CLAIMS

1. A pharmaceutical composition comprising an RNA-based expression system, further comprising a buffer, an amino acid, and a cryoprotectant.

2. The pharmaceutical composition of claim 1, wherein the RNA-based expression system is selected from the group consisting of a messenger RNA (mRNA)-based expression system, a circular (circRNA)-based expression system, single guide RNA (sgRNA)-based expression system, and a self-amplifying RNA (samRNA) expression system.

3. The pharmaceutical composition of claim 1, wherein the RNA-based expression system is a self-amplifying RNA (samRNA) expression system.

4. The pharmaceutical composition of any of claims 1-2, wherein the amino acid is selected from histidine, lysine, arginine, glutamine, and arginine or a pharmaceutically acceptable salt thereof.

5. The pharmaceutical composition of claim 6, wherein the amino acid is arginine.

6. The pharmaceutical composition of any of claims 1-5, wherein the amino acid has a concentration of 10-140 mM.

7. The pharmaceutical composition of any of claims 1-5, wherein the amino acid has a concentration of 25-125 mM.

8. The pharmaceutical composition of any of claims 1-5, wherein the amino acid has a concentration of 50-100 mM.

9. The pharmaceutical composition of any of claims 1-5, wherein the amino acid has a concentration of 70-80 mM.

10. The pharmaceutical composition of any of claims 1-5, wherein the amino acid has a concentration of about 75 mM.

11. The pharmaceutical composition of claims 1-10, wherein the composition has a pH of 6.5-9.1.

12. The pharmaceutical composition of claim 11, wherein the pH is 7.3-8.3.

13. The pharmaceutical composition of claim 11, wherein the pH is about 7.8.

14. The pharmaceutical composition of any of claims 1-13, wherein the buffer is selected from the group consisting of a buffer is selected from the group consisting of citrate, succinate, malate, phosphate, histidine, glycine, MOPS, HEPES, Tris, and Bis-Tris.

15. The pharmaceutical composition of claim 14, wherein the buffer is Tris.

16. The pharmaceutical composition of claim 14, wherein the buffer is not a phosphate buffer.

17. The pharmaceutical composition of any of claims 1-15, wherein the buffer has a concentration of 1-15 mM.

18. The pharmaceutical composition of any of claims 1-15, wherein the buffer has a concentration of 3-12 mM.

19. The pharmaceutical composition of any of claims 1-15, wherein the buffer has a concentration of 6-10 mM.

20. The pharmaceutical composition of any of claims 1-15, wherein the buffer has a concentration of about 8.0 mM.

21. The pharmaceutical composition of any of claims 1-15, wherein the buffer has a concentration greater than 5.0 mM

22. The pharmaceutical composition of any of claims 1-20, wherein the pharmaceutical composition comprises a cryoprotectant.

23. The pharmaceutical composition of claim 22, wherein the cryoprotectant is selected from the group consisting of ethanol, sucrose, maltose, lactose, glucose, galactose, trehalose, raffinose, other polyols and polyhydric alcohols.

24. The pharmaceutical composition of any of claims 22-23, wherein the cryoprotectant is 6-18 wt% of the pharmaceutical composition.

25. The pharmaceutical composition of any of claims 22-23, wherein the cryoprotectant is 11-15 wt% of the pharmaceutical composition.

26. The pharmaceutical composition of any of claims 22-23, wherein the cryoprotectant is about 13 wt% of the pharmaceutical composition.

27. The pharmaceutical composition of claims 1-26, wherein the cryoprotectant is sucrose.

28. A pharmaceutical composition comprising a samRNA-based expression system, and further comprising

    70-80 mM arginine;

    11-15 wt% Sucrose; and

    6-10 mM Tris;

    wherein the pharmaceutical composition has a pH of 7.6-8.0.

29. A pharmaceutical composition comprising a samRNA-based expression system, and further comprising

    about 75 mM arginine;

    about 13 wt% Sucrose; and

    about 8 mM Tris;

    wherein the pharmaceutical composition has a pH of about 7.8.

30. The pharmaceutical composition of any of claims 1-29, wherein stability of the pharmaceutical composition remains at a temperature of at least -20 ºC, at least 5 ºC, or at least 25 ºC.

31. The pharmaceutical composition of claim 30, wherein the stability is assessed by one or more assays comprising particle size, PDI, samRNA concentration, percent encapsulation, Full Length Profile (FLP) of samRNA and potency.

32. The pharmaceutical composition of claim 31, wherein the potency of the RNA-based expression system of the pharmaceutical composition is above about 40% of potency after storage as compared to potency before storage.

33. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is stored at about -20 ºC.

34. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is stored at about 5 ºC.

35. The pharmaceutical composition of claim 32, wherein the pharmaceutical composition is stored at about 25 ºC.

36. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 1 day.

37. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 3 days.

38. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 5 days.

39. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 1 week.

40. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 2 weeks.

41. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 1 month.

42. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 3 months.

43. The pharmaceutical composition of any of claims 32-35, wherein the pharmaceutical composition is stored for at least 6 months.

44. The pharmaceutical composition of any of claims 1-43, wherein the buffer is not a phosphate buffer.

45. The pharmaceutical composition of any of claims 1-43, wherein the pharmaceutical composition does not comprise a phosphate buffer.

46. The pharmaceutical composition of any of claims 1-45, wherein the pharmaceutical composition is in a liquid formulation.

47. The pharmaceutical composition of any of claims 1-45, wherein the pharmaceutical composition is in a lyophilized formulation.

48. The pharmaceutical composition of any of claim 47, wherein water content of the pharmaceutical composition is <5 wt%.

49. The pharmaceutical composition of any of claims 1-48, wherein the pharmaceutical composition is stored in a container comprising SiO_2.

50. Use of the composition of any of claims 1-48 in the manufacture of a medicament for inducing an immune response in a subject, wherein the composition is to be administered to the subject.

51. The use according to claim 50, wherein the composition is to be administered intramuscularly (IM), intradermally (ID), subcutaneously (SC), or intravenously (IV).

52. The use according to claim 51, wherein the composition is to be administered intramuscularly.

53. The use according to any of claims 50-52, wherein the subject is to be further administered with one or more immune modulators, optionally wherein the immune modulator is to be administered before, concurrently with, or after administration of the composition or pharmaceutical composition.

54. The use according to claim 53, wherein the one or more immune modulators are selected from the group consisting of: an anti-CTLA4 antibody or an antigen-binding fragment thereof, an anti-PD-1 antibody or an antigen-binding fragment thereof, an anti-PD-L1 antibody or an antigen-binding fragment thereof, an anti-4-1BB antibody or an antigen-binding fragment thereof, or an anti-OX-40 antibody or an antigen-binding fragment thereof.

55. The use according to any of claims 53 or 54, wherein the immune modulator is to be administered intravenously (IV), intramuscularly (IM), intradermally (ID), or subcutaneously (SC).

56. The use according to claim 55, wherein the subcutaneous administration is near the site of the composition or pharmaceutical composition administration or in close proximity to one or more vector or composition draining lymph nodes.

57. The use according to any of claims 49-56, wherein the subject is to be further administered with a second vaccine composition.

58. The use according to claim 57, wherein the second vaccine composition is to be administered prior to the administration of the composition of any of claims 1-48.

59. The use according to claim 57, wherein the second vaccine composition is to be administered subsequent to the administration of the composition of any of claims 1-48.

60. The use according to any of claims 57-59, wherein the second vaccine composition is the same as the composition of any of claims 1-48.

61. The use according to any of claims 57-59, wherein the second vaccine composition is different from the composition any of claims 1-48.

62. A method of preparing the pharmaceutical composition of any of claims 1-48, comprising a step of lyophilization.

63. The method of claim 62, wherein the step of lyophilization comprises a secondary drying step.

64. The method of claim 63, wherein conditions for the secondary drying step comprise a shelf temperature of about 10 °C.

65. The method of claim 63, wherein conditions for the secondary drying step comprise a shelf temperature between 5-15 °C.

66. The method of any of claims 62-65, wherein conditions for the secondary drying step comprise a vacuum of about 50 mTorr.

67. The method of any of claims 62-65, wherein conditions for the secondary drying step comprise a vacuum between 25-75 mTorr.

68. The method of claim 63, wherein conditions for the secondary drying step comprise

a) a shelf temperature of about 10 °C;

b) a vacuum of about 50 mTorr;

c) a ramp rate of about 0.1 °C/min; and

d) a duration of about 1040 min.

69. The method of any of claims 63-68, wherein the step of lyophilization comprises a primary drying step.

70. The method of claim 69, wherein conditions for the primary drying step comprise a shelf temperature of about -37 °C.

71. The method of claim 69, wherein conditions for the primary drying step comprise a shelf temperature between -62 to -12 °C.

72. The method of any of claims 69-71, wherein conditions for the primary drying step comprise a vacuum of about 50 mTorr.

73. The method of any of claims 69-71, wherein conditions for the primary drying step comprise a vacuum between 25-75 mTorr.

74. The method of any of claims 63-73, wherein the step of lyophilization comprises a freezing step.

75. The method of claim 74, wherein conditions for the freezing step comprise a shelf temperature of about -55 °C.

76. The method of claim 74, wherein conditions for the freezing step comprise a shelf temperature between -60 to -35 °C.

77. The method of claim 69, wherein conditions for the primary drying step comprise

a) a shelf temperature of about -37 °C;

b) a vacuum of about 50 mTorr;

c) a ramp rate of about 0.5 °C/min; and

d) a duration of about 4800 min.

78. A composition prepared by the method of any of claims 63-77.

79. The composition of any of claims 1-48, for use in therapy.