

CLAIMS

1. A method of modifying an immune cell, the method comprising the steps of:
(a) modifying a messenger RNA (mRNA) encoding a chimeric antigen receptor (CAR),
(b) purifying the mRNA, and
(c) delivering the mRNA to the immune cell,
wherein the immune cell comprises a macrophage, a monocyte or a dendritic cell, and
wherein the modified immune cell comprises a CAR.
2. The method of claim 1, wherein the step of modifying comprises causing the mRNA to include a modified nucleotide, an alteration to the 5' or 3' untranslated region (UTR), a cap structure, and/or a poly(A) tail.
3. The method of claim 2, wherein the cap structure comprises AGCap1, m6AGCap1, or Anti-Reverse Cap Analog (ARCA).
4. The method of any one of claims 1-3, wherein the modified nucleotide comprises pseudouridine (PsU), 5-methoxyuridine (5moU), 5-methylcytidine/pseudouridine (5meC PsU), N1-methyl-pseudouridine (N1mPsU), or combinations thereof.
5. The method of any one of claims 1-4, wherein the step of purifying comprises silica membrane purification and/or high performance liquid chromatography (HPLC).
6. The method of any one of claims 1-5, wherein the step of delivering comprises transfection.
7. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include AGCap1 and 5moU, the step of purifying comprises silica membrane purification, and the step of delivering comprises electroporation.

8. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include AGCap1 and PsU, the step of purifying comprises HPLC, and the step of delivering comprises electroporation.
9. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include AGCap1 and N1mPsU, the step of purifying comprises HPLC, and the step of delivering comprises electroporation.
10. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include m6-AGCap1 and N1mPsU, the step of purifying comprises HPLC, and the step of delivering comprises electroporation.
11. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include m6-AGCap1 and PsU, the step of purifying comprises HPLC, and the step of delivering comprises electroporation.
12. The method of any one of claims 1-6, wherein the step of modifying comprises modifying the mRNA to include AGCap1 and PsU, the step of purifying comprises HPLC, and the step of delivering comprises transfection.
13. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include m6-AGCap1 and PsU, the step of purifying comprises HPLC, and the step of delivering comprises transfection.
14. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include m6-AGCap1 and N1mPsU, the step of purifying comprises HPLC, and the step of delivering comprises transfection.
15. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include AGCap1 and 5moU.

16. The method of any one of claims 1-6, wherein the step of modifying comprises causing the mRNA to include m6AGCap1 and 5moU.
17. The method of any one of claims 1-16, further comprising a step of treating the immune cell with an RNaseL inhibitor.
18. The method of claim 17, wherein the RNaseL inhibitor comprises sunitinib.
19. The method of claim 17, wherein the RNaseL inhibitor comprises ABCE1.
20. The method of claim 17, wherein the step of treating occurs before the step of delivering.
21. The method of any one of claims 1-20, further comprising a step of culturing the immune cell with a cytokine or immune stimulating recombinant protein.
22. The method of claim 21, wherein the cytokine comprises IFN- α , IFN- β , IFN- γ , TNF α , IL-6, STNGL, LPS, a CD40 agonist, a 4-1BB ligand, a recombinant 4-1BB receptor, a TLR agonist, beta-glucan, IL-4, IL-13, IL-10, TGF- β , a glucocorticoid, an immune complex, or a combination thereof.
23. The method of claim 21 or claim 22, wherein the cytokine comprises IFN- β .
24. The method of any one of claims 21-23, wherein the step of culturing occurs after the step of delivering.
25. The method of any one of claims 1-24, wherein the modified immune cell expresses the CAR.
26. The method of claim 25, wherein CAR expression is increased relative to CAR expression in a modified immune cell of the same type wherein unmodified mRNA encoding the CAR was delivered.

27. The method of claim 25, wherein the modified immune cell exhibits increased effector activity relative to effector activity in a modified immune cell of the same type wherein unmodified mRNA encoding the CAR was delivered.
28. A modified immune cell made by the method of any one of claims 1-27.
29. The modified immune cell of claim 28, wherein the modified immune cell exhibits increased viability relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
30. The modified immune cell of claim 28 or claim 29, wherein the modified immune cell exhibits increased expression of the mRNA encoding the CAR relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
31. The modified immune cell of any one of claims 28-30, wherein the modified immune cell exhibits increased CAR expression relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
32. The modified immune cell of any one of claims 28-31, wherein the modified immune cell exhibits increased longevity of the mRNA encoding the CAR relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
33. The modified immune cell of any one of claims 28-32, wherein the modified immune cell exhibits increased longevity of the CAR relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
34. The modified immune cell of any one of claims 28-33, wherein the modified immune cell exhibits increased effector activity relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.

35. The modified immune cell of any one of claims 28-34, wherein the modified immune cell exhibits increased M1 polarization relative to a modified immune cell of the same type comprising unmodified mRNA encoding the CAR.
36. A composition comprising:
one or more modified mRNA, wherein the one or more modified mRNA comprise a modified nucleotide, an alteration to the 5' or 3' untranslated region (UTR), a cap structure, a poly A tail, or a combination thereof, and
one or more RNaseL inhibitors.
37. The composition of claim 36, wherein the cap structure comprises AGCap1 or m6AGCap1.
38. The method of claim 36 or claim 37, wherein the modified nucleotide comprises pseudouridine (PsU), 5-methoxyuridine (5moU), 5-methylcytidine/pseudouridine (5meC PsU), or N1-methyl-pseudouridine (N1mPsU).
39. The method of any one of claims 36-38, wherein the one or more RNaseL inhibitors comprise sunitinib.
40. The method of any one of claims 36-38, wherein the one or more RNaseL inhibitors comprise ABCE1.