renal cancer, multiple myeloma, and pancreatic tumors, as examples. Expression of CD70 in normal tissue is limited to a subset of T cells and dendritic cells (DC).

[0007] ~~Embodiment~~Examples of the disclosure encompass a variety of novel, specific CAR constructs incorporating CD70 scFv heterologously fused to one or more signaling domains (including, for example, those comprising cytoplasmic portions of CD247 (also known as CD3ζ) and one or more of CD28, DAP10, DAP12, and NKG2D. The scFv may comprise a fusion of the variable fragments derived from the heavy (V_H) and light (V_L) chains of a murine antibody with specificity for human CD70 antigen, in some cases. The vector also may comprise one or more cytokine genes, including the gene to produce human interleukin 15 (IL-15) , IL-2, IL-21, IL-12, IL-7, and/or IL-18 that aids in the survival and maintenance of the NK cells. As one example, CB-NK cells, thus modified, comprise a vector encoding CD70 scFv in a CAR that includes CD28 and CD3z in addition to IL15 that is produced as a separate molecule from the CAR.

[0008] Although in some ~~embodiment~~examples the methods and compositions are utilized to treat individuals with CD70-positive cancers, in other cases the methods and compositions are utilized to ablate CD70-expressing (non-cancerous) immunoregulatory cells, such as T regulatory cells (Tregs) as checkpoints. In specific ~~embodiment~~examples, there are methods of targeting CD70-expressing non-cancerous cells in an individual comprising delivering to the individual an effective amount of CD70 CAR-expressing cells.

[0009] Particular ~~embodiment~~examples of the disclosure allow for the use of off-the-shelf immune cells, including at least NK cells, that are allogeneic with respect to a recipient individual, that target CD70-positive cells of any kind, and that also may or may not be transduced to express one or more cytokines, such as IL15, IL-2, IL21, IL-12, IL-7, and/or IL-18.

[0010] In specific ~~embodiment~~examples of the disclosure, expression of one or more endogenous genes in the immune cell has been modified, for example the expression may be partially or fully

reduced in expression. Although the modification may occur by any means, in specific ~~embodiment~~examples expression of the one or more genes has been modified, such as by being reduced in expression levels, and this may occur by any suitable means including at least CRISPR. Merely as examples, the endogenous gene may be selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, CTLA-4, TDAG8, CD38, and a combination thereof.

[0010a] In one aspect, the present disclosure refers to an expression construct comprising sequence that encodes a CD70-specific engineered receptor and that encodes one or both of the following: (a) a suicide gene; and (b) a cytokine.

[0011] In one ~~embodiment~~example, there is an expression construct comprising sequence that encodes a CD70-specific engineered receptor and that encodes one or both of the following: (a) a suicide gene; and (b) a cytokine. In specific cases, the CD70-specific engineered receptor is a chimeric antigen receptor (CAR) or a T cell receptor. The CD70-specific CAR may comprise a scFv having a heavy chain and a light chain, and wherein the heavy chain in the sequence that encodes the CAR is upstream of the light chain in a 5′ to 3′ direction. In other cases, the CD70-specific CAR comprises a scFv having a heavy chain and a light chain, and wherein the heavy chain in the sequence that encodes the CAR is downstream of the light chain in a 5′ to 3′ direction. In any case herein, the CD70-specific CAR does or does not comprise a codon optimized scFv. In any case herein, the CD70-specific CAR does or does not comprise a humanized scFv. In any case herein, the CD70-specific CAR does or does not comprise a signaling peptide, such as one from CD8alpha, Ig heavy chain, or granulocyte-macrophage colony-stimulating factor receptor or a signal peptide derived from one or more other surface receptors. In particular ~~embodiment~~examples, the CD70-specific CAR comprises one or more costimulatory domains, such as one or more costimulatory domains selected from the group consisting of CD28, CD27, OX-40 (CD134), DAP10, DAP12, 4-1BB (CD137), CD40L, 2B4, DNAM, CS1, CD48, NKG2D, NKp30, NKp44, NKp46, NKp80, or a combination thereof.

[0012] Any CD70 -specific CAR may or may not comprise CD3zeta and/or a hinge between the scFv and a transmembrane domain. In specific cases, the hinge is CD8-alpha hinge, the hinge comprises an artificial spacer comprised of Gly3, or the hinge comprises CH1, CH2, and/or CH3 domains of IgGs. In specific ~~embodiment~~examples, the cytokine is IL-15, IL-12, IL-2, IL-18, IL-21, IL-7, or a combination thereof. In cases wherein a suicide gene is employed, the suicide gene may be a mutant TNF-alpha (such as an engineered nonsecretable mutant), inducible caspase 9, HSV-thymidine kinase, CD19, CD20, CD52, or EGFRv3.

[0013] ~~Embodiment~~Examples of the disclosure include expression constructs that comprise any one or more of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

[0014] ~~Embodiment~~Examples of the disclosure include immune cells of any kind comprising any expression encompassed herein. In one aspect, the present disclosure refers to an immune cell, comprising any one of the expression constructs disclosed herein. In specific ~~embodiment~~examples, the immune cell is a NK cell, T cell, gamma delta T cell, invariant NKT (iNKT) cell, B cell, macrophage, MSC, or dendritic cell. In cases wherein the immune cell is an NK cell, the NK cell may be derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, or from a cell line. In specific ~~aspect~~examples, the NK cell line is NK-92 cell line or another NK cell line derived from a tumor or from a healthy NK cell or a progenitor cell.

[0015] In specific ~~embodiment~~examples, the immune cell is an NK cell, such as one derived from cord blood, such as from a cord blood mononuclear cell. The NK cell may be a CD56+ NK cell, in specific cases. The NK cells may express one or more exogenously provided cytokines, such as IL-15, IL-2, IL-12, IL-18, IL-21, IL-7, or a combination thereof. Particular ~~embodiment~~examples include populations of immune cells of any kind of the disclosure, and the cells may be present in a suitable medium or a suitable carrier of any kind. In one aspect, the present disclosure refers to a population of any one of the immune cells disclosed herein, said cells present in a suitable medium.

[0015a] In one aspect, the present disclosure refers to use of cells harboring any one of the expression constructs disclosed herein in the manufacture of a medicament for killing CD70-positive cells in an individual.

[0016] In one ~~embodiment~~example, there is a method of killing CD70-positive cells in an individual, comprising the step of administering to the individual an effective amount of cells harboring any expression construct encompassed by the disclosure. In specific ~~embodiment~~examples, the cells are NK cells, T cells, gamma delta T cells, invariant NKT (iNKT) cells, B cells, macrophages, gamma delta T cells, or dendritic cells. NK cells may be derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, or from a cell line. NK cells may be derived from cord blood mononuclear cells. In some cases, the CD70-positive cells are not cancer cells, although in other cases they are cancer cells. The CD70-positive cells may be T regulatory cells. In particular ~~embodiment~~examples, the individual has acute myeloid leukemia, lymphoma, lung cancer, renal cancer, bladder cancer, melanoma, glioblastoma, breast cancer, head and neck cancer, mesothelioma, or a combination thereof. The cells may be allogeneic or autologous with respect to the individual, who may or may not be a human. The cells may be administered to the individual by injection, intravenously, intraarterially, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, intracranially,

percutaneously, subcutaneously, regionally, by perfusion, in a tumor microenvironment, or a combination thereof.

[0017] In particular ~~embodiment~~examples of the methods, the cells may be administered to the individual once or more than once. The duration of time between administrations of the cells to the individual may be 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or 1 or more years. The methods may further comprise the step of providing to the individual an effective amount of an additional therapy, such as surgery, radiation, gene therapy, immunotherapy, and/or hormone therapy. The additional therapy may comprise one or more antibodies or antibody-based agents, in some cases. In some ~~aspect~~examples to the methods, they may further comprising the step of identifying CD70-positive cells in the individual.

[0018] In specific ~~embodiment~~examples there is a composition of matter that comprises the sequences of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQID NO:12, and SEQ ID NO:13. In one aspect, the present disclosure refers to as a composition of matter, the sequences of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQID NO:12, and SEQ ID NO:13.

[0019] It is specifically contemplated that any limitation discussed with respect to one ~~embodiment~~example of the ~~invention~~disclosure may apply to any other ~~embodiment~~example of the ~~invention~~disclosure. Furthermore, any composition of the ~~invention~~disclosure may be used in any method of the ~~invention~~disclosure, and any method of the ~~invention~~disclosure may be used to produce or to utilize any composition of the ~~invention~~disclosure. Aspects of an ~~embodiment~~example set forth in the Examples are also ~~embodiment~~examples that may be implemented in the context of ~~embodiments~~ examples discussed elsewhere in a different Example or elsewhere in the application, such as in the ~~Brief~~ Summary, Detailed Description, Claims, and Brief Description of the Drawings.

[0020] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific ~~embodiments~~ examples disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the

CLAIMS

What is claimed is:

1. An expression construct comprising sequence that encodes a CD70-specific engineered receptor and that encodes one or both of the following:

(a) a suicide gene; and

(b) a cytokine.

2. The construct of claim 1, wherein the CD70-specific engineered receptor is a chimeric antigen receptor (CAR) or a T cell receptor.

3. The expression construct of claim 2, wherein the CD70-specific CAR comprises a scFv having a heavy chain and a light chain, and wherein the heavy chain in the sequence that encodes the CAR is upstream of the light chain in a 5′ to 3′ direction.

4. The expression construct of claim 2, wherein the CD70-specific CAR comprises a scFv having a heavy chain and a light chain, and wherein the heavy chain in the sequence that encodes the CAR is downstream of the light chain in a 5′ to 3′ direction.

5. The expression construct of any one of claims 1-4, wherein the CD70-specific CAR comprises a codon optimized scFv.

6. The expression construct of any one of claims 1-4, wherein the CD70-specific CAR comprises a humanized scFv.

7. The expression construct of any one of claims 1-6, wherein the CD70-specific CAR comprises a signaling peptide.

8. The expression construct of claim 7, wherein the signaling peptide is from CD8alpha, Ig heavy chain, or granulocyte-macrophage colony-stimulating factor receptor or a signal peptide derived from one or more other surface receptors.

9. The expression construct of any one of claims 1-8, wherein the CD70-specific CAR comprises one or more costimulatory domains.

10. The expression construct of claim 9, wherein the costimulatory domain is selected from the group consisting of CD28, CD27, OX-40 (CD134), DAP10, DAP12, 4-1BB (CD137), CD40L, 2B4, DNAM, CS1, CD48, NKG2D, NKp30, NKp44, NKp46, NKp80, and a combination thereof.

11. The expression construct of any one of claims 1-10, wherein the CD70-specific CAR comprises CD3zeta.

12. The expression construct of any one of claims 1-11, wherein the CD70-specific CAR comprises a hinge between the scFv and a transmembrane domain.

13. The expression construct of claim 12, wherein the hinge is CD8-alpha hinge, the hinge comprises an artificial spacer comprised of Gly3, or the hinge comprises CH1, CH2, and/or CH3 domains of IgGs.

14. The expression construct of any one of claims 1-13, wherein the cytokine is IL-15, IL-12, IL-2, IL-18, IL-21, IL-7, or a combination thereof.

15. The expression construct of any one of claims 1-14, wherein the suicide gene is a mutant TNF-alpha, inducible caspase 9, HSV-thymidine kinase, CD19, CD20, CD52, or EGFRv3.

16. The expression construct of claim 14, wherein the mutant TNF-alpha is an engineered nonsecretable mutant TNF-alpha.

17. An expression construct of any one of claims 1-16, wherein the expression construct comprises any one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

18. An immune cell, comprising the expression construct of any one of claims 1-17.

19. The immune cell of claim 18, wherein the immune cell is a natural killer (NK) cell, T cell, gamma delta T cells, invariant NKT (iNKT) cell, B cell, macrophage, MSCs, or dendritic cell.

20. The immune cell of claim 18 or 19, wherein the immune cell is a NK cell.

21. The immune cell of claim 19 or 20, wherein the NK cell is derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, or from a cell line.

22. The immune cell of claim 21, wherein the NK cell line is NK-92 cell line or another NK cell line derived from a tumor or from a healthy NK cell or a progenitor cell.

23. The immune cell of any one of claims 19-22, wherein the NK cell is a cord blood mononuclear cell.

24. The immune cell of any one of claims 19-23, wherein the NK cell is a CD56+ NK cell.

25. The immune cell of any one of claims 19-24, wherein the NK cells express one or more exogenously provided cytokines.

26. The immune cell of claim 25, wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-7, or a combination thereof.

27. The immune cell of any one of claims 18-26, wherein expression of one or more endogenous genes in the immune cell has been modified.

28. The immune cell of claim 27, wherein the expression has been partially or fully reduced in expression.

29. The immune cell of claim 27 or 28, wherein expression of the one or more gene has been modified using CRISPR.

30. The immune cell of any one of claims 27-29, wherein the gene is selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, CTLA-4, TDAG8, CD38, and a combination thereof.

31. A population of immune cells of any one of claims 18-30, said cells present in a suitable medium.

32. The population of claim 31, wherein the immune cells are NK cells.

33. ~~A method of killing CD70 positive cells in an individual, comprising the step of administering to the individual an effective amount~~Use of cells harboring the expression construct of any one of claims 1-17 in the manufacture of a medicament for killing CD70-positive cells in an individual.

34. The use~~method~~ of claim 33, wherein the cells are NK cells, T cells, gamma delta T cells, induced NKT (iNKT) cells, B cells, macrophages, gamma delta T cells, or dendritic cells.

35. The ~~method~~use of claim 34, wherein the NK cells are derived from cord blood, peripheral blood, induced pluripotent stem cells, bone marrow, or from a cell line.

36. The ~~method~~use of any one of claims 34-35, wherein the NK cells are derived from cord blood mononuclear cells.

37. The ~~method~~use of any one of claims 33-36, wherein the CD70-positive cells are not cancer cells.

38. The ~~method~~use of claim 37, wherein the CD70-positive cells are T regulatory cells.

39. The ~~method~~use of any one of claims 33-36, wherein the individual has acute myeloid leukemia, lymphoma, lung cancer, renal cancer, bladder cancer, melanoma, glioblastoma, breast cancer, head and neck cancer, mesothelioma, multiple myeloma, pancreatic cancer or a combination thereof.

40. The ~~method~~use of any one of claims 33-39, wherein the cells are allogeneic with respect to the individual.

41. The ~~method~~use of any one of claims 33-39, wherein the cells are autologous with respect to the individual.

42. The ~~method~~use of any one of claims 33-41, wherein the individual is a human.

43. The ~~method~~use of any one of claims 32-42, wherein the cells are to be administered to the individual once or more than once.

44. The ~~method~~use of claim 43, wherein the duration of time between administrations of the cells to the individual is 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or one or more years.

45. The ~~method~~use of any one of claims 33-44, ~~further comprising the step of providing to the individual~~wherein an effective amount of an additional therapy is to be provided to the individual.

46. The ~~method~~use of claim 45, wherein the additional therapy comprises surgery, radiation, gene therapy, immunotherapy, or hormone therapy.

47. The ~~method~~use of claim 45 or 46, wherein the additional therapy comprises one or more antibodies.

48. The ~~method~~use of any one of claims 33-47, wherein the cells are to be administered to the individual by injection, intravenously, intraarterially, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, intracranially, percutaneously, subcutaneously, regionally, by perfusion, in a tumor microenvironment, or a combination thereof.

49. The ~~method~~use of any one of claims 33-48, ~~further comprising the step of identifying~~ wherein CD70-positive cells in the individual are to be identified.

50. The ~~method~~use of any one of claims 33-49, further comprising the step of producing the cells harboring the expression construct.

51. As a composition of matter, the sequences of SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQID NO:12, and SEQ ID NO:13.