CLAIMS

1. A polypeptide comprising an effector module, said effector module comprising:

i) a stimulus response element (SRE), wherein the SRE comprises a drug responsive domain (DRD), said DRD comprising human carbonic anhydrase 2 (CA2; SEQ ID NO. 5810) or a region thereof, and further comprising one or more mutations relative to the amino acid sequence of SEQ ID NO. 5810; and

ii) at least one payload which is operably linked to the SRE, wherein:

(a) the payload comprises CD40L (SEQ ID NO. 6) or a portion thereof; or

(b) the payload comprises CD40L (SEQ ID NO. 6) or a portion thereof comprising one or more mutations relative to the amino acid sequence of SEQ ID NO. 6.

2. The polypeptide of claim 1, wherein the DRD comprises a H122Y mutation in the amino acid at position 122 (H122) of SEQ ID NO. 5810.

3. The polypeptide of claim 2, wherein the DRD further comprises:

(i) a R27L mutation in the amino acid at position 27 (R27) of SEQ ID NO. 5810;

(ii) a T87I mutation in the amino acid at position 87 (T87) of SEQ ID NO. 5810;

(iii) a N252D mutation in the amino acid at position 252 (N252) of SEQ ID NO. 5810; or

(iv) a combination of (i), (ii) and/or (iii).

4. The polypeptide of claim 1, wherein the DRD comprises a E106D mutation in the amino acid at position 106 (E106) of SEQ ID NO. 5810.

5. The polypeptide of claim 1, wherein the DRD comprises a W208S mutation in the amino acid at position 208 (W208) of SEQ ID NO. 5810.

6. The polypeptide of claim 4 or 5, wherein the DRD further comprises a C205S mutation in the amino acid at position 205 (C205) of SEQ ID NO. 5810.

7. The polypeptide of claim 1, wherein the DRD comprises a I59N mutation in the amino acid at position 59 (I59) of SEQ ID NO. 5810.

8. The polypeptide of claim 7, wherein the DRD further comprises a G102R mutation in the amino acid at position 102 (G102) of SEQ ID NO. 5810.

9. The polypeptide of claim 1, wherein the DRD comprises a L156H mutation in the amino acid at position 156 (L156) of SEQ ID NO. 5810.

10. The polypeptide of claim 9, wherein the DRD further comprises:

(i) a W4Y mutation in the amino acid at position 4 (W4) of SEQ ID NO. 5810;

(ii) a F225L mutation in the amino acid at position 225 (F225) of SEQ ID NO. 5810;

(iii) deletion of amino acids at positions 257-260 of SEQ ID NO. 5810;

(iv) deletion of amino acids at positions 1-5 of SEQ ID NO. 5810; or

(v) deletion of amino acids G234, E235 and P236 of SEQ ID NO. 5810.

11. The polypeptide of claim 9, wherein the DRD comprises four mutations relative to SEQ ID NO. 5810, said mutations corresponding to:

(i) L156H, S172C, F178Y, and E186D; or

(ii) D70N, D74N, D100N, and L156H.

12. The polypeptide of claim 1, wherein the DRD comprises a first mutation and a second mutation relative to SEQ ID NO. 5810, wherein:

(i) the first mutation is a S73N mutation in the amino acid at position 73 (S73) of SEQ ID NO. 5810; and

(ii) the second mutation is a substitution of F or Y at the amino acid position 89 (R89) of SEQ ID NO. 5810.

13. The polypeptide of claim 1, wherein the DRD comprises a substitution of N or F at the amino acid position 56 (S56) of SEQ ID NO. 5810.

14. The polypeptide of claim 13, wherein the DRD comprises a S56N mutation in the amino acid at position 56 (S56) of SEQ ID NO. 5810.

15. The polypeptide of claim 13, wherein the DRD comprises two substitutions relative to SEQ ID NO. 5810 that correspond to S56F and D71S.

16. The polypeptide of claim 1, wherein the DRD comprises one or more substitutions relative to SEQ ID NO. 5810, wherein at least one substitution is a substitution of D or N at the amino acid position 63 (G63) of SEQ ID NO. 5810, and wherein the one or more substitutions correspond to:

G63D;

G63D and M240L;

G63D, E69V and N231I; or

T55K, G63N and Q248N.

17. The polypeptide of claim 1, wherein the DRD comprises two or more substitutions relative to SEQ ID NO. 5810, wherein one of the two or more substitutions is a substitution of L or K at the amino acid position 71 (D71) of SEQ ID NO. 5810, and wherein said two or more substitutions correspond to:

D71L and T87N;

D71L and L250R;

D71L, T87N and L250R; or

D71K and T192F.

18. The polypeptide of claim 1, wherein the DRD comprises two or more substitutions relative to SEQ ID NO. 5810, wherein at least one of the two or more substitutions is:

(i) a substitution of F at the amino acid position 241 (V241) of SEQ ID NO. 5810; or

(ii) a substitution of F or L at the amino acid position 249 (P249) of SEQ ID NO. 5810; and

wherein the two or more substitutions correspond to:

D72F and V241F;

D72F and P249L;

D72F and P249F;

D72F, V241F and P249L;
A77I and P249F; or
V241F and P249L.

19. The polypeptide of claim 1, wherein the DRD comprises one or more substitutions relative to SEQ ID NO. 5810, selected from Y51T, L183S, Y193I, L197P and the combination of V134F and L228F.

20. The polypeptide of any one of claims 1-19, wherein the DRD comprises the region of human CA2 corresponding to amino acids 2 to 260 of SEQ ID NO. 5810.

21. The polypeptide of any one of claims 1-19, wherein the DRD comprises the region of human CA2 corresponding to the full-length CA2 comprising amino acids 1 to 260 of SEQ ID NO. 5810.

22. The polypeptide of any one of claims 1-21, wherein the SRE is responsive to one or more stimuli.

23. The polypeptide of claim 22, wherein the stimulus is a small molecule, wherein the small molecule is selected from Acetazolamide, Celecoxib, Valdecoxib, Rofecoxib, Methazolamide, Dorzolamide, Brinzolamide, Diclofenamide, Ethoxzolamide, Zonisamide, Dansylamide, or Dichlorphenamide.

24. The polypeptide of claim 23, wherein the small molecule is Acetazolamide.

25. The polypeptide of any one of claims 1-24, wherein the payload is CD40L (SEQ ID NO. 6) or a portion thereof comprising at least one mutation.

26. The polypeptide of any one of claims 1-24, wherein the payload is CD40L (SEQ ID NO. 6) or a portion thereof comprising at least two mutations, wherein the at least two mutations are selected from:
(i) H224G and G226F;
(ii) H224G and G226H;
(iii) Y172G and G226F;
(iv) H125 and G227;

(v) Y120G, H224G and G226W; and

(vi) S110G, F111G, E112S, M113G, Q114G, and K115S.

27. The polypeptide of any one of claims 1-24, wherein the payload is CD40L (SEQ ID NO: 6).

28. The polypeptide of any one of claims 1-27, wherein the DRD is N-terminally located to the payload.

29. The polypeptide of any one of claims 1-28, wherein the DRD is separated from the payload by a linker.

30. The polypeptide of any one of claims 1-29, wherein the effector module further comprises a signal peptide, a targeting and/or penetrating peptide, a linker, a protein tag, and/or a protein cleavage site.

31. A composition comprising the polypeptide of any one of claims 1-30.

32. A polynucleotide encoding the polypeptide of any one of claims 1-30, wherein the polynucleotide is a DNA molecule or an RNA molecule.

33. The polynucleotide of claim 32, wherein the polynucleotide is monocistronic, bicistronic or multicistronic.

34. The polynucleotide of claim 33, wherein the polynucleotide is bicistronic and encodes a second polypeptide, wherein said second polypeptide comprises an immunotherapeutic agent selected from an antibody and fragments and variants thereof, a T cell receptor (TCR) and variants thereof, a chimeric antigen receptor (CAR), a chimeric switch receptor, an inhibitor of a co-inhibitory receptor or ligand, an agonist of a co-stimulatory receptor and ligand, a cytokine, chemokine, a cytokine receptor, a chemokine receptor, cytokine-cytokine receptor fusion, a soluble growth factor, a metabolic factor, a suicide gene, or a homing receptor.

35. The polynucleotide of claim 33, wherein the polynucleotide is multicistronic and encodes at least two additional polypeptides, wherein said at least two additional polypeptides comprise an immunotherapeutic agent selected from an antibody and fragments and variants thereof, a T cell receptor (TCR) and variants thereof, a chimeric antigen receptor (CAR), a chimeric switch receptor, an inhibitor of a co-inhibitory receptor or ligand, an agonist of a co-stimulatory

receptor and ligand, a cytokine, chemokine, a cytokine receptor, a chemokine receptor, cytokine-cytokine receptor fusion, a soluble growth factor, a metabolic factor, a suicide gene, or a homing receptor.

36. The polynucleotide of claim 34 or 35, wherein the second polypeptide is or the at least two additional polypeptides are operably linked to a second SRE.

37. The polynucleotide of claim 34 or 35, wherein the second polypeptide is not, or the at least two additional polypeptides are not, operably linked to any SRE.

38. A vector comprising the polynucleotide of any one of claims 32-37; wherein, optionally, the vector is a viral vector or a plasmid.

39. The vector of claim 38, which is a viral vector and wherein the viral vector is a retroviral vector, a lentiviral vector, a gamma-retroviral vector, a recombinant AAV vector, an adenoviral vector, or an oncolytic viral vector.

40. A cell comprising at least one of: the effector module of any one of claims 1-30, the polynucleotide of any one of claims 32-37, or the vector of claim 38 or 39.

41. A cell transduced or transfected with the vector of claim 38 or 39.

42. The cell of claim 40 or 41, wherein:

(i) said cell is an immune cell for adoptive cell transfer (ACT); or

(ii) said cell is a CD8+ T cell, a CD4+ T cell, a helper T cell, a natural killer (NK) cell, a NKT cell, a cytotoxic T lymphocyte (CTL), a tumor infiltrating lymphocyte (TIL), a memory T cell, a regulatory T (Treg) cell, a cytokine-induced killer (CIK) cell, a dendritic cell, lymphokine activated killer (LAK) cells, a human embryonic stem cell, a mesenchymal stem cell, a hematopoietic stem cell, or a mixture thereof.

43. The cell of claim 42, wherein said cell is modified to express a chimeric antigen receptor (CAR) or an antigen-specific T cell receptor (TCR).

44. The cell of claim 43, wherein said cell is a T cell or NK cell.

45. A cell which expresses the effector module of any one of claims 1-30, and/or comprises the polynucleotide of any one of claims 32-37, and/or is infected or transfected with the vector of claim 38 or 39, wherein, said cell is a T cell modified to express an antigen-specific T cell receptor (TCR) or an antigen-specific chimeric antigen receptor (CAR).

46. A pharmaceutical composition comprising:

(i) the polypeptide of any one of claims 1-30;

(ii) the polynucleotide of any one of claims 32-37;

(iii) the vector of claim 38 or 39; or

(iv) the cell of any one of claims 40-45; and

a pharmaceutically acceptable excipient.

47. A method of producing a modified cell, said method comprising introducing into a cell a nucleic acid molecule comprising the polynucleotide of any one of claims 32-37.

48. A method of modulating expression, function, and/or level of a payload in the cell of any one of claims 40-45, said method comprising administering to the cell a stimulus, wherein the SRE is responsive to the stimulus and wherein the expression, function, and/or level of the at least one payload is modulated in response to the stimulus.

49. A method of treating a disease and/or inducing an immune response in a subject in need thereof, said method comprising:

(a) administering to the subject a therapeutically effective amount of any of the polypeptides of claims 1-30, the polynucleotide of any one of claims 32-37, the vector of claim 38 or 39, or the cell of any one of claims 40-45; and

(b) administering to the subject a therapeutically effective amount of a stimulus,

wherein the SRE is responsive to the stimulus and wherein expression of the at least one payload is modulated in response to the stimulus to thereby treat the disease and/or induce an immune response.

50. The method of claim 49, wherein the disease is cancer.

51. The method according to claim 49 or 50, wherein the stimulus is selected from Acetazolamide, Celecoxib, Valdecoxib, Rofecoxib, Methazolamide, Dorzolamide, Brinzolamide, Diclofenamide, Ethoxzolamide, Zonisamide, Dansylamide, or Dichlorphenamide.

52. An engineered cell comprising:

i) a first polynucleotide which encodes a first polypeptide, said first polypeptide comprising:

a. a first stimulus response element (SRE), wherein the first SRE comprises a drug responsive domain (DRD), said DRD comprising human carbonic anhydrase 2 (CA2; SEQ ID NO. 5810) or a region thereof, and further comprising one or more mutations relative to the amino acid sequence of SEQ ID NO. 5810; and

b. a first payload which is operably linked to the first SRE, wherein: (I) the first payload comprises CD40L (SEQ ID NO. 6) or a portion thereof; or (II) the first payload comprises CD40L (SEQ ID NO. 6) or a portion thereof comprising one or more mutations relative to the amino acid sequence of SEQ ID NO. 6; and

ii) a second polynucleotide which encodes one or more additional polypeptides, said one or more additional polypeptides comprising an immunotherapeutic agent selected from the group consisting of: a T cell receptor (TCR) and variants thereof or a chimeric antigen receptor (CAR);

wherein the DRD and the first payload are destabilized in the absence of a first stimulus and wherein the DRD and the first payload are stabilized in the presence of the first stimulus, and the one or more additional polypeptides are expressed independently of the first payload.

53. The engineered cell according to claim 52, wherein the first payload is:

a) CD40L (SEQ ID NO. 6) or a portion thereof comprising at least one mutation; or

b) CD40L (SEQ ID NO. 6) or a portion thereof comprising at least two mutations, optionally, wherein the at least two mutations are selected from:

(i) H224G and G226F;

(ii) H224G and G226H;

(iii) Y172G and G226F;

(iv) H125 and G227;

(v) Y120G, H224G and G226W; and

(vi) S110G, F111G, E112S, M113G, Q114G, and K115S.

54. The engineered cell according to claim 52 or 53, wherein the one or more additional polypeptides are linked to a second SRE comprising a second DRD, wherein the second DRD is the same or different as the DRD in the first SRE, the second DRD and the one or more additional polypeptides are destabilized in the absence of the first or a second stimulus and wherein the second DRD and the one or more additional polypeptides are stabilized in the presence of the first or the second stimulus.