

CLAIMS

What is claimed is:

1. A method of producing a T-cell line for use in an allogeneic application, comprising:
 - a. introducing a nucleic acid molecule encoding an engineered nuclease under the control of a controllable promoter into a T-cell line;
 - b. integrating the nucleic acid molecule into the genome of the T-cell line; and
 - c. expanding the T-cell line.
2. The method of claim 1, wherein the engineered nuclease is selected from the group consisting of a meganuclease, a zinc finger nuclease, a transcription activator-like effector-based nuclease, and a CRISPR-associated nuclease.
3. The method of claim 2, wherein the CRISPR-associated nuclease is a Cas9 nuclease or a Cas12 nuclease.
4. The method of any one of claims 1-3, wherein the controllable promoter is an inducible promoter.
5. The method of claim 4, wherein the inducible promoter is a 4HT inducible promoter, a rapamycin inducible promoter, a hormone response element, or a glutamate inducible promoter.
6. The method of any one of claims 1-3, wherein the controllable promoter is a derepressible promoter.
7. The method of claim 6, wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).

8. The method of claim 7, wherein the nucleic acid molecule further includes a tetracycline repressor protein.
9. The method of any one of claims 1-3, wherein the controllable promoter comprises a Tet-on system.
10. The method of any one of claims 1-9, further comprising freezing the expanded T-cell line following the expanding.
11. The method of any one of claims 1-10, wherein the nucleic acid molecule is introduced into the T-cell line using a lentiviral vector.
12. The method of any one of claims 1-11, wherein the T-cell line comprises at least about 10^9 T-cells.
13. A method of producing a genetically modified T-cell line, comprising:
 - a. introducing a nucleic acid molecule encoding a CRISPR-associated nuclease under the control of a controllable promoter into a T-cell line;
 - b. integrating the nucleic acid molecule into the genome of the T-cell line;
 - c. expanding the T-cell line;
 - d. inducing expression of the CRISPR-associated nuclease by activating the controllable promoter;
 - e. introducing a guide-RNA and a gene of interest into the expanded T-cell line;
 - f. knocking out expression of a T-cell receptor and introducing the gene of interest into the genome of the T-cell line; and
 - g. recovering the genetically modified T-cell line.
14. The method of claim 13, wherein the CRISPR-associated nuclease is a Cas9 nuclease or a Cas12 nuclease.

15. The method of claim 13 or claim 14, wherein the controllable promoter is an inducible promoter.
16. The method of claim 15, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.
17. The method of claim 13 or claim 14, wherein the controllable promoter is a derepressible promoter.
18. The method of claim 17, wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).
19. The method of claim 18, wherein the nucleic acid molecule further includes a tetracycline repressor protein.
20. The method of claim 18 or claim 19, wherein the activating the derepressible promoter comprises adding doxycycline to the T-cell line.
21. The method of claim 13 or claim 14, wherein the controllable promoter comprises a Tet-on system.
22. The method of claim 21, wherein the activating the Tet-on system comprises adding doxycycline to the T-cell line.
23. The method of any one of claims 13-22, wherein the gene of interest encodes a chimeric antigen receptor (CAR).
24. The method any one of claims 13-23, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.
25. The method of any one of claims 13-24, wherein the genetically modified T-cell line comprises at least about 10^9 T-cells.
26. A method of producing a chimeric antigen receptor (CAR) T-cell line, comprising:

- a. introducing a nucleic acid molecule encoding a Cas9 nuclease or a Cas12 nuclease under the control of a controllable promoter into a T-cell line;
 - b. integrating the nucleic acid molecule into the genome of the T-cell line;
 - c. expanding the T-cell line;
 - d. inducing expression of the Cas9 nuclease or Cas12 nuclease by activating the controllable promoter;
 - e. introducing a guide-RNA and a nucleic acid encoding a chimeric antigen receptor (CAR) into the expanded T-cell line;
 - f. knocking out expression of a T-cell receptor and introducing the nucleic acid encoding the CAR into the genome of the T-cell line; and
 - g. recovering the CAR T-cell line.
27. The method of claim 26, wherein the controllable promoter is an inducible promoter.
 28. The method of claim 27, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.
 29. The method of claim 26, wherein the controllable promoter is a derepressible promoter.
 30. The method of claim 29 wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).
 31. The method of claim 30, wherein the nucleic acid molecule further includes a tetracycline repressor protein.
 32. The method of claim 30 or claim 31, wherein the activating the derepressible promoter comprises adding doxycycline to the T-cell line.
 33. The method of claim 26, wherein the controllable promoter comprises a Tet-on system.

34. The method of claim 33, wherein the activating the Tet-on system comprises adding doxycycline to the T-cell line.
35. The method any one of claims 26-34, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.
36. The method of any one of claims 26-35, wherein the CAR T-cell line comprises at least about 10^9 T-cells.
37. An allogeneic T-cell line, comprising a CRISPR-associated (Cas) nuclease under the control of a controllable promoter integrated into the genome of the T-cell line.
38. The allogeneic T-cell line of claim 37, wherein the CRISPR-associated nuclease is a Cas9 nuclease or a Cas12 nuclease.
39. The allogeneic T-cell line of claim 37 or claim 38, wherein the controllable promoter is an inducible promoter.
40. The allogeneic T-cell line of claim 39, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.
41. The allogeneic T-cell line of claim 37 or claim 38, wherein the controllable promoter is a derepressible promoter.
42. The allogeneic T-cell line of claim 41, wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).
43. The allogeneic T-cell line of claim 42, wherein T-cell further includes a nucleic acid encoding a tetracycline repressor protein.
44. The allogeneic T-cell line of claim 37 or 38, wherein the controllable promoter comprises a Tet-on system.

45. The allogeneic T-cell line of any one of claims 37-44, comprising at least about 10^9 T-cells.
46. The allogeneic T-cell line of claim 45, comprising at least about 10^{10} T-cells.