

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.~~
- 13.1. A method of producing a genetically modified T-cell line, comprising:
- introducing a nucleic acid molecule encoding a CRISPR-associated nuclease under the control of a controllable promoter into a T-cell line;
 - integrating the nucleic acid molecule into the genome of the T-cell line;
 - expanding the T-cell line;
 - inducing expression of the CRISPR-associated nuclease by activating the controllable promoter;
 - introducing a guide-RNA and a gene of interest into the expanded T-cell line;
 - knocking out expression of a T-cell receptor and introducing the gene of interest into the genome of the T-cell line; and
 - recovering the genetically modified T-cell line.
- 14.2. The method of claim 13.1, wherein the CRISPR-associated nuclease is a Cas9 nuclease or a Cas12 nuclease.

~~15.3.~~ The method of claim ~~13~~1 or claim ~~14~~2, wherein the controllable promoter is an inducible promoter.

~~16.4.~~ The method of claim ~~15~~3, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.

~~17.5.~~ The method of claim ~~13~~1 or claim ~~14~~2, wherein the controllable promoter is a derepressible promoter.

~~18.6.~~ The method of claim ~~17~~5, wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).

~~19.7.~~ The method of claim ~~18~~6, wherein the nucleic acid molecule further includes a tetracycline repressor protein.

~~20.8.~~ The method of claim ~~18~~6 or claim ~~19~~7, wherein the activating the derepressible promoter comprises adding doxycycline to the T-cell line.

~~21.9.~~ The method of claim ~~13~~1 or claim ~~14~~2, wherein the controllable promoter comprises a Tet-on system.

~~22.10.~~ The method of claim ~~21~~9, wherein the activating the Tet-on system comprises adding doxycycline to the T-cell line.

~~23.11.~~ The method of any one of claims ~~13~~1-~~22~~10, wherein the gene of interest encodes a chimeric antigen receptor (CAR).

~~24.12.~~ The method any one of claims ~~13~~1-~~23~~11, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.

~~25.13.~~ The method of any one of claims ~~13~~1-~~24~~12, wherein the genetically modified T-cell line comprises at least about 10^9 T-cells.

~~26.14.~~ 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.

~~27~~15. The method of claim ~~26~~14, wherein the controllable promoter is an inducible promoter.

~~28~~16. The method of claim ~~27~~15, wherein the inducible promoter is a 4HT inducible promoter or a glutamate inducible promoter.

~~29~~17. The method of claim ~~26~~14, wherein the controllable promoter is a derepressible promoter.

~~30~~18. The method of claim ~~29~~17 wherein the derepressible promoter includes one or more tetracycline operator sequences (TetO).

~~31~~19. The method of claim ~~30~~18, wherein the nucleic acid molecule further includes a tetracycline repressor protein.

~~32~~20. The method of claim ~~30~~18 or claim ~~31~~19, wherein the activating the derepressible promoter comprises adding doxycycline to the T-cell line.

~~33~~21. The method of claim ~~26~~14, wherein the controllable promoter comprises a Tet-on system.

~~34.22.~~ The method of claim ~~3321~~, wherein the activating the Tet-on system comprises adding doxycycline to the T-cell line.

~~35.23.~~ The method any one of claims ~~2614-3422~~, further comprising freezing the T-cell line following the expanding in c, and thawing prior to the inducing in d.

~~36.24.~~ The method of any one of claims ~~2614-3523~~, 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.