

What is claimed is:

1. A method for producing a modified immune cell, or a population of modified immune cells, with reduced immunogenicity and/or increased anti-neoplasia activity by multiplexed editing, the method comprising:

modifying a target nucleobase in at least four genes or regulatory elements thereof in an immune cell, thereby generating the modified immune cell or the population of modified immune cells with reduced immunogenicity and/or increased anti-neoplasia activity,

wherein the at least four genes or regulatory elements thereof include: CD7, CD52, PDCD1, and TRAC.

2. The method of claim 1, wherein at least one of the four genes is a checkpoint inhibitor gene, an immune response regulation gene, or an immunogenic gene.

3. The method of any one of claims 1 or 2, wherein expression of at least one of the four genes is reduced by at least 50% or by at least 80% as compared to a control cell without the modification.

4. The method of any one of claims 1 to 3, wherein the at least four genes comprise a gene that encodes a TCR complex polypeptides ~~that form a TCR complex~~.

5. The method of claim 1, wherein one of the at least four genes encodes a polypeptide selected from the group consisting of TRAC, a check point inhibitor, PDCD1, a T cell marker, CD2, CD52, CD7, CD3 epsilon, CD3 gamma, CD3 delta, TRBC1, TRBC2, CD4, CD5, ~~CD7~~, CD30, CD33, ~~CD52~~, CD70, B2M, HLA-DR, and CIITA.

6. The method of any one of claims 1 to 5, wherein the modifying comprises deaminating the single target nucleobase.

7. The method of claim 6, wherein the deaminating is performed by a polypeptide comprising a deaminase.

8. The method of claim 7, wherein the deaminase is associated with a nucleic acid programmable DNA binding protein (napDNAbp) to form a base editor.
9. The method of claim 8, wherein the deaminase is fused to the nucleic acid programmable DNA binding protein (napDNAbp).
10. The method of claim 9, wherein the napDNAbp comprises a Cas9 nickase or nuclease dead Cas9.
11. The method of ~~claim 9~~any one of claims 7-10, wherein the deaminase is a cytidine deaminase that converts a cytosine (C) to a thymine (T) or an adenosine deaminase that converts an adenosine (A) to a guanine (G).
12. The method of claim 11, wherein the deaminase is a cytidine deaminase and the base editor further comprises a uracil glycosylase inhibitor.
13. The method of any one of claims 1 to 12, wherein the modifying comprises contacting the immune cell or the population of immune cells with a base editor and a guide nucleic acid sequence.
14. The method of claim 13, wherein the guide nucleic acid sequence comprises a sequence selected from the group consisting of UUCGUAUCUGUAAAACCAAG, CCUACCUGUCACCAGGACCA, CUCUUACCUGUACCAUAACC, CACCUACCUAAGAACCAUCC, ACUCACGCUGGAUAGCCUCC, ACUCACCCAGCAUCCCCAGC, CACUCACCUUAGCCUGAGCA, and CACGCACCUGGACAGCUGAC.
15. The method of any one of claims 1 to 14, wherein the target nucleobase is in an exon, a splice donor site, or a splice acceptor site.

16. The method of claim 1, wherein the target nucleobase is in a splice acceptor or splice donor of a TRAC, PDCD1, CD52, CD7, B2M, CD2, CD5, or CIITA gene.

17. The method of any one of claims 1 to 16, wherein the immune cell is a human cytotoxic T cell, a regulatory T cell, a T helper cell, a dendritic cell, a B cell, or a NK cell, or a population thereof.

18. The method of claim 17, wherein the immune cell or the population of immune cells is derived from a single human donor.

19. The method of any one of claims 1 to 18, further comprising contacting the immune cell or the population of immune cells with a lentivirus comprising a polynucleotide that encodes an exogenous functional chimeric antigen receptor (CAR) or a functional fragment thereof.

20. The method of claim 19, wherein the CAR or the functional fragment thereof specifically binds a marker associated with neoplasia.

21. The method of claim 20, wherein the neoplasia is a T cell cancer, a B cell cancer, a lymphoma, a leukemia, or a multiple myeloma.

22. The method of claim 21, wherein the CAR or the functional fragment thereof specifically binds CD7 or BCMA.

23. A modified immune cell with reduced immunogenicity or increased anti-neoplasia activity produced according to the method of any one of claims 1 to 22,
wherein the modified immune cell comprises a modification of a target nucleobase in at least four genes or regulatory elements thereof,
wherein the at least four genes or regulatory elements include: CD7, CD52, PDCD1, and TRAC.

24. A modified immune cell with reduced immunogenicity or increased anti-neoplasia activity, wherein the modified immune cell comprises a single target nucleobase modification in each one of at least four gene sequences or regulatory elements thereof,

wherein the gene sequences are selected from the group consisting of CD3, CD5, CD52, CD7, CD2, TRAC, CD3 epsilon, CD3 gamma, CD3 delta, TRBC1, TRBC2, CD4, CD30, CD33, CD70, B2M, PDCD1, and CIITA or a regulatory element of each thereof,

wherein the at least four gene sequences or regulatory elements thereof include: CD7, CD52, PDCD1, and TRAC; and

wherein the immune cell is a human immune cell selected from the group consisting of a cytotoxic T cell, a regulatory T cell, a T helper cell, a dendritic cell, a B cell, and a NK cell.

25. The modified immune cell of claim 24, wherein the immune cell further comprises a polynucleotide that encodes an exogenous functional chimeric antigen receptor (CAR) ~~of or~~ a functional fragment thereof; wherein

the CAR specifically binds a marker associated with a neoplasia;

the CAR specifically binds a marker associated with a neoplasia selected from a T cell cancer, a B cell cancer, a lymphoma, a leukemia, or a multiple myeloma; and/or

the CAR specifically binds CD7 or BCMA.

~~26. — A composition comprising a base editor comprising a nucleic acid programmable DNA binding protein (napDNAbp), a deaminase, a uracil glycosylate inhibitor, and a guide nucleic acid sequence, wherein the guide nucleic acid sequence comprises a sequence selected from the group consisting of UUCGUAUCUGUAAAACCAAG, CCUACCUGUCACCAGGACCA, CUCUUACCUGUACCAUAACC, CACCUACCUAAGAACCAUCC, ACUCACGCUGGAUAGCCUCC, ACUCACCCAGCAUCCCCAGC, CACUCACCUUAGCCUGAGCA, and CACGCACCUGGACAGCUGAC.~~

~~27. — The composition of claim 26, wherein the napDNAbp comprises a Cas9 nickase or nuclease dead Cas9 and wherein the deaminase is a cytidine or adenosine deaminase.~~

~~28. — A method for producing a modified immune cell with reduced immunogenicity and/or increased anti-neoplasia activity, the method comprising:~~

—— a) modifying a single target nucleobase in a first gene sequence or a regulatory element thereof in an immune cell;

b) modifying a second gene sequence or a regulatory element thereof in the immune cell with a Cas12 polypeptide, wherein the Cas12 polypeptide generates a site-specific cleavage in the second gene sequence; wherein each of the first gene and the second gene is an immunogenic gene, a checkpoint inhibitor gene, or an immune response regulation gene; and

e) contacting the modified immune cell with a lentivirus comprising a polynucleotide encoding an exogenous functional chimeric antigen receptor (CAR) or a functional fragment thereof, thereby generating a modified immune cell with reduced immunogenicity and/or increased anti-neoplasia activity.

29. — The method of claim 28, wherein the polynucleotide encoding the CAR or the functional fragment thereof is inserted into the site-specific cleavage generated by the Cas12 polypeptide.

30. — The method of claim 29, wherein the Cas12 polypeptide is a Cas12b polypeptide.

31. — The method of any one of claims 28 to 30, wherein the first gene is selected from the group consisting of CD3 epsilon, CD3 gamma, CD3 delta, CD4, TRAC, TRBC1, TRBC2, PDCD1-CD30, CD33, CD7, CD52, B2M, CD70, CHTA, CD2 and CD5; and/or the first gene or the second gene is selected from the group consisting of TRAC, CHTA, CD2, CD5, CD7 and CD52.

32. — A modified immune cell with reduced immunogenicity and/or increased anti-neoplasia activity, the modified immune cell comprising:

a) a single target nucleobase modification in a first gene sequence or a regulatory element thereof in an immune cell; and

b) a modification in a second gene sequence or a regulatory element thereof, wherein the modification is an insertion of an exogenous chimeric antigen receptor (CAR) or a functional fragment thereof or an exogenous T cell receptor or a functional fragment thereof;

—— wherein each of the first gene and the second gene is a immunogenic gene, a checkpoint inhibitor gene, or immune response regulation gene.

33. — ~~A method for producing a modified immune cell with increased anti-neoplasia activity, the method comprising: modifying a single target nucleobase in a Cbl Proto-Oncogene B (CBLB) gene sequence or a regulatory element thereof in an immune cell, wherein the modification reduces an activation threshold of the immune cell compared with an immune cell lacking the modification; thereby generating a modified immune cell with increased anti-neoplasia activity.~~

34. — ~~A composition comprising the modified immune cell of claim 33.~~

35. — ~~A composition comprising a polynucleotide encoding a base editor polypeptide, wherein the base editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and an adenosine or cytidine deaminase and at least four different guide nucleic acid sequences for base editing.~~

36. — ~~The composition of claim 35, wherein the polynucleotide encoding the base editor is an mRNA sequence.~~

37. — ~~The composition of claim 35 or 36, wherein the at least four guide nucleic acid sequences comprise a sequence selected from the group consisting of
UUCGUAUCUGUAAAACCAAG, CCUACCUGUCACCAGGACCA,
CUCUUACCUGUACCAUAACC, CACCUACCUAAGAACCAUCC,
ACUCACGCUGGAUAGCCUCC, ACUCACCCAGCAUCCCCAGC,
CACUCACCUUAGCCUGAGCA, and CACGCACCUGGACAGCUGAC.~~

38. — ~~An immune cell comprising the composition of claim 35, 36, or 37, wherein the composition is introduced into the immune cell by electroporation, nucleofection, viral transduction, lipid nanoparticle, or a combination thereof.~~