

CLAIMS

What is claimed is:

1. A nucleic acid molecule comprising a polynucleotide encoding a recombinant protein comprising a drug responsive domain (DRD) operably linked to an IL15 payload, wherein said DRD is derived from human carbonic anhydrase II (CA2) and comprises one, two, three, four or more mutations relative to SEQ ID NO:1 or SEQ ID NO:2.
2. The nucleic acid molecule of claim 1, wherein the DRD comprises one, two, three or four amino acid additions, substitutions and/or deletions relative to SEQ ID NO:1 or SEQ ID NO:2.
3. The nucleic acid molecule of claim 2, wherein the DRD comprises the amino acid sequence of SEQ ID NO:4.
4. The nucleic acid molecule of claim 3, wherein the DRD consists of the amino acid sequence of SEQ ID NO:4.
5. The nucleic acid molecule of any of claims 1-4, wherein the IL15 payload comprises the amino acid sequence of SEQ ID NO:8.
6. The nucleic acid molecule of any one of claims 1-5, wherein the IL15 payload is N-terminal to the DRD.
7. The nucleic acid molecule of claim 6, wherein the IL15 payload is a membrane-bound IL15 polypeptide.
8. The nucleic acid molecule of claim 7, wherein the membrane-bound IL15 polypeptide comprises an IL15 polypeptide component comprising the amino acid sequence of SEQ ID NO:8, a transmembrane domain and an intracellular tail, wherein the transmembrane domain is C-terminal to the IL15 polypeptide component and the intracellular tail is C-terminal to the transmembrane domain.

9. The nucleic acid molecule of claim 8, wherein the membrane-bound IL15 polypeptide further comprises a linker between the IL15 polypeptide component and the transmembrane domain.
10. The nucleic acid molecule of any one of claims 1-7, wherein the IL15 payload further comprises one or more components selected from the group consisting of:
- (a) a leader sequence;
 - (b) a GS linker;
 - (c) a hinge domain;
 - (d) a transmembrane domain; and
 - (e) an intracellular tail.
11. The nucleic acid molecule of any one of claims 1-7, wherein the IL15 payload further comprises:
- (a) a leader sequence;
 - (b) a GS linker;
 - (c) a hinge domain;
 - (d) a transmembrane domain; and
 - (e) an intracellular tail.
12. The nucleic acid molecule of claim 11, wherein the polynucleotide encodes the amino acid sequence of SEQ ID NO:24.
13. The nucleic acid molecule of claim 12, wherein the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:25.
14. The nucleic acid molecule of claim 11, wherein the polynucleotide encodes the amino acid sequence of SEQ ID NO:28.
15. The nucleic acid molecule of claim 14, wherein the polynucleotide comprises the nucleic acid sequence of SEQ ID NO:29.
16. The nucleic acid molecule of any one of claims 1-15, wherein the nucleic acid molecule further comprises a second polynucleotide encoding a chimeric antigen receptor (CAR) or T cell

receptor (TCR), wherein the CAR or TCR comprises an antigen-binding domain specific to an antigen of interest.

17. The nucleic acid molecule of claim 16, wherein the second polynucleotide encodes a CAR comprising an antigen-binding domain specific to an antigen of interest.

18. The nucleic acid molecule of claim 17, wherein the CAR comprises an antigen-binding domain specific to CD19.

19. A vector comprising the nucleic acid molecule of any one of claims 1-18.

20. The vector of claim 19, wherein the vector is a plasmid or a viral vector.

21. The vector of claim 20, wherein the vector is a viral vector derived from an adenovirus, adeno-associated virus (AAV), alphavirus, flavivirus, herpes virus, measles virus, rhabdovirus, retrovirus, lentivirus, Newcastle disease virus (NDV), poxvirus, or picornavirus.

22. The vector of claim 21, wherein the viral vector is selected from a lentiviral vector, adenoviral vector, AAV vector, herpes simplex viral vector, retroviral vector or oncolytic viral vector.

23. The vector of claim 22, wherein the viral vector is selected from a lentiviral vector or a gamma retroviral vector.

24. A recombinant protein encoded by the nucleic acid molecule of any one of claims 1-15.

25. A cell comprising the nucleic acid molecule of any one of claims 1-18, the vector of any one of claims 18-23, or the recombinant protein of claim 24.

26. The cell of claim 25, wherein the cell is a bacterial cell.

27. The cell of claim 25, wherein the cell is a mammalian cell.

28. The cell of claim 27, wherein the mammalian cell is a human cell.

29. The cell of claim 28, wherein the human cell is a T cell, natural killer (NK) cell, or tumor infiltrating lymphocyte (TIL).
30. The cell of claim 29, wherein the cell is a CD4⁺ or CD8⁺ T cell.
31. The cell of claim 29, wherein the cell is isolated.
32. The cell of claim 29, wherein the human cell is a T cell or an NK cell, and wherein the human T cell or the human NK cell further comprises a second polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR), wherein the CAR or TCR comprises an antigen-binding domain specific to an antigen of interest.
33. The cell of claim 32, wherein the second polynucleotide encodes a CAR comprising an antigen-binding domain specific to an antigen of interest.
34. The cell of claim 33, wherein the CAR comprises an antigen-binding domain specific to CD19.
35. A pharmaceutical composition comprising the cell of any one of claims 25-34 and a pharmaceutically acceptable carrier.
36. The pharmaceutical composition of claim 35, wherein the cell is a human T cell, human NK cell or human TIL.
37. A method of modulating the expression, function, and/or level of IL15 in the cell of any one of claims 25-34, said method comprising administering to the cell a stimulus to which the DRD is responsive, wherein the stimulus is administered in an amount sufficient to modulate the expression, function and/or level of IL15.
38. The method of claim 37, wherein the stimulus is selected from acetazolamide, celecoxib, valdecoxib, rofecoxib, methazolamide, dorzolamide, brinzolamide, diclofenamide, ethoxzolamide, zonisamide, dansylamide, or dichlorphenamide.
39. The method of claim 38, wherein the stimulus is acetazolamide.

40. The method of claim 37, wherein the cell is a human T cell or a human NK cell, and wherein the human T cell or the human NK cell further comprises a second polynucleotide encoding a chimeric antigen receptor (CAR) or T cell receptor (TCR), wherein the CAR or TCR comprises an antigen-binding domain specific to an antigen of interest.
41. The method of claim 40, wherein the second polynucleotide encodes a CAR comprising an antigen-binding domain specific to an antigen of interest.
42. The method of claim 41, wherein the CAR comprises an antigen-binding domain specific to CD19.
43. A method of treating a disease or disorder responsive to regulated IL15 in a subject in need thereof, said method comprising:
- (a) administering to the subject a therapeutically effective amount of the nucleic acid molecule of any one of claims 1-18, the vector of any one of claims 19-23, the recombinant protein of claim 24, the cell of any one of claims 25-34 or the pharmaceutical composition of any one of claims 35-36; and
 - (b) administering a therapeutically effective amount of a stimulus to the subject, wherein the DRD is responsive to the stimulus and wherein expression of the IL15 payload is modulated in response to the stimulus.
44. The method of claim 43, wherein the stimulus is selected from acetazolamide, celecoxib, valdecoxib, rofecoxib, methazolamide, dorzolamide, brinzolamide, diclofenamide, ethoxzolamide, zonisamide, dansylamide, or dichlorphenamide.
45. The method of claim 44, wherein the stimulus is acetazolamide.
46. The method of any of claims 43-45, wherein the disease or disorder is cancer.
47. A method of treating a malignant tumor in a subject in need thereof, wherein said tumor expresses a tumor-associated antigen, said method comprising:
- (a) administering to the subject a therapeutically effective amount of the human T cell or the human NK cell of any one of claims 32-34, or a pharmaceutical composition thereof,

wherein the CAR or TCR comprises an antigen-binding domain specific to the tumor-associated antigen; and

(b) administering a therapeutically effective amount of a stimulus to the subject, wherein the DRD is responsive to the stimulus and wherein expression of the IL15 payload is modulated in response to the stimulus.

48. The method according to claim 48, wherein the subject is administered a therapeutically effective amount of the human T cell comprising a CAR, or a pharmaceutical composition thereof.

49. The method according to either of claims 47 or 48, wherein the stimulus is selected from acetazolamide, celecoxib, valdecoxib, rofecoxib, methazolamide, dorzolamide, brinzolamide, diclofenamide, ethoxzolamide, zonisamide, dansylamide, or dichlorphenamide.

50. The method of claim 49, wherein the stimulus is acetazolamide.

51. A method of producing a genetically engineered T cell, natural killer (NK) cell or tumor infiltrating lymphocyte (TIL), comprising introducing into the T cell, NK cell or TIL a polynucleotide encoding a protein comprising a drug responsive domain (DRD) operably linked to an IL15 payload, wherein the polynucleotide encodes an amino acid sequence of SEQ ID NOS:24 or 28.

52. The method of claim 51, wherein the polynucleotide comprises a nucleotide sequence of SEQ ID NOS:25 or 29.

53. The method of claim 52, wherein the polynucleotide is introduced into the T cell, NK cell or TIL by lentiviral transduction.

54. The method of claim 52, wherein the polynucleotide is introduced into the T cell, NK cell or TIL by a non-viral vector delivery method.