

CLAIMS

What is claimed is:

1. 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.
2. The method of claim 1, wherein the CRISPR-associated nuclease is a Cas9 nuclease or a Cas12 nuclease.
3. The method of claim 1 or claim 2, wherein the controllable promoter is an inducible promoter.
4. The method of claim 3, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.
5. The method of claim 1 or claim 2, wherein the controllable promoter is a derepressible promoter.
6. The method of claim 5, wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).

7. The method of claim 6, wherein the nucleic acid molecule further includes a sequence encoding a tetracycline repressor protein.
8. The method of claim 6 or claim 7, wherein the activating the derepressible promoter comprises adding doxycycline to the T-cell line.
9. The method of claim 1 or claim 2, wherein the controllable promoter comprises a Tet-on system.
10. The method of claim 9, wherein the activating the Tet-on system comprises adding doxycycline to the T-cell line.
11. The method of any one of claims 1-10, wherein the gene of interest encodes a chimeric antigen receptor (CAR).
12. The method any one of claims 1-11, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.
13. The method of any one of claims 1-12, wherein the genetically modified T-cell line comprises at least 10^9 T-cells.
14. 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.
- 15. The method of 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 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 sequence encoding 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 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 any one of claims 14-22, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.
- 24. The method of any one of claims 14-23, wherein the CAR T-cell line comprises at least 10^9 T-cells.