Claims

1. Nucleic acid sequence comprising or coding for

a) a coding region, preferably encoding a peptide or protein, provided that the coding region does not code for histone proteins, reporter proteins selected from EGFP and Luciferase and marker or selection proteins selected from alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT),

b) at least one histone stem-loop, and

c) a poly(A) sequence or a polyadenylation signal.

2. Nucleic acid according to claim 1, 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 viral nucleic acid sequence, a histone stem-loop processing signal, in particular a histon-stem loop processing sequence derived from mouse histon H2A614 gene, a Neo gene, an inactivated promoter sequence and an inactivated enhancer sequence.

3. Nucleic acid according to claim 1 or 2, wherein the nucleic acid does not contain a ribozyme, preferably a self-splicing ribozyme, and one of the group consisting of: a Neo gene, an inactivated promotor sequence, an inactivated enhancer sequence, a histon stem-loop processing signal, in particular a histon-stem loop processing sequence derived from mouse histon H2A614 gene.

4. Nucleic acid according to claim 1 to 3, wherein the nucleic acid is an RNA, preferably an mRNA.

5. Nucleic acid according to any of claims 1 to 4, wherein the at least one histone stem-loop is selected from following formulae (I) or (II):

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

$$\underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}$$

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

$$\underbrace{N_{1-6}}_{\text{stem1 bordering element}}\ \underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}\ \underbrace{N_{1-6}}_{\text{stem2 bordering element}}$$

wherein:

stem1 or stem2 bordering element 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.

6. Nucleic acid according to claim 5, 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)

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

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

7. Nucleic acid according to any of claims 1 to 6, 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.

8. Nucleic acid according to any of claims 1 to 7, wherein the polyadenylation signal comprises the consensus sequence NNUANA, preferably AAUAAA or AUUAAA.

9. Nucleic acid according to any of claims 1 to 8, wherein the nucleic acid encodes a therapeutically active protein or peptide, an adjuvant protein, an antigen, a tumour antigen, a pathogenic antigen, an animal antigen, a viral antigen, a protozoal antigen, a bacterial antigen, an allergenic antigen, an autoimmune antigen, an allergen, an antibody, an immunostimulatory protein or peptide, or an antigen-specific T-cell receptor.

10. Nucleic acid according to any of claims 1 to 9, wherein the nucleic acid is a monocistronic, bicistronic or even multicistronic nucleic acid.

11. Use of a nucleic acid as defined according to any of claims 1 to 10 for increasing the expression level of an encoded peptide or protein.

12. Nucleic acid as defined according to any of claims 1 to 10 for use as a medicament.

13. Pharmaceutical composition comprising a nucleic acid as defined according to any of claims 1 to 10 and optionally a pharmaceutically acceptable carrier.

14. Vaccine comprising a nucleic acid according to any of claims 1-10 coding for an antigen or comprising an antigen.

15. Vaccine according to claim 14, wherein the antigen is selected from tumour antigens, allergenic antigens, auto-immune self-antigens, pathogenic antigens, viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens, or allergenic antigens.

16. Use of a nucleic acid as defined according to any of claims 1 to 10 for increasing the expression of a protein in treatment of cancer or tumour diseases, infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, diseases which have a genetic inherited background and which are typically caused by a defined gene defect and are inherited according to Mendel's laws, cardiovascular diseases, neuronal diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, neoplasms, immune deficiencies, endocrine, nutritional and metabolic diseases, eye diseases or ear diseases.

17. *In vitro* or *ex vivo* method for increasing the expression of a protein comprising the steps:

a) providing the nucleic acid as defined according to any of claims 1 to 10,

b) applying or administering the nucleic acid to a cell-free expression system, a cell (e.g. an expression host cell or a somatic cell), a tissue or an organism.

Claims

1. Nucleic acid sequence comprising or coding for

a) a coding region encoding a therapeutically active protein or peptide, an adjuvant protein, an antigen, a tumour antigen, a pathogenic antigen, an animal antigen, a viral antigen, a protozoal antigen, a bacterial antigen, an allergenic antigen, an autoimmune antigen, an allergen, an antibody, an immunostimulatory protein or peptide, or an antigen-specific T-cell receptor; provided that the coding region does not code for histone proteins, reporter proteins selected from EGFP and Luciferase and marker or selection proteins selected from alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase,

b) at least one histone stem-loop, and

c) a poly(A) sequence or a polyadenylation signal,

for use for increasing the expression of said protein encoded by the coding region as defined by (a) in the treatment of cancer diseases, infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, genetic diseases , cardiovascular diseases, neuronal diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, immune deficiencies, endocrine, nutritional and metabolic diseases, eye diseases or ear diseases.

2. Nucleic acid for use according to claim 1 in the treatment of viral, bacterial or protozoological infectious diseases.

3. Nucleic acid for use according to claim 1 or 2, 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 or a self-splicing ribozyme, a viral nucleic acid sequence, a histon stem-loop processing signal, in particular a histon-stem loop processing sequence derived from mouse histon H2A614 gene, a Neo gene, an inactivated promoter sequence and an inactivated enhancer sequence.

4. Nucleic acid for use according to claim 1 to 3, wherein the nucleic acid does not contain a ribozyme, preferably a self-splicing ribozyme, and one of the group consisting of: a Neo gene, an inactivated promotor sequence, an inactivated enhancer sequence, a histone

stem-loop processing signal, in particular a histon-stem loop processing sequence derived from mouse histon H2A614 gene.

5. Nucleic acid for use according to claim 1 to 4, wherein the nucleic acid is an RNA, preferably an mRNA.

6. Nucleic acid for use according to any of claims 1 to 5, wherein the at least one histone stem-loop is selected from following formulae (I) or (II):

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

$$\underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}$$

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

$$\underbrace{N_{1-6}}_{\text{stem1}}\ \underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}\ \underbrace{N_{1-6}}_{\text{stem2}}$$

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.

7. Nucleic acid for use according to claim 6, 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)

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

bordering element bordering element

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

8. Nucleic acid for use according to any of claims 1 to 7, 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.

9. Nucleic acid for use according to any of claims 1 to 7, wherein the polyadenylation signal comprises the consensus sequence NNUANA, preferably AAUAAA or AUUAAA.

10. Nucleic acid for use according to any of claims 1 to 9, wherein the nucleic acid is a monocistronic, bicistronic or even multicistronic nucleic acid.

11. Pharmaceutical composition comprising a nucleic acid as defined according to any of claims 1 to 10 for use for increasing the expression of a protein in the treatment of cancer diseases, infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, genetic diseases, cardiovascular diseases, neuronal diseases, diseases of the respiratory system, diseases of the digestive system, diseases of the skin, musculoskeletal disorders, disorders of the connective tissue, immune deficiencies, endocrine, nutritional and metabolic diseases, eye diseases or ear diseases.

12. Pharmaceutical composition for use according to claim 11 comprising a pharmaceutically acceptable carrier.

13. Pharmaceutical composition for use according to claim 11 or 12 in the treatment of viral, bacterial or protozoological infectious diseases.

14. Vaccine comprising a nucleic acid according to any of claims 1 to 13 coding for an antigen or comprising an antigen for use for increasing the expression of a protein in the treatment of cancer diseases, infectious diseases, allergies or allergic diseases or autoimmune diseases.

15. Vaccine for use according to claim 14 in the treatment of viral, bacterial or protozoological infectious diseases.

16. Vaccine for use according to claim 14 or 15, wherein the antigen is selected from tumour antigens, allergenic antigens, auto-immune self-antigens, pathogenic antigens, viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens, or allergenic antigens.