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VON DER MÜLBE et al.(10) **Pub. No.: US 2010/0239608 A1**(43) **Pub. Date: Sep. 23, 2010**(54) **PHARMACEUTICAL COMPOSITION
CONTAINING A STABILISED MRNA
OPTIMISED FOR TRANSLATION IN ITS
CODING REGIONS**(75) Inventors: **FLORIAN VON DER MÜLBE,**
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A61P 31/22 (2006.01)(52) **U.S. Cl. 424/207.1; 536/23.72; 536/23.5;**
514/44 R; 424/204.1; 514/12; 424/228.1;
424/225.1; 424/227.1; 424/231.1; 424/230.1;
424/218.1; 424/209.1(57) **ABSTRACT**

The present invention relates to a pharmaceutical composi-
tion containing an mRNA that is stabilised by sequence modi-
fications in the translated region and is optimised for the
translation. The pharmaceutical composition according to the
invention is particularly suitable as an inoculating agent as
well as a therapeutic agent for tissue regeneration. In addition
a process is described for determining sequence modifica-
tions that serve for the stabilisation and translation optimisa-
tion of mRNA.

Influenza matrix: wild type gene (for comparison)

agatctaaagatgaggtcttcttaaccgaggtcgaaacgtacgttctctcta
tcatcccgtcaggccccctcaaagccgagatcgacacagagacttgaagat
gtctttgacagggaagaacaccgatcttgaggttctcatggaatggctaaa
gacaagaccaatcctgtcacctctgactaaggggatttttaggatttgtgt
tcacgctcacggtgccagtgagcagaggactgcagcgtagacgctttgtc
caaatgcccttaatgggaacggggatccaaataacatggacaaagcagt
taaactgtataggaagctcaagagggagataacattccatggggccaaag
aatctcactcagttattctgctggtgcacttgccagttgtatgggcctc
atatacaacaggatgggggctgtgaccactgaagtggcatttggcctggt
atgtgcaacctgtgaacagattgctgactcccagcatcggtctcataggc
aatggtgacaacaaccaaccactaatcagacatgagaacagaatgggtt
ttagccagcactacagctaaggctatggagcaaattggctggatcgagtga
gcaagcagcagaggccatggaggttgctagtcaggctaggcaaattgggtgc
aagcgatgagaaccattgggaactcatcctagctccagtgctggtctgaaa
aatgatcttcttgaaaatttgcaggcctatcagaaacgaatgggggtgca
gatgcaacggttcaagtgaactag

Fig. 1A

Influenza matrix: wild type gene (for comparison)

agatctaaag**atg**agtccttctaaccgaggtcgaaacgtacgttctctcta
tcatcccgtcaggccccctcaaagccgagatcgcacagagacttgaagat
gtctttgcaggaagaacaccgatcttgaggttctcatggaatggctaaa
gacaagaccaatcctgtcacctctgactaaggggatttttaggatttgtgt
tcacgctcacctgtcccagtgagcgaggactgcagcgtagacgctttgtc
caaatgcccttaatgggaacggggatccaaataacatggacaaagcagt
taaactgtataggaagctcaagagggagataacattccatggggccaaag
aaatctcactcagttattctgctggtgcacttgccagttgtatgggcctc
atatacaacaggatgggggctgtgaccactgaagtggcattttggcctggt
atgtgcaacctgtgaacagattgctgactcccagcatcgggtctcataggc
aaatggtgacaacaaccaaccactaatcagacatgagaacagaatgggtt
ttagccagcactacagctaaggctatggagcaaattggctggatcgagtga
gcaagcagcagaggccatggaggttgctagtcaggctaggcaaattggtgc
aagcgatgagaaccattgggactcatcctagctccagtgctggtctgaaa
aatgatcttcttgaaaatttgcaggcctatcagaaacgaatgggggtgca
gatgcaacgggttcaag**tga**actag

Fig. 1B

Influenza matrix: protein sequence

MSLLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRP
ILSPLTKGILGFVFTLTVPSEGLQRRRFVQNALNGNGDPNNMDKAVKLY
RKLKREITFHGAKEISLSYSAGALASCMGLIYNRMGAVTTEVAFGLVCAT
CEQIADSQHRSHRQMVTNTNPLIRHENRMVLASTTAKAMEQMAGSSEQAA
EAMEVASQARQMVQAMRTIGTHPSSAGLKNDDLLENLQAYQKRMGVQMQR
FK*

Fig. 1C

Influenza matrix: gene with increased G/C content

agatctaaagatgagCctGctGaccgaggtGgaGacCtacgtGctGAGCa
tcatcccCAGCggccccctGaaGgccgagatcgcCagagGctGgaGgaC
gtGttCgcCggCaagaacaccgaCctGgaggtGctGatggaGtggctGaa
gacCagGccCatcctgAGCccCctgacCaagggCatCCTGggCttCgtgt
tcacCctGaccgtgccagCgagcgCggCctgcagcgCCGCcgcttCgtG
caGaaCgcccctGaaCggCaacggCgaCccCaaCaacatggacaaGgcCgt
GaaGctgttaCaggaagctGaagagggagatCacCttccaCggCgcaaGg
aGatcAGCctGagCtaAGCgcCggCgcCctGgccagCtgCatgggcctG
atCtacaacaggatgggCgcCgtgaccacCgaGgtggcCttCggcctggt
GtgCgcCacctgCgaGcagatCgcCgacAGCagcaCcgCAGCcaCaggc
aGatggtgacCacCaccaacccCctGatcagGcaCgagaacagGatggtG
CTGgccagcacCacCgcCaagggCatggagcaGatggcCggCAGCaGCga
gcaGgcCgcCgagggcatggaggtGgcCagCaggcCaggcaGatggtgc
aGgcCatgagGaccatCggCacCcaCccCagcAGCagCgcCggCctgaaG
aaCgaCctGctGgaGaaCCTGcaggcctaCcagaaGcgCatgggCgtgca
gatgcaGcgCttcaagtgaactagt

Fig. 1D

Influenza matrix: gene for secreted form (with N-terminal
signal sequence) with increased G/C content

AgatctaaagatgCCGTCATGGCCCCCGCACCCCTGGTGCTGCTGCTGA
GCGGCGCCCTGGCCCTGACCCAGACCTGGGCTagCctGctGaccgaggtG
gaGacCtacgtGctGAGCatcatcccCAGCggccccctGaaGgccgagat
cgcCagagGctGgaGgaCgtGttCgcCggCaagaacaccgaCctGgagg
tGctGatggaGtggctGaagacCagGccCatcctgAGCccCctgacCaag
ggCatCCTGggCttCgtgttcacCctGaccgtgccagCgagcgCggCct
gcagcgCCGCcgcttCgtGcaGaaCgcccctGaaCggCaacggCgaCccCa
aCaacatggacaaGgcCgtGaaGctgttaCaggaagctGaagagggagatC
acCttccaCggCgcaaGgaGatcAGCctGagCtaAGCgcCggCgcCct
GgccagCtgCatgggcctGatCtacaacaggatgggCgcCgtgaccacCg
aGgtggcCttCggcctggtGtgCgcCacctgCgaGcagatCgcCgacAGC
cagcaCcgCAGCcaCaggcaGatggtgacCacCaccaacccCctGatcag
GcaCgagaacagGatggtGCTGgccagcacCacCgcCaagggCatggagc
aGatggcCggCAGCaGCgagcaGgcCgcCgagggcatggaggtGgcCagC
caggcCaggcaGatggtgcaGgcCatgagGaccatCggCacCcaCccCag
cAGCagCgcCggCctgaaGaaCgaCctGctGgaGaaCCTGcaggcctaCc
agaaGcgCatgggCgtgcagatgcaGcgCttcaagtgaactagt

Fig. 1E

Influenza matrix: mRNA with stabilisation sequences

```
GCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACUUUGGCagauc
uaaagaugagucucuaaaccgaggucgaaacguacguucucucuaucauc
ccgucaggcccccucaaagccgagaucgcacagagacuugaagaugucuu
ugcaggggaagaacaccgaucuuagguucucauggaauaggcuaaagacaa
gaccaauccugucaccucugacuaaggggaauuuaggauuuguguucacg
cucaccgugcccagugagcgaggacugcagcguagacgcuuuguccaaaa
ugcccuuaaugggaacggggauccaaauaacauggacaaagcaguuaaac
uguauaggaagcucaagagggagauaacaauuccauggggccaaagaaauc
ucacucaguuaucugcugggugcacuugccaguuguauggggccucauaa
caacaggauuggggcugugaccacugaaguggcauuuggccugguaugug
caaccugugaacagauugcugacucccagcaucggucucauaggcaaaug
gugacaacaaccaacccacuaaucagacaugagaacagaaugguuuagc
cagcacuacagcuaaggcuauaggagcaaauggcuggaucgagugagcaag
cagcagaggccauggagguugcuagucaggcuaggcaaauggugcaagcg
augagaaccauuggggacucauccuagcuccagugcuggucugaaaauga
ucuucugaaaaauuugcaggccuaucaagaacgaauugggggugcagaugc
aacgguucaagugaACUAGUGACUGACUAGCCCGCUGGGCCUCCCAACGG
GCCCUCUCCCCUCCUUGCACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

Fig. 1F

Influenza matrix: mRNA with increased G/C content and stabilisation sequences

```
GCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACUUUGGCagauc
uaaagaugagCcuGcuGaccgagguGgaGacCuacguGcuGAGCaucauc
ccCAGCggecccccGaaGgcccagagaucgcCagagGcuGgaGgaCguGuu
CgcCggCaagaacaccgaCcuGgagguGcuGauggaGuggcuGaagacCa
gGccCauccugAGCcccCugacCaagggCauCCUGggCuuCguguucacC
cuGaccgugcccagCgagcgCggCcuGcagcgCCGCcgcuuCguGcaGaa
CgcccGaaCggCaacggCgaCccCaaCaacauggacaaGgcCguGaaGc
uguaCaggaagcuGaagagggagauCacCuuccaCggCgccaGgaGauc
AGCcuGagCuaCAGCgcCggCgcCcuGgccagCugCauggggccuGauCua
caacaggauugggCgcCgugaccacCgaGguggcCuuCggccugguGugCg
cCaccugCgaGcagauCgcCgacAGCcagcaCcgCAGCcaCaggcaGaug
gugacCacCaccaaccccCcuGaucagGcaCgagaacagGaugguGCUGgc
cagcacCacCgcCaagggCauggagcaGaugggCggCAGCaGCgagcaGg
cCgcCgaggccauggagguGgcCagCcaggcCaggcaGauggugcaGgcC
augagGaccuGggCacCcaCccCagcAGCagCgcCggCcuGaaGaaCga
CcuGcuGgaGaaCCUGcaggccuaCcagaaGcgCaugggCgugcagaugc
aGcgCucaagugaACUAGUGACUGACUAGCCCGCUGGGCCUCCCAACGG
GCCCUCUCCCCUCCUUGCACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

[illegible]

Fig. 2A

MAGE1: wild type gene (for comparison)

```
catcatgtctcttgagcagaggagtctgcactgcaagcctgaggaagccc
ttgaggcccaacaagagggccctgggcctggtgtgtgtgcaggtgccacc
tcctcctcctctcctctggtcctgggcacccctggaggaggtgccactgc
tgggtcaacagatcctccccagagtcctcagggagcctccgcctttccca
ctaccatcaacttcactcgacagaggcaacccagtgagggttccagcagc
cgtgaagaggaggggccaagcacctcttgatcctggagtccttggtccg
agcagtaatcactaagaaggtggctgatttggttggttttctgtcctca
aatatcgagccaggaggagccagtcacaaaggcagaaatgctggagagtgtc
atcaaaaaattacaagcactgttttctgagatcttcggcaaagcctctga
gtccttgacagctggtctttggcattgacgtgaaggaagcagacccaccg
gccactcctatgtccttgtcacctgcctaggtctctcctatgatggcctg
ctgggtgataatcagatcatgcccagacaggcttcctgataattgtcct
ggatcatgattgcaatggaggggcgccatgctcctgaggaggaaatctggg
aggagctgagtgtgatggaggtgtatgatgggaggagcacagtgccctat
ggggagcccaggaagctgctcacccaagatttggtgcaggaaaagtacct
ggagtagccggcaggtgccggacagtgatcccgacgctatgagttcctgt
gggggtccaaggggccctcgctgaaaccagctatgtgaaagtccttgagtat
gtgatcaaggctcagtgaagagttcgctttttcttcccatccctgcgtga
agcagctttgagagaggagggaagagggaggtctgagcatga
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Fig. 2B

MAGE1: protein sequence

SER, LEU, GLU, GLN, ARG, SER, LEU, HIS, CYS, LYS, PRO, GLU, GLU, ALA, LEU, GLU, ALA, GLN, GLN, GLU, ALA, LEU, GLY, LEU, VAL, CYS, VAL, GLN, ALA, ALA, THR, SER, SER, SER, SER, PRO, LEU, VAL, LEU, GLY, THR, LEU, GLU, GLU, VAL, PRO, THR, ALA, GLY, SER, THR, ASP, PRO, PRO, GLN, SER, PRO, GLN, GLY, ALA, SER, ALA, PHE, PRO, THR, THR, ILE, ASN, PHE, THR, ARG, GLN, ARG, GLN, PRO, SER, GLU, GLY, SER, SER, SER, ARG, GLU, GLU, GLU, GLY, PRO, SER, THR, SER, CYS, ILE, LEU, GLU, SER, LEU, PHE, ARG, ALA, VAL, ILE, THR, LYS, LYS, VAL, ALA, ASP, LEU, VAL, GLY, PHE, LEU, LEU, LEU, LYS, TYR, ARG, ALA, ARG, GLU, PRO, VAL, THR, LYS, ALA, GLU, MET, LEU, GLU, SER, VAL, ILE, LYS, ASN, TYR, LYS, HIS, CYS, PHE, PRO, GLU, ILE, PHE, GLY, LYS, ALA, SER, GLU, SER, LEU, GLN, LEU, VAL, PHE, GLY, ILE, ASP, VAL, LYS, GLU, ALA, ASP, PRO, THR, GLY, HIS, SER, TYR, VAL, LEU, VAL, THR, CYS, LEU, GLY, LEU, SER, TYR, ASP, GLY, LEU, LEU, GLY, ASP, ASN, GLN, ILE, MET, PRO, LYS, THR, GLY, PHE, LEU, ILE, ILE, VAL, LEU, VAL, MET, ILE, ALA, MET, GLU, GLY, GLY, HIS, ALA, PRO, GLU, GLU, GLU, ILE, TRP, GLU, GLU, LEU, SER, VAL, MET, GLU, VAL, TYR, ASP, GLY, ARG, GLU, HIS, SER, ALA, TYR, GLY, GLU, PRO, ARG, LYS, LEU, LEU, THR, GLN, ASP, LEU, VAL, GLN, GLU, LYS, TYR, LEU, GLU, TYR, ARG, GLN, VAL, PRO, ASP, SER, ASP, PRO, ALA, ARG, TYR, GLU, PHE, LEU, TRP, GLY, PRO, ARG, ALA, LEU, ALA, GLU, THR, SER, TYR, VAL, LYS, VAL, LEU, GLU, TYR, VAL, ILE, LYS, VAL, SER, ALA, ARG, VAL, ARG, PHE, PHE, PHE, PRO, SER, LEU, ARG, GLU, ALA, ALA, LEU, ARG, GLU, GLU, GLU, GLY, VAL, STP - , ALA, STP

Fig. 2C

MAGE1: mRNA with increased G/C content

augagccuggagcagcgcagccugcacugcaagccggaggaggcgcuggaggcgcagcagga
ggcgcuggggccuggucugcguccaggcggcgacgagcagcagcagcccgucgguccugggca
cgcuggaggaggucccgacggcgggcagcacggaccccgccgagagcccgaggggcgcgagc
gcuucccgacgacgaucaacuucacgcgccagcgccagccgagcgcaggggcagcagcagccg
cgaggaggaggggcccgagcacgagcugcauccuggagagccuguuccgcgcggucaucacga
agaaggucgcggaccuggucggcuuccugcugcugaaguaccgcgcgcgcgagccggucacg
aaggcggagaugcuggagagcgucaucaagaacuacaagcacugcuucccgagauucgg
caaggcgagcgcagagccugcagcuggucuuccggcaucgacgucaaggaggcggacccgacgg
gccacagcuacguccuggucacgugccuggggccugagcuacgacggccugcuggggcgacaac
cagaucaugccgaagacgggcuuccugaucaucguccuggucaugaucgcgauggaggggcg
ccacgcgcggaggaggagauucgggaggagcugagcgucauggaggucuaacgacggccgcg
agcacagcgcguacggcgcgagccgcgcaagcugcugacgcaggaccugguccaggagaaguac
cuggaguaccggccaggucccgacagcgaccccgcgcgcuacgaguuccuguggggcccgcg
cgcgucggcgagacgagcuacgucaaggucucggaguacgucaucaaggucagcgcgcgcg
uccgcuucuuucccgagccugcgcgaggcgcgucgcgcgaggaggaggaggggcgucuga
gcgugauga

Fig. 2D

MAGE1: mRNA with alternative codon usage

augagccuggagcagcgcagccugcacugcaagcccgaggaggcccgaggaggccagcagga
ggcccuggggccuggugugcgugcaggcccgccaccagcagcagcagcccccuggugcugggca
cccuggaggaggugcccaaccgcccgcagcacccgacccccccagagccccaggggcgccagc
gccuuccccaccaccaucaacuucacccgccagcgcgccagccagcgcaggggcagcagcagccg
cgaggaggaggggccccagcacccagcugcauccuggagagccuguuccgcgcggugauacca
agaagguggccgaccuggugggcuuccugcugcugaaguaccgcgcggcgagcccgugacc
aaggccgagaugcuggagagcgugaaucaagaacuacaagcacugcuuccccgagauucgg
caaggccagcgcagagccugcagcuggugucggcaucgacgugaaggaggccgacccccaccg
gccacagcuacgugcuggugaccuqccuggggccugagcuacgacggccugcuggggcgacaac
cagaucaugcccaagaccggcuuccugaucaucgugcuggugaugaucgccauggaggggcg
ccacgcccccgaggaggagauucgggaggagcugagcgugauggagguguaacgacggccgcg
agcacagcgccuacggcgagccccgcaagcugcugacccaggaccuggugcaggagaaguac
cuggaguaccggccaggugcccgcagcgaccccgcccgcuaacgaguuccuguggggcccccg
cgcccuggccgagaccagcuacgugaaggugcuggaguacgucaucaaggugagcggccgcg
ugcgcuucuuucccgagccugcgcgaggcccgcccugcgcgaggaggaggaggggcguguga
gccugauga

PHARMACEUTICAL COMPOSITION CONTAINING A STABILISED MRNA OPTIMISED FOR TRANSLATION IN ITS CODING REGIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This Application is a Continuation of application Ser. No. 10/729,830 filed on Dec. 5, 2003, which is a continuation-in-part of PCT Application No. PCT/EP02/06180 filed Jun. 5, 2002, which in turn, claims priority from German Application 10127283.9, filed Jun. 5, 2001. Applicants claim benefit under 35 U.S.C. §120 as to the U.S. application and the PCT application and under 35 U.S.C. §119 to the German application, and the disclosures of all of said applications are incorporated herein by reference.

SUBMISSION OF SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is filed in electronic format via EFS-Web and hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is Sequence_Listing_22122_00009_CON2. The size of the text file is 19 KB, and the text file was created on May 19, 2010.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention relates to a pharmaceutical composition containing an mRNA that is stabilised by sequence modifications in the translated region and is optimised for translation. The pharmaceutical composition according to the invention is suitable in particular as an inoculating agent and also as a therapeutic agent for tissue regeneration. Furthermore a process for determining sequence modifications that stabilise and optimise mRNA translation is disclosed.

[0005] 2. Description of the Prior Art

[0006] Gene therapy and genetic vaccination are tools of molecular medicine whose use in the treatment and prevention of diseases has considerable potential. Both of these approaches are based on the incorporation of nucleic acids into a patient's cells or tissue as well as on the subsequent processing of the information coded by the incorporated nucleic acids, i.e. the expression of the desired polypeptides.

[0007] The conventional procedure involved in previous processes of gene therapy and genetic vaccination is the use of DNA in order to incorporate the required genetic information into the cell. In this connection various processes for the incorporation of DNA into cells have been developed, such as for example calcium phosphate transfection, polybrene transfection, protoplast fusion, electroporation, microinjection and lipofection, in which connection lipofection in particular has proved to be a suitable process.

[0008] A further process that has been suggested in particular in the case of genetic vaccination involves the use of DNA viruses as DNA vehicles. Because such viruses are infectious, a very high transfection rate can be achieved when using DNA viruses as vehicles. The viruses used are genetically altered so that no functional infectious particles are formed in the transfected cell. Despite this precautionary measure, however the risk of uncontrolled propagation of the introduced

therapeutic gene as well as viral genes remains due to the possibility of recombination events.

[0009] Normally DNA incorporated into a cell is integrated to a certain extent into the genome of the transfected cell. On the one hand this phenomenon can exert a desirable effect, since in this way a long-lasting action of the introduced DNA can be achieved. On the other hand the integration into the genome brings with it a significant risk for gene therapy. Such integration events may, for example, involve an insertion of the incorporated DNA into an intact gene, which produces a mutation that interferes with or completely ablates the function of the endogenous gene. As a result of such integration events, enzyme systems that are important for cellular viability may be switched off. Alternatively, there is also the risk of inducing transformation of the transfected cell if the integration site occurs in a gene that is critical for regulating cell growth. Accordingly, when using DNA viruses as therapeutic agents and vaccines, a carcinogenic risk cannot be excluded. In this connection it should also be borne in mind that, in order to achieve effective expression of the genes incorporated into the cell, the corresponding DNA vehicles contain a strong promoter, for example the viral CMV promoter. The integration of such promoters into the genome of the treated cell may, however, lead to undesirable changes in the regulation of the gene expression in the cell.

[0010] A further disadvantage of the use of DNA as a therapeutic agent or vaccine is the induction of pathogenic anti-DNA antibodies in the patient, resulting in a potentially fatal immune response.

[0011] In contrast to DNA, the use of RNA as a therapeutic agent or vaccine is regarded as significantly safer. In particular, use of RNA is not associated with a risk of stable integration into the genome of the transfected cell. In addition, no viral sequences such as promoters are necessary for effective transcription of RNA. Beyond this, RNA is degraded rapidly in vivo. Indeed, the relatively short half-life of RNA in circulating blood, as compared to that of DNA, reduces the risks associating with developing pathogenic anti-RNA antibodies. Indeed, anti-RNA antibodies have not been detected to date. For these reasons RNA may be regarded as the molecule of choice for molecular medicine therapeutic applications.

[0012] However, some basic problems still have to be solved before medical applications based on RNA expression systems can be widely employed. One of the problems in the use of RNA is the reliable, cell-specific and tissue-specific efficient transfer of the nucleic acid. Since RNA is normally found to be very unstable in solution, up to now RNA could not be used or used only very inefficiently as a therapeutic agent or inoculating agent in the conventional applications designed for DNA use.

[0013] Enzymes that break down RNA, so-called RNases (ribonucleases), are responsible in part for the instability. Even minute contamination by ribonucleases is sufficient to degrade down RNA completely in solution. Moreover, the natural decomposition of mRNA in the cytoplasm of cells is exquisitely regulated. Several mechanisms are known which contribute to this regulation. The terminal structure of a functional mRNA, for example, is of decisive importance. The so-called "cap structure" (a modified guanosine nucleotide) is located at the 5' end and a sequence of up to 200 adenosine nucleotides (the so-called poly-A tail) is located at the 3' end. The RNA is recognised as mRNA by virtue of these structures and these structures contribute to the regulatory machinery controlling mRNA regulation. In addition there are further

mechanisms that stabilise or destabilise RNA. Many of these mechanisms are still unknown, although often an interaction between the RNA and proteins appears to be important in this regard. For example, an mRNA surveillance system has been described (Hellerin and Parker, *Annu. Rev. Genet.* 1999, 33: 229 to 260), in which incomplete or nonsense mRNA is recognised by specific feedback protein interactions in the cytosol and is made accessible to decomposition. Exonucleases appear to contribute in large measure to this process.

[0014] Certain measures have been proposed in the prior art in order to improve the stability of RNA and thereby enable its use as a therapeutic agent or RNA vaccine.

[0015] In EP-A-1083232 a process for the incorporation of RNA, in particular mRNA, into cells and organisms has been proposed in order to solve the aforementioned problem of the instability of RNA *ex vivo*. As described therein, the RNA is present in the form of a complex with a cationic peptide or protein.

[0016] WO 99/14346 describes further processes for stabilising mRNA. In particular modifications of the mRNA are proposed that stabilise the mRNA species against decomposition by RNases. Such modifications may involve stabilisation by sequence modifications, in particular reduction of the C content and/or U content by base elimination or base substitution. Alternatively, chemical modifications may be used, in particular the use of nucleotide analogues, as well as 5' and 3' blocking groups, an increased length of the poly-A tail as well as the complexing of the mRNA with stabilising agents, and combinations of the aforementioned measures.

[0017] In U.S. Pat. No. 5,580,859 and U.S. Pat. No. 6,214,804 mRNA vaccines and mRNA therapeutic agents are disclosed *inter alia* within the scope of "transient gene therapy" (TGT). Various measures are described therein for enhancing the translation efficiency and mRNA stability that relate in particular to the composition of the non-translated sequence regions.

[0018] Bieler and Wagner (in: Schleef (Ed.), *Plasmids for Therapy and Vaccination*, Chapter 9, pp. 147 to 168, Wiley-VCH, Weinheim, 2001) report on the use of synthetic genes in combination with gene therapy methods employing DNA vaccines and lentiviral vectors. The construction of a synthetic gag-gene derived from HIV-1 is described, in which the codons have been modified with respect to the wild type sequence (alternative codon usage) in such a way as to correspond to frequently used codons found in highly expressed mammalian genes. In this way, in particular, the A/T content compared to the wild type sequence was reduced. Moreover, the authors found an increased rate of expression of the synthetic gag gene in transfected cells. Furthermore, increased antibody formation against the gag protein was observed in mice immunised with the synthetic DNA construct. An increase in cytokine release *in vitro* in the case of transfected spleen cells of such mice was also observed. Finally, an induction of a cytotoxic immune response in mice immunised with the gag expression plasmid was also found. The authors of this article attribute the improved properties of their DNA vaccine to a change in the nucleocytoplasmic transport of the mRNA expressed by the DNA vaccine, which was due to the optimised codon usage. The authors maintain that the effect of the altered codon usage on the translation efficiency was only slight.

SUMMARY OF THE INVENTION

[0019] The object of the present invention is to provide a new system for gene therapy and genetic vaccination that

overcomes the disadvantages associated with the properties of DNA therapeutic agents and DNA vaccines and that increases the effectiveness of therapeutic agents based on RNA species.

[0020] This object is achieved by the embodiments of the present invention characterised in the claims.

[0021] In particular, a modified mRNA, as well as a pharmaceutical composition containing at least one modified mRNA of the present invention and a pharmaceutically compatible carrier and/or vehicle are provided. The modified mRNA encodes at least one biologically active or antigenic peptide or polypeptide, wherein the sequence of the mRNA comprises at least one modification as set forth herein below as compared to the wild type mRNA. Such modifications may be located in the region coding for the at least one peptide or polypeptide, or in untranslated regions.

[0022] In one aspect, the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is increased relative to that of the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide. The encoded amino acid sequence, however, remains unchanged compared to the wild type (i.e. silent with respect to the encoded amino acid sequence).

[0023] This modification is based on the fact that, for efficient translation of an mRNA, the sequence of the region of the mRNA to be translated is essential. In this connection the composition and the sequence of the various nucleotides play an important role. In particular sequences with an increased G (guanosine)/C (cytosine) content are more stable than sequences with an increased A (adenosine)/U (uracil) content. In accordance with the invention, the codons are varied compared to the wild type mRNA, while maintaining the translated amino acid sequence, so that they contain increased amounts of G/C nucleotides. Since several different codons can encode the same amino acid, due to degeneracy of the genetic code, the codons most favourable for the stability of the modified mRNA can be determined and incorporated (alternative codon usage).

[0024] Depending on the amino acid to be coded by the modified mRNA, various possibilities for modifying the mRNA sequence compared to the wild type sequence are feasible. In the case of amino acids that are encoded by codons that contain exclusively G or C nucleotides, no modification of the codon is necessary. Thus, the codons for Pro (CCC or CCG), Arg (CGC or CGG), Ala (GCC or GCG) and Gly (GGC or GGG) do not require any alteration since no A or U is present.

[0025] In the following cases the codons that contain A and/or U nucleotides are altered by substituting other codons that code for the same amino acids, but do not contain A and/or U. Examples include: the codons for Pro, which may be changed from CCU or CCA to CCC or CCG; the codons for Arg, which may be changed from CGU or CGA or AGA or AGG to CGC or CGG; the codons for Ala, which may be changed from GCU or GCA to GCC or GCG; the codons for Gly, which may be changed from GGU or GGA to GGC or GGG.

[0026] In other cases, although A and/or U nucleotides may not be eliminated from the codons, it is however possible to reduce the A and U content by using codons that contain fewer A and/or U nucleotides. For example: the codons for Phe, which may be changed from UUU to UUC; the codons for Leu may be changed from UUA, CUU or CUA to CUC or CUG; the codons for Ser, which may be changed from UCU

or UCA or AGU to UCC, UCG or AGC; the codon for Tyr, which may be changed from UAU to UAC; the stop codon UAA, which may be changed to UAG or UGA; the codon for Cys, which may be changed from UGU to UGC; the codon for His, which may be changed from CAU to CAC; the codon for Gln, which may be changed from CAA to CAG; the codons for Ile, which may be changed from AUU or AUA to AUC; the codons for Thr, which may be changed from ACU or ACA to ACC or ACG; the codon for Asn may be changed from AAU to AAC; the codon for Lys, which may be changed from AAA to AAG; the codons for Val, which may be changed from GUU or GUA to GUC or GUG; the codon for Asp, which may be changed from GAU to GAC; the codon for Glu, which may be changed from GAA to GAG.

[0027] In the case of the codons for Met (AUG) and Trp (UGG) there is however no possibility of modifying the sequence.

[0028] The substitutions listed above may be used individually and in all possible combinations in order to increase the G/C content of a modified mRNA compared to the original sequence. Thus for example all codons for Thr occurring in the original (wild type) sequence can be altered to ACC (or ACG). Preferably, however, combinations of the substitution possibilities given above are employed, for example: substitution of all codons coding in the original sequence for Thr to ACC (or ACG) and substitution of all codons coding for Ser to UCC (or UCG or AGC); substitution of all codons coding in the original sequence for Ile to AUC and substitution of all codons coding for Lys to AAG and substitution of all codons coding originally for Tyr to UAC; substitution of all codons coding in the original sequence for Val to GUC (or GUG) and substitution of all codons coding for Glu to GAG and substitution of all codons coding for Ala to GCC (or GCG) and substitution of all codons coding for Arg to CGC (or CGG); substitution of all codons coding in the original sequence for Val to GUC (or GUG) and substitution of all codons coding for Glu to GAG and substitution of all codons coding for Ala to GCC (or GCG) and substitution of all codons coding for Gly to GGC (or GGG) and substitution of all codons coding for Asn to AAC; substitution of all codons coding in the original sequence for Val to GUC (or GUG) and substitution of all codons coding for Phe to UUC and substitution of all codons for Cys to UGC and substitution of all codons coding for Leu to CUG (or CUC) and substitution of all codons coding for Gln to CAG and substitution of all codons encoding Pro to CCC (or CCG); etc.

[0029] Preferably the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is increased by at least 7%, more preferably by at least 15%, and particularly preferably by at least 20% compared to the G/C content of the coded region of the wild type mRNA encoding for the polypeptide.

[0030] In this connection it is particularly preferred to maximize the G/C content of the modified mRNA as compared to that of the wild type sequence. For some applications, it may be particularly advantageous to maximise the G/C content of the modified mRNA in the region encoding the at least one peptide or polypeptide.

[0031] In accordance with the invention, a further modification of the mRNA comprised in the pharmaceutical composition of the present invention is based on an understanding that the translation efficiency is also affected by the relative abundance of different tRNAs in various cells. A high frequency of so-called "rare" codons in an RNA sequence,

which are recognized by relatively rare tRNAs, tends to decrease the translational efficiency of the corresponding mRNA, whereas a high frequency of codons recognized by relatively abundant tRNAs tends to enhance the translational efficiency of a corresponding mRNA.

[0032] Thus, according to the invention, the modified mRNA (which is contained in the pharmaceutical composition) comprises a region coding for the peptide or polypeptide which is changed compared to the corresponding region of the wild type mRNA so as to replace at least one codon of the wild type sequence that is recognized by a rare cellular tRNA with a codon recognized by an abundant cellular tRNA, wherein the abundant and rare cellular tRNAs recognize the same amino acid. In other words, the substituted codon in the modified mRNA, which is recognized by a relatively frequent tRNA, encodes the same amino acid as the wild type (unmodified) codon.

[0033] Through such modifications, the RNA sequences are modified so that codons are inserted/substituted that are recognized by abundantly expressed cellular tRNAs. Modifications directed to altering codon usage in a nucleic acid sequence to optimise expression levels of polypeptides encoded therefrom are generally referred to in the art as "codon optimisation."

[0034] Those tRNAs which are abundant or rare in a particular cell are known to a person skilled in the art; see for example Akashi, *Curr. Opin. Genet. Dev.* 2001, 11(6): 660-666. Each organism has a preferred choice of nucleotide or codon usage to encode any particular amino acid. Different species vary in their codon preferences for translating mRNA into protein. The codon preferences of a particular species in which a modified mRNA of the present invention is to be expressed will, therefore, at least in part dictate the parameters of codon optimisation for a nucleic acid sequence.

[0035] By means of this modification, according to the invention all codons of the wild type sequence that are recognized by a relatively rare tRNA in a cell may in each case be replaced by a codon that is recognized by a relatively abundant tRNA. As described herein, however, the coding sequence of the peptide or polypeptide is preserved. That is, a relatively abundant tRNA species, which replaces a relatively rare tRNA species in a modified mRNA of the invention, recognizes an amino acid identical to that recognized by the rare tRNA species.

[0036] According to the invention, it is particularly preferred to couple the sequential increase in the G/C fraction of a modified mRNA (particularly, for example, a maximally modified G/C content), with an increase in the number of codons recognized by abundant tRNAs, wherein the amino acid sequence of the peptide or polypeptide (one or more) encoded by the mRNA remains unaltered. This preferred embodiment provides a particularly preferred mRNA species, possessing properties of efficient translation and improved stability. Such preferred mRNA species are well suited, for example, for the pharmaceutical compositions of the present invention.

[0037] Sequences of eukaryotic mRNAs frequently include destabilising sequence elements (DSE) to which signal proteins can bind and thereby regulate the enzymatic degradation of the mRNA in vivo. Accordingly, for the further stabilisation of a modified mRNA of the invention, which may be a component of a pharmaceutical composition of the invention, one or more changes may be made in the wild type mRNA sequence encoding the at least one peptide or polypeptide, so

as to reduce the number of destabilising sequence elements present. In accordance with the invention, DSEs located anywhere in an mRNA, including the coding region and in the non-translated regions (3' and/or 5' UTR), may be mutated or changed to generate a modified mRNA having improved properties.

[0038] Such destabilising sequences are for example AU-rich sequences ("AURES") that occur in 3'-UTR regions of a number of unstable mRNAs (Caput et al., Proc. Natl. Acad. Sci. USA 1986, 83: 1670-1674). The RNA molecules contained in the pharmaceutical composition according to the invention are therefore preferably altered as compared to the wild type mRNA so as to reduce the number of or eliminate these destabilising sequences. Such an approach also applies to those sequence motifs recognised by potential endonucleases. Such sequences include, for example, GAACAAG, which is found in the 3'UTR of the gene encoding the transferring receptor (Binder et al., EMBO J. 1994, 13: 1969-1980). Sequence motifs recognized by endonucleases are also preferably reduced in number or eliminated in the modified mRNA of the pharmaceutical composition according to the invention.

[0039] Various methods are known to the person skilled in the art that are suitable for the substitution of codons in the modified mRNA according to the invention. In the case of relatively short coding regions (that code for biologically active or antigenic peptides), the whole mRNA may, for example, be chemically synthesised using standard techniques.

[0040] Preferably, however, base substitutions are introduced using a DNA matrix for the production of modified mRNA with the aid of techniques routinely employed in targeted mutagenesis; see Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd Edition, Cold Spring Harbor, N.Y., 2001.

[0041] In this method, a corresponding DNA molecule is therefore transcribed in vitro for the production of the mRNA. This DNA matrix has a suitable promoter, for example a T7 or SP6 promoter, for in vitro transcription, followed by the desired nucleotide sequence for the mRNA to be produced and a termination signal for the in vitro transcription. According to the invention the DNA molecule that forms the matrix of the RNA construct to be produced is prepared as part of a plasmid replicable in bacteria, wherein the plasmid is replicated or amplified during the course of bacterial replication and subsequently isolated by standard techniques. Plasmids suitable for use in the present invention include, but are not limited to pT7Ts (GenBank Accession No. U26404; Lai et al., Development 1995, 121: 2349-2360), the pGEM® series, for example pGEM®-1 (GenBank Accession No. X65300; from Promega) and pSP64 (GenBank-Accession No. X65327); see also Mezei and Storts, Purification of PCR Products, in: Griffin and Griffin (Eds.), PCR Technology: Current Innovation, CRC Press, Boca Raton, Fla., 2001.

[0042] Thus, by using short synthetic DNA oligonucleotides that comprise short single-strand transitions at the corresponding cleavage sites, or by means of genes produced by chemical synthesis, the desired nucleotide sequence can be cloned into a suitable plasmid by molecular biology methods known to the person skilled in the art (see Maniatis et al., above). The DNA molecule is then excised from the plasmid, in which it may be present as a single copy or multiple copies, by digestion with restriction endonucleases.

[0043] The modified mRNA that is contained in the pharmaceutical composition according to the invention may furthermore have a 5' cap structure (a modified guanosine nucleotide). Examples of suitable cap structures include, but are not limited to m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G).

[0044] According to a further preferred embodiment of the present invention the modified mRNA comprises a poly-A tail of at least 50 nucleotides, preferably at least 70 nucleotides, more preferably at least 100 nucleotides and particularly preferably at least 200 nucleotides.

[0045] For efficient translation of the mRNA a productive binding of the ribosomes to the ribosome binding site [Kozak sequence: GCCGCCACCAUGG (SEQ ID NO: 13), the AUG forms the start codon] is generally required. In this regard it has been established that an increased A/U content around this site facilitates more efficient ribosome binding to the mRNA.

[0046] In addition, it is possible to introduce one or more so-called IRES ("internal ribosomal entry site") into the modified mRNA. An IRES may act as the sole ribosome binding site, or may serve as one of the ribosome binding sites of an mRNA. An mRNA comprising more than one functional ribosome binding site may encode several peptides or polypeptides that are translated independently by the ribosomes ("multicistronic mRNA"). Examples of IRES sequences that can be used according to the invention include without limitation, those from picornaviruses (e.g. FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

[0047] According to a further preferred embodiment of the present invention the modified mRNA comprises in the 5' non-translated and/or 3' non-translated regions stabilisation sequences that are capable of increasing the half-life of the mRNA in the cytosol.

[0048] These stabilisation sequences may exhibit 100% sequence homology with naturally occurring sequences that are present in viruses, bacteria and eukaryotic cells, or may be derived from such naturally occurring sequences (i.e., may comprise, e.g., mutations substitutions, or deletions in these sequences). Stabilising sequences that may be used in the present invention include, by way of non-limiting example, the untranslated sequences (UTR) of the β -globin gene of *Homo sapiens* or *Xenopus laevis*. Another example of a stabilisation sequence has the general formula (C/U)CCAN_xCCC(U/A)Py_xUC(C/U)CC, which is contained in the 3'UTR of the very stable mRNAs that encode α -globin, α -(I)-collagen, 15-lipoxygenase, or tyrosine hydroxylase (C. F. Holcik et al., Proc. Natl. Acad. Sci. USA 1997, 94: 2410-2414). Obviously such stabilisation sequences may be used individually or in combination, as well as in combination with other stabilisation sequences known to a person skilled in the art.

[0049] For the further stabilisation of the modified mRNA it is preferred that the modified mRNA comprises at least one analogue of a naturally occurring nucleotide. This approach is based on the understanding that RNA-decomposing enzymes present in a cell preferentially recognise RNA comprising naturally occurring nucleotides as a substrate. The insertion of nucleotide analogues into an RNA molecule, therefore,

retards decomposition of the RNA molecule so modified, whereas the effect of such analogs on translational efficiency, particularly when inserted into the coding region of the mRNA, may result in either an increase or decrease in translation of the modified RNA molecule.

[0050] The following is a non-limiting list of nucleotide analogues that can be used in accordance with the invention: phosphorus amidates, phosphorus thioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to the person skilled in the art, for example from U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642. According to the invention such analogues may be present in non-translated and/or translated regions of the modified mRNA.

[0051] Furthermore the effective transfer of the modified mRNA into the cells to be treated or into the organism to be treated may be improved if the modified mRNA is associated with a cationic peptide or protein, or is bound thereto. In particular in this connection the use of protamine as polycationic, nucleic acid-binding protein is particularly effective. It is also possible to use other cationic peptides or proteins such as poly-L-lysine or histones. Procedures for stabilising mRNA are described in EP-A-1083232, whose relevant disclosure is incorporated herein in its entirety.

[0052] For gene therapy applications, for example, wherein a pharmaceutical composition of the invention is used, the modified mRNA therein codes for at least one biologically active peptide or polypeptide that is not formed or is only insufficiently or defectively formed in the patient to be treated. Administration of a modified mRNA encoding the at least one biologically active peptide or polypeptide or a composition thereof to such a patient, therefore, at least partially restores the expression and/or activity of the at least one biologically active peptide or polypeptide in the patient and thereby complements the patient's genetic defect. The direct introduction of a normal, functional gene into a living animal has been studied as a means for replacing defective genetic information. In such studies, nucleic acid sequences are introduced directly into cells of a living animal. The following references pertain to methods for the direct introduction of nucleic acid sequences into a living animal: Nabel et al., (1990) *Science* 249:1285-1288; Wolfe et al., (1990) *Science* 247:1465-1468; Acsadi et al. (1991) *Nature* 352:815-818; Wolfe et al. (1991) *BioTechniques* 11(4):474-485; and Felgner and Rhodes, (1991) *Nature* 349:351-352, which are incorporated herein by reference.

[0053] Accordingly, examples of polypeptides coded by a modified mRNA of the invention include, without limitation, dystrophin, the chloride channel, which is defectively altered in cystic fibrosis; enzymes that are lacking or defective in metabolic disorders such as phenylketonuria, galactosaemia, homocystinuria, adenosine deaminase deficiency, etc.; enzymes that are involved in the synthesis of neurotransmitters such as dopamine, norepinephrine and GABA, in particular tyrosine hydroxylase and DOPA decarboxylase, and α -1-antitrypsin, etc. Pharmaceutical compositions of the invention may also be used to effect expression of cell surface receptors and/or binding partners of cell surface receptors if the modified mRNA contained therein encodes for such biologically active proteins or peptides. Examples of such pro-

teins that act in an extracellular manner or that bind to cell surface receptors include for example tissue plasminogen activator (TPA), growth hormones, insulin, interferons, granulocyte-macrophage colony stimulating factor (GM-CSF), and erythropoietin (EPO), etc. By choosing suitable growth factors, the pharmaceutical composition of the present invention may, for example, be used for tissue regeneration. In this way diseases that are characterised by tissue degeneration, for example neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc. and other degenerative conditions, such as arthrosis, can be treated. In these cases the modified mRNA, in particular that contained in the pharmaceutical composition of the present invention, preferably encodes, without limitation, a TGF- β family member, EGF, FGF, PDGF, BMP, GDNF, BDNF, GDF and neurotrophic factors such as NGF, neutrophines, etc.

[0054] A further area of application of the present invention is vaccination, i.e. the use of a modified mRNA for inoculation or the use of a pharmaceutical composition comprising a modified mRNA as an inoculating agent, or the use of a modified mRNA in the preparation of the pharmaceutical composition for inoculation purposes. Vaccination is based on introducing an antigen into an organism or subject, in particular into a cell of the organism or subject. In the context of the present invention, the genetic information encoding the antigen is introduced into the organism or subject in the form of a modified mRNA encoding the antigen. The modified mRNA contained in the pharmaceutical composition is translated into the antigen, i.e. the polypeptide or antigenic peptide coded by the modified mRNA is expressed, and an immune response directed against the polypeptide or antigenic peptide is stimulated. For vaccination against a pathogenic organism, e.g., a virus, a bacterium, or a protozoan, a surface antigen of such an organism may be used as an antigen against which an immune response is elicited. In the context of the present invention, a pharmaceutical composition comprising a modified mRNA encoding such a surface antigen may be used as a vaccine. In applications wherein a genetic vaccine is used for treating cancer, the immune response is directed against tumour antigens by generating a modified mRNA encoding a tumour antigen(s), in particular a protein which is expressed exclusively on cancer cells. Such a modified mRNA encoding a tumour antigen may be used alone or as a component of a pharmaceutical composition according to the invention, wherein administration of either the modified mRNA or a composition thereof results in expression of the cancer antigen(s) in the organism. An immune response to such a vaccine would, therefore, confer to the vaccinated subject a degree of protective immunity against cancers associated with the immunizing cancer antigen. Alternatively, such measures could be used to vaccinate a cancer patient with a modified mRNA encoding a tumour antigen(s) expressed on the patient's cancer cells so as to stimulate the cancer patient's immune response to attack any cancer cells expressing the encoded antigen.

[0055] In its use as a vaccine the pharmaceutical composition according to the invention is suitable in particular for the treatment of cancers (in which the modified mRNA codes for a tumour-specific surface antigen (TSSA), for example for treating malignant melanoma, colon carcinomas, lymphomas, sarcomas, small-cell lung carcinomas, blastomas, etc. A non-limiting list of specific examples of tumour antigens include, inter alia, 707-AP, AFP, ART-4, BAGE, β -catenin/m, Bcr-abl, CAMEL, CAP-1, CASP-8, CDC27/m, CDK4/m, CEA,

CT, Cyp-B, DAM, ELF2M, ETV6-AML1, G250, GAGE, GnT-V, Gp100, HAGE, HER-2/neu, HLA-A*0201-R1701, HPV-E7, HSP70-2M, HAST-2, hTERT (or hTRT), iCE, KIAA0205, LAGE, LDLR/FUT, MAGE, MART-1/melan-A, MC1R, myosin/m, MUC1, MUM-1, -2, -3, NA88-A, NY-ESO-1, p190 minor bcr-abl, Pml/RAR α , PRAMS, PSA, PSM, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, TEUAML1, TPI/m, TRP-1, TRP-2, TRP-2/INT2 and WT1. In addition to the above application, the pharmaceutical composition of the invention may be used to treat infectious diseases, for example, viral infectious diseases such as AIDS (HIV), hepatitis A, B or C, herpes, herpes zoster (chicken pox), German measles (rubella virus), yellow fever, dengue fever etc. (flavi viruses), flu (influenza viruses), haemorrhagic infectious diseases (Marburg or Ebola viruses), bacterial infectious diseases such as Legionnaires' disease (*Legionella*), gastric ulcer (*Helicobacter*), cholera (*Vibrio*), *E. coli* infections, staphylococcal infections, *salmonella* infections or streptococcal infections, tetanus (*Clostridium tetani*), or protozoan infectious diseases (malaria, sleeping sickness, leishmaniasis, toxoplasmosis, i.e. infections caused by *plasmodium*, trypanosomes, *leishmania* and *toxoplasma*). Preferably also in the case of infectious diseases the corresponding surface antigens with the strongest antigenic potential are encoded by the modified mRNA. With the aforementioned genes of pathogenic vectors or organisms, in particular in the case of viral genes, this is typically a secreted form of a surface antigen. Moreover, according to the invention mRNAs preferably coding for polypeptides are employed, because polypeptides generally comprise multiple epitopes (polyepitopes). Polypeptides comprising polyepitopes include but are not limited to, surface antigens of pathogenic vectors or organisms, or of tumour cells, preferably secreted protein forms.

[0056] Moreover, the modified mRNA according to the invention may comprise in addition to the antigenic or therapeutically active peptide or polypeptide, at least one further functional region that encodes, for example, a cytokine that promotes the immune response (e.g., a monokine, lymphokine, interleukin or chemokine, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, INF- α , INF- γ , GM-CSF, LT- α or growth factors such as hGH).

[0057] Furthermore, in order to increase immunogenicity, the pharmaceutical composition according to the invention may contain one or more adjuvants. The term "adjuvant" is understood in this context to denote any chemical or biological compound that promotes or augments a specific immune response. Various mechanisms may be involved in this connection, depending on the various types of adjuvants. For example, compounds that promote endocytosis of the modified mRNA contained in the pharmaceutical composition by dendritic cells (DC) form a first class of usable adjuvants. Other compounds that activate or accelerate maturation of DC (for example, lipopolysaccharides, TNF- α or CD40 ligand) comprise a second class of suitable adjuvants. In general, any agent which is recognized as a potential "danger signal" by the immune system (LPS, GP96, oligonucleotides with the CpG motif) or cytokines such as GM-CSF, may be used as an adjuvant. Co-administration of an adjuvant enhances an immune response generated against an antigen encoded by the modified mRNA. The aforementioned cytokines are particularly preferred in this aspect. Other known adjuvants include aluminium hydroxide, and Freund's adjuvant, as well as the aforementioned stabilising cationic pep-

tides or polypeptides such as protamine. In addition, lipopeptides such as Pam3Cys are also particularly suitable for use as adjuvants in the pharmaceutical composition of the present invention; see Deres et al, Nature 1989, 342: 561-564.

[0058] The pharmaceutical composition according to the invention comprises, in addition to the modified mRNA, a pharmaceutically compatible carrier and/or a pharmaceutically compatible vehicle. Appropriate methods for achieving a suitable formulation and preparation of the pharmaceutical composition according to the invention are described in "Remington's Pharmaceutical Sciences" (Mack Pub. Co., Easton, Pa., 1980), which is herein incorporated by reference in its entirety. For parenteral administration suitable carriers include for example sterile water, sterile saline solutions, polyalkylene glycols, hydrogenated naphthalene and in particular biocompatible lactide polymers, lactide/glycolide copolymers or polyoxyethylene/polyoxypropylene copolymers. Compositions according to the invention may contain fillers or substances such as lactose, mannitol, substances for the covalent coupling of polymers such as for example polyethylene glycol to inhibitors according to the invention, complexing with metal ions or incorporation of materials in or on special preparations of polymer compound, such as for example polylactate, polyglycolic acid, hydrogel or on liposomes, microemulsions, microcells, unilamellar or multilamellar vesicles, erythrocyte fragments or spheroplasts. The respective modifications of the compositions are chosen depending on physical properties such as, for example, solubility, stability, bioavailability or degradability. Controlled or constant release of the active component according to the invention in the composition includes formulations based on lipophilic depot substances (for example fatty acids, waxes or oils). Coatings of substances or compositions according to the invention containing such substances, namely coatings with polymers (for example poloxamers or poloxamines), are also disclosed within the scope of the present invention. Moreover substances or compositions according to the invention may contain protective coatings, for example protease inhibitors or permeability enhancers. Preferred carriers are typically aqueous carrier materials, in which water for injection (WFI) or water buffered with phosphate, citrate or acetate, etc., is used, and the pH is typically adjusted to 5.0 to 8.0, preferably 6.0 to 7.0. The carrier or the vehicle will in addition preferably contain salt constituents, for example sodium chloride, potassium chloride or other components that for example make the solution isotonic. In addition the carrier or the vehicle may contain, besides the aforementioned constituents, additional components such as human serum albumin (HSA), polysorbate 80, sugars or amino acids.

[0059] The concentration of the modified mRNA in such formulations may therefore vary within a wide range from 1 μ g to 100 mg/ml. The pharmaceutical composition according to the invention is preferably administered parenterally, for example intravenously, intraarterially, subcutaneously or intramuscularly to the patient. It is also possible to administer the pharmaceutical composition topically or orally.

[0060] The invention thus also provides a method for the treatment of the aforementioned medical conditions or an inoculation method for the prevention of the aforementioned conditions, which comprises the administration of the pharmaceutical composition according to the invention to a subject or patient, in particular a human patient.

[0061] A typical regimen for preventing, suppressing, or treating a pathology related to a viral, bacterial, or protozoan

infection, may comprise administration of an effective amount of a vaccine composition as described herein, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including between one week and about 24 months, or any range or value therein.

[0062] According to the present invention, an “effective amount” of a vaccine composition is one that is sufficient to achieve a desired biological effect. It is understood that nature and manner of the administration and the effective dosage may be determined by a medical practitioner based on a number of variables including the age, sex, health, and weight of the recipient, the medical condition to be treated and its stage of progression, the kind of concurrent treatment, if any, frequency of treatment, and the nature of the desired outcome. The ranges of effective doses provided below are not intended to limit the invention, but are provided as representative preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art, without undue experimentation. See, e.g., Berkow et al., eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Goodman et al., eds., *Goodman and Gilman’s The Pharmacological Basis of Therapeutics*, 8th edition, Pergamon Press, Inc., Elmsford, N.Y., (1990); Avery’s *Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, Md. (1987), Ebadi, *Pharmacology*, Little, Brown and Co., Boston, Mass. (1985); and Katzung, ed. *Basic and Clinical Pharmacology*, Fifth Edition, Appleton and Lange, Norwalk, Conn. (1992), which references and references cited therein, are entirely incorporated herein by reference.

[0063] The present invention relates to the use of genetic material (e.g., nucleic acid sequences) as immunizing agents. In one aspect, the present invention relates to the introduction of exogenous or foreign modified DNA or RNA molecules into an individual’s tissues or cells, wherein these molecules encode an exogenous protein capable of eliciting an immune response to the protein. The exogenous nucleic acid sequences may be introduced alone or in the context of an expression vector wherein the sequences are operably linked to promoters and/or enhancers capable of regulating the expression of the encoded proteins. The introduction of exogenous nucleic acid sequences may be performed in the presence of a cell stimulating agent capable of enhancing the uptake or incorporation of the nucleic acid sequences into a cell. Such exogenous nucleic acid sequences may be administered in a composition comprising a biologically compatible or pharmaceutically acceptable carrier. The exogenous nucleic acid sequences may be administered by a variety of means, as described herein, and well known in the art.

[0064] Such methods may be used to elicit immunity to a pathogen, absent the risk of infecting an individual with the pathogen. The present invention may be practiced using procedures known in the art, such as those described in PCT International Application Number PCT/US90/01515, wherein methods for immunizing an individual against pathogen infection by directly injecting polynucleotides into the individual’s cells in a single step procedure are presented.

[0065] In one aspect, the present invention relates to methods for eliciting immune responses in an individual or subject which can protect the individual from pathogen infection. Accordingly, genetic material that encodes an immunogenic protein is introduced into a subject’s cells either in vivo or ex vivo. The genetic material is expressed by these cells, thereby

producing immunogenic target proteins capable of eliciting an immune response. The resulting immune response is broad based and involves activation of the humoral immune response and both arms of the cellular immune response.

[0066] This approach is useful for eliciting a broad range of immune responses against a target protein. Target proteins may be proteins specifically associated with pathogens or the individual’s own “abnormal” or infected cells. Such an approach may be used advantageously to immunize a subject against pathogenic agents and organisms such that an immune response against a pathogen protein provides protective immunity against the pathogen. This approach is particularly useful for protecting an individual against infection by non-encapsulated intracellular pathogens, such as a virus, which produce proteins within the host cells. The immune response generated against such proteins is capable of eliminating infected cells with cytotoxic T cells (CTLs).

[0067] The immune response elicited by a target protein produced by vaccinated cells in a subject is a broad-based immune response which includes B cell and T cell responses, including CTL responses. It has been observed that target antigen produced within the cells of the host are processed intracellularly into small peptides, which are bound by Class I MHC molecules and presented in the context of Class I on the cell surface. The Class I MHC-target antigen complexes are capable of stimulating CD8⁺T cells, which are predominantly CTLs. Notably, genetic immunization according to the present invention is capable of eliciting CTL responses (killer cell responses).

[0068] The CTL response is crucial in protection against pathogens such as viruses and other intracellular pathogens which produce proteins within infected cells. Similarly, the CTL response can be utilized for the specific elimination of deleterious cell types, which may express aberrant cell surface proteins recognizable by Class I MHC molecules.

[0069] The genetic vaccines of the present invention may be administered to cells in conjunction with compounds that stimulate cell division and facilitate uptake of genetic constructs. This step provides an improved method of direct uptake of genetic material. Administration of cell stimulating compounds results in a more effective immune response against the target protein encoded by the genetic construct.

[0070] According to the present invention, modified DNA or mRNA that encodes a target protein is introduced into the cells of an individual where it is expressed, thus producing the target protein. The modified DNA or RNA may be operably linked to regulatory elements (e.g., a promoter) necessary for expression in the cells of the individual. Other elements known to skilled artisans may also be included in genetic constructs of the invention, depending on the application.

[0071] As used herein, the term “genetic construct” refers to the modified DNA or mRNA molecule that comprises a nucleotide sequence which encodes the target protein and which may include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal (for modified DNA) capable of directing expression in the cells of the vaccinated individual. As used herein, the term “expressible form” refers to gene constructs which contain the necessary regulatory elements operably linked to a coding sequence of a target protein, such that when present in the cell of the individual, the coding sequence is expressed. As used herein, the term “genetic vaccine” refers to a pharmaceutical preparation that comprises a genetic construct.

[0072] The present invention provides genetic vaccines, which include genetic constructs comprising DNA or RNA which encode a target protein. As used herein, the term “target protein” refers to a protein capable of eliciting an immune response. The target protein is an immunogenic protein derived from the pathogen or undesirable cell-type, such as an infected or transformed cell. In accordance with the invention, target proteins may be pathogen-associated proteins or tumour-associated proteins. The immune response directed against the target protein protects the individual against the specific infection or disease with which the target protein is associated. For example, a genetic vaccine comprising a modified DNA or RNA molecule that encodes a pathogen-associated target protein is used to elicit an immune response that will protect the individual from infection by the pathogen.

[0073] DNA and RNA-based vaccines and methods of use are described in detail in several publications, including Leitner et al. (1999, *Vaccines* 18:765-777), Nagashunmugam et al. (1997, *AIDS* 11: 1433-1444), and Fleeton et al. (2001, *J Infect Dis* 183:1395-1398) the entire contents of each of which is incorporated herein by reference.

[0074] In order to test expression, genetic constructs can be tested for expression levels in vitro using cells maintained in culture, which are of the same type as those to be vaccinated. For example, if the genetic vaccine is to be administered into human muscle cells, muscle cells grown in culture such as solid muscle tumor cells of rhabdomyosarcoma may be used as an in vitro model for measuring expression levels. One of ordinary skill in the art could readily identify a model in vitro system which may be used to measure expression levels of an encoded target protein.

[0075] In accordance with the invention, multiple inoculants can be delivered to different cells, cell types, or tissues in an individual. Such inoculants may comprise the same or different nucleic acid sequences of a pathogenic organism. This allows for the introduction of more than a single antigen target and maximizes the chances for developing immunity to the pathogen in a vaccinated subject.

[0076] According to the invention, the genetic vaccine may be introduced in vivo into cells of an individual to be immunized or ex vivo into cells of the individual which are re-implanted after incorporation of the genetic vaccine. Either route may be used to introduce genetic material into cells of an individual. As described herein above, preferred routes of administration include intramuscular, intraperitoneal, intradermal, and subcutaneous injection. Alternatively, the genetic vaccine may be introduced by various means into cells isolated from an individual. Such means include, for example, transfection, electroporation, and microprojectile bombardment. These methods and other protocols for introducing nucleic acid sequences into cells are known to and routinely practiced by skilled practitioners. After the genetic construct is incorporated into the cells, they are re-implanted into the individual. Prior to re-implantation, the expression levels of a target protein encoded by the genetic vaccine may be assessed. It is contemplated that otherwise non-immunogenic cells that have genetic constructs incorporated therein can be implanted into autologous or heterologous recipients.

[0077] The genetic vaccines according to the present invention comprise about 0.1 to about 1000 micrograms of nucleic acid sequences (i.e., DNA or RNA). In some preferred embodiments, the vaccines comprise about 1 to about 500 micrograms of nucleic acid sequences. In some preferred

embodiments, the vaccines comprise about 25 to about 250 micrograms of nucleic acid sequences. Most preferably, the vaccines comprise about 100 micrograms nucleic acid sequences.

[0078] The genetic vaccines according to the present invention are formulated according to the mode of administration to be used. One having ordinary skill in the art can readily formulate a genetic vaccine that comprises a genetic construct. In cases where intramuscular injection is the chosen mode of administration, for example, an isotonic formulation is generally used. As described in detail herein above, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. Isotonic solutions such as phosphate buffered saline are preferred. Stabilizers can include gelatin and albumin.

[0079] In some embodiments of the invention, the individual is administered a series of vaccinations to produce a comprehensive immune response. According to this method, at least two and preferably four injections are given over a period of time. The period of time between injections may include from 24 hours apart to two weeks or longer between injections, preferably one week apart. Alternatively, at least two and up to four separate injections may be administered simultaneously to different parts of the body.

[0080] While this disclosure generally discusses immunization or vaccination in the context of prophylactic methods of protection, the terms “immunizing” or “vaccinating” are meant to refer to both prophylactic and therapeutic methods. Thus, a method for immunizing or vaccinating includes both methods of protecting an individual from pathogen challenge, as well as methods for treating an individual suffering from pathogen infection. Accordingly, the present invention may be used as a vaccine for prophylactic protection or in a therapeutic manner; that is, as a reagent for immunotherapeutic methods and preparations.

[0081] The amount of a modified nucleic acid sequence generated using the methods of the invention which provides a therapeutically effective dose in the treatment of a patient with, for example, cancer or a pathogen-related disorder can be determined by standard clinical techniques based on the present description. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally directed to achieve a concentration of about 20-500 micrograms of polypeptide encoded by the modified nucleic acid per kilogram body weight. Suitable dosage ranges for intranasal administration are generally directed to achieve a concentration of about 0.01 pg to 1 mg of polypeptide encoded by the modified nucleic acid per kg body weight. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0082] The compositions comprising the modified nucleic acid molecules of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, compositions are administered to a patient already suffering from a hyperproliferative disorder (such as, e.g., cancer) in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as a “therapeu-

tically effective amount or dose.” Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient.

[0083] Compositions comprising modified nucleic acid molecules of the invention can be administered alone, or in combination, and/or in conjunction with known therapeutic agents/compounds used for the treatment of a patient with a particular disorder. For the treatment of a patient with cancer, for example, a composition comprising at least one modified nucleic acid of the invention which encodes a tumour antigen, may be used in conjunction with one or more known cancer therapeutics, such as those described in the *Physicians' Desk Reference*, 54th Edition (2000) or in *Cancer: Principles & Practice of Oncology*, DeVita, Jr., Hellman, and Rosenberg (eds.) 2nd edition, Philadelphia, Pa.: J. B. Lippincott Co., 1985, wherein standard treatment protocols and dosage formulations are presented.

[0084] In addition a method is also provided for determining how to modify the sequence of an mRNA so as to generate a modified mRNA having altered properties, which may be used alone or in a pharmaceutical composition of the invention. In this connection, and in accordance with the invention, the modification of an RNA sequence is carried out with two different optimisation objectives: to maximize G/C content, and to maximize the frequency of codons that are recognized by abundantly expressed tRNAs. In the first step of the process a virtual translation of an arbitrary RNA (or DNA) sequence is carried out in order to generate the corresponding amino acid sequence. Starting from the amino acid sequence, a virtual reverse translation is performed that provides, based on degeneracy of the genetic code, all of the possible choices for the corresponding codons. Depending on the required optimisation or modification, corresponding selection lists and optimisation algorithms are used for choosing suitable codons. The algorithms are executed on a computer, normally with the aid of suitable software. In accordance with the present invention, a suitable software program comprises a source code of Appendix I. Thus, the optimised mRNA sequence is generated and can be output, for example, with the aid of a suitable display device and compared with the original (wild type) sequence. The same also applies with regard to the frequency of the individual nucleotides. The changes compared to the original nucleotide sequence are preferably emphasised. Furthermore, according to a preferred embodiment, naturally occurring stable sequences are incorporated therein to produce an RNA stabilised by the presence of natural sequence motifs. A secondary structural analysis may also be performed that can analyse, on the basis of structural calculations, stabilising and destabilising properties or regions of the RNA.

[0085] Also encompassed by the present invention are modified nucleic acid sequences generated using the above computer-based method. Exemplary modified nucleic acid sequences of the invention include SEQ ID NOs: 3-7, 10 and 11. The present invention also includes pharmaceutical compositions of modified nucleic acid sequences of the invention, including SEQ ID NOs: 3-7, 10 and 11.

BRIEF DESCRIPTION OF THE DRAWINGS

[0086] FIG. 1 shows wild type sequences and modified sequences for the influenza matrix protein.

[0087] FIG. 1A (SEQ ID NO: 1) shows the wild type gene and FIG. 1B (SEQ ID NO: 2) shows the amino acid sequence derived therefrom (1-letter code). FIG. 1C (SEQ ID NO: 3)

shows a gene sequence coding for the influenza matrix protein, whose G/C content is increased as compared to that of the wild type sequence. FIG. 1D (SEQ ID NO: 4) shows the sequence of a gene that codes for a secreted form of the influenza matrix protein (including an N-terminal signal sequence), wherein the G/C content of the sequence is increased relative to that of the wild type sequence. FIG. 1E (SEQ ID NO: 5) shows an mRNA coding for the influenza matrix protein, wherein the mRNA comprises stabilising sequences not present in the corresponding wild type mRNA. FIG. 1F (SEQ ID NO: 6) shows an mRNA coding for the influenza matrix protein that in addition to stabilising sequences also contains an increased G/C content. FIG. 1G (SEQ ID NO: 7) likewise shows a modified mRNA that codes for a secreted form of the influenza matrix protein and comprises, as compared to the wild type, stabilising sequences and an elevated G/C content. In FIG. 1A and FIGS. 1C to 1G the start and stop codons are shown in bold type. Nucleotides that are changed relative to the wild type sequence of FIG. 1A are shown in capital letters in 1C to 1G.

[0088] FIG. 2 shows wild type sequences and modified sequences according to the invention that encode for the tumour antigen MAGE1.

[0089] FIG. 2A (SEQ ID NO: 8) shows the sequence of the wild type gene and FIG. 2B (SEQ ID NO: 9) shows the amino acid sequence derived therefrom (3-letter code). FIG. 2C (SEQ ID NO: 10) shows a modified mRNA coding for MAGE1, whose G/C content is increased as compared to the wild type. FIG. 2D (SEQ ID NO: 11) shows the sequence of a modified mRNA encoding MAGE1, in which the codon usage has been optimised as frequently as possible with respect to the tRNA present in the cell and to the coding sequence in question. Start and stop codons are shown in each case in bold type.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0090] The following examples describe the invention in more detail and in no way are to be construed as restricting the scope thereof.

Example 1

[0091] As an exemplary embodiment of the process for determining the sequence of a modified mRNA according to the invention, a computer program was established that modifies the nucleotide sequence of an arbitrary mRNA in such a way as to maximise the G/C content of the nucleic acid, and maximise the presence of codons recognized by abundant tRNAs present in a particular cell(s). The computer program is based on an understanding of the genetic code and exploits the degenerative nature of the genetic code. By this means a modified mRNA having desirable properties is obtained, wherein the amino acid sequence encoded by the modified mRNA is identical to that of the unmodified mRNA sequence. Alternatively, the invention may encompass alterations in either the G/C content or codon usage of an mRNA to produce a modified mRNA.

[0092] The source code in Visual Basic 6.0 (program development environment employed: Microsoft Visual Studio Enterprise 6.0 with Servicepack 3) is given in the Appendix I.

Example 2

[0093] An RNA construct with a sequence of the lac-Z gene from *E. coli* optimised with regard to stabilisation and trans-

lational efficiency was produced with the aid of the computer program of Example 1. A G/C content of 69% (compared to the wild type sequence of 51%; C. F. Kalnins et al., EMBO J. 1983, 2(4): 593-597) was achieved in this manner. Through the synthesis of overlapping oligonucleotides that comprise the modified sequence, the optimised sequence was produced according to methods known in the art. The terminal oligonucleotides have the following restriction cleavage sites: at the 5' end an EcoRV cleavage site, and at the 3' end a BglII cleavage site. The modified lacZ sequence was incorporated into the plasmid pT7Ts (GenBank Accession No. U26404; C. F. Lai et al., see above) by digestion with EcoRV/BglII. pT7Ts contains untranslated region sequences from the β -globin gene of *Xenopus laevis* at the 5' and 3' ends. The plasmid was cleaved with the aforementioned restriction enzymes to facilitate insertion of the modified lacZ sequence having compatible 5' and 3' termini.

[0094] The pT7Ts-lac-Z construct was propagated in bacteria and purified by phenol-chloroform extraction. 2 μ g of the construct were transcribed in vitro using methods known to a skilled artisan and the modified mRNA was produced.

Example 3

[0095] The gene for the influenza matrix protein (wild type sequence, see FIG. 1A; derived amino acid sequence, see FIG. 1B) was optimised with the aid of the computer program according to the invention of Example 1. The G/C-rich sequence variant shown in FIG. 1C (SEQ ID NO: 3) was thereby formed. A G/C-rich sequence coding for a secreted form of the influenza matrix protein, which includes an N-terminal signal sequence was also determined (see FIG. 1D;

SEQ ID NO: 4). The secreted form of the influenza matrix protein has the advantage of increased immunogenicity as compared to that of the non-secreted form.

[0096] Corresponding mRNA molecules were designed starting from the optimised sequences. The mRNA for the influenza matrix protein, optimised with regard to G/C content and codon usage, was additionally provided with stabilising sequences in the 5' region and 3' region (the stabilisation sequences derive from the 5'-UTRs and 3'-UTRs of the β -globin-mRNA of *Xenopus laevis*; pT7Ts-Vektor in C. F. Lai et al., see above). See also FIG. 1E; SEQ ID NO: 5, which includes only stabilising sequences and 1F; SEQ ID NO: 6, which includes both increased G/C content and stabilising sequences. The mRNA coding for the secreted form of the influenza matrix protein was likewise also sequence optimised in the translated region and provided with the aforementioned stabilising sequences (see FIG. 1G; SEQ ID NO: 7).

Example 4

[0097] The mRNA encoding the tumour antigen MAGE1 was modified with the aid of the computer program of Example 1. The sequence shown in FIG. 2C (SEQ ID NO: 10) was generated in this way, and has a 24% higher G/C content (351 G, 291 C) as compared to the wild type sequence (275 G, 244 C). In addition, by means of alternative codon usage, the wild type sequence was improved with regard to translational efficiency by substituting codons corresponding to tRNAs that are more abundant in a target cell (see FIG. 2D; SEQ ID NO: 11). The G/C content was likewise raised by 24% by the alternative codon usage.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 13

<210> SEQ ID NO 1

<211> LENGTH: 774

<212> TYPE: DNA

<213> ORGANISM: Influenza virus

<220> FEATURE:

<223> OTHER INFORMATION: Influenza matrix: wildtype gene (for comparison)

<220> FEATURE:

<223> OTHER INFORMATION: Start codon: atg (nucleotides 11 to 13), stop codon: tga (nucleotides 767 to 769)

<400> SEQUENCE: 1

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agatctaaag atgagtcctc taaccgaggt cgaaacgtac gttctctcta tcatcccgtc      60
aggcccccctc aaagccgaga tcgcacagag acttgaagat gtctttgcag ggaagaacac      120
cgatcttgag gttctcatgg aatggctaaa gacaagacca atcctgtcac ctctgactaa      180
ggggatttta ggatttgtgt tcacgctcac cgtgccaggt gagcgaggac tgcagcgtag      240
acgctttgtc caaaatgccc ttaatgggaa cggggatcca aataacatgg acaaagcagt      300
taaaactgtat aggaagctca agaggagat aacattccat ggggccaaag aaatctcact      360
cagttattct gctgggtcac ttgccagttg tatgggcctc atatacaaca ggatgggggc      420
tgtgaccact gaagtggcat ttggcctggt atgtgcaacc tgtgaacaga ttgctgactc      480
ccagcatcgg tctcataggc aaatgggtgac aacaaccaac ccactaatca gacatgagaa      540

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cagaatgggtt ttagccagca ctacagctaa ggctatggag caaatggctg gatcgagtga    600
gcaagcagca gaggccatgg aggttgctag tcaggctagg caaatgggtgc aagcgatgag    660
aaccattggg actcacccta gctccagtgc tggctctgaaa aatgatcttc ttgaaaattt    720
gcaggcctat cagaaacgaa tgggggtgca gatgcaacgg ttcaagtga ctag          774

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<210> SEQ ID NO 2
<211> LENGTH: 252
<212> TYPE: PRT
<213> ORGANISM: Influenza virus

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<400> SEQUENCE: 2

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Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro
1      5      10      15
Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
20     25     30
Ala Gly Lys Asn Thr Asp Leu Glu Val Leu Met Glu Trp Leu Lys Thr
35     40     45
Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
50     55     60
Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65     70     75     80
Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala
85     90     95
Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
100    105    110
Lys Glu Ile Ser Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
115    120    125
Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
130    135    140
Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg
145    150    155    160
Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
165    170    175
Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
180    185    190
Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
195    200    205
Ala Arg Gln Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser
210    215    220
Ser Ser Ala Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225    230    235    240
Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
245    250

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<210> SEQ ID NO 3
<211> LENGTH: 775
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Influenza
matrix: gene with increased G/C-content
<220> FEATURE:
<223> OTHER INFORMATION: Start codon: atg (nucleotides 11 to 13), stop
codon: tga (nucleotides 767 to 769)

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-continued

<400> SEQUENCE: 3

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agatctaaag atgagcctgc tgaccgaggt ggagacctac gtgctgagca tcatccccag    60
cggccccctg aaggccgaga tcgccagag gctggaggac gtgttcgccg gcaagaacac    120
cgacctggag gtgctgatgg agtggctgaa gaccaggccc atcctgagcc cctgaccaa    180
gggcatcctg ggcttcgtgt tcacctgac cgtgccagc gagcgcgcc tgcagcgccg    240
ccgcttcgtg cagaacgccc tgaacggcaa cggcgacccc acaacatgg acaaggccgt    300
gaagctgtac aggaagtga agagggagat caccttcac ggcgccaagg agatcagcct    360
gagctacagc gccggcgccc tggccagctg catgggcctg atctacaaca ggatgggagc    420
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ccagcaccgc agccacagcg agatgggtgac caccaccaac cccctgatca ggcacgagaa    540
caggatgggtg ctggccagca ccaccgcaa ggccatggag cagatggccg gcagcagcga    600
gcaggccgcc gaggccatgg aggtggccag ccaggccagg cagatgggtg aggccatgag    660
gaccatcggc acccacccca gcagcagcgc cggcctgaag aacgacctgc tggagaacct    720
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<210> SEQ ID NO 4

<211> LENGTH: 844

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Influenza matrix: gene for secreted form (with N-terminal signal sequence) with increased G/C-content

<220> FEATURE:

<223> OTHER INFORMATION: Start codon: atg (nucleotides 11 to 13), stop codon: tga (nucleotides 836 to 838)

<400> SEQUENCE: 4

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agatctaaag atggccgtca tggccccccg caccctggtg ctgctgctga gcggcgccct    60
ggcctgacc cagacctggg ctagcctgct gaccgaggtg gagacctacg tgctgagcat    120
catccccagc ggccccctga aggccgagat cgcccagagg ctggaggacg tgttcgccg    180
caagaacacc gacctggagg tgctgatgga gtggtgaag accaggccca tctgagccc    240
cctgaccaag ggcacctcgt gcttcgtgtt caccctgacc gtgccagcgc agcgcgccct    300
gcagcgccgc cgcttcgtgc agaacgcctt gaacggcaac ggcgacccca acaacatgga    360
caaggccgtg aagctgtaca ggaagctgaa gagggagatc accttcacg gcgccaagga    420
gatcagcctg agctacagcg ccggcgccct ggccagctgc atgggcctga tctacaacag    480
gatgggcccgt gtgaccaccg aggtggcctt cggcctggtg tgcgccacct gcgagcagat    540
cgccgacagc cagcaccgca gccacaggca gatggtgacc accaccaacc cctgatcag    600
gcacgagaac aggatggtgc tggccagcac caccgccaag gccatggagc agatggccgg    660
cagcagcgag caggcccgcc aggccatgga ggtggccagc caggccaggc agatggtgca    720
ggccatgagg accatcgga cccaccccag cagcagcgcc ggctgaaga acgacctgct    780
ggagaacctg caggcctacc agaagcgcat gggcgtgcag atgcagcgct tcaagtgaac    840
tagt                                                844

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<210> SEQ ID NO 5

<211> LENGTH: 942

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Influenza
matrix: mRNA with stabilisation sequences
<220> FEATURE:
<223> OTHER INFORMATION: The stabilisation sequences are derived from
5'- and 3'-UTRs of a-globin-mRNA from Xenopus laevis,
respectively.
<220> FEATURE:
<223> OTHER INFORMATION: Start codon: aug (nucleotides 56 to 58), stop
codon: uga (nucleotides 812 to 814)

<400> SEQUENCE: 5
gcuuguucuu uuugcagaag cucagaauaa acgcucaacu uuggcagauc uaaagaugag      60
ucuucuaacc gaggucgaaa cguacguucu cucuaucauc ccgucaggcc ccucuaaagc     120
cgagaucgca cagagacuug aagaugucuu ugcaggggaag aacaccgauc uugaggguucu     180
cauggaaugg cuaaagacaa gaccaauccu gucaccucug acuaagggga uuuuaggauu     240
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ugcccuuaau gggaacgggg auccaaauaa cauggacaaa gcaguuuaac uguauaggaa     360
gcuaagagg gagauaacau uccauggggc caaagaaauc ucacucaguu auucugcugg      420
ugcacuugcc aguuguauug gccucauaua caacaggauug ggggcuguga ccacugaagu     480
ggcauuuggc cugguauug caaccuguga acagauugcu gacucccagc aucggucuca     540
uaggcaaaug gugacaacaa ccaaccacac aaucagacau gagaacagaa ugguuuuagc     600
cagcacuaca gcuaaggcua uggagcaaa ugcuggaucg agugagcaag cagcagaggc     660
cauggagguu gcuagucagg cuaggcaaa uggugcaagcg augagaacca uggggacuca     720
uccuagcucc agugcugguc ugaaaaauga ucuucuugaa aauuugcagg ccuauacagaa     780
acgaaugggg gugcagaugc aacgguuca gugaacuagu gacugacuag cccgcugggc     840
cuccaacgg gccuccucc ccuccuugca ccaaaaaaaaa aaaaaaaaaa aaaaaaaaaa     900
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa                          942

<210> SEQ ID NO 6
<211> LENGTH: 942
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Influenza
matrix: mRNA with increased G/C-content and
stabilisation sequences
<220> FEATURE:
<223> OTHER INFORMATION: The stabilisation sequences are derived from 5'
-and 3'-UTRs of a-globin-mRNA from Xenopus laevis,
respectively.
<220> FEATURE:
<223> OTHER INFORMATION: Start codon: aug (nucleotides 56 to 58), stop
codon: uga (nucleotides 812 to 814)

<400> SEQUENCE: 6
gcuuguucuu uuugcagaag cucagaauaa acgcucaacu uuggcagauc uaaagaugag      60
ccugcugacc gagguggaga ccuacgugcu gagcauaua cccagcggcc ccugaaggc     120
cgagaucgcc cagaggcugg aggacguguu cgccggcaag aacaccgacc uggaggugcu     180
gauggagugg cugaagacca ggcccauccu gagccccug accaagggca uccugggcuu     240
cguguuacac cugaccgugc ccagcgagcg cggccugcag cgccgccgcu ucgugcagaa     300

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cgcccggaac ggcaacggcg accccaacaa cauggacaag gccgugaagc uguacaggaa 360
gcugaagagg gagaucaccu uccacggcgc caaggagauc agccugagcu acagcgccgg 420
cgcccgggc agcugcaugg gccugaucua caacaggau ggcccguga ccaccgaggu 480
ggccuucggc cuggugugcg ccaccugcga gcagaucgcc gacagccagc accgcagcca 540
caggcagaug gugaccacca ccaacccccc gaucaggcac gagaacagga uggugcuggc 600
cagcaccacc gccaaggcca uggagcagau ggccggcagc agcgagcagg ccgcccaggc 660
cauggaggug gccagccagg ccaggcagau ggugcaggcc augaggacca ucggcaccca 720
ccccagcagc agcgccggcc ugaagaacga ccugcuggag aaccugcagg ccuaccagaa 780
gcgcaugggc gugcagaugc agcgcucaa gugaacuagu gacugacuag cccgcugggc 840
cucccaacgg gccuccucc ccuccuugca ccaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 900
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 942

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<210> SEQ ID NO 7

<211> LENGTH: 1011

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Influenza matrix: mRNA coding for secreted form with increased G/C-content and stabilisation sequences

<220> FEATURE:

<223> OTHER INFORMATION: Start codon: aug (nucleotides 56 to 58), stop codon: uga (nucleotides 881 to 883)

<400> SEQUENCE: 7

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gcuugucuu uuugcagaag cucagaauaa acgcucaacu uggcagauc uaaagauggc 60
cgucauggcc ccccgacccc uggugcugcu gcugagcggc gcccuggccc ugaccagac 120
cugggccagc cugcugaccg agguggagac cuacgugcug agcaucaucc ccagcgccc 180
ccugaaggcc gagaucgccc agaggcugga ggacguguuc gccggcaaga acaccgaccu 240
ggaggugcug auggaguggc ugaagaccag gcccauccug agccccuga ccaagggaau 300
ccugggcuuc guguuacccc ugaccgugcc cagcgagcgc ggccugcagc gccgcccguu 360
cgugcagaac gcccgaaac gcaacggcga cccaacaac auggacaagg ccgugaagcu 420
guacaggaag cugaagaggg agaucaccuu ccacggcgcc aaggagauca gccugagcua 480
cagcgccggc gcccugcca gcugcauggg ccugaucua aacaggauug gcgccgugac 540
caccgaggug gccuucggcc uggugugcgc caccugcgag cagaucgccg acagccagca 600
ccgcagccac aggcagaugg ugaccaccac caaccccug aucaggcacg agaacaggau 660
ggugcuggcc agcaccaccg ccaaggccau ggagcagaug gccggcagca gcgagcaggc 720
cgccgaggcc auggaggugg ccagccaggc caggcagaug gugcaggcca uaggaccacu 780
cggcaccac cccagcagca gcgcccggcc gaagaacgac cugcuggaga accugcaggc 840
cuaccagaag cgcauggggc ugcagaugca gcgcuucaag ugaacuagug acugacuagc 900
ccgcuggggc uccaacggg ccuccuccc cuccuugcac caaaaaaaaa aaaaaaaaaa 960
aaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 1011

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<210> SEQ ID NO 8

<211> LENGTH: 940

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

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<220> FEATURE:
<223> OTHER INFORMATION: MAGE1: wildtype-gene (for comparison)
<220> FEATURE:
<223> OTHER INFORMATION: Start codon: atg (nucleotides 5 to 7), stop
        codon: tga (nucleotides 932 to 934)

<400> SEQUENCE: 8

catcatgtct cttgagcaga ggagtctgca ctgcaagcct gaggaagccc ttgaggccca    60
acaagaggcc ctgggcctgg tgtgtgtgca ggctgccacc tcctcctcct ctctcttggt    120
cctgggcacc ctggaggagg tgcccactgc tgggtcaaca gatcctcccc agagtctctca    180
gggagcctcc gcctttccca ctaccatcaa cttcactcga cagaggcaac ccagtgaggg    240
ttccagcagc cgtgaagagg aggggccaag cacctcttgt atcctggagt ccttgttccg    300
agcagtaatc actaagaagg tggtctgatt ggttggtttt ctgctcctca aatatcgagc    360
cagggagcca gtcacaaagg cagaaatgct ggagagtgtc atcaaaaatt acaagcactg    420
ttttcttgag atcttcggca aagcctctga gtccttgag ctggtctttg gcattgacgt    480
gaaggaagca gacccccacc gccactccta tgccttgtc acctgcctag gtctctccta    540
tgatggcctg ctgggtgata atcagatcat gccaagaca ggcttcctga taattgtcct    600
ggtcattgatt gcaatggagg gcggccatgc tcctgaggag gaaatctggg aggagctgag    660
tgtgatggag gtgtatgatg ggagggagca cagtgcctat ggggagccca ggaagctgct    720
cacccaagat ttggtgcagg aaaagtacct ggagtaccgg caggtgccgg acagtgatcc    780
cgacgctat  gagttctctg tgggtccaag ggccctcgct gaaaccagct atgtgaaagt    840
ccttgagtat gtgatcaagg tcagtgaag agttcgcttt ttcttcccat ccctgcgtga    900
agcagctttg agagaggagg aagagggagt ctgagcatga    940

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<210> SEQ ID NO 9
<211> LENGTH: 308
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Tumor antigen MAGE1: protein sequence

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<400> SEQUENCE: 9

Ser Leu Glu Gln Arg Ser Leu His Cys Lys Pro Glu Glu Ala Leu Glu
1             5             10            15

Ala Gln Gln Glu Ala Leu Gly Leu Val Cys Val Gln Ala Ala Thr Ser
20            25            30

Ser Ser Ser Pro Leu Val Leu Gly Thr Leu Glu Glu Val Pro Thr Ala
35            40            45

Gly Ser Thr Asp Pro Pro Gln Ser Pro Gln Gly Ala Ser Ala Phe Pro
50            55            60

Thr Thr Ile Asn Phe Thr Arg Gln Arg Gln Pro Ser Glu Gly Ser Ser
65            70            75            80

Ser Arg Glu Glu Glu Gly Pro Ser Thr Ser Cys Ile Leu Glu Ser Leu
85            90            95

Phe Arg Ala Val Ile Thr Lys Lys Val Ala Asp Leu Val Gly Phe Leu
100           105           110

Leu Leu Lys Tyr Arg Ala Arg Glu Pro Val Thr Lys Ala Glu Met Leu
115           120           125

Glu Ser Val Ile Lys Asn Tyr Lys His Cys Phe Pro Glu Ile Phe Gly
130           135           140

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Lys Ala Ser Glu Ser Leu Gln Leu Val Phe Gly Ile Asp Val Lys Glu
 145 150 155 160
 Ala Asp Pro Thr Gly His Ser Tyr Val Leu Val Thr Cys Leu Gly Leu
 165 170 175
 Ser Tyr Asp Gly Leu Leu Gly Asp Asn Gln Ile Met Pro Lys Thr Gly
 180 185 190
 Phe Leu Ile Ile Val Leu Val Met Ile Ala Met Glu Gly Gly His Ala
 195 200 205
 Pro Glu Glu Glu Ile Trp Glu Glu Leu Ser Val Met Glu Val Tyr Asp
 210 215 220
 Gly Arg Glu His Ser Ala Tyr Gly Glu Pro Arg Lys Leu Leu Thr Gln
 225 230 235 240
 Asp Leu Val Gln Glu Lys Tyr Leu Glu Tyr Arg Gln Val Pro Asp Ser
 245 250 255
 Asp Pro Ala Arg Tyr Glu Phe Leu Trp Gly Pro Arg Ala Leu Ala Glu
 260 265 270
 Thr Ser Tyr Val Lys Val Leu Glu Tyr Val Ile Lys Val Ser Ala Arg
 275 280 285
 Val Arg Phe Phe Phe Pro Ser Leu Arg Glu Ala Ala Leu Arg Glu Glu
 290 295 300
 Glu Glu Gly Val
 305

<210> SEQ ID NO 10

<211> LENGTH: 939

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: MAGE1: mRNA with increased G/C-content

<220> FEATURE:

<223> OTHER INFORMATION: Start codon: aug (nucleotides 1 to 3), stop codon: uga (nucleotides 937 to 939)

<400> SEQUENCE: 10

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augagccugg agcagcgag ccugcacugc aagccggagg aggcgcugga ggcgcagcag      60
gaggcgcuugg gccuggucug cguccaggcg ggcagagaca gcagcagccc gcugguccug    120
ggcacgcugg agggaguccc gacggcgggc agcacggacc cggcgagag cccgcagggc      180
gcgagcgcuu ucccagcagc gaucacuuu acgcgccagc gccagccgag cgagggcagc      240
agcagccgag agggaggggg cccgagcagc agcugcaucc uggagagccu guuccgcgag      300
gucaucacga agaaggucgc ggaccugguc ggcuuccugc ugcugaagua ccgcgcgcg      360
gagccgguca cgaaggcgga gaugcuggag agcgucauca agaacuaca gcacugcuuc      420
ccggagauau ucggcaaggc gagcgagagc cugcagcugg ucuucggcau cgacgucaag      480
gaggcggaac cgacgggcca cagcuacguc cuggucacgu gccugggccc gagcuacgac      540
ggcucgcuug ggcacaacca gaucgucgg aagacgggcu uccugaucau cguccugguc      600
augaucgcau uggaggggcg ccacgcgccg gaggaggaga ucugggagga gcugagcguc      660
auggaggucu acgacggcgg cgagcacagc gcguacggcg agccgcgcaa gcugcugacg      720

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caggaccugg uccaggagaa guaccuggag uaccgccagg ucccgacag cgacccggcg 780
cgcuacgagu uccugugggg cccgcgcgcg cuggccgaga cgagcuacgu caagguccug 840
gaguacguca ucaaggucag cgcgcgcguc cgcuuuuu ucccagaccu gcgcgaggcg 900
gcgcugcgcg aggaggagga gggcgucuga gcgugauga 939

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<210> SEQ ID NO 11
<211> LENGTH: 939
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: MAGE1: mRNA
with alternative use of codon
<220> FEATURE:
<223> OTHER INFORMATION: Start codon: aug (nucleotides 1 to 3), stop
codon: uga (nucleotides 937 to 939)

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<400> SEQUENCE: 11

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augagccugg agcagcgag ccugcacugc aagcccagg aggccugga ggcccagcag 60
gaggccugg gccugugug cgugcaggcc gccaccagca gcagcagccc ccuggugcug 120
ggcaccugg aggaggugcc caccgcgcgc agcaccgacc ccccccagag ccccccaggc 180
gccagcgccu uccccaccac caucaacu acccgccagc gccagcccag cgagggcagc 240
agcagccgcg aggaggagg cccagcacc agcugcaucc uggagagccu guuccgcgc 300
gugaucacca agaaggugc cgaccuggug ggcuuucugc ugcugaagua ccgcgcccgc 360
gagcccguga ccaaggccga gaugcuggag agcgugauga agaacucaa gcacugcuuc 420
cccgaagcu ucggcaaggc cagcgagagc cugcagcugg uguucggcau cgacgugaag 480
gaggccgacc ccaccggcca cagcuacgug cuggugaccu gccugggccc gagcuacgac 540
ggccugcugg gcgacaacca gaucaugccc aagaccggcu uccugaucan cgugcuggug 600
augaucgcca uggaggggcg ccacgcccc gagggaggaga ucugggagga gcugagcgug 660
auggaggugu acgacggccg cgagcacagc gccuacggcg agccccgcaa gcugcugacc 720
caggaccugg ugcaggagaa guaccuggag uaccgccagg ugcccagacag cgaccccgcc 780
cgcuacgagu uccugugggg ccccgcgccc cuggccgaga ccagcuacgu gaaggugcug 840
gaguacguga ucaaggugag cgcgcgcgug cgcuuuuu ucccagaccu gcgcgaggcg 900
gcccgcgcg aggaggagga gggcguguga gccugauga 939

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<210> SEQ ID NO 12
<211> LENGTH: 7
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Sequence
motif recognizable for an endonuclease contained in the
3'UTR-segment of the gene coding for the
transferrin receptor (see p.10 of description)

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<400> SEQUENCE: 12

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gaacaag

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7

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<210> SEQ ID NO 13
<211> LENGTH: 13
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Kozak sequence, ribosome binding site (see p. 12 of description)

<400> SEQUENCE: 13

gccgccacca ugg

13

1. Modified mRNA coding for at least one viral peptide or polypeptide, characterised in that the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is increased compared to the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide, and the encoded amino acid sequence is unchanged as compared to the wild type.

2. Modified mRNA according to claim 1, characterised in that the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is increased by at least 7% points, preferably at least 15% points, compared to the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide.

3. Modified mRNA according to claim 1, characterised in that the modified mRNA comprises a 5' cap structure and/or a poly-A tail of at least 70 nucleotides and/or an IRES and/or a 5' stabilisation sequence and/or a 3' stabilisation sequence.

4. Modified mRNA according to claim 1, characterised in that the modified mRNA comprises at least one analogue of naturally occurring nucleotides.

5. Modified mRNA according to claim 4, characterised in that the analogue is selected from the group consisting of phosphorus thioates, phosphorus amidates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine.

6. Modified mRNA according to claim 1, characterised in that the viral antigen derives from the secreted form of a surface antigen.

7. Modified mRNA according to claim 1, characterised in that the mRNA codes for a surface antigen of a pathogenic viral germ.

8. Modified mRNA according to claim 1, characterised in that the mRNA is associated with a cationic peptide or protein or is bound thereto.

9. Modified mRNA according to claim 8, characterised in that the cationic peptide or protein is selected from the group consisting of protamine, poly-L lysine, and histones.

10. Modified mRNA according to claim 1, characterised in that the polypeptide is a polypeptide of viral antigens.

11. Modified mRNA according to claim 1, characterised in that the modified mRNA is a multicistronic mRNA.

12. Modified mRNA according to claim 1, characterised in that the mRNA in addition codes for at least one cytokine.

13. Modified mRNA according to claim 1, characterised in that the multicistronic RNA comprises more than one IRES sequence, wherein the IRES sequences are in particular selected from picorna viruses (e.g. FMDV), plague viruses (CFFV), polio viruses (PV), encephalo myocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classic swine fever viruses (CSFV), murine leukemia virus (MLV), simian immunodeficiency viruses (SIV), or cricket paralysis viruses (CrPV).

14. Pharmaceutical composition characterized in that it contains a modified mRNA according to claim 1 in combination with a pharmaceutically acceptable carrier and/or vehicle.

15. Pharmaceutical composition according to claim 14, characterised in that the pharmaceutical composition contains at least one immune response stimulating adjuvant.

16. Pharmaceutical composition according to claim 14, which in addition contains at least one cytokine.

17. A vaccine for inoculation against viral infectious diseases comprising a modified mRNA according to claim 1.

18. Use of a pharmaceutical composition according to claim 17 for the preparation of a vaccine for inoculation against infections caused by AIDS, hepatitis A, B or C, Herpes, Herpes zoster, Dengue, haemorrhagic infection, Yellow fever and influenza.

* * * * *