However, it also could result in a T cell lymphoma, or other adverse effect, as the modified T cells now lack part of the normal cellular control; these therapeutic T cells could themselves become malignant. Transducing these modified T cells with an inducible CSM polypeptide-based safety switch as presented herein, would provide a safety switch that could avoid this result.

Thus, featured in some embodiments is a composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, a multimerizing region and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40. In some embodiments, the membrane-targeting region is selected from the group consisting of myristoylation-targeting sequence, palmitoylation-targeting sequence, prenylation sequences (i.e., farnesylation, geranyl-geranylation, CAAX Box), protein-protein interaction motifs and transmembrane sequences (utilizing signal peptides) from receptors. In certain aspects, the membrane-targeting region is a myristoylation targeting sequence. In some embodiments, the inducible chimeric signaling molecule further comprises a second co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40. In some embodiments, the co-stimulatory polypeptide cytoplasmic signaling regions comprise a CD28 cytoplasmic signaling region and a 4-1BB cytoplasmic signaling region. In some embodiments, wherein the co-stimulatory polypeptide cytoplasmic signaling regions comprise an OX40 cytoplasmic signaling region polypeptide and a 4-1BB cytoplasmic signaling region polypeptide. In some embodiments, the inducible chimeric signaling molecule further comprises a CD3ζ polypeptide.

In some embodiments, multimerizing region is selected from the group consisting of FKBP, cyclophilin receptor, steroid receptor, tetracycline receptor, heavy chain antibody subunit, light chain antibody subunit, and mutated sequences thereof. In some embodiments, the multimerizing region is an FKBP12 region. In some embodiments, the FKB12 region is an FKB12v36 region. In some embodiments, the multimerizing region is Fv'Fvls. In some embodiments, the multimerizing region binds a ligand selected from the group consisting of an FK506 dimer and a dimeric FK506 analog ligand. In some embodiments, the ligand is AP1903 or AP20187. In some embodiments, the multimerizing region has an amino acid sequence of SEQ ID NO: 58 or a functional fragment thereof. In some embodiments, the multimerizing region is encoded by a nucleotide sequence in SEQ ID NO: 57, or a functional fragment thereof.

However, it also could result in a T cell lymphoma, or other adverse effect, as the modified T cells now lack part of the normal cellular control; these therapeutic T cells could themselves become malignant. Transducing these modified T cells with an inducible CSM polypeptide-based safety switch as presented herein, would provide a safety switch that could avoid this result.

In one aspect, there is provided a composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, an intracellular multimerizing region comprising an FK506 ligand binding region variant, that binds to a multimeric ligand, and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40, and a second co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40.

In one aspect, there is provided a cell transduced or transfected with a composition as described herein, a vector as described herein, or a plasmid as described herein, wherein the cell is selected from the group consisting of T cells, NK cells, and NK-T cells.

In one aspect, there is provided the use of the cell as described herein in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of a blood or bone marrow disease, a solid tumor, and cancer present in the blood or bone marrow.

In one aspect, there is provided the use of the cell as described herein in the manufacture of a medicament for treating leukemia in a subject, wherein the single chain variable fragment binds to CD19.and wherein a multimeric ligand is to be administered to the subject.

In one aspect, there is provided a cell transduced or transfected with a nucleic acid that comprises
(a) a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region,
(b) an intracellular multimerizing region comprising an FK506 ligand binding region variant, that binds to a multimeric ligand, and

(c) a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, and OX40 wherein the cell is selected from the group consisting of T cells, NK cells, and NK-T cells.

In one aspect, there is provided the use of the cell as described herein in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of sickle cell anemia, metachromatic leukodystroph, a primary immune deficiency disorder, hemophagocytosis lymphohistiocytosis (HLH) or other hemophagocytic disorder, an inherited marrow failure disorder, a hemoglobinopathy, a metabolic disorder, and an osteoclast disorder.

In one aspect, there is provided the use of the cell as described herein in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of Severe Combined Immune Deficiency (SCID), Combined Immune Deficiency (CID), Congenital T-cell Defect/Deficiency, Common Variable Immune Deficiency (CVID), Chronic Granulomatous Disease, IPEX (Immune deficiency, polyendocrinopathy, enteropathy, X-linked) or IPEX-like, Wiskott-Aldrich Syndrome, CD40 Ligand Deficiency, Leukocyte Adhesion Deficiency, DOCK 8 Deficiency, IL-10 Deficiency/IL-10 Receptor Deficiency, GATA 2 deficiency, X-linked lymphoproliferative disease (XLP), Cartilage Hair Hypoplasia, Shwachman Diamond Syndrome, Diamond Blackfan Anemia, Dyskeratosis Congenita, Fanconi Anemia, Congenital Neutropenia, Sickle Cell Disease, Thalassemia, Mucopolysaccharidosis, Sphingolipidoses, and Osteopetrosis.

Thus, featured in some embodiments is a composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, a multimerizing region and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40. In some embodiments, the membrane-targeting region is selected from the group consisting of myristoylation-targeting sequence, palmitoylation-targeting sequence, prenylation sequences (i.e., farnesylation, geranyl-geranylation, CAAX Box), protein-protein interaction motifs and transmembrane sequences (utilizing signal peptides) from receptors. In certain aspects, the membrane-targeting region is a myristoylation targeting sequence. In some embodiments, the inducible chimeric signaling molecule further comprises a second co-stimulatory polypeptide

cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40. In some embodiments, the co-stimulatory polypeptide cytoplasmic signaling regions comprise a CD28 cytoplasmic signaling region and a 4-1BB cytoplasmic signaling region. In some embodiments, wherein the co-stimulatory polypeptide cytoplasmic signaling regions comprise an OX40 cytoplasmic signaling region polypeptide and a 4-1BB cytoplasmic signaling region polypeptide. In some embodiments, the inducible chimeric signaling molecule further comprises a CD3ζ polypeptide.

In some embodiments, multimerizing region is selected from the group consisting of FKBP, cyclophilin receptor, steroid receptor, tetracycline receptor, heavy chain antibody subunit, light chain antibody subunit, and mutated sequences thereof. In some embodiments, the multimerizing region is an FKBP12 region. In some embodiments, the FKB12 region is an FKB12v36 region. In some embodiments, the multimerizing region is Fv'Fvls. In some embodiments, the multimerizing region binds a ligand selected from the group consisting of an FK506 dimer and a dimeric FK506 analog ligand. In some embodiments, the ligand is AP1903 or AP20187. In some embodiments, the multimerizing region has an amino acid sequence of SEQ ID NO: 58 or a functional fragment thereof. In some embodiments, the multimerizing region is encoded by a nucleotide sequence in SEQ ID NO: 57, or a functional fragment thereof.

What is claimed is:

1. A composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, a multimerizing region and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, and OX40.

2. The composition of claim 1, wherein the inducible chimeric signaling molecule further comprises a second co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, and OX40.

3. A composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, a multimerizing region, and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40, and a second co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40.

4. The composition of any one of claims 1-3, wherein the inducible chimeric signaling molecule further comprises a CD3ζ polypeptide.

5. The composition of any one of claims 1-4, wherein the multimerizing region is an FKBP12 region.

6. The composition of claim 5, wherein the FKB12 region is an FKB12v36 region.

7. The composition of any one of claims 1-4, wherein the multimerizing region is Fv'Fvls.

8. A cell transformed or transfected with a composition of any one of claims 1-7.

9. The cell of claim 8, wherein the cell is further transformed or transduced with a nucleic acid comprising a polynucleotide that encodes a chimeric polypeptide comprising a signal peptide, a single chain variable fragment, a CH2-CH3 hinge region and a CD3ζ polypeptide.

10. The cell of claim 9, wherein the single chain variable fragment binds to an antigen on a tumor cell or to an antigen on a cell involved in a hyperproliferative disease.

11. The cell of any one of claims 9-10, wherein the single chain variable fragment is selected from the group consisting of αPSMA, αPSCA, αMUC1, αCD19, αROR1, αMesothelin, αGD2, αCD123, αMUC16, and αHer2/Neu single chain variable fragments.

12. The use of the cell of claim 8 in the manufacture of a medicament for inducing an immune response.

13. The use of claim 12, wherein the cell contacts a ligand that binds to the multimerizing region resulting in multimerization of the inducible chimeric signaling molecule.

14. The use of any one of claims 12-13, wherein the cell is to be administered to a subject.

15. The use of claim 14, wherein the subject has a solid tumor.

16. The use of the cell of claim 11 in the manufacture of a medicament for treating leukemia in a subject, wherein the single chain variable fragment binds to CD19 and wherein a multimeric ligand is to be administered to the subject.

17. The use of claim 16, wherein the level of CD19-expressing B cells is decreased following the administration of the multimeric ligand relative to the level of CD19-expressing B cells before administration of the multimeric ligand.

18. The use of any one of claims 13-17, wherein the multimeric ligand is AP1903 or AP20187.

CLAIMS

1. A composition, comprising a nucleic acid that comprises a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region, an intracellular multimerizing region comprising an FK506 ligand binding region variant, that binds to a multimeric ligand, and a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40, and a second co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, CD40, RANK/TRANCE-R, CD3 zeta chain, and OX40.

2. The composition of claim 1, wherein the inducible chimeric signaling molecule further comprises a CD3ζ polypeptide.

3. The composition of any one of claims 1-2, wherein the co-stimulatory polypeptide cytoplasmic signaling regions are selected from the group consisting of CD27, CD28, ICOS, 4-1BB, and OX40.

4. A cell transduced or transfected with a composition of any one of claims 1-3 or 23-30, a vector of claim 31, or a plasmid of claim 32, wherein the cell is selected from the group consisting of T cells, NK cells, and NK-T cells.

5. The cell of any one of claims 4 or 15-22, wherein the cell is further transfected-or transduced with a nucleic acid comprising a polynucleotide that encodes a chimeric polypeptide comprising a signal peptide, a single chain variable fragment, a CH2-CH3 hinge region and a CD3ζ polypeptide.

6. The cell of claim 5, wherein the single chain variable fragment binds to an antigen on a tumor cell or to an antigen on a cell involved in a hyperproliferative disease.

7. The cell of any one of claims 5-6, wherein the single chain variable fragment is selected from the group consisting of αPSMA, αPSCA, αMUC1, αCD19, αROR1, αMesothelin, αGD2, αCD123, αMUC16, and αHer2/Neu single chain variable fragments.

8. The use of the cell of any one of claims 4 or 15-22 in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of a blood or bone marrow disease, a solid tumor, and cancer present in the blood or bone marrow.

9. The use of claim 8, wherein the cell contacts a ligand that binds to the multimerizing region resulting in multimerization of the inducible chimeric signaling molecule.

10. The use of any one of claims 8-9, wherein the medicament is to be administered to a subject.

11. The use of claim 10, wherein the subject has a solid tumor.

12. The use of the cell of claim 7 in the manufacture of a medicament for treating leukemia in a subject, wherein the single chain variable fragment binds to CD19.and wherein a multimeric ligand is to be administered to the subject.

13. The use of claim 12, wherein the level of CD19-expressing B cells is decreased following the administration of the multimeric ligand relative to the level of CD19-expressing B cells before administration of the multimeric ligand.

14. The use of any one of claims 9-13, wherein the multimeric ligand is AP1903 or AP20187.

15. A cell transduced or transfected with a nucleic acid that comprises

(a) a polynucleotide encoding an inducible chimeric signaling molecule, wherein the inducible chimeric signaling molecule comprises a membrane-targeting region,

(b) an intracellular multimerizing region comprising an FK506 ligand binding region variant, that binds to a multimeric ligand, and

(c) a co-stimulatory polypeptide cytoplasmic signaling region selected from the group consisting of CD27, CD28, ICOS, 4-1BB, and OX40 wherein the cell is selected from the group consisting of T cells, NK cells, and NK-T cells.

16. The cell of claim 15, wherein the multimerizing region is an FKBP12 region variant.

17. The cell of any one of claims 15-16, wherein the multimerizing region binds a ligand selected from the group consisting of an FK506 dimer and a dimeric FK506 analog ligand.

18. The cell of claim 17, wherein the ligand is AP1903 or AP20187.

19. The cell of any one of claims 15-18, wherein the multimerizing region is an FKB12v36 region.

20. The cell of any one of claims 15-18 wherein the multimerizing region is Fv'Fvls.

21. The cell of any one of claims 15-20, wherein the membrane-targeting region is selected from the group consisting of myristoylation-targeting sequence, palmitoylation-targeting sequence, prenylation sequences (i.e., farnesylation, geranyl-geranylation, CAAX Box), protein-protein interaction motifs and transmembrane sequences (utilizing signal peptides) from receptors.

22. The cell of any one of claims 15-21, wherein the membrane-targeting region is a myristoylation targeting sequence.

23. The composition of any one of claims 1-3, wherein the multimerizing region is an FKBP12 region variant.

24. The composition of any one of claims 1-2, or 23, wherein the multimerizing region is an FKBP12v36 region.

25. The composition of any one of claims 1-2, or 23, wherein the multimerizing region is Fv'Fvls.

26. The composition of any one of claims 1-2, or 23-25, wherein the multimerizing region binds a ligand selected from the group consisting of an FK506 dimer and a dimeric FK506 analog ligand.

27. The composition of claim 26, wherein the ligand is AP1903 or AP20187.

28. The composition of any of claims 1-2, or 23-27, wherein the membrane-targeting region is selected from the group consisting of myristoylation-targeting sequence, palmitoylation-targeting sequence, prenylation sequences (i.e., farnesylation, geranyl-geranylation, CAAX Box), protein-protein interaction motifs and transmembrane sequences (utilizing signal peptides) from receptors.

29. The composition of claim 28, wherein the membrane-targeting region is a myristoylation targeting sequence.

30. The composition of any one of claims 1-2, or 23-29, wherein the nucleic acid comprises a promoter sequence operably linked to the polynucleotide.

31. The composition of any one of claims 1-2, or 23-30, wherein the nucleic acid is contained within a viral vector.

32. The composition of any one of claims 1-2, or 23-30, wherein the nucleic acid is contained within a plasmid.

33. The use of the cell of any one of claims 4 or 15-22 in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of sickle cell anemia, metachromatic leukodystroph, a primary immune deficiency disorder, hemophagocytosis lymphohistiocytosis (HLH) or other hemophagocytic disorder, an inherited marrow failure disorder, a hemoglobinopathy, a metabolic disorder, and an osteoclast disorder.

34. The use of the cell of any one of claims 4 or 15-22 in the manufacture of a medicament for inducing an immune response in a subject that has been diagnosed with a condition selected from the group consisting of Severe Combined Immune Deficiency

(SCID), Combined Immune Deficiency (CID), Congenital T-cell Defect/Deficiency, Common Variable Immune Deficiency (CVID), Chronic Granulomatous Disease, IPEX (Immune deficiency, polyendocrinopathy, enteropathy, X-linked) or IPEX-like, Wiskott-Aldrich Syndrome, CD40 Ligand Deficiency, Leukocyte Adhesion Deficiency, DOCK 8 Deficiency, IL-10 Deficiency/IL-10 Receptor Deficiency, GATA 2 deficiency, X-linked lymphoproliferative disease (XLP), Cartilage Hair Hypoplasia, Shwachman Diamond Syndrome, Diamond Blackfan Anemia, Dyskeratosis Congenita, Fanconi Anemia, Congenital Neutropenia, Sickle Cell Disease, Thalassemia, Mucopolysaccharidosis, Sphingolipidoses, and Osteopetrosis.