Claims

1. Nucleic acid sequence comprising or coding in 5' to 3'-direction for

i) - a coding region, encoding at least one peptide or protein;
- at least one histone stem-loop, and
- a poly(A) sequence;

or

ii) - a coding region, encoding at least one peptide or protein;
- a poly(A) sequence; and
- at least one histone stem-loop;

wherein the at least one histone stem-loop in i) or ii) is selected from following formulae (I) or (II):

formula (I) (stem-loop sequence without stem bordering elements):

$$[N_{0-2}GN_{3-5}]\ [N_{0-4}(U/T)N_{0-4}]\ [N_{3-5}CN_{0-2}]$$

stem1 loop stem2

formula (II) (stem-loop sequence with stem bordering elements):

$$N_{1-6}\ [N_{0-2}GN_{3-5}]\ [N_{0-4}(U/T)N_{0-4}]\ [N_{3-5}CN_{0-2}]\ N_{1-6}$$

stem1 stem1 loop stem2 stem2

bordering element bordering element

wherein:

stem1 or stem2 bordering elements N_{1-6}	is a consecutive sequence of 1 to 6, preferably of 2 to 6, more preferably of 2 to 5, even more preferably of 3 to 5, most preferably of 4 to 5 or 5 N, wherein each N is

independently from another selected from a nucleotide selected from A, U, T, G and C, or a nucleotide analogue thereof;

stem1 $[N_{0-2}GN_{3-5}]$ is reverse complementary or partially reverse complementary with element stem2, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof, and

wherein G is guanosine or an analogue thereof, and may be optionally replaced by a cytidine or an analogue thereof, provided that its complementary nucleotide cytidine in stem2 is replaced by guanosine;

loop sequence $[N_{0-4}(U/T)N_{0-4}]$ is located between elements stem1 and stem2, and is a consecutive sequence of 3 to 5 nucleotides, more preferably of 4 nucleotides;

wherein each N_{0-4} is independent from another a consecutive sequence of 0 to 4, preferably of 1 to 3, more preferably of 1 to 2 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein U/T represents uridine, or optionally thymidine;

stem2 $[N_{3-5}CN_{0-2}]$ is reverse complementary or partially reverse complementary with element stem1, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein C is cytidine or an analogue thereof, and may be optionally replaced by a guanosine or an analogue thereof provided that its complementary nucleotide guanosine in stem1 is replaced by cytidine;

wherein

stem1 and stem2 are capable of base pairing with each other

forming a reverse complementary sequence, wherein base pairing may occur between stem1 and stem2, or

forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2;

wherein the at least one histone stem-loop binds to stem-loop binding protein (SLBP); and

wherein said peptide or protein comprises a tumour antigen,

or

a fragment of said tumour antigen, wherein the fragment has a length of at least six amino acid residues, and

wherein the tumour antigen is selected from the list of:

5T4, 707-AP, 9D7, AFP, AlbZIP HPG1, alpha-5-beta-1-integrin, alpha-5-beta-6-integrin, alpha-actinin-4/m, alpha-methylacyl-coenzyme A racemase, ART-4, ARTC1/m, B7H4, BAGE-1, BCL-2, bcr/abl, beta-catenin/m, BING-4, BRCA1/m, BRCA2/m, CA 15-3/CA 27-29, CA 19-9, CA72-4, CA125, calreticulin, CAMEL, CASP-8/m, cathepsin B, cathepsin L, CD19, CD20, CD22, CD25, CDE30, CD33, CD4, CD52, CD55, CD56, CD80, CDC27/m, CDK4/m, CDKN2A/m, CEA, CLCA2, CML28, CML66, COA-1/m, coactosin-like protein, collage XXIII, COX-2, CT-9/BRD6, Cten, cyclin B1, cyclin D1, cyp-B, CYPB1, DAM-10, DAM-6, DEK-CAN, EFTUD2/m, EGFR, ELF2/m, EMMPRIN, EpCam, EphA2, EphA3, ErbB3, ETV6-AML1, EZH2, FGF-5, FN, Frau-1, G250, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE7b, GAGE-8, GDEP, GnT-V, gp100, GPC3, GPNMB/m, HAGE, HAST-2, hepsin, Her2/neu, HERV-K-MEL, HLA-A*0201-R17I, HLA-A11/m, HLA-A2/m, HNE, homeobox NKX3.1, HOM-TES-14/SCP-1, HOM-TES-85, HPV-E6, HPV-E7, HSP70-2M, HST-2, hTERT, iCE, IGF-1R, IL-13Ra2, IL-2R, IL-5, immature laminin receptor, kallikrein-2, kallikrein-4, Ki67, KIAA0205, KIAA0205/m, KK-LC-1, K-Ras/m, LAGE-A1, LDLR-FUT, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-A10, MAGE-A12, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E2, MAGE-F1, MAGE-H1, MAGEL2, mammaglobin A, MART-1/melan-A, MART-2, MART-2/m, matrix protein 22, MC1R, M-CSF, ME1/m, mesothelin, MG50/PXDN, MMP11, MN/CA IX-antigen, MRP-3, MUC-1, MUC-2, MUM-1/m, MUM-2/m, MUM-3/m, myosin class I/m, NA88-A, N-acetylglucosaminyltransferase-V, Neo-PAP, Neo-PAP/m, NFYC/m, NGEP, NMP22, NPM/ALK, N-Ras/m, NSE, NY-ESO-B, OA1, OFA-iLRP, OGT, OGT/m, OS-9, OS-9/m, osteocalcin, osteopontin, p15, p190 minor bcr-abl, p53, p53/m, PAGE-4, PAI-1, PAI-2, PAP, PART-1, PATE, PDEF, Pim-1-Kinase, Pin-1, Pml/PARalpha, POTE, PRAME, PRDX5/m, prostein, proteinase-3, PSA, PSCA, PSGR, PSM, PSMA, PTPRK/m, RAGE-1, RBAF600/m, RHAMM/CD168, RU1, RU2, S-100, SAGE, SART-1, SART-2, SART-3, SCC, SIRT2/m, Sp17, SSX-1, SSX-2/HOM-MEL-40, SSX-4, STAMP-1, STEAP-1, survivin, survivin-2B, SYT-SSX-1, SYT-SSX-2, TA-90, TAG-72, TARP, TEL-AML1, TGFbeta, TGFbetaRII, TGM-4, TPI/m, TRAG-3, TRG, TRP-1, TRP-2/6b, TRP/INT2, TRP-p8, tyrosinase, UPA, VEGFR1, VEGFR-2/FLK-1, WT1 and a immunoglobulin idiotype of a lymphoid blood cell or a T cell receptor idiotype of a lymphoid blood cell.

2. The nucleic acid sequence according to claim 1, wherein the histone stem loop is a metazoan histone stem loop sequence.

3. The nucleic acid sequence according to claim 1 or 2, wherein the tumour antigen is selected from survivin or an antigen from the MAGE-family.

4. The nucleic acid sequence according to any of claims 1 to 3, wherein the at least one histone stem loop is heterologous to the coding region encoding the at least one peptide or protein, preferably, wherein the coding region does not encode a histone protein or fragment thereof having histone or histone-like function, wherein the fragment has a length of at least six amino acid residues.

5. The nucleic acid sequence of any of claims 1 to 4, wherein its coding region does not encode a reporter protein or a marker or selection protein, wherein the reporter protein, marker or selection protein is not a tumour antigen.

6. The nucleic acid sequence according to any of claims 1 to 5, wherein the nucleic acid does not contain one or two or at least one or all but one or all of the components of the group consisting of: a sequence encoding a ribozyme (preferably a self-splicing ribozyme), a histone stem-loop processing signal, in particular a histone-stem loop processing sequence derived from mouse histone H2A614 gene and a Neo gene.

7. The nucleic acid sequence according to any of claims 1 to 5, wherein the nucleic acid does not contain a mouse histone stem-loop sequence.

8. Nucleic acid sequence according to any of claims 1 to 7, wherein the nucleic acid is an RNA, preferably an mRNA.

9. The nucleic acid sequence according to any of claims 1 to 8, wherein the at least one histone stem-loop is selected from at least one of following formulae (Ia) or (IIa):

$$\underbrace{[N_{0-1}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{1-3}(U/T)N_{0-2}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-1}]}_{\text{stem2}}$$

formula (Ia) (stem-loop sequence without stem bordering elements)

$$\underbrace{N_{2\text{-}5}}_{\text{stem1}}\ \underbrace{[N_{0\text{-}1}GN_{3\text{-}5}]}_{\text{stem1}}\ \underbrace{[N_{1\text{-}3}(U/T)N_{0\text{-}2}]}_{\text{loop}}\ \underbrace{[N_{3\text{-}5}CN_{0\text{-}1}]}_{\text{stem2}}\ \underbrace{N_{2\text{-}5}}_{\text{stem2}}$$

bordering element — bordering element

formula (IIa) (stem-loop sequence with stem bordering elements)

10. The nucleic acid sequence according to any of claims 1 to 9, wherein the poly(A) sequence comprises a sequence of about 25 to about 400 adenosine nucleotides, preferably a sequence of about 50 to about 400 adenosine nucleotides, more preferably a sequence of about 50 to about 300 adenosine nucleotides, even more preferably a sequence of about 50 to about 250 adenosine nucleotides, most preferably a sequence of about 60 to about 250 adenosine nucleotides.

11. Nucleic acid sequence according to any of claims 1 to 10, wherein the nucleic acid sequence is a modified nucleic acid, in particular a stabilized nucleic acid.

12. A composition comprising at least one type of nucleic acid sequences according to any of claims 1 to 11.

13. The composition according to claim 12, wherein the composition comprises at least two types of nucleic acid sequences wherein each type of nucleic acid sequence encodes for a different peptide or protein, preferably for a different tumour antigen.

14. The composition according to claim 12 or 13, wherein one type of the contained nucleic acid sequences encodes for PSA, PSMA, PSCA, STEAP-1, NY-ESO-1, 5T4, Survivin, MAGE-C1, or MAGE-C2.

15. The composition according to any of claims 12 to 14, wherein the nucleic acid sequence does not encode for NY-ESO1, provided that the composition contains only one type of nucleic acid sequence.

16. The composition according to any of claims 12 to 15 comprising at least:

a) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen PSA, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues; and

b) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen PSMA, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues; and

c) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen PSCA, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues; and

d) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen STEAP-1, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues.

17. The composition according to any of claims 12 to 15 comprising at least:

a) a nucleic acid sequence comprising or coding for

i. a coding region, encoding at least one peptide or protein which comprises the tumour antigen NY-ESO-1, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues,

ii. at least one histone stem-loop, and

iii. a poly(A) sequence or a polyadenylation signal;

b) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen 5T4, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues; and

c) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen Survivin, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues.

18. The composition according to any of claims 12 to 15 further comprising at least:

a) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen MAGE-C1, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues; and

b) a nucleic acid sequence of any of claims 1 to 11 wherein said encoded peptide or protein comprises the tumour antigen MAGE-C2, or a fragment thereof, wherein the fragment has a length of at least six amino acid residues.

19. Pharmaceutical composition comprising a nucleic acid sequence as defined according to any of claims 1 to 11, or a composition as defined according to any of claims 12 to 18 and optionally a pharmaceutically acceptable carrier.

20. Use of a nucleic acid sequence as defined according to any of claims 1 to 11, or a composition as defined according to any of claims 12 to 18 for increasing the expression of said encoded peptide or protein in vitro.

21. An in vitro method for increasing the expression of an encoded peptide or protein comprising the steps:

a) providing the nucleic acid sequence as defined according to any of claims 1 to 12 or the composition as defined according to any of claims 12 to 18,

b) applying or administering the nucleic acid sequence or the composition to a cell-free expression system, a cell or a tissue.