during long term storage in a liquid state at temperatures ranging from just above freezing to ambient temperatures would be operationally advantageous as well as greatly increase convenience and flexibility for the health care provider and patient. The invention fulfills this need by providing such compositions.

SUMMARY OF THE INVENTION

[0007] In one aspect, the present invention refers to a live virus composition comprising a herpes simplex virus, a protein, at least one sugar, sodium chloride and sodium phosphate at pH 7-8. In another aspect, the present invention refers to a live virus composition comprising a herpes simplex virus, a protein, at least one sugar, and phosphate buffer at pH 7-8. In still another e aspect, the present invention refers to a live virus composition comprising: a herpes simplex 1 virus; partially hydrolyzed gelatin at a concentration of 3% (w/v); sorbitol at a concentration of 3% (w/v); sodium chloride at a concentration of 50 to 250 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a frozen live virus composition comprising: a herpes simplex 1 virus; partially hydrolyzed gelatin at a concentration of 3% (w/v); sorbitol at a concentration of 3% (w/v); sodium chloride at a concentration of 50 to 250 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); sorbitol at a concentration of 3% (w/v); sodium chloride at a concentration of 50 to 250 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a frozen live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); sorbitol at a concentration of 3% (w/v); sodium chloride at a concentration of 50 to 250 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a frozen live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); sorbitol at a concentration of 3% (w/v); sodium chloride at a concentration of 50 to 250 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a frozen live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); trehalose at a concentration of 15% (w/v); sodium chloride at a concentration of 137 mM; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to a live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); sorbitol at a concentration of 0.5% to 3% (w/v); myo-inositol at a concentration of 4% (w/v); sodium chloride; and wherein said composition is at pH 7.4. In another aspect, the present invention

refers to a frozen live virus composition comprising: a herpes simplex 1 virus; human serum albumin at a concentration of 0.25% to 2% (w/v); sorbitol at a concentration of 0.5% to 3% (w/v); myo-inositol at a concentration of 4% (w/v); sodium chloride; and wherein said composition is at pH 7.4. In another aspect, the present invention refers to the use of a composition according to the present invention in the manufacture of a medicament for killing tumor cells. In another aspect, the present invention refers to a composition according to the present invention for use in therapy. In one example, disclosed herein is a live virus composition comprising a herpes simplex virus, a protein, at least one sugar, sodium chloride and sodium phosphate at pH 7.4, wherein the composition is frozen.

[0008] In one example the composition may be thawed and stored at 2°C to at least 25°C. In a related example following thawing at 2°C to at least 25°C, the live virus composition is frozen again and stored at a temperature of at least -30°C.

[0009] In another example the composition may be thawed and stored at 2°C to 8°C. In a related example following thawing at 2°C to 8°C, the live virus composition is frozen again and stored at a temperature of at least -30°C.

[0010] In another example the protein is partially hydrolyzed gelatin or human serum albumin.

[0011] In another example the concentration of partially hydrolyzed gelatin is from 0.01% to 1% (w/v).

[0012] In another example the concentration of human serum albumin is from 0.25% to 1%.

[0013] In another example at least one sugar is sorbitol, myo-inositol or sucrose. In a related example the concentration of sorbitol is 2% (w/v). In a related example the concentration of myo-inositol is 4% (w/v). In a related example the concentration of sucrose is 9% (w/v) to 15% (w/v).

[0014] In another example the concentration of sodium chloride is 145 mM.

[0015] In another example the concentration of sodium chloride is about 145 mM.

[0016] In another example the concentration of sodium phosphate is 100 mM.

[0017] In another example the concentration of sodium phosphate is about 100 mM.

[0018] In another example the concentration of sodium phosphate is 102 mM.

[0019] In another example the concentration of sodium phosphate is about 102 mM.

[0020] In another example the partially hydrolyzed gelatin is porcine.

[0021] In another example the virus is a herpes simplex virus 1.

[0022] In another example the herpes simplex virus is a clinical isolate.

[0023] In another example the herpes simplex virus is a clinical isolate from a recurrent cold sore.

[0024] In another example the herpes simplex virus 1 strain is selected from the group consisting of strain JS1, strain 17+, strain F, and strain KOS.

[0025] In another example the herpes simples lacks one or more functional genes. In a related example the herpes simplex virus lacks a functional ICP34.5-encoding gene. In a related example the herpes simplex virus lacks a functional ICP47-encoding gene. In a related example the herpes simplex virus further lacks a functional ICP6–encoding gene, a functional glycoprotein H-encoding gene or a functional thymidine kinase-encoding gene. In a related example the herpes simplex virus lacks a functional vhs–encoding gene. In another related example the herpes simplex virus lacks a functional UL43-encoding gene. In a related example the herpes simplex virus lacks a functional VMW-encoding gene, a functional ICPO-encoding gene, a functional ICP4-encoding gene, a functional ICP22-encoding gene, or a functional ICP27-encoding gene. In a related example a modification to the herpes simplex virus has been made such that the Us11 gene is expressed as an early gene. In a related example the herpes simplex virus comprises one or more heterologous genes and/or viral genes. In a related example the heterologous gene and/or viral gene is selected from the group consisting of a gene encoding a cytotoxin, an immunomodulatory protein, a tumor antigen, prodrug activator, a tumor suppressor, a prodrug converting enzyme, proteins capable of causing cell to cell fusion, a TAP inhibitor, viral protein Us11, antisense RNA molecule, or a ribozyme. In another related example the heterologous gene and/or viral gene is selected from the group consisting of a gene encoding IL-12, granulocyte macrophage colony stimulating factor (GM-CSF), cytosine deaminase, gibbon ape leukaemia fusogenic glycoprotein, bovine herpesvirus (BHV) UL49.5 polypeptide or viral protein Us11.

[0026] In another example the herpes simplex virus is selected from the group consisting of talimogene laherparepvec, Seprehvir™ (HSV-1716), G207, OrienX010, NV1020, M032, ImmunoVEX, NSC-733972, HF-10, BV-2711, JX-594, Myb34.5, AE-618, Brainwel™, Heapwel™, and OncoVEX$^{GALV/CD}$.

[0027] In another example the method for killing tumor cells in a patient comprising administering to a subject in need thereof a live virus composition described above under conditions effective to kill tumor cells in the patient. In a related example the live virus composition is administered in combination with a check point inhibitor. In a related example the live virus composition is administered prior to, simultaneously with or following the checkpoint inhibitor. In a related example the tumor cells are selected from the

group consisting of astrocytoma, oligodendroglioma, meningioma, neurofibroma,glioblastoma, ependymoma, Schwannoma, neurofibrosarcoma, medulloblastoma, melanoma cells, pancreatic cancer cells, prostate carcinoma cells, breast cancer cells, lung cancer cells, colon cancer cells, hepatoma cells, mesothelioma, bladder cancer cells, and epidermoid carcinoma cells. In a related example the patient is a human. In a related example the administration is carried out by injection.

[0028] In another example infectivity is increased compared to the same live virus composition lacking a protein.

FIGURES

[0029] Fig. 1. Effect of buffer and salt content on freeze/thaw stability. Solid diamond, solid line: 10 mM Naphos, Open circle, dashed line, 10mM Kphos, Solid circle, solid line: 100 mM Kphos; Solid square, solid line: 73 mM NaCl; Open square, dashed line: 0 mM NaCl, and Solid diamond, dashed line: control.

[0030] Fig. 2A. Effect of sugar concentration on freeze/thaw stability. Solid square, solid line: 9% sorbitol, Open square, dashed line: 15% sorbitol, and Solid diamond, dashed line: control.

[0031] Fig 2B. Effect of sugar concentration on freeze/thaw stability. Solid square, solid line, 9% sorbitol, Solid triangle, solid line: 15% sucrose, Open square, dashed line: 9% trehalose, and Open triangle, dashed line: 15% trehalose.

[0032] Fig. 3 Effect of protein/sugar combinations on stability during freeze/thaw. Solid square, solid line: 9% sucrose, 2% anti-streptavidin mAb, Open square, dashed line: 9% sucrose, 2% phGelatin, Solid circle, solid line: 4% rHSA, Open circle, dashed line: 4% phGelatin, and Solid diamond, dashed line: control.

[0033] Fig. 4A Effect of sugar and protein excipients on liquid stability at 2-8°C. Solid square, solid line: 2% rHSA, Open square, dashed line: 2% phGelatin; Solid circle, solid line: 15% trehalose, Open circle, dashed line: 15% sucrose, Solid triangle, solid line: 9% sucrose, 2% rHSA, and Solid diamond, dashed line: control.

[0034] Fig. 4B Effect of sugar and protein excipients on liquid stability at 25°C. Solid square, solid line: 2% rHSA, Open square, dashed line: 2% phGelatin, Open circle, dashed line: 15% sucrose, Solid triangle, solid line: 9% sucrose and 2% rHSA, and Solid diamond, dashed line: control.

[0035] Fig 5 The effect of rHSA and phGelatin concentration on stability during freeze/thaw cycles. Solid square, solid line: 1% rHSA, Solid triangle, solid line: 2% rHSA, Solid

Claims

1. A live virus composition comprising a herpes simplex virus, a protein, at least one sugar, sodium chloride and sodium phosphate at pH 7-8.

2. The live virus composition according to claim 1, wherein the composition is frozen.

3. The live virus composition according to claim 1 or 2, wherein the composition is at pH 7.4.

4. A live virus composition comprising a herpes simplex virus, a protein, at least one sugar, and phosphate buffer at pH 7-8.

5. The live virus composition of claim 4, wherein the phosphate buffer comprises potassium phosphate.

6. The live virus composition according to claim 4 or 5, wherein the composition is frozen.

7. The live virus composition according to any one of claims 4-6, wherein the composition is at pH 7.4.

8. The live virus composition according to any one of claims 1-7, wherein said herpes simplex virus is a herpes simplex 1 virus.

9. The live virus composition according to any one of claims 1-7, wherein the herpes simplex virus is selected from the group consisting of talimogene laherparepvec, Seprehvir™ , G207, OrienX010, NV1020, M032, ImmunoVEX and OncoVEX$^{GALV/CD}$.

10. The live virus composition according to any one of claims 1-7, wherein the herpes simplex virus is talimogene laherparepvec.

11. The live virus composition of any one of claims 1-10, wherein the composition may be thawed and stored at 2°C to at least 25°C.

12. The live virus of claim 11, wherein following thawing, the live virus composition is frozen again and stored at a temperature of at least -30°C.

13. The live virus composition of any one of claims 1-10, wherein the composition may be thawed and stored at 2°C to 8°C.

14. The live virus of claim 13, wherein following thawing, the live virus composition is frozen again and stored at a temperature of at least -30°C.

15. The live virus composition according to any one of claims 1-10, wherein the protein is partially hydrolyzed gelatin or human serum albumin.

16. The live virus composition according to claim 15, wherein said composition comprises partially hydrolyzed gelatin at a concentration of 0.1% to 3% (w/v).17. The live virus composition according to claim 15, wherein said composition comprises partially hydrolyzed gelatin at a concentration of 0.01% to 1% (w/v).

18. The live virus composition according to claim 15, wherein said composition comprises partially hydrolyzed gelatin at a concentration of 3% (w/v).

19. The live virus composition according to claim 15, wherein said composition comprises human serum albumin at a concentration of 0.25% to 2.5%.

20. The live virus composition according to claim 15, wherein said composition comprises human serum albumin at a concentration of 0.25% to 2% (w/v).

21. The live virus composition according to claim 15, wherein said composition comprises human serum albumin at a concentration of 0.25% to 1% (w/v).

22. The live virus composition according to any one of claim 1-10, wherein the at least one sugar is sorbitol, myo-inositol, trehalose or sucrose.

23. The live virus composition according to claim 22, wherein said composition comprises sorbitol at a concentration of 0.5% to 4% (w/v).

24. The live virus composition according to claim 22, wherein said composition comprises sorbitol at a concentration of 0.5% to 3% (w/v).

25. The live virus composition according to claim 22, wherein said composition comprises sorbitol at a concentration of 3% (w/v).

26. The live virus composition according to claim 22, wherein said composition comprises sorbitol at a concentration of 2% (w/v).

27. The live virus composition according to claim 22, wherein said composition comprises myo-inositol at a concentration of 4% (w/v).

28. The live virus composition according to claim 22, wherein said composition comprises trehalose at a concentration of 9% or 15% (w/v).

29. The live virus composition according to claim 22, wherein said composition comprises trehalose at a concentration of 15% (w/v).

30. The live virus composition according to claim 22, wherein said composition comprises sucrose at a concentration of 9% (w/v) to 15% (w/v).

31. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium chloride at a concentration of 130 to 160 mM.

32. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium chloride at a concentration of 50 to 250 mM.

33. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium chloride at a concentration of 137 mM.

34. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium chloride at a concentration of 145 mM.

35. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium phosphate at a concentration of 10 to 300 mM.

36. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium phosphate at a concentration of 70 to 130 mM.

37. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium phosphate at a concentration of 80 to 120 mM.

38. The live virus composition according to any one of claims 1-10, wherein said composition comprises sodium phosphate at a concentration of 102 mM.

39. The live virus composition according to any one of claims 15-18, wherein the partially hydrolyzed gelatin is porcine.

40. A live virus composition comprising:
a herpes simplex 1 virus;
partially hydrolyzed gelatin at a concentration of 3% (w/v);
sorbitol at a concentration of 3% (w/v);
sodium chloride at a concentration of 50 to 250 mM; and
wherein said composition is at pH 7.4.

41. A frozen live virus composition comprising:
a herpes simplex 1 virus;
partially hydrolyzed gelatin at a concentration of 3% (w/v);
sorbitol at a concentration of 3% (w/v);
sodium chloride at a concentration of 50 to 250 mM; and
wherein said composition is at pH 7.4.

42. A live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
sorbitol at a concentration of 3% (w/v);
sodium chloride at a concentration of 50 to 250 mM; and
wherein said composition is at pH 7.4.

43. A frozen live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
sorbitol at a concentration of 3% (w/v);
sodium chloride at a concentration of 50 to 250 mM; and
wherein said composition is at pH 7.4.

44. A live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
trehalose at a concentration of 15% (w/v);
sodium chloride at a concentration of 137 mM; and
wherein said composition is at pH 7.4.

45. A frozen live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
trehalose at a concentration of 15% (w/v);
sodium chloride at a concentration of 137 mM; and
wherein said composition is at pH 7.4.

46. A live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
sorbitol at a concentration of 0.5% to 3% (w/v);
myo-inositol at a concentration of 4% (w/v);
sodium chloride; and
wherein said composition is at pH 7.4.

47. A frozen live virus composition comprising:
a herpes simplex 1 virus;
human serum albumin at a concentration of 0.25% to 2% (w/v);
sorbitol at a concentration of 0.5% to 3% (w/v);
myo-inositol at a concentration of 4% (w/v);
sodium chloride; and
wherein said composition is at pH 7.4.

48. The live virus composition according to any one of claims 40-47, wherein the herpes simplex virus is talimogene laherparepvec.

48. Use of a composition according to any one of claims 1-48 in the manufacture of a medicamentfor killing tumor cells.

49. The use according to claim 48, wherein said composition further comprises a check point inhibitor.

50. The use according to claim 48 or 49, wherein said tumor cells are astrocytoma, oligodendroglioma, meningioma, neurofibroma,glioblastoma, ependymoma, Schwannoma, neurofibrosarcoma, medulloblastoma, melanoma cells, pancreatic cancer cells, prostate carcinoma cells, breast cancer cells, lung cancer cells, colon cancer cells, hepatoma cells, mesothelioma, bladder cancer cells, or epidermoid carcinoma cells.

51. A composition according to any one of claims 1-48 for use in therapy.