

SUMMARY

[0006] The present disclosure is directed to compositions and methods related to cells utilized for adoptive transfer to control pathogenic conditions due to immune activation. The composition and methods apply to autologous and allogeneic cells. Although some steps may be taken to reduce the reactivity of allogeneic cells in the recipient individual, such cells would still be targeted by the immune system of the recipient (primarily T- and NK-cells), which would recognize them as foreign leading to rejection and limiting therapeutic benefit.

[0007] The present disclosure overcomes this problem by modifying adoptive therapy cells to target activated pathogenic T, NK-T, and NK cells to prevent or treat medical conditions associated with their presence. In particular examples, the compositions and methods utilize adoptive T-cell transfer with cells that express receptors that selectively target pathogenic T cells while sparing resting T cells. In specific examples, the adoptive T-cells for transfer are engineered to express chimeric molecules that target pathogenic T cells that express certain target molecules whose presence on T cells is indicative of pathogenic T cells. In particular examples the disclosure concerns auto/allo-immune defense receptors (ADRs) for the selective targeting of pathogenic T-cells.

[0008] Particular examples of the disclosure include methods of protecting engineered allogeneic T cells from elimination in a host individual by providing to the individual cells armed with ADRs. Examples also include methods that avoid allo-immune reactions in individuals receiving tissue or organ transplants, for example.

[0009] In particular examples, cells encompassed by the disclosure have been modified or can be modified to allow them to survive in a recipient, including an allogeneic recipient. In specific cases, cells for adoptive cell therapy (including T cells, NKT cells, and so forth) are suitable for being utilized “Off-the-shelf”, which herein refers to cells kept in a repository, or bank, that may be provided (with or without further modification) to an individual in need thereof for a specific purpose. The individual in many cases is not the individual from which the cells were originally derived. The cells utilized in such a manner may be pre-manufactured to express an ADR, although in some cases the cells are obtained from a bank and afterwards are modified to express an ADR. The banked cells may or may not also express a CAR or recombinant TCR, or the cells obtained from the bank may afterwards be modified to express a CAR or recombinant TCR. Such practices allow for ease of use of third party-derived

therapeutic cells without immune rejection by a host and without having to manufacture a patient-specific produce every time one is needed.

[0010] In particular examples, there is an isolated polynucleotide, comprising sequence encoding: (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation. The polynucleotide may comprise an OX40-specific ligand, a 4-1BB-specific ligand, or a CD40L-specific ligand. The OX40-specific ligand may be OX40L, an antibody that targets OX40, an OX40L-Fc fusion, or a combination thereof, or any other engineered protein capable of specific binding to OX40. The 4-1BB-specific ligand may be 4-1BBL, an antibody that targets 4-1BB, a 4-1BBL-Fc fusion, or a combination thereof, or any other engineered protein capable of specific binding to 4-1BB. The CD40L-specific ligand may be CD40, an antibody that targets CD40L, a CD40-Fc fusion, or any other engineered protein capable of specific binding to CD40L or combination thereof. In at least certain cases, the polynucleotide further comprises sequence that encodes a spacer between (1) and (2), such as between 10 and 220 amino acids in length, for example. The spacer may have sequence that facilitates surface detection with an antibody, such as the spacer being detectable with an anti-Fc Ab. The spacer may comprise an IgG Fc portion.

[0010a] In one aspect, the present disclosure refers to an isolated polynucleotide, comprising sequence encoding a polypeptide, wherein said polypeptide comprises: (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation.

[0011] In particular examples, polynucleotides of the disclosure may further encode a chimeric antigen receptor, a T-cell receptor, or both. The polynucleotide may be in any form including present on a vector, such as a viral vector (retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector) or non-viral vector (plasmid, transposon, *etc.*). In particular cases, the polynucleotide is present in a cell, including a eukaryotic cell or a bacterial cell. The cell may be an immune cell, such as a T cell. The cell may be engineered. The cell may comprise one or more chimeric antigen receptors (CARs) and/or one or more engineered T cell receptors (TCRs).

[0012] Polypeptides expressed by any polynucleotide encompassed by the disclosure are included as part of the disclosure. In another aspect, the present disclosure refers to a polypeptide, comprising: (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, and CD40; that is operably linked to (2) a signaling domain promoting T-cell activation. The signaling domain promoting T-cell activation may be from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.

[0012a] In yet another aspect, the present disclosure refers to a polypeptide expressed by a polynucleotide as disclosed herein.

[0013] Any cell encompassed by the disclosure is part of the disclosure. In specific examples, any chimeric receptor-expressing cell is part of the disclosure, including cells, comprising any polynucleotide contemplated herein and/or any polypeptides contemplated herein. The cell may be an engineered cell. The cell may be an immune cell, such as a T cell, including a CAR-transduced T cell and/or a T cell receptor (TCR)-transduced T cell. In specific examples, the cell is engineered to lack endogenous expression of one or more genes, such as lack one or more of 4-1BB, OX40 and/or CD40L. The cell may be engineered using CRISPR/Cas9, zinc finger nucleases, TALE nucleases, or meganucleases. Alternatively, the cell may be engineered to prevent surface expression of ADR ligands, for example, by trapping the ADR ligand with a specific antibody or a receptor anchored in the endoplasmic reticulum or another intracellular compartment.

[0013a] In yet another aspect, the present disclosure refers to a chimeric receptor-expressing cell, comprising the polynucleotide or the polypeptide as disclosed herein.

[0013b] In yet another aspect, the present disclosure refers to a method of preparing cells for cell therapy, comprising the step of transfecting immune effector cells with the polynucleotide as disclosed herein.

[0013c] In yet another aspect, the present disclosure refers to use of the cells prepared by the method as disclosed herein, in the manufacture of a medicament for providing an effective amount of the cells to an individual in need thereof.

[0013d] In yet another aspect, the present disclosure refers to use of allogeneic therapeutic cells, in the manufacture of a medicament for treating an individual for a medical condition, wherein said cells express a polypeptide comprising (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation.

[0014] In one example there is a method of avoiding rejection of allogeneic cells, tissue, or organs in an individual, comprising the step of delivering to the individual an effective amount of allogeneic immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that comprises CD3 zeta, wherein the delivering step results in the following in the individual: (1) inhibition of endogenous alloreactive T cells in the individual; and/or (2) suppression of NK cell activation in the individual. In specific examples, the allogeneic cells are the allogeneic immune cells expressing the chimeric receptor. The allogeneic cells may express a chimeric antigen receptor and/or an engineered T cell receptor. The allogeneic immune cells may be delivered to the individual before, during, and/or after tissue and/or organ transplantation in the individual. In specific cases, the activated T cells are pathogenic T cells.

[0014a] In yet another aspect, the present disclosure refers to use of allogeneic immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that comprises CD3 zeta, in the manufacture of a medicament for avoiding rejection of allogeneic cells, tissue, or organs in an individual, wherein the delivering step medicament results in the following in the individual:

- (1) inhibition of endogenous alloreactive T cells in the individual; and/or
- (2) suppression of NK cell activation in the individual.

[0015] In one example, there is a method of selectively targeting activated T cells in an individual, comprising the step of providing to the individual an effective amount of cells expressing an engineered chimeric receptor, said chimeric receptor comprising: (1) an extracellular domain that targets compounds that are selectively present on activated T cells; and (2) a signaling domain promoting T-cell activation. The signaling domain promoting T-cell activation may be derived from CD3 zeta subunit, DAP12, an Fc receptor, any ITAM-comprising sequence, or a combination thereof. In specific cases, the activated T cells are pathogenic T cells.

[0015a] In yet another aspect, the present disclosure refers to use of immune cells expressing an engineered chimeric receptor, said chimeric receptor comprising:

(1) an extracellular domain that targets compounds that are selectively present on activated T cells; and

(2) a signaling domain promoting T-cell activation.,

in the manufacture of a medicament for selectively targeting activated T cells in an individual.

[0016] In a certain example, there is a method of preventing or treating a medical condition related to activated T cells in an individual, comprising the step of delivering to the individual an effective amount of immune cells expressing an engineered chimeric receptor that selectively targets said activated T cells, said chimeric receptor comprising: (1) an extracellular domain that targets compounds that are selectively present on activated T cells; and (2) a signaling domain promoting T-cell activation. The medical condition may be an autoimmune disorder, such as graft rejection, graft-versus-host disease, type I diabetes, multiple sclerosis, autoimmune colitis, or a combination thereof, for example.

[0016a] In yet another aspect, the present disclosure refers to use of immune cells expressing an engineered chimeric receptor that selectively targets said activated T cells, said chimeric receptor comprising:

(1) an extracellular domain that targets compounds that are selectively present on activated T cells; and

(2) a signaling domain promoting T-cell activation.,

in the manufacture of a medicament for preventing or treating a medical condition related to activated T cells in an individual.

[0017] In one example, there is a method of avoiding NK cell-mediated host rejection of allogeneic T cells, tissues, or organs in an individual, comprising the step of providing to the individual an effective amount of immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that also comprises a signaling domain promoting T-cell activation. The immune cells expressing the engineered chimeric receptor are the allogeneic T cells, in certain cases. The immune cells may express a chimeric antigen receptor and/or an engineered T cell receptor. The amount of immune cells expressing the engineered chimeric receptor that are provided to the individual may be in a range of 10^2 - 10^{12} per m^2 . The cells expressing the chimeric receptor may be provided to the individual systemically or locally. The immune cells may be T cells. The immune cells may be delivered to the individual once or more than once.

[0017a] In yet another aspect, the present disclosure refers to use of immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that also comprises a signaling domain promoting T-cell activation, in the manufacture of a medicament for avoiding NK cell-mediated host rejection of allogeneic T cells, tissues, or organs in an individual.

[0018] 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 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 following description when considered in connection with the accompanying figures. It is to be

CLAIMS

What is claimed is:

1. An isolated polynucleotide, comprising sequence encoding a polypeptide, wherein said polypeptide comprises:

(1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to

(2) a signaling domain promoting T-cell activation.

2. The polynucleotide of claim 1, wherein the polypeptide comprises an OX40-specific ligand.

3. The polynucleotide of claim 1, wherein the polypeptide comprises a 4-1BB-specific ligand.

4. The polynucleotide of claim 1, wherein the polypeptide comprises CD40L-specific ligand.

5. The polynucleotide of any one of claims 1-2, wherein the OX40-specific ligand is OX40L, an antibody that targets OX40, an OX40L-Fc fusion, or a combination thereof.

6. The polynucleotide of any one of claims 1 and 3, wherein the 4-1BB-specific ligand is 4-1BBL, an antibody that targets 4-1BB, a 4-1BBL-Fc fusion, or a combination thereof.

7. The polynucleotide of any one of claims 1 and 4, wherein the CD40L-specific ligand is CD40, an antibody that targets CD40L, a CD40-Fc fusion, or any other engineered protein capable of specific binding to CD40L.

8. The polynucleotide of any one of claims 1-7, wherein the polypeptide further comprises 1, 2, or more costimulatory domains.

9. The polynucleotide of any one of claims 1-8, wherein the polynucleotide further comprises sequence that encodes a spacer between (1) and (2).

10. The polynucleotide of claim 9, wherein the spacer is between 10 and 220 amino acids in length.
11. The polynucleotide of claim 10, wherein the spacer has sequence that facilitates surface detection with an antibody.
12. The polynucleotide of claim 11, wherein the spacer is detectable with an anti-Fc Ab.
13. The polynucleotide of claim 12, wherein the spacer comprises IgG Fc portion.
14. The polynucleotide of any one of claims 1-13, wherein the polynucleotide further encodes a chimeric antigen receptor, a T-cell receptor, or both.
15. The polynucleotide of claim 14, wherein there is a 2A element or IRES element on the polynucleotide that encodes the polypeptide of claim 1 and the polynucleotide that encodes the chimeric antigen receptor, a T-cell receptor, or both.
16. The polynucleotide of claim 15, wherein the chimeric antigen receptor comprises 1, 2, or more costimulatory domains.
17. The polynucleotide of any one of claims 1-16, wherein the polynucleotide is present on a vector.
18. The polynucleotide of claim 17, wherein the vector is a viral vector or non-viral vector.
19. The polynucleotide of claim 18, wherein the viral vector is a retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector.
20. The polynucleotide of any one of claims 1-19, wherein the polynucleotide is present in a cell.
21. The polynucleotide of claim 20, wherein the cell is a eukaryotic cell or a bacterial cell.
22. The polynucleotide of claim 20 or 21, wherein the cell is an immune cell.
23. The polynucleotide of any one of claims 20-22, wherein the cell is engineered.
24. The polynucleotide of claim 22, wherein the immune cell is a T cell.

25. The polynucleotide of claim 24, wherein the T cell comprises one or more chimeric antigen receptors.
26. The polynucleotide of claim 25, wherein the chimeric antigen receptor comprises 1, 2, or more costimulatory domains.
27. The polynucleotide of claim 24, wherein the T cell comprises one or more engineered T cell receptors (TCRs).
28. A polypeptide expressed by a polynucleotide of any one of claims 1-27.
29. A polypeptide, comprising:
- (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, and CD40; that is operably linked to
 - (2) a signaling domain promoting T-cell activation.
30. The polypeptide of claim 29, wherein the signaling domain promoting T-cell activation is, from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.
31. The polypeptide of claim 29 or 30, wherein the polypeptide further comprises 1, 2, or more costimulatory domains.
32. A chimeric receptor-expressing cell, comprising the polynucleotide of any one of claims 1-27 or the polypeptide of any one of claims 29-31.
33. The cell of claim 32, wherein the cell is an immune cell.
34. The cell of claim 32 or 33, wherein the cell is engineered.
35. The cell of claim 33, wherein the immune cell is a T cell.
36. The cell of claim 35, wherein the T cell is a CAR-transduced T cell.
37. The cell of claim 35, wherein the T cell is a T cell receptor (TCR)-transduced T cell.
38. The cell of any one of claims 32-37, wherein the cell is engineered to lack endogenous expression of one or more genes.

39. The cell of claim 38, wherein the cell is engineered to lack endogenous expression of 4-1BB, OX40 and/or CD40L.
40. The cell of claim 38 or 39, wherein the cell is engineered using CRISPR/Cas9, zinc finger nucleases, TALE nucleases, or meganucleases.
41. The cell of any one of claims 32-40, wherein the cell is housed in a cell repository.
42. A method of preparing cells for cell therapy, comprising the step of transfecting immune effector cells with the polynucleotide of any one of claims 1-27.
43. The method of claim 42, further comprising the step of depositing the cells in a cell repository.
44. The method of claim 42 or 43, further comprising the step of modifying the cells to express one or more chimeric antigen receptors and/or one or more recombinant T-cell receptors.
45. Use of the cells prepared by the method of any one of claims 42-44, in the manufacture of a medicament for providing an effective amount of the cells to an individual in need thereof.
46. The use of claim 45, wherein the cells are allogeneic with respect to the individual.
47. Use of allogeneic therapeutic cells, in the manufacture of a medicament for treating an individual for a medical condition, wherein said cells express a polypeptide comprising (1) one or more of an OX40-specific ligand, a 4-1BB-specific ligand, CD40L-specific ligand, or functional derivatives thereof; that is operably linked to (2) a signaling domain promoting T-cell activation.
48. The use of claim 47, wherein the cells are obtained from a cell repository.
49. The use of claim 47 or 48, wherein the cells express one or more chimeric antigen receptors and/or one or more recombinant T-cell receptors.
50. Use of allogeneic immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that comprises CD3 zeta, in the manufacture of a medicament for avoiding rejection of allogeneic cells, tissue, or organs in an individual,

wherein the medicament results in the following in the individual:

(1) inhibition of endogenous alloreactive T cells in the individual; and/or

(2) suppression of NK cell activation in the individual.

51. The use of claim 50, wherein the allogeneic cells are the allogeneic immune cells expressing the chimeric receptor.

52. The use of claim 50 or 51, wherein the allogeneic cells express a chimeric antigen receptor or an engineered T cell receptor.

53. The use of claim 50, wherein the allogeneic immune cells are to be delivered to the individual before, during, and/or after tissue and/or organ transplantation in the individual.

54. The use of any one of claims 50-53, wherein the activated T cells are pathogenic T cells.

55. Use of immune cells expressing an engineered chimeric receptor, said chimeric receptor comprising:

(1) an extracellular domain that targets compounds that are selectively present on activated T cells; and

(2) a signaling domain promoting T-cell activation,

in the manufacture of a medicament for selectively targeting activated T cells in an individual.

56. The use of claim 55, wherein the signaling domain promoting T-cell activation is derived from CD3 zeta subunit, DAP12, an Fc receptor, or a combination thereof.

57. The use of claim 55 or 56, wherein the activated T cells are pathogenic T cells.

58. Use of immune cells expressing an engineered chimeric receptor that selectively targets said activated T cells, said chimeric receptor comprising:

(1) an extracellular domain that targets compounds that are selectively present on activated T cells; and

(2) a signaling domain promoting T-cell activation,

in the manufacture of a medicament for preventing or treating a medical condition related to activated T cells in an individual.

59. The use of claim 58, wherein the chimeric receptor further comprises 1, 2, or more costimulatory domains.

60. The use of claim 58 or 59, wherein the medical condition is an autoimmune disorder.

61. The use of any one of claims 58-60, wherein the medical condition comprises graft rejection, graft-versus-host disease, type I diabetes, multiple sclerosis, autoimmune colitis, or a combination thereof.

62. Use of immune cells expressing an engineered chimeric receptor that comprises an extracellular domain that targets compounds that are selectively present on activated T cells and that also comprises a signaling domain promoting T-cell activation, in the manufacture of a medicament for avoiding NK cell-mediated host rejection of allogeneic T cells, tissues, or organs in an individual.

63. The use of claim 62, wherein the immune cells expressing the engineered chimeric receptor are the allogeneic T cells.

64. The use of claim 62 or 63, wherein the immune cells express a chimeric antigen receptor or an engineered T cell receptor.

65. The use of any one of claims 62-64, wherein the amount of immune cells expressing the engineered chimeric receptor that are to be provided to the individual are in the range of 10^2 - 10^{12} per m^2 .

66. The use of any one of claims 47-65, wherein the cells expressing the chimeric receptor are to be provided to the individual systemically or locally.

67. The use of any one of claims 47-66, wherein the immune cells are T cells.

68. The use of any one of claims 47-67, wherein the immune cells are to be delivered to the individual once or more than once.