CLAIMS

1. A composition for inducing an immune response in a cell or a subject comprising a first effector module, said effector module comprising a first stimulus response element (SRE) operably linked to at least one immunotherapeutic agent.

2. The composition of claim 1, wherein said at least one immunotherapeutic agent is selected from a chimeric antigen receptor (CAR) and an antibody.

3. The composition of claim 2, wherein said first SRE is responsive to or interacts with at least one stimulus.

4. The composition of claim 3, wherein said first SRE is a destabilizing domain (DD).

5. The composition of claim 4, wherein the DD is derived from a parent protein or a mutant protein having one, two, three or more amino acid mutations compared to said parent protein, wherein the parent protein is selected from:

(a) human protein FKBP comprising the amino acid sequence of SEQ ID NO. 3,
(b) human DHFR (hDHFR) comprising the amino acid sequence of SEQ ID NO. 1,
(c) E. coli DHFR (ecDHFR) comprising the amino acid sequence of SEQ ID NO. 2,
(d) PDE5 comprising the amino acid sequence of SEQ ID NO. 4,
(e) PPAR gamma comprising the amino acid sequence of SEQ ID NO. 5,
(f) CA2 comprising the amino acid sequence of SEQ ID NO. 6, and
(g) NQO2 comprising the amino acid sequence of SEQ ID NO. 7.

6. The composition of claim 5, wherein the parent protein is hDHFR and the DD comprises a mutant protein having:

(a) a single mutation selected from hDHFR (I17V), hDHFR (F59S), hDHFR (N65D), hDHFR (K81R), hDHFR (A107V), hDHFR (Y122I), hDHFR (N127Y), hDHFR (M140I), hDHFR (K185E), hDHFR (N186D), hDHFR (M140I), hDHFR (Amino acid 2-187 of WT; N127Y), hDHFR (Amino acid 2-187 of WT; I17V), hDHFR (Amino acid 2-187 of WT; Y122I), and hDHFR (Amino acid 2-187 of WT; K185E);

(b) a double mutation selected from hDHFR (C7R, Y163C), hDHFR (A10V, H88Y), hDHFR (Q36K, Y122I), hDHFR (M53T, R138I), hDHFR (T57A, I72A), hDHFR (E63G, I176F), hDHFR (G21T, Y122I), hDHFR (L74N, Y122I), hDHFR (V75F, Y122I), hDHFR (L94A, T147A), DHFR (V121A, Y22I), hDHFR (Y122I, A125F), hDHFR (H131R, E144G), hDHFR (T137R, F143L), hDHFR (Y178H, E18IG), hDHFR (Y183H, K185E), hDHFR (E162G, I176F) hDHFR (Amino acid 2-187 of WT; I17V, Y122I), hDHFR (Amino acid 2-187 of WT; Y122I, M140I), hDHFR (Amino acid 2-187 of WT; N127Y, Y122I), hDHFR (Amino acid 2-187 of WT; E162G, I176F), and hDHFR (Amino acid 2-187 of WT; H131R, E144G), and hDHFR (Amino acid 2-187 of WT; Y122I, A125F);

(c) a triple mutation selected from hDHFR (V9A, S93R, P150L), hDHFR (I8V, K133E, Y163C), hDHFR (L23S, V121A, Y157C), hDHFR (K19E, F89L, E181G), hDHFR (Q36F, N65F, Y122I), hDHFR (G54R, M140V, S168C), hDHFR (V110A, V136M, K177R), hDHFR (Q36F, Y122I, A125F), hDHFR (N49D, F59S, D153G), hDHFR (G21E, I72V, I176T), hDHFR (Amino acid 2-187 of WT; Q36F, Y122I, A125F), hDHFR (Amino acid 2-187 of WT; Y122I, H131R, E144G), hDHFR (Amino acid 2-187 of WT; E31D, F32M, V116I), and hDHFR (Amino acid 2-187 of WT; Q36F, N65F, Y122I); or

(d) a quadruple or higher mutation selected from hDHFR (V2A, R33G, Q36R, L100P, K185R), hDHFR (Amino acid 2-187 of WT; D22S, F32M, R33S, Q36S, N65S), hDHFR (I17N, L98S, K99R, M112T, E151G, E162G, E172G), hDHFR (G16S, I17V, F89L, D96G, K123E, M140V, D146G, K156R), hDHFR (K81R, K99R, L100P, E102G, N108D, K123R, H128R, D142G, F180L, K185E), hDHFR (R138G, D142G, F143S, K156R, K158E, E162G, V166A, K177E, Y178C, K185E, N186S), hDHFR (N14S, P24S, F35L, M53T, K56E, R92G, S93G, N127S, H128Y, F135L, F143S, L159P, L160P, E173A, F180L), hDHFR (F35L, R37G, N65A, L68S, K69E, R71G, L80P, K99G, G117D, L132P, I139V, M140I, D142G, D146G, E173G, D187G), hDHFR (L28P, N30H, M38V, V44A, L68S, N73G, R78G, A97T, K99R, A107T, K109R, D111N, L134P, F135V, T147A, I152V, K158R, E172G, V182A, E184R), hDHFR (V2A, I17V, N30D, E31G, Q36R, F59S, K69E, I72T, H88Y, F89L, N108D, K109E, V110A, I115V, Y122D, L132P, F135S, M140V, E144G, T147A, Y157C, V170A, K174R, N186S), hDHFR

(L100P, E102G, Q103R, P104S, E105G, N108D, V113A, W114R, Y122C, M126I, N127R, H128Y, L132P, F135P, I139T, F148S, F149L, I152V, D153A, D169G, V170A, I176A, K177R, V182A, K185R, N186S), and hDHFR (A10T, Q13R, N14S, N20D, P24S, N30S, M38T, T40A, K47R, N49S, K56R, I61T, K64R, K69R, I72A, R78G, E82G, F89L, D96G, N108D, M112V, W114R, Y122D, K123E, I139V, Q141R, D142G, F148L, E151G, E155G, Y157R, Q171R, Y183C, E184G, K185del, D187N).

7. The composition of claim 6, wherein the stimulus is selected from the group consisting of Trimethoprim (TMP) and Methotrexate (MTX).

8. The composition of claim 2, wherein the immunotherapeutic agent is a chimeric antigen receptor (CAR).

9. The composition of claim 8, wherein the chimeric antigen receptor (CAR) comprises

(a) an extracellular target moiety;

(b) a transmembrane domain;

(c) an intracellular signaling domain; and

(d) optionally, one or more co-stimulatory domains.

10. The composition of claim 9, wherein the CAR is a standard CAR, a split CAR, an off-switch CAR, an on-switch CAR, a first-generation CAR, a second-generation CAR, a third-generation CAR, or a fourth-generation CAR.

11. The composition of claim 9, wherein the extracellular target moiety recognizes a target molecule on the surface of a cancer cell, wherein said target molecule on the surface of the cancer cell is selected from a cancer antigen, a plasma membrane lipid, a receptor and a membrane bound glycoprotein.

12. The composition of any of claims 9-11, wherein the extracellular target moiety is selected from any of:

i. an Ig NAR,

ii. a Fab fragment,
iii. a Fab' fragment,
iv. a F(ab)'2 fragment,
v. a F(ab)'3 fragment,
vi. an Fv,
vii. a single chain variable fragment (scFv),
viii. a bis-scFv, a (scFv)2,
ix. a minibody,
x. a diabody,
xi. a triabody,
xii. a tetrabody,
xiii. an intrabody,
xiv. a disulfide stabilized Fv protein (dsFv),
xv. a unibody,
xvi. a nanobody, and
xvii. an antigen binding region derived from an antibody that specifically binds to any of a protein of interest, a ligand, a receptor, a receptor fragment or a peptide aptamer.

13. The composition of claim 12, wherein the extracellular target moiety is a scFv derived from an antibody that specifically binds a CD19 antigen.

14. The composition of claim 13, wherein the scFv is a CD19 scFv is selected from one that comprises:

(a) a heavy chain variable region having an amino acid sequence independently selected from the group consisting of SEQ ID NO: 49-80, and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122; or

(b) an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267 and 624.

15. The composition of claim 9, wherein

(a) the intracellular signaling domain of the CAR is the signaling domain derived from T cell receptor CD3zeta or a cell surface molecule selected from the group consisting of FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d; and

(b) the co-stimulatory domain is present and is selected from the group consisting of 2B4, HVEM, ICOS, LAG3, DAP10, DAP12, CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD30, CD40, ICOS (CD278), glucocorticoid-induced tumor necrosis factor receptor (GITR), lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3.

16. The composition of claim 15, wherein the intracellular signaling domain of the CAR is a T cell receptor CD3zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 339.

17. The composition of claim 9, wherein the intracellular signaling domain of the CAR is a T cell receptor CD3zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 626 and the co-stimulatory domain is present, said co-stimulatory domain being selected from amino acid sequence of any of SEQ ID NOs: 268-374.

18. The composition of claim 9, wherein the transmembrane domain is derived from any of the members of the group consisting of:

(a) a transmembrane region of an alpha, beta or zeta chain of a T-cell receptor;

(b) the CD3 epsilon chain of a T-cell receptor;

(c) a molecule selected from CD4, CD5, CD8, CD8α, CD9, CD16, CD22, CD33, CD28, CD37, CD45, CD64, CD80, CD86, CD148, DAP 10, EpoRI, GITR, LAG3, ICOS, Her2, OX40 (CD134), 4-1BB (CD137), CD152, CD154, PD-1, or CTLA-4; and

(d) an immunoglobulin selected from IgG1, IgD, IgG4, and an IgG_4 Fc region.

19. The composition of claim 9, wherein the transmembrane domain comprises an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 375-425 and 897-907.

20. The composition of claim 9, wherein the CAR further comprises

(e) a hinge region near the transmembrane domain, said hinge region comprising an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 426-504.

21. The composition of claim 2, wherein the immunotherapeutic agent is an antibody that is specifically immunoreactive to an antigen selected from a tumor specific antigen (TSA), a tumor associated antigen (TAA), or an antigenic epitope.

22. The composition of claim 21, wherein the antigen is an antigenic epitope and said antigenic epitope is CD19.

23. The composition of claim 22, wherein the antibody is selected from one that comprises

(a) a heavy chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 49-80 and a light chain variable region having an amino acid sequence independently selected from the group consisting of any of SEQ ID NOs: 81-122; or

(b) an amino acid sequence selected from the group consisting of any of SEQ ID NOs: 123-267.

24. The composition of claim 1 wherein said first effector module comprises the amino acid sequence of any of SEQ ID NO: 635-649, 1005-1010, 1015-1018, and 1215-1231.

25. The composition of claim 24, wherein said first SRE of the effector module stabilizes the immunotherapeutic agent by a stabilization ratio of 1 or more, wherein the stabilization ratio comprises the ratio of expression, function or level of the immunotherapeutic agent in the presence of the stimulus to the expression, function or level of the immunotherapeutic agent in the absence of the stimulus.

26. The composition of any of claims 24-25, wherein the SRE destabilizes the immunotherapeutic agent by a destabilization ratio between 0, and 0.09, wherein the destabilization ratio comprises the ratio of expression, function or level of the

immunotherapeutic agent in the absence of the stimulus specific to the SRE to the expression, function or level of the immunotherapeutic agent that is expressed constitutively, and in the absence of the stimulus specific to the SRE.

27. A polynucleotide encoding any of the ~~compositions~~ effector modules of any one of claims 1-26.

~~28. The polynucleotide of claim 27, wherein the polynucleotide is a DNA molecule, or a RNA molecule.~~

~~29. The polynucleotide of claim 28, wherein the polynucleotide is an RNA molecule and said RNA molecule is a messenger RNA.~~

~~30. The polynucleotide of claim 29, which is chemically modified.~~

~~31. The polynucleotide of claim 28, which comprises spatiotemporally selected codons.~~

~~32. The polynucleotide of claim 29, further encoding a promoter, a linker, a signal peptide, a tag, a cleavage site and/or a targeting peptide.~~

~~33.~~ 28. A vector comprising a polynucleotide of ~~any of~~ claim 27 ~~claims 27-32~~.

~~34.~~ 29. The vector of claim ~~33~~ 28, wherein the vector is a viral vector, or a plasmid.

~~35.~~ 30. The vector of claim ~~34~~ 29, 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 adeno viral vector, or an oncolytic viral vector.

~~36.~~ 31. An immune cell for adoptive cell transfer (ACT), which expresses any of the compositions of any of claims 1-26, the polynucleotides of ~~any of claims 27-32~~ claim 27, and/or is infected or transfected with the vector of any of claims ~~33-35~~ 28-30.

~~37.~~ 32. The immune cell of claim ~~36~~ 31, wherein the immune 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, a human embryonic stem cell, a mesenchymal stem cell, a hematopoietic stem cell, or a mixture thereof.

~~38.~~ 33. The immune cell of claim ~~36~~ 31, wherein the immune cell ~~49~~which further expresses a composition comprising a second effector module, said second effector module comprising a second SRE linked to a second immunotherapeutic agent wherein the second immunotherapeutic agent is selected from a cytokine, and a cytokine- cytokine receptor fusion.

~~39.~~ 34. The immune cell of claim ~~38~~ 33, wherein the second immunotherapeutic agent is a cytokine.

~~40.~~ 35. The immune cell of claim ~~39~~ 34, wherein the cytokine is IL12 or IL15.

~~41.~~ 36. The immune cell of claim ~~38~~ 33, wherein the second immunotherapeutic agent is a cytokine- cytokine receptor fusion polypeptide.

~~42.~~ 37. The immune cell of claim ~~41~~36, wherein the cytokine-cytokine receptor fusion polypeptide is selected from a IL12-IL12 receptor fusion polypeptide, a IL15-IL15 receptor fusion polypeptide, and a IL15-IL15 receptor sushi domain fusion polypeptide.

~~43.~~ 38. The immune cell of claim ~~36~~31 or ~~37~~32, wherein the immune cell is autologous, allogeneic, syngeneic, or xenogeneic in relation to a particular individual subject.

~~44. A method of reducing a tumor volume or burden in a subject, comprising contacting said subject with a composition of any of claims 1-26, the polynucleotides of any of claims 27-32, the vectors of any of claims 33-35 or the immune cells of any of claims 36-43, wherein the SRE responds to a stimulus and regulates the expression and function of the immunotherapeutic agent.~~

39. The composition of any of claims 1-26, the polynucleotides of claim 27, the vectors of any of claims 28-30 or the immune cells of any of claims 31-38 for use in reducing a tumor volume or burden in a subject, wherein the SRE responds to a stimulus and regulates the expression and function of the immunotherapeutic agent.

40. The composition of any of claims 1-26, the polynucleotides of claim 27, the vectors of any of claims 28-30 or the immune cells of any of claims 31-38 for use in inducing an immune response in a subject.

~~45. A method of inducing an immune response in a subject comprising administering to the subject an effective amount of any of the compositions of claims 1-26, the polynucleotides of any of claims 27-32, the vectors of any of claims 33-35 or the immune cells of any of claims 36-43.~~

~~46. A method of identifying a domain of a CD19 antigen which will not bind the FMC63 antibody (FMC63-distinct CD19 binding domain), said method comprising:~~

~~(a) preparing a composition comprising a CD19 antigen,~~

~~(b) contacting the composition in (a) with saturating levels of FMC63 antibody,~~

~~(c) contact the composition of step (b) with one or more selected members of a library of potential CD19 binders; and~~

~~(d) identifying a binding domain on the CD19 antigen based on the differential binding of the selected members of the library of CD19 binders compared to the binding of FMC63.~~

~~47. The method of claim 46, wherein said binding domains of the library are generated using phage display techniques with the CD19 antigen as the seed sequence.~~

~~48. The method of claim 47, wherein the binding domain is selected from a Fab fragment, a Fab' fragment, a F(ab)'2 fragment, a F(ab)'3 fragment, Fv, a single chain variable fragment (scFv), a bis-scFv, a (scFv)2, a minibody, a diabody, a triabody, a tetrabody, a disulfide stabilized Fv protein (dsFv), a unibody, a nanobody, or an antigen binding region of an antibody, and an antibody fragment.~~

~~49. The method of claim 48, wherein the CD19 antigen is selected from a whole or a portion of a human CD19 antigen, and a whole or a portion of a Rhesus CD19 antigen.~~

~~50. A chimeric antigen receptor comprising the FMC63-distinct CD19 binding domain obtained according to the method of any of claims 46-49.~~

~~51. An effector module comprising a stimulus response element (SRE) operably lined to the chimeric antigen receptor of claim 50.~~