CLAIMS

What is claimed is:

1. A method for the *ex-vivo* genetic modification of a plurality of immune cells comprising:

delivering to the plurality of immune cells a nucleic acid sequence comprising a sequence encoding a transposase enzyme, wherein the nucleic acid sequence encoding the transposase enzyme is an RNA sequence and a recombinant and non-naturally occurring DNA sequence comprising a DNA sequence encoding a transposon;

wherein the delivering step comprises electroporation or nucleofection of the plurality of immune cells,

wherein a total amount of DNA comprising an amount of a DNA sequence encoding the transposon is less than 10 µg per 100 µl of an electroporation or nucleofection reaction, and

wherein a concentration of the total amount of DNA comprising the amount of the DNA sequence encoding the transposon in the electroporation or nucleofection reaction less than 10 µg/mL.

2. The method of claim 1, further comprising culturing the plurality of immune cells with at least one of bafilomycin A1, chloroquine, quinacrine and AC-YVAD-CMK after electroporation or nucleofection.

3. The method of any one of claims 1-2, wherein the method further comprises the step of stimulating the immune cell with one or more cytokine(s).

4. The method of claim 3, wherein the step of stimulating the immune cell with one or more cytokine(s) occurs following the delivering step.

5. The method of claim 3, wherein the step of stimulating the immune cell with one or more cytokine(s) occurs prior to the delivering step.

6. The method of any one of claims 3-5, wherein the one or more cytokine(s) comprise(s) IL-2, IL-21, IL-7 and/or IL-15.

7. The method of any one of claims 1-6, wherein the plurality of immune cells comprise at least one T-lymphocyte.

8. The method of any one of claims 1-6, wherein the plurality of immune cells comprise at least one Natural Killer (NK) cell, at least one Cytokine-induced Killer (CIK) cell, at least one Natural Killer T (NKT) cell or any combination thereof.

9. The method of any one of the preceding claims, wherein the transposase enzyme is a Super piggyBac (sPBo) transposase enzyme.

10. The method of claim 9, wherein the Super piggyBac (PB) transposase enzyme comprises an amino acid sequence at least 75% identical to:
MGSSLDDEHILSALLQSDDELVGEDSDSEVSDHVSEDDVQSDTEEAFIDEVHEVQPTSSG
SEILDEQNVIEQPGSSLASNRILTLPQRTIRGKNKHCWSTSKSTRRSRVSALNIVRSQRGPT
RMCRNIYDPLLCFKLFFTDEIISEIVKWTNAEISLKRRESMTSATFRDTNEDEIYAFFGILV
MTAVRKDNHMSTDDLFDRSLSMVYVSVMSRDRFDFLIRCLRMDDKSIRPTLRENDVFT
PVRKIWDLFIHQCIQNYTPGAHLTIDEQLLGFRGRCPFRVYIPNKPSKYGIKILMMCDSGT
KYMINGMPYLGRGTQTNGVPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQEP
YKLTIVGTVRSNKREIPEVLKNSRSRPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDE
DASINESTGKPQMVMYYNQTKGGVDTLDQMCSVMTCSRKTNRWPMALLYGMINIACI
NSFIIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRDNISNILPKE
VPGTSDDSTEEPVMKKRTYCTYCPSKIRRKANASCKKCKKVICREHNIDMCQSCF (SEQ
ID NO: 1).

11. The method of any one of claims 1-8, wherein the transposase enzyme is a Sleeping Beauty transposase enzyme or a hyperactive Sleeping Beauty SB100X transposase.

12. The method of any one of the preceding claims, wherein the recombinant and non-naturally occurring DNA sequence encoding a transposon is contained in a plasmid vector.

13. The method of any one of claims 1-11, wherein the recombinant and non-naturally occurring DNA sequence encoding a transposon is contained in a minicircle DNA vector.

14. The method of any one of claims 1-11, wherein the recombinant and non-naturally occurring DNA sequence encoding a transposon is linear.

15. The method of any one of the preceding claims, wherein the immune cell is isolated or derived from a human.

16. The method of any one of the preceding claims, wherein the recombinant and non-naturally occurring DNA sequence encoding a transposon further comprises a sequence encoding a chimeric antigen receptor or a portion thereof.

17. The method of claim 16, wherein the sequence encoding a chimeric antigen receptor or a portion thereof comprises a sequencing encoding an antigen recognition region.

18. The method of claim 16 or 17, wherein the antigen recognition region comprises an antibody, an antibody mimetic, a protein scaffold or a fragment thereof.

19. The method of claim 18, wherein the antibody is a chimeric antibody, a recombinant antibody, a humanized antibody or a human antibody.

20. The method of claim 18, wherein the antibody comprises or consists of a single-chain variable fragment (scFv), a VHH, a single domain antibody (sdAB), a small modular immunopharmaceutical (SMIP) molecule or a nanobody.

21. The method of claim 20, wherein the VHH is camelid.

22. The method of claim 20 or 21, wherein the VHH is humanized.

23. The method of claim 20, wherein the scFV is a humanized scFv.

24. The method of claim 18, wherein the antibody mimetic comprises or consists of an affibody, an afflilin, an affimer, an affitin, an alphabody, an anticalin, an avimer, a DARPin, a Fynomer, a Kunitz domain peptide, or a monobody.

25. The method of claim 18, wherein the protein scaffold comprises or consists of Centyrin.

26. The method of any one of the preceding claims,

wherein a total amount of DNA comprising an amount of a DNA sequence encoding the transposon is equal to or less than 7.5 μg per 100 μL of an electroporation or nucleofection reaction, and

wherein a concentration of the total amount of DNA comprising the amount of the DNA sequence encoding the transposon in the electroporation or nucleofection reaction is equal to or less than 75 μg/mL.

27. The method of any one of the preceding claims

wherein a total amount of DNA comprising an amount of a DNA sequence encoding the transposon is equal to or less than 1.67 μg per 100 μL of an electroporation or nucleofection reaction, and

wherein a concentration of the total amount of DNA comprising the amount of the DNA sequence encoding the transposon in the electroporation or nucleofection reaction is equal to or less than 16.7 µg/mL.

28. An immune cell modified according to the method of any one of the preceding claims.

29. The immune cell of claim 27, wherein the immune cell is a T-lymphocyte, a Natural Killer (NK) cell, a Cytokine-induced Killer (CIK) cell or a Natural Killer T (NKT) cell.

30. The immune cell of claim 27 or 28, further modified by a second gene editing tool.

31. The immune cell of claim 29, wherein the second gene editing tool comprises an endonuclease operably-linked to either a Cas9 or a TALE sequence.

32. The immune cell of claim 30, wherein the Cas9 is an inactivated Cas9 (dCas9).

33. A composition comprising the immune cell according to any one of claims 27-31.

34. The composition according to claim 32 for use in therapy.

35. The composition of claim 33, for use in the treatment of cancer.

36. The composition of claim 33, for use in the treatment of an infectious disease.

37. The composition of any one of claims 33-36, wherein the immune cell is autologous.

38. The composition of any one of claims 33-36, wherein the immune cell is allogeneic.