

CLAIMS

1. A T cell wherein a recombinant nucleic acid sequence encoding a chimeric antigen receptor (CAR) is integrated at a first site within the genome of the cell such that the CAR is expressed by the cell at the surface of the cell, wherein integration of the nucleic acid encoding the CAR at said first site reduces or prevents expression of a functional T cell receptor (TCR) complex at the surface of the cell, and wherein expression of the integrated nucleic acid sequence in the T cell is under the control of an endogenous promoter of a TCR complex.

2. The T cell of claim 1, wherein the nucleic acid sequence encoding the CAR is integrated at a single site within the genome.

3. The T cell of claim 1, wherein the nucleic acid sequence encoding the CAR is integrated at two sites within the genome of the cell.

4. The T cell of claim 3, wherein the first site is in an exon of the gene encoding a protein of the TCR complex.

5. The T cell of any one of claims 1-4, wherein integration of the nucleic acid sequence encoding the CAR at the first site reduces or prevents expression of a protein selected from the group consisting of T cell receptor alpha chain, T cell receptor beta chain, CD3 gamma chain, CD3 delta chain, CD3 epsilon chain, and CD3 zeta chain.

6. The T cell of claim 1, wherein the endogenous promoter is a T cell receptor complex promoter.

7. The T cell of claim 1, wherein the endogenous promoter is a promoter of a gene encoding a T cell receptor alpha chain, T cell receptor beta chain, CD3 gamma chain, CD3 delta chain, CD3 epsilon chain, or CD3 zeta chain.

8. The T cell of any one of claims 1-7, wherein the CAR binds to a cancer antigen.

9. The T cell of claim any one of claims 1-8 which is derived from a human.

10. The T cell of any one of claims 1-9, wherein the nucleic acid sequence encoding the CAR is integrated into the first site by targeted homologous recombination.

11. The T cell of claim 10, wherein the nucleic acid sequence encoding the CAR is integrated at a plurality of sites within the genome of the cell, and such that expression of the nucleic acid sequence encoding the CAR at said plurality of sites is under the control of a different endogenous promoter.

12. The T cell of any one of claims 1-11, wherein said nucleic acid sequence encoding a CAR is also integrated at a second site within the genome of the cell such that the CAR is expressed by the cell at the surface of the cell.

13. The T cell of claim 12, wherein integration of the nucleic acid encoding the CAR at said second site also reduces or prevents expression of a functional TCR complex at the surface of the cell, wherein said first site and said second site are in different genes.

14. The T cell of any one of claims 1-13, wherein a second nucleic acid sequence encoding a second CAR is integrated at a second site within the genome of the cell such that the second CAR is expressed by the cell at the surface of the cell, and such that expression of the second nucleic acid sequence is under the control of an endogenous promoter at said second site, wherein said first site and said second site are in different genes.

15. A human T cell wherein a promotor-less recombinant nucleic acid sequence encoding a CAR is integrated at a site in the genome of the cell, said site being the first exon of the T cell receptor (TCR) alpha chain, such that the CAR is expressed under control of the endogenous TCR alpha chain promoter, to produce said CAR at the surface of the cell, and wherein integration of the CAR at said site reduces or prevents expression of a functional TCR alpha chain.

16. The human T cell of claim 15, wherein the CAR binds to CD19.

17. An isolated population of T cells, which comprises a plurality of the cell of any one of claims 1-16.

18. A pharmaceutical composition comprising a therapeutically effective amount of the cell of any one of claims 1-16; and a pharmaceutically acceptable carrier.

19. A pharmaceutical composition comprising a therapeutically effective amount of a population of T cells, which population comprises a plurality of the cell of any one of claims 1-16; and a pharmaceutically acceptable carrier.

20. Use of an effective amount of the cell of any one of claims 1-16 in the manufacture of a medicament for treating a subject with CAR therapy in need thereof, wherein the medicament is to be administered to the subject.

21. Use of the pharmaceutical composition of claim 18 in the manufacture of a medicament for treating a subject with CAR therapy in need thereof, wherein the medicament is to be administered to the subject.

22. Use of a therapeutically effective amount of the cell population of claim 17 in the manufacture of a medicament for treating a subject with CAR therapy in need thereof, wherein the medicament is to be administered to the subject.

23. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating a subject with CAR therapy in need thereof, wherein the medicament is to be administered to the subject.

24. Use of an effective amount of the cell of any one of claims 1-16 in the manufacture of a medicament for treating a subject having cancer, wherein the CAR binds to a cancer antigen of the cancer, wherein the medicament is to be administered to the subject.

25. Use of the pharmaceutical composition of claim 18 in the manufacture of a medicament for treating a subject having cancer, wherein the CAR binds to a cancer antigen of the cancer, wherein the medicament is to be administered to the subject.

26. Use of a therapeutically effective amount of the cell population of claim 17 in the manufacture of a medicament for treating a subject having cancer, wherein the CAR binds to a cancer antigen of the cancer, wherein the medicament is to be administered to the subject.

27. Use of the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating a subject having cancer, wherein the CAR binds to a cancer antigen of the cancer, wherein the medicament is to be administered to the subject.

28. The use of any one of claims 20-27, wherein the subject is a human, and wherein the cell is derived from a human.

29. An in vitro method of generating a T cell that expresses a chimeric antigen receptor (CAR) and lacks a functional T cell receptor (TCR) complex, comprising:

introducing into a T cell:

(i) a nucleic acid sequence encoding a CAR, and

(ii) a homologous recombination system suitable for targeted integration of the nucleic acid sequence at a site within the genome of the cell, such that the expression of the nucleic acid is under the control of an endogenous promoter of a TCR complex, whereby the homologous recombination system integrates the nucleic acid sequence encoding the CAR at said site within the genome of the cell such that integration of the CAR at said site reduces or prevents expression of a functional T cell receptor complex at the surface of the cell, thereby generating a T cell that expresses the CAR and lacks a functional TCR complex.