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Abstract

Pharmaceutical Composition containing a stabilised mRNA optimised for translation in its Coding Regions

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 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 modifications that serve for the stabilisation and translation optimisation of mRNA.

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Pharmaceutical Composition containing a stabilised mRNA optimised for translation in the Coding Regions thereof

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 the translation. The pharmaceutical composition according to the invention is suitable in particular as an inoculating agents and also as a therapeutic agent for tissue regeneration. Furthermore a process for determining sequence modifications that serve for the stabilisation and optimisation of the translation of mRNA is disclosed.

Gene therapy and genetic vaccination are molecular medicine processes whose use in the treatment and prevention of diseases will have considerable effects on medical practice. Both processes 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.

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, polyprene transfection, protoplast fusion, electroporation, microinjection and lipofection, in which connection lipofection in particular has proved to be a suitable process.

A further process that has been suggested in particular in the case of genetic vaccination processes is the use of DNA viruses as DNA vehicle. Such viruses have the advantage that, on account of their infectious properties, a very high transfection rate can be achieved. The viruses used are genetically altered so that no functional infectious particles are formed in the transfected cell. Despite this precautionary measure however a certain risk of the uncontrolled propagation of the introduced genetically therapeutically active as well as viral genes cannot be excluded on account of possible recombination events.

Normally the DNA incorporated into the 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 the gene therapy. Thus, this may for example result in an insertion of the incorporated DNA into an intact gene, which produces a mutation that interferes with or indeed completely switches off the function of the endogenous gene. As a result of such integration events enzyme systems that are important for cellular life may on the one hand be switched off, while on the other hand there is also the danger of a transformation of the thus altered cell into a degenerate state if, due to the integration of

the foreign DNA, a gene that is decisive for regulating cell growth is altered. Accordingly, when using DNA viruses as gene 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 the 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 lead to undesirable changes in the regulation of the gene expression in the cell.

A further disadvantage of the use of DNA as gene therapeutic agents and vaccines is the induction of pathogenic anti-DNA antibodies in the patient, resulting in a possible fatal immune response.

In contrast to DNA the use of RNA as a gene therapeutic agent or vaccine is regarded as being significantly safer. In particular RNA does not bring with it the danger of becoming stably integrated into the genome of the transfected cell. In addition, no viral sequences such as promoters are necessary for the effective transcription. Over and above this, RNA is degraded significantly more readily *in vivo*. Indeed, on account of the relatively short half-life of RNA in the blood circulation compared to DNA, up to now no anti-RNA antibodies have been detected. For this reason RNA may be regarded as the molecule of choice for molecular medicine therapeutic processes.

However, some basic problems still have to be solved before medical processes 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 processes that are used for DNA.

Enzymes that break down RNA, so-called RNAases (ribonucleases), are responsible for the instability. Even minute contamination by ribonucleases is sufficient to break down RNA completely in solution. The natural decomposition of mRNA in the cytoplasm of cells is extremely finely regulated. Several mechanisms are known in this respect. Thus, for a functional mRNA the terminal structure 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 these structures and the decomposition is regulated. In addition there are further processes that stabilise or destabilise RNA. Many of these processes are still unknown, though often an interaction between the RNA and proteins appears to be decisive in this regard. For example, an mRNA surveillance system has recently 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, a main part of these processes being completed by exonucleases.

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

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, in which the RNA is present in the form of a complex with a cationic peptide or protein.

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 involve on the one hand stabilisation by sequence modifications, in particular reduction of the C content and/or U content by base elimination or base substitution. On the other hand chemical modifications are proposed, 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.

In US patents US 5,580,859 and US 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 for raising the translation efficiency and the mRNA stability, that relate in particular to the non-translated sequence regions.

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 that it corresponded to the use of codons that can be found in highly expressed mammalian genes. In this way in particular the A/T content compared to the wild type sequence was reduced. The authors find in particular an increased rate of expression of the synthetic *gag* gene in transfected cells. Furthermore, in mice an increased antibody formation against the *gag* protein was observed in the case of mice immunised with the synthetic DNA construct and also an increased cytokine release in vitro in the case of transfected spleen cells of mice. 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 basically to a change of the nucleocytoplasmic transport of the mRNA expressed by the DNA vaccine, brought about by the optimised codon usage. On the other hand the authors maintain that the effect of the altered codon usage on the translation efficiency is only slight.

The object of the present invention is accordingly 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.

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

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In particular a modified mRNA as well as a pharmaceutical composition containing at least an mRNA modified in this way in combination with a pharmaceutically compatible carrier and/or vehicle are provided, in which the modified mRNA codes for at least one biologically active or antigenic peptide or polypeptide, wherein the sequence of the mRNA, in particular in the region coding for the at least one peptide or polypeptide, comprises the following modifications compared to the wild type mRNA, which may be present either individually or in combination.

On the one hand the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is larger than the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide, the coded amino acid sequence being unchanged compared to the wild type.

This modification is based on the fact that, for an 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 rôle. In particular sequences with an increased G(guanosine)/C(cytosine) content are more stable than sequences with an increased A(adenosine)/U(uracil) content. Accordingly, according to 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 codons code for one and the same amino acid (degeneration of the genetic code), the

codons most favourable for the stability can be determined (alternative codon usage).

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 coded 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.

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 may be changed from CCU or CCA to CCC or CCG;

the codons for Arg may be changed from CGU or CGA or AGA or AGG to CGC or CGG;

the codons for Ala may be changed from GCU or GCA to GCC or GCG;

the codons for Gly may be changed from GGU or GGA to GGC or GGG.

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 may be changed from UUU to UUC;

the codons for Leu may be changed from UUA, CUU or CUA to CUC or CUG;

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

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

The substitutions listed above may obviously be used individually but also in all possible combinations in order to increase the G/C content ratio of the 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 originally 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

originally 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 originally for Glu to GAG and substitution of all codons coding originally for Ala to GCC (or GCG) and substitution of all codons coding originally 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 originally for Glu to GAG and substitution of all codons coding originally for Ala to GCC (or GCG) and substitution of all codons coding originally for Gly to GGC (or GGG) and substitution of all codons coding originally 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 originally for Phe to UUC and substitution of all codons coding originally for Cys to UGC and substitution of all codons coding originally for Leu to CUG (or CUC) and substitution of all codons coding originally for Gln to CAG and substitution of all codons coding originally for Pro to CCC (or CCG); etc.

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

In this connection it is particularly preferred to increase by the maximum possible amount the G/C content of the

modified mRNA, in particular in the region coding for the at least one peptide or polypeptide, compared to the wild type sequence.

The further modification according to the invention of the mRNA contained in the pharmaceutical composition characterised in the present invention is based on the knowledge that the translation efficiency is also determined by a different frequency in the occurrence of tRNAs in cells. If therefore so-called "rare" codons are frequently present in an RNA sequence, then the corresponding mRNA is translated significantly worse than in the case when codons coding for relatively "frequent" tRNAs are present.

Thus, according to the invention in the modified mRNA (which is contained in the pharmaceutical composition) the region coding for the peptide or polypeptide is changed compared to the corresponding region of the wild type mRNA in such a way that at least one codon of the wild type sequence that codes for a relatively rare tRNA in the cell is exchanged for a codon that codes for a relatively frequent tRNA in the cell that carries the same amino acid as the relatively rare tRNA.

Through this modification the RNA sequences are modified so that codons are inserted that are available for the frequently occurring tRNAs.

Which tRNAs occur relatively frequently in the cell and which on the other hand occur relatively seldom is known to the person skilled in the art; see for example Akashi, Curr. Opin. Genet. Dev. 2001, 11(6): 660-666.

By means of this modification, according to the invention all codons of the wild type sequence that code for a relatively rare tRNA in the cell may in each case be exchanged for a codon that codes for a relatively frequent tRNA in the cell that in each case carries the same amino acid as the relatively rare tRNA.

According to the invention it is particularly preferred to couple the sequential G/C fraction that according to the invention is increased, in particular maximally increased, in the modified mRNA, with the "frequent" codons, without changing the amino acid sequence of the peptide or polypeptide (one or more) coded by the coding region of the mRNA. This preferred embodiment provides a particularly efficiently translated and stabilised mRNA, for example for the pharmaceutical composition according to the invention.

In the sequences of eukaryotic mRNAs there are destabilising sequence elements (DSE) to which signal proteins bind and regulate the enzymatic decomposition of the mRNA *in vivo*. Accordingly, for the further stabilisation of the modified mRNA contained in the pharmaceutical composition according to the invention one or more changes compared to the corresponding region of the wild type mRNA are carried out if necessary in the region coding for the at least one peptide or polypeptide, so that no destabilising sequence elements are contained. Obviously it is also preferred according to the invention to eliminate from the mRNA DSE possibly present in the non-translated regions (3' and/or 5' UTR).

Such destabilising sequences are for example AU-rich sequence ("AURES") that occur in 3'-UTR sections of numerous unstable mRNAs (Caput et al., Proc. Natl. Acad. Sci. USA 1986, 83: 1670 to 1674). The RNA molecules contained in the pharmaceutical composition according to the invention are therefore preferably altered compared to the wild type mRNA so that they do not have any such destabilising sequences. This also applies to those sequence motifs that are recognised by possible endonucleases, for example the sequence GAACAAG, that is contained in the 3'UTR segment of the gene coding for the transferin receptor (Binder et al., EMBO J. 1994, 13: 1969 to 1980). These sequence motifs too are preferably eliminated in the modified mRNA of the pharmaceutical composition according to the invention.

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), for example the whole mRNA may be chemically synthesised using standard techniques.

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

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 the *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 by fermentative replication and subsequent isolation as part of a plasmid replicable in bacteria. As plasmids suitable for the present invention there may for example be mentioned the plasmids pT7Ts (GeneBank Accession No. U26404; Lai et al., Development 1995, 121: 2349 to 2360), the pGEM® series, for example pGEM®-1 (GeneBank Accession No. X65300; from Promega) and pSP64 (GeneBank-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, FL, 2001.

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 or multiple copy, by digestion with restriction endonucleases.

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). As examples of cap structures there may be mentioned m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G.

According to a further preferred embodiment of the present invention the modified mRNA contains 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.

For an efficient translation of the mRNA an effective binding of the ribosomes to the ribosome binding site (Kozak sequence: GCCGCCACC**AUG**G, the **AUG** forms the start codon) is furthermore necessary. In this regard it has been established that an increased A/U content around this site permits a more efficient ribosome binding to the mRNA.

In addition it is possible to introduce one or more so-called IRES ("internal ribosomal entry site") into the modified mRNA. An IRES may thus act as the sole ribosome binding site, but may however also serve for the provision of an mRNA that codes several peptides or polypeptides that are to be translated independently of one another by the ribosomes ("multicistronic mRNA"). Examples of IRES sequences that can be used according to the invention are 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 leukoma virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

According to a further preferred embodiment of the present invention the modified mRNA contains 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.

These stabilisation sequences may exhibit a 100% sequence homology with naturally occurring sequences that are present in viruses, bacteria and eukaryotic cells, but may however also be partly or completely synthetic. As an example of stabilising sequences that may be used in the present invention, the non-translated sequences (UTR) of the β-globin gene, for example of *Homo sapiens* or *Xenopus laevis*, may be mentioned. 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 mRNA that codes for α-globin, α-(I)-collagen, 15-lipoxygenase or for tyrosine hydroxylase (c.f. Holcik et al., Proc. Natl. Acad. Sci. USA 1997, 94: 2410 to 2414). Obviously such stabilisation sequences may be used individually or in combination with one another as well as in combination with other stabilisation sequences known to the person skilled in the art.

For the further stabilisation of the modified mRNA it is also preferred if this contains at least one analogue of naturally occurring nucleotides. That is based on the fact that the RNA-decomposing enzymes present in the cells recognise as substrate preferably naturally occurring nucleotides. By insertion of nucleotide analogues the RNA decomposition can therefore be made more difficult, whereby the effect on the translation efficiency when inserting these analogues, in particular into the coding region of the mRNA, may have a positive or negative effect on the translation efficiency.

In a by no means exhaustive list the following may be mentioned as examples of nucleotide analogues that can be used according to 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 US patents 4,373,071, US 4,401,796, US 4,415,732, US 4,458,066, US 4,500,707, US 4,668,777, US 4,973,679, US 5,047,524, US 5,132,418, US 5,153,319, US 5,262,530 and 5,700,642. According to the invention such analogues may be present in non-translated and translated regions of the modified mRNA.

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. In addition it is also possible to use other cationic peptides or proteins such as poly-L-lysine or histones. This procedure for the stabilisation of the modified mRNA is described in EP-A-1083232, whose relevant disclosure is included in full in the present invention.

In the gene therapy use of the pharmaceutical composition according to the invention (or in the use of the mRNA for gene therapy or in the use of the mRNA for the preparation of a pharmaceutical composition for gene therapy) the modified mRNA 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. Accordingly, examples of polypeptides coded by

the mRNA according to the invention include 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, α-1-antitrypsin, etc. Besides this the pharmaceutical composition may be used for the release of cell surface receptors and molecules that bind to such receptors if the modified mRNA contained in the pharmaceutical composition codes for such a biologically active protein or peptide. Examples of such proteins 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-CFS), 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 a tissue degeneration, for example neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc. and other degenerative conditions, for example arthrosis, can be treated. In these cases the modified mRNA, in particular that contained in the pharmaceutical composition of the present invention, preferably codes for growth factors from the TGF-β family, in which in particular EGF, FGF, PDGF, BMP, GDNF, BDNF, GDF and neurotrophic factors such as NGF, neutrophines, etc., may be mentioned.

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A further area of application of the present invention is vaccination, i.e. the use of the modified mRNA for inoculation or the use of the pharmaceutical composition as an inoculating agent or the use of the modified mRNA for the preparation of the pharmaceutical composition for inoculation purposes. Vaccination is based on introducing an antigen, in the present case the genetic information for the antigen in the form of the modified mRNA coding for the antigen, into the organism, in particular into the cell. 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, whereby an immune response directed against this polypeptide or antigenic peptide is stimulated. In vaccination against a pathogenic organism, i.e. a virus, a bacterium or a protozoological organism, a surface antigen of such an organism is therefore used for the vaccination with the aid of the pharmaceutical composition according to the invention containing the modified mRNA coding for the said surface antigen. In case of use as a genetic vaccine for treating cancer, the immune response is achieved by incorporating the genetic information for tumour antigens, in particular proteins that are expressed exclusively on cancer cells, in which a pharmaceutical composition according to the invention is administered that contains an mRNA coding for such a cancer antigen. In this way the cancer antigen(s) is/are expressed in the organism, whereby an immune response is produced that is directed effectively against the cancer cells.

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 carcinoma, lymphomas, sarcomas, small-cell lung carcinomas, blastomas, etc. 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-R170I, 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/RARα, PRAME, PSA, PSM, RAGE, RU1 or RU2, SAGE, SART-1 or SART-3, TEL/AML1, TPI/m, TRP-1, TRP-2, TRP-2/INT2 and WT1. In addition to this the pharmaceutical composition according to the invention is 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), or protozoological 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 coded by the modified mRNA. With the aforementioned genes of pathogenic germs 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, these polypeptides being polyepitopes, for example of the aforementioned antigens, in particular surface antigens of pathogenic germs or organisms, or tumour cells, preferably secreted protein forms.

In addition to this, the modified mRNA according to the invention may contain, besides the antigenic or the genetically therapeutically active peptide or polypeptide, also at least one further functional section that codes for example for a cytokine promoting the immune response (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-CFS, LT-α or growth factors such as hGH).

Furthermore, in order to increase the 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 a specific immune response. Various mechanisms may be involved in this connection, depending on the various types of adjuvants. For example, compounds that promote through dentritic cells (DC) an endocytosis of the modified mRNA contained in the pharmaceutical composition form a first class of usable adjuvants. Other compounds that permit the maturation of the DC, for example lipopolysaccharides, TNF-α or CD40 ligand, comprise a further class of suitable adjuvants. In general each agent influencing in the manner of a "danger signal" the immune system (LPS, GP96, oligonucleotides with the CpG motif) or cytokines such as GM-CSF, may be used as

adjuvant, which enables an immune response against an antigen that is coded by the modified mRNA to be increased and/or specifically influenced. The aforementioned cytokines are particularly preferred in this connection. Further known adjuvants include aluminium hydroxide, Freund's adjuvant as well as the aforementioned stabilising cationic peptides or polypeptides such as protamine. In addition to this 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.

The pharmaceutical composition according to the invention contains, apart from the modified mRNA, also 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), all of which form part of the disclosure of the present invention. 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

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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 the physical behaviour, for example with regard to 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 depôt 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.

The nature and manner of the administration and the dosage of the pharmaceutical composition according to the invention depend on the medical condition to be treated and its stage of progression, as well as on the body weight, age and sex of the patient.

The concentration of the modified mRNA in such formulations may therefore vary within a wide range from 1 µg 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.

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 patient, in particular a human patient.

In addition a method is also provided that serves for the determination of the modified sequence of the mRNA contained in the pharmaceutical composition according to the invention. In this connection, according to the invention the adaptation of the RNA sequences is carried out with two different optimisation objectives: on the one hand with the largest possible G/C content, and on the other hand taking into account as far as possible the frequency of the tRNAs according to codon usage. 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, on account of the degenerated genetic code, possible choices for the corresponding codons. Depending on the required optimisation or

modification, corresponding selection lists and optimisation algorithms are used for choosing the suitable codons. The algorithms are executed on a computer, normally with the aid of suitable software. 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 as regards the frequency of the individual nucleotides. The changes compared to the original nucleotide sequence are preferably emphasised. Furthermore, according to a preferred embodiment stable sequences known in nature are read in, which can form the basis for an RNA stabilised according to natural sequence motifs. A secondary structure analysis may also be provided that can analyse, on the basis of structural calculations, stabilising and destabilising properties or regions of the RNA.

In the Figures:

Fig. 1 shows wild type sequences and modified sequences for the influenza matrix protein.
Fig. 1A shows the wild type gene and Fig. 1B shows the amino acid sequence derived therefrom (1-letter code).
Fig. 1C shows a gene sequence coding for the influenza matrix protein, whose G/C content is raised compared to the wild type sequence. Fig. 1D shows the sequence of a gene that codes for a secreted form of the influenza matrix protein (including an N-terminal signal sequence), the G/C content of the sequence being raised compared to the wild type sequence. Fig. 1E shows an mRNA coding for the influenza matrix protein that contains stabilising sequences compared to the wild type mRNA. Fig. 1F shows an

mRNA coding for the influenza matrix protein that in addition to stabilising sequences also contains an increased G/C content.

Fig. 1G likewise shows a modified mRNA that codes for the secreted form of the influenza matrix protein and comprises, compared to the wild type, stabilising sequences and a raised 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 compared to the wild type sequence of Fig. 1A are shown in capital letters in 1C to 1G.

Fig. 2 shows wild type sequences and modified sequences according to the invention that code for the tumour antigen MAGE1.

Fig. 2A shows the sequence of the wild type gene and Fig. 2B shows the amino acid sequence derived therefrom (3-letter code). Fig. 2C shows a modified mRNA coding for MAGE1, whose G/C content is raised compared to the wild type. Fig. 2D shows the sequence of a modified mRNA coding for MAGE1, in which the codon usage has been optimised as frequently as possible in the tRNA present in the cell, with regard to the coding. Start and stop codons are shown in each case in bold type.

The following examples describe the invention in more detail without however restricting the scope thereof.

Example 1

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, with the

aid of the genetic code or its degenerative nature, the nucleotide sequence of an arbitrary mRNA in such a way that a maximum G/C content in conjunction with the use of codons that code for tRNAs occurring as frequently as possible in the cell, is obtained, in which the amino acid sequence coded by the modified mRNA is identical compared to the unmodified sequence. Alternatively only the G/C content or only the codon usage compared to the original sequence may also be modified.

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 Annexe..

Example 2

An RNA construct with a sequence of the lac-Z gene from *E.coli* optimised as regards stabilisation and translation 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) could be achieved in this way. Through the synthesis of overlapping oligonucleotides that comprise the modified sequence, the optimised sequence was produced according to methods known in the prior art. The terminal oligonucleotides have the following restriction cleavage sites: at the 5' end an *Eco*RV cleavage site, and at the 3' end a *Bgl*II cleavage site. The modified lacZ sequence was incorporated into the plasmid pT7Ts (GeneBank Accession No. U 26404; c.f. Lai et al., see above) by digestion with *Eco*RV/*Bgl*II. pT7Ts contains as non-translated regions sequences from the β-globin gene of *Xenopus laevis*, in each case, at 5' and 3'. The plasmid

was cleaved with the aforementioned restriction enzymes before the insertion of the modified lacZ sequence.

The pT7Ts-lac-Z construct was propagated in bacteria and purified by phenol-chloroform extraction. 2 µg of the construct were transcribed *in vitro* by means of methods known to the person skilled in the art, whereby the modified mRNA was produced.

Example 3

The gene for the influenza matrix protein (wild type sequence, see Fig. 1A, derived amino acid sequence 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 was thereby formed. A G/C-rich sequence coding for the secreted form of the influenza matrix protein and which codes for an N-terminal signal sequence was also determined (see Fig. 1D). The secreted form of the influenza matrix protein has the advantage of an increased immunogenicity compared to the non-secreted form.

Corresponding mRNA molecules were designed starting from the optimised sequences. The mRNA for the influenza matrix protein, optimised as regards 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 *Xenupus laevis*; c.f. pT7Ts-Vektor in Lai et al., see above) (see Figs. 1E and 1F). 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).

Example 4

The mRNA coding for the tumour antigen MAGE1 was modified with the aid of the computer program of Example 1. The sequence shown in Fig. 2C was determined in this way, and has a 24% higher G/C content (351 G, 291 C) compared to the wild type sequence (275 G, 244 G). In addition, by means of alternative codon usage, the wild type sequence was improved as regards the translation efficiency on the basis of the coding of tRNAs occurring more frequently in the cell (see Fig. 2D). The G/C content was likewise raised by 24% by the alternative codon usage.

Appendix: Source text of a computer program according to the invention

Curevac_Genetic_Controls with the following modules

Name: Curevac_Genetic_Controls.vbp
Code:

```
Type=Control
Reference=*\G{00020430-0000-0000-C000-000000000046}#2.0#0#..\..\WINNT\System32\STDOLE2.TLB#OLE Automation
Object={0D452EE1-E08F-101A-852E-02608C4D0BB4}#2.0#0; FM20.DLL
Object={F9043C88-F6F2-101A-A3C9-08002B2F49FB}#1.2#0; COMDLG32.OCX
UserControl=Curevac_Amino.ctl
Startup="(None)"
HelpFile=""
Title="Curevac Genetic Controls"
Command32=""
Name="Curevac_Genetic_Controls"
HelpContextID="0"
CompatibleMode="1"
MajorVer=1
MinorVer=0
RevisionVer=0
AutoIncrementVer=0
ServerSupportFiles=0
VersionComments="the RNA people"
VersionCompanyName="CureVac GmbH"
VersionFileDescription="Controls for Handling of Nucleotids"
VersionLegalCopyright="by Christian Klump"
VersionLegalTrademarks="Curevac Genetic Controls(tm)"
VersionProductName="Curevac Genetic Controls"
CompilationType=0
OptimizationType=0
FavorPentiumPro(tm)=0
CodeViewDebugInfo=0
NoAliasing=0
BoundsCheck=0
OverflowCheck=0
FlPointCheck=0
FDIVCheck=0
UnroundedFP=0
StartMode=1
Unattended=0
Retained=0
ThreadPerObject=0
MaxNumberOfThreads=1
ThreadingModel=1
```

```
DebugStartupOption=1
DebugStartupComponent=Curevac_Amino
```

Name: Curevac_Amino.ctl
Code:

```
VERSION 5.00
Object = "{0D452EE1-E08F-101A-852E-02608C4D0BB4}#2.0#0"; "FM20.DLL"
Begin VB.UserControl Curevac_Amino
   CanGetFocus    =  0  'False
   ClientHeight   =  690
   ClientLeft     =  0
   ClientTop      =  0
   ClientWidth    =  1200
   ScaleHeight    =  690
   ScaleWidth     =  1200
   Begin VB.Line linLower
      X1          =  0
      X2          =  1080
      Y1          =  600
      Y2          =  600
   End
   Begin VB.Line linUpper
      X1          =  120
      X2          =  1080
      Y1          =  0
      Y2          =  0
   End
   Begin MSForms.Label lblAminoAcid
      DragMode    =  1  'Automatic
      Height      =  255
      Left        =  120
      TabIndex    =  1
      Top         =  360
      Width       =  975
      Size        =  "1720;450"
      SpecialEffect =  1
      FontName    =  "Lucida Sans Unicode"
      FontEffects =  1073741826
      FontHeight  =  165
      FontCharSet =  0
      FontPitchAndFamily=  2
      ParagraphAlign =  3
   End
   Begin MSForms.Label lblTriplett
      DragMode    =  1  'Automatic
      Height      =  255
      Left        =  120
```

```
        TabIndex      = 0
        Top           = 120
        Width         = 975
        Size          = "1720;450"
        SpecialEffect = 1
        FontHeight    = 165
        FontCharSet   = 0
        FontPitchAndFamily= 2
        ParagraphAlign = 3
    End
End
Attribute VB_Name = "Curevac_Amino"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = True
Attribute VB_PredeclaredId = False
Attribute VB_Exposed = True
Option Explicit

Private msAAShortcut As String
Private msAAName As String
Private msBestTriplett As String
Private msSecondBest As String
Private msThirdBest As String
Private msTriplett As String
Private msBackColor As Long
Private mbShowOriginal As Boolean


Public Enum enuAminoAcid
    amaGlycin
    amaAlanin
    amaValin
    amaLeucin
    amaIsoLeucin
    amaPhenylalanin
    amaTyrosin
    amaTryptophan
    amaAsparaginAcid
    amaAsparagin
    amaGlutaminAcid
    amaGlutamin
    amaSerin
    amaThreonin
    amaCystein
    amaMethionin
    amaProlin
    amaHistidin
    amaLysin
    amaStop
```

```
    amaStart
End Enum

Private Sub Main()

    Call UserControl_Resize

End Sub

Public Sub Translation(ByVal sTriplett As String)

    msTriplett = sTriplett

    Select Case sTriplett
        Case "GGU", "GGC", "GGA", "GGG"
            msAAShortcut = "GLY"
        Case "GCU", "GCC", "GCA", "GCG"
            msAAShortcut = "ALA"
        Case "GUU", "GUC", "GUA", "GUG"
            msAAShortcut = "VAL"
        Case "UUA", "UUG", "CUU", "CUA", "CUG", "CUC"
            msAAShortcut = "LEU"
        Case "AUA", "AUU", "AUC"
            msAAShortcut = "ILE"
        Case "UUU", "UUC"
            msAAShortcut = "PHE"
        Case "UAU", "UAC"
            msAAShortcut = "TYR"
        Case "UGG"
            msAAShortcut = "TRP"
        Case "GAU", "GAC"
            msAAShortcut = "ASP"
        Case "AAU", "AAC"
            msAAShortcut = "ASN"
        Case "GAA", "GAG"
            msAAShortcut = "GLU"
        Case "CAA", "CAG"
            msAAShortcut = "GLN"
        Case "AGU", "AGC", "UCA", "UCU", "UCG", "UCC"
            msAAShortcut = "SER"
        Case "ACA", "ACU", "ACG", "ACC"
            msAAShortcut = "THR"
        Case "UGU", "UGC"
            msAAShortcut = "CYS"
        Case "AUG"
            msAAShortcut = "MET"
        Case "CCA", "CCU", "CCG", "CCC"
            msAAShortcut = "PRO"
        Case "CAU", "CAC"
```

```
            msAAShortcut = "HIS"
          Case "AGA", "AGG", "CGA", "CGU", "CGG", "CGC"
            msAAShortcut = "ARG"
          Case "AAA", "AAG"
            msAAShortcut = "LYS"
          Case "UAA", "UAG", "UGA"
            msAAShortcut = "STP"
        End Select
        Call Synthesis(msAAShortcut)
        Call Show

    End Sub

    Public Sub Synthesis(ByVal sShortCut As String)

        msAAShortcut = sShortCut 'nur falls die Sub extern angesprochen wird

        Select Case msAAShortcut
          Case "GLY"
            msAAName = "Glycin"
            msBestTriplett = "GGC"
            msSecondBest = "GGG"
            msBackColor = 8421631
          Case "ALA"
            msAAName = "Alanin"
            msBestTriplett = "GCG"
            msSecondBest = "GCC"
            msBackColor = 8454143
          Case "VAL"
            msAAName = "Valin"
            msBestTriplett = "GUC"
            msSecondBest = "GUG"
            msBackColor = 8454016
          Case "LEU"
            msAAName = "Leucin"
            msBestTriplett = "CUG"
            msSecondBest = "CUC"
            msBackColor = 16777088
          Case "ILE"
            msAAName = "Isoleucin"
            msBestTriplett = "AUC"
            msBackColor = 12615935
          Case "PHE"
            msAAName = "Phenylalanin"
            msBestTriplett = "UUC"
            msBackColor = 255
          Case "TYR"
            msAAName = "Tyrosin"
            msBestTriplett = "UAC"
```

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```
        msBackColor = 4210816
      Case "TRP"
        msAAName = "Tryptophan"
        msBestTriplett = "UGG"
        msBackColor = 4227327
      Case "ASP"
        msAAName = "Asparaginsäure"
        msBestTriplett = "GAC"
        msBackColor = 8388863
      Case "ASN"
        msAAName = "Asparagin"
        msBestTriplett = "AAC"
        msBackColor = 4227200
      Case "GLU"
        msAAName = "Glutaminsäure"
        msBestTriplett = "GAG"
        msBackColor = 32768
      Case "GLN"
        msAAName = "Glutamin"
        msBestTriplett = "GGC"
        msBackColor = 8421440
      Case "SER"
        msAAName = "Serin"
        msBestTriplett = "AGC"
        msSecondBest = "UCG"
        msThirdBest = "UCC"
        msBackColor = 16512
      Case "THR"
        msAAName = "Threonin"
        msBestTriplett = "ACG"
        msBackColor = 16711680
      Case "CYS"
        msAAName = "Cystein"
        msBestTriplett = "UGC"
        msBackColor = 6932960
      Case "MET"
        msAAName = "Methionin"
        msBestTriplett = "AUG"
        msBackColor = 10417643
      Case "PRO"
        msAAName = "Prolin"
        msBestTriplett = "CCG"
        msSecondBest = "CCC"
        msBackColor = 12898746
      Case "HIS"
        msAAName = "Histidin"
        msBestTriplett = "CAC"
        msBackColor = 12898746
      Case "ARG"
```

```
            msAAName = "Arginin"
            msBestTriplett = "CGC"
            msSecondBest = "CGG"
            msBackColor = 6174925
        Case "LYS"
            msAAName = "Lysin"
            msBestTriplett = "AAG"
            msBackColor = 14141641
        Case "STP"
            msAAName = "Stop"
            msBestTriplett = "UGA"
            msSecondBest = "UAG"
            msBackColor = 11332093
    End Select

End Sub

Public Sub Show()

    lblAminoAcid.Caption = msAAShortcut
    If mbShowOriginal = True Then
        lblTriplett.Caption = msTriplett
    Else
        lblTriplett.Caption = msBestTriplett
    End If
    lblAminoAcid.BackColor = msBackColor
    lblTriplett.BackColor = msBackColor

End Sub

Public Function ChooseBestGC(sShortCut As String)

    Select Case sShortCut
        Case "GLY"
            msAAName = "Glycin"
            msBestTriplett = "GGC"
            msSecondBest = "GGG"
            msBackColor = 8421631
        Case "ALA"
            msAAName = "Alanin"
            msBestTriplett = "GCG"
            msSecondBest = "GCC"
            msBackColor = 8454143
        Case "VAL"
            msAAName = "Valin"
            msBestTriplett = "GUC"
            msSecondBest = "GUG"
            msBackColor = 8454016
        Case "LEU"
```

```
            msAAName = "Leucin"
            msBestTriplett = "CUG"
            msSecondBest = "CUC"
            msBackColor = 16777088
        Case "ILE"
            msAAName = "Isoleucin"
            msBestTriplett = "AUC"
            msBackColor = 12615935
        Case "PHE"
            msAAName = "Phenylalanin"
            msBestTriplett = "UUC"
            msBackColor = 255
        Case "TYR"
            msAAName = "Tyrosin"
            msBestTriplett = "UAC"
            msBackColor = 4210816
        Case "TRP"
            msAAName = "Tryptophan"
            msBestTriplett = "UGG"
            msBackColor = 4227327
        Case "ASP"
            msAAName = "Asparaginsäure"
            msBestTriplett = "GAC"
            msBackColor = 8388863
        Case "ASN"
            msAAName = "Asparagin"
            msBestTriplett = "AAC"
            msBackColor = 4227200
        Case "GLU"
            msAAName = "Glutaminsäure"
            msBestTriplett = "GAG"
            msBackColor = 16711808
        Case "GLN"
            msAAName = "Glutamin"
            msBestTriplett = "GGC"
            msBackColor = 12632256
        Case "SER"
            msAAName = "Serin"
            msBestTriplett = "AGC"
            msSecondBest = "UCG"
            msThirdBest = "UCC"
            msBackColor = 8421504
        Case "THR"
            msAAName = "Threonin"
            msBestTriplett = "ACG"
            msBackColor = &HD4C0FF
        Case "CYS"
            msAAName = "Cystein"
            msBestTriplett = "UGC"
```

```
            msBackColor = &HD5C0FF
        Case "MET"
            msAAName = "Methionin"
            msBestTriplett = "AUG"
            msBackColor = &HD6C0FF
        Case "PRO"
            msAAName = "Prolin"
            msBestTriplett = "CCG"
            msSecondBest = "CCC"
            msBackColor = &HD7C0FF
        Case "HIS"
            msAAName = "Histidin"
            msBestTriplett = "CAC"
            msBackColor = &HD8C0FF
        Case "ARG"
            msAAName = "Arginin"
            msBestTriplett = "CGC"
            msSecondBest = "CGG"
            msBackColor = &HD9C0FF
        Case "LYS"
            msAAName = "Lysin"
            msBestTriplett = "AAG"
            msBackColor = &HE0C0FF
        Case "STP"
            msAAName = "Stop"
            msBestTriplett = "UGA"
            msSecondBest = "UAG"
            msBackColor = &HE1C0FF
    End Select

End Function


Public Property Let ShowOriginal(ByVal bNewValue As Boolean)
    mbShowOriginal = bNewValue
End Property

Public Property Get ShowOriginal() As Boolean
    ShowOriginal = mbShowOriginal
End Property

Public Property Get AAShortcut() As String
    AAShortcut = msAAShortcut
End Property

Public Property Get AAName() As String
    AAName = msAAName
```

```
End Property

Public Property Get BestTriplett() As String
  BestTriplett = msBestTriplett
End Property

Public Property Get SecondBest() As String
  SecondBest = msSecondBest
End Property

Public Property Get ThirdBest() As String
  ThirdBest = msThirdBest
End Property

Public Property Get BackColor() As String
  BackColor = msBackColor
End Property


Private Property Get Triplett() As String
  Triplett = msTriplett
End Property

Private Sub UserControl_Resize()

Dim lOuterWidth As Long
Dim lDistance As Long

  lDistance = 30

  lOuterWidth = ScaleWidth + lDistance


  With lblTriplett
    .Top = 0
    .Left = lDistance / 2
    .Width = ScaleWidth - lDistance
    .Height = ScaleHeight / 2 + 30
  End With
  With lblAminoAcid
    .Top = ScaleHeight / 2 - 30
    .Left = lDistance / 2
    .Width = ScaleWidth - lDistance
    .Height = ScaleHeight / 2
  End With
  With linUpper
    .X1 = ScaleLeft
    .Y1 = 0
    .X2 = ScaleWidth
```

```
    .Y2 = 0
  End With
  With linLower
    .X1 = ScaleLeft
    .Y1 = ScaleHeight - 20
    .X2 = ScaleWidth
    .Y2 = ScaleHeight - 20
  End With

End Sub
```

Curevac_RNA_Optimizer with following modules

Name: Curevac_RNA_Optimizer.vbp
Code:

```
Type=Exe
Reference=*\G{00020430-0000-0000-C000-
000000000046}#2.0#0#..\..\WINNT\System32\STDOLE2.TLB#OLE Automation
Object={F9043C88-F6F2-101A-A3C9-08002B2F49FB}#1.2#0; COMDLG32.OCX
Module=basMain; basMain.bas
Form=mdiMain.frm
Object=*\ACurevac_Genetic_Controls.vbp
Object={38911DA0-E448-11D0-84A3-00DD01104159}#1.1#0; COMCT332.OCX
Object={3B7C8863-D78F-101B-B9B5-04021C009402}#1.2#0; RICHTX32.OCX
Form=frmOutPut.frm
Form=frmStatistics.frm
Module=basPublics; basPublics.bas
Form=frmOptions.frm
Form=frmInput.frm
Form=frmDisplay.frm
Form=frmAbout.frm
Startup="mdiMain"
HelpFile=""
Title="RNA-Optimizer"
Command32=""
Name="RNA_Optimizer"
HelpContextID="0"
CompatibleMode="0"
MajorVer=1
MinorVer=0
RevisionVer=0
AutoIncrementVer=0
ServerSupportFiles=0
VersionComments="the RNA people"
VersionCompanyName="CureVac GmbH"
VersionFileDescription="Application for Optimization of RNA"
```

```
VersionLegalCopyright="by Christian Klump"
VersionLegalTrademarks="Curevac RNA-Optimizer(tm)"
VersionProductName="Curevac RNA-Optimizer"
CompilationType=0
OptimizationType=0
FavorPentiumPro(tm)=0
CodeViewDebugInfo=0
NoAliasing=0
BoundsCheck=0
OverflowCheck=0
FlPointCheck=0
FDIVCheck=0
UnroundedFP=0
StartMode=0
Unattended=0
Retained=0
ThreadPerObject=0
MaxNumberOfThreads=1
DebugStartupOption=0
```

Name: basMain.bas
Code:

```
Attribute VB_Name = "basMain"
Option Explicit

Private Type udtAminoAcid
   sShortcut As String
   sName As String
   sBestTriplett As String
   sSecondBest As String
   sThirdBest As String
End Type

Private mastrAminoAcids() As udtAminoAcid

Public Sub RebuildChain(bShowOriginal As Boolean)

Dim iLoop As Integer

   For iLoop = 1 To glAminoCounter
      With frmDisplay.Curevac_Amino1(iLoop)
         .ShowOriginal = bShowOriginal
         .Show
      End With
   Next iLoop

End Sub
```

```
    Public Sub ResetPublics()
      gsOriginalCode = ""
      gsFormattedCode = ""
      glCodeLength = ""
5   End Sub


    Public Sub BuildAminoChain(ByVal sTempCode As String, bGraphical As Boolean)

10  Dim lVerticalOffset As Long
    Dim lHorizontalOffset As Long
    Dim lTop As Long

    Dim asTripletts() As String
15  Dim sAminoChain As String
    Dim lLoop As Long

      If bGraphical = True Then
        lTop = frmDisplay.optNucleotids(0).Height + 40
20    End If
      ReDim mastrAminoAcids(glAminoCounter)
      asTripletts = Split(sTempCode)
      For lLoop = 0 To glAminoCounter - 1
        If bGraphical = True Then
25        Load frmDisplay.Curevac_Amino1(lLoop + 1)
          With frmDisplay.Curevac_Amino1(lLoop + 1)

            Call .Translation(asTripletts(lLoop))

30          .Left = lLoop * .Width - lHorizontalOffset
            .Top = lVerticalOffset + lTop
            If .Left + 2 * .Width > frmDisplay.ScaleWidth Then
              lVerticalOffset = lVerticalOffset + .Height + 150
              lHorizontalOffset = lLoop * .Width + .Width
35          End If

            sAminoChain = sAminoChain + .aaShortcut + ","
            .Visible = True
          End With
40      Else
          mastrAminoAcids(lLoop) = Translation(asTripletts(lLoop))
'         sTest = sTest + mastrAminoAcids(iLoop).sName + ","
        End If
      Next
45    If bGraphical = True Then
        frmDisplay.Show
      Else
        Call frmOutPut.Show
      End If
```

```
    Debug.Print sAminoChain
End Sub

Private Function Translation(ByVal sTriplett As String) As udtAminoAcid

    Dim stiTemp As udtAminoAcid

    If mdiMain.optKindOfOptimize(0) = True Then
      'Optimization for Most GC
      Select Case sTriplett
        Case "GGU", "GGC", "GGA", "GGG"
          stiTemp.sShortcut = "GLY"
          stiTemp.sName = "Glycin"
          stiTemp.sBestTriplett = "GGC"
          stiTemp.sSecondBest = "GGG"
        Case "GCU", "GCC", "GCA", "GCG"
          stiTemp.sShortcut = "ALA"
          stiTemp.sName = "Alanin"
          stiTemp.sBestTriplett = "GCG"
          stiTemp.sSecondBest = "GCC"
        Case "GUU", "GUC", "GUA", "GUG"
          stiTemp.sShortcut = "VAL"
          stiTemp.sName = "Valin"
          stiTemp.sBestTriplett = "GUC"
          stiTemp.sSecondBest = "GUG"
        Case "UUA", "UUG", "CUU", "CUA", "CUG", "CUC"
          stiTemp.sShortcut = "LEU"
          stiTemp.sName = "Leucin"
          stiTemp.sBestTriplett = "CUG"
          stiTemp.sSecondBest = "CUC"
        Case "AUA", "AUU", "AUC"
          stiTemp.sShortcut = "ILE"
          stiTemp.sName = "Isoleucin"
          stiTemp.sBestTriplett = "AUC"
        Case "UUU", "UUC"
          stiTemp.sShortcut = "PHE"
          stiTemp.sName = "Phenylalanin"
          stiTemp.sBestTriplett = "UUC"
        Case "UAU", "UAC"
          stiTemp.sShortcut = "TYR"
          stiTemp.sName = "Tyrosin"
          stiTemp.sBestTriplett = "UAC"
        Case "UGG"
          stiTemp.sShortcut = "TRP"
          stiTemp.sName = "Tryptophan"
          stiTemp.sBestTriplett = "UGG"
        Case "GAU", "GAC"
          stiTemp.sShortcut = "ASP"
          stiTemp.sName = "Asparaginsäure"
```

```
            stiTemp.sBestTriplett = "GAC"
        Case "AAU", "AAC"
            stiTemp.sShortcut = "ASN"
            stiTemp.sName = "Asparagin"
            stiTemp.sBestTriplett = "AAC"
        Case "GAA", "GAG"
            stiTemp.sShortcut = "GLU"
            stiTemp.sName = "Glutaminsäure"
            stiTemp.sBestTriplett = "GAG"
        Case "CAA", "CAG"
            stiTemp.sShortcut = "GLN"
            stiTemp.sName = "Glutamin"
            stiTemp.sBestTriplett = "CAG"
        Case "AGU", "AGC", "UCA", "UCU", "UCG", "UCC"
            stiTemp.sShortcut = "SER"
            stiTemp.sName = "Serin"
            stiTemp.sBestTriplett = "AGC"
            stiTemp.sSecondBest = "UCG"
            stiTemp.sThirdBest = "UCC"
        Case "ACA", "ACU", "ACG", "ACC"
            stiTemp.sShortcut = "THR"
            stiTemp.sName = "Threonin"
            stiTemp.sBestTriplett = "ACG"
        Case "UGU", "UGC"
            stiTemp.sShortcut = "CYS"
            stiTemp.sName = "Cystein"
            stiTemp.sBestTriplett = "UGC"
        Case "AUG"
            stiTemp.sShortcut = "MET"
            stiTemp.sName = "Methionin"
            stiTemp.sBestTriplett = "AUG"
        Case "CCA", "CCU", "CCG", "CCC"
            stiTemp.sShortcut = "PRO"
            stiTemp.sName = "Prolin"
            stiTemp.sBestTriplett = "CCG"
            stiTemp.sSecondBest = "CCC"
        Case "CAU", "CAC"
            stiTemp.sShortcut = "HIS"
            stiTemp.sName = "Histidin"
            stiTemp.sBestTriplett = "CAC"
        Case "AGA", "AGG", "CGA", "CGU", "CGG", "CGC"
            stiTemp.sShortcut = "ARG"
            stiTemp.sName = "Arginin"
            stiTemp.sBestTriplett = "CGC"
            stiTemp.sSecondBest = "CGG"
        Case "AAA", "AAG"
            stiTemp.sShortcut = "LYS"
            stiTemp.sName = "Lysin"
            stiTemp.sBestTriplett = "AAG"
```

```
        Case "UAA", "UAG", "UGA"
          stiTemp.sShortcut = "STP"
          stiTemp.sName = "Stop"
          stiTemp.sBestTriplett = "UGA"
          stiTemp.sSecondBest = "UAG"
      End Select
      Translation = stiTemp
    Else   'Optimization for best frequency
      Select Case sTriplett
        Case "GGU", "GGC", "GGA", "GGG"
          stiTemp.sShortcut = "GLY"
          stiTemp.sName = "Glycin"
          stiTemp.sBestTriplett = "GGC"
          stiTemp.sSecondBest = "GGU"
        Case "GCU", "GCC", "GCA", "GCG"
          stiTemp.sShortcut = "ALA"
          stiTemp.sName = "Alanin"
          stiTemp.sBestTriplett = "GCC"
          stiTemp.sSecondBest = "GCU"
        Case "GUU", "GUC", "GUA", "GUG"
          stiTemp.sShortcut = "VAL"
          stiTemp.sName = "Valin"
          stiTemp.sBestTriplett = "GUG"
          stiTemp.sSecondBest = "GUC"
        Case "UUA", "UUG", "CUU", "CUA", "CUG", "CUC"
          stiTemp.sShortcut = "LEU"
          stiTemp.sName = "Leucin"
          stiTemp.sBestTriplett = "CUG"
          stiTemp.sSecondBest = "CUC"
        Case "AUA", "AUU", "AUC"
          stiTemp.sShortcut = "ILE"
          stiTemp.sName = "Isoleucin"
          stiTemp.sBestTriplett = "AUC"
        Case "UUU", "UUC"
          stiTemp.sShortcut = "PHE"
          stiTemp.sName = "Phenylalanin"
          stiTemp.sBestTriplett = "UUC"
        Case "UAU", "UAC"
          stiTemp.sShortcut = "TYR"
          stiTemp.sName = "Tyrosin"
          stiTemp.sBestTriplett = "UAC"
        Case "UGG"
          stiTemp.sShortcut = "TRP"
          stiTemp.sName = "Tryptophan"
          stiTemp.sBestTriplett = "UGG"
        Case "GAU", "GAC"
          stiTemp.sShortcut = "ASP"
          stiTemp.sName = "Asparaginsäure"
          stiTemp.sBestTriplett = "GAC"
```

```
        Case "AAU", "AAC"
          stiTemp.sShortcut = "ASN"
          stiTemp.sName = "Asparagin"
          stiTemp.sBestTriplett = "AAC"
        Case "GAA", "GAG"
          stiTemp.sShortcut = "GLU"
          stiTemp.sName = "Glutaminsäure"
          stiTemp.sBestTriplett = "GAG"
        Case "CAA", "CAG"
          stiTemp.sShortcut = "GLN"
          stiTemp.sName = "Glutamin"
          stiTemp.sBestTriplett = "CAG"
        Case "AGU", "AGC", "UCA", "UCU", "UCG", "UCC"
          stiTemp.sShortcut = "SER"
          stiTemp.sName = "Serin"
          stiTemp.sBestTriplett = "AGC"
          stiTemp.sSecondBest = "UCC"
          stiTemp.sThirdBest = "UCU"
        Case "ACA", "ACU", "ACG", "ACC"
          stiTemp.sShortcut = "THR"
          stiTemp.sName = "Threonin"
          stiTemp.sBestTriplett = "ACC"
        Case "UGU", "UGC"
          stiTemp.sShortcut = "CYS"
          stiTemp.sName = "Cystein"
          stiTemp.sBestTriplett = "UGC"
        Case "AUG"
          stiTemp.sShortcut = "MET"
          stiTemp.sName = "Methionin"
          stiTemp.sBestTriplett = "AUG"
        Case "CCA", "CCU", "CCG", "CCC"
          stiTemp.sShortcut = "PRO"
          stiTemp.sName = "Prolin"
          stiTemp.sBestTriplett = "CCC"
          stiTemp.sSecondBest = "CCU"
        Case "CAU", "CAC"
          stiTemp.sShortcut = "HIS"
          stiTemp.sName = "Histidin"
          stiTemp.sBestTriplett = "CAC"
        Case "AGA", "AGG", "CGA", "CGU", "CGG", "CGC"
          stiTemp.sShortcut = "ARG"
          stiTemp.sName = "Arginin"
          stiTemp.sBestTriplett = "CGC"
          stiTemp.sSecondBest = "AGG"
        Case "AAA", "AAG"
          stiTemp.sShortcut = "LYS"
          stiTemp.sName = "Lysin"
          stiTemp.sBestTriplett = "AAG"
        Case "UAA", "UAG", "UGA"
```

```
            stiTemp.sShortcut = "STP"
            stiTemp.sName = "Stop"
            stiTemp.sBestTriplett = "UGA"
            stiTemp.sSecondBest = "UAG"
        End Select
        Translation = stiTemp
    End If

End Function

Public Function ReverseTranscription() As String
    Dim sTempCode As String
    Dim iLoop As Integer
    For iLoop = 0 To glAminoCounter - 1
        sTempCode = sTempCode + mastrAminoAcids(iLoop).sBestTriplett
    Next
    ReverseTranscription = sTempCode
End Function

Public Sub CountBases(ByVal sTempCode As String)

Dim lLoop As Long
Dim sFormattedCode As String
Dim sActualBase As String
Dim lCodeLength As Long

    lCodeLength = Len(sTempCode)
    For lLoop = 1 To CLng(lCodeLength)
        sActualBase = Mid(sTempCode, lLoop, 1)
        Select Case sActualBase
            Case "A", "a"
                glOptAdenin = glOptAdenin + 1
            Case "U", "u", "T", "t"
                glOptThymin = glOptThymin + 1
            Case "G", "g"
                glOptGuanin = glOptGuanin + 1
            Case "C", "c"
                glOptCytosin = glOptCytosin + 1
        End Select
    Next lLoop
End Sub
```

Name: basPublics.bas
Code:

```
Attribute VB_Name = "basPublics"
Option Explicit
```

```
Public gsOriginalCode As String
Public gsFormattedCode As String
Public glCodeLength As Long

Public glAminoCounter As Long
Public glAdenin As Long
Public glThymin As Long
Public glGuanin As Long
Public glCytosin As Long
Public glOptAdenin As Long
Public glOptThymin As Long
Public glOptGuanin As Long
Public glOptCytosin As Long

Public gbSequenceChanged As Boolean
```

Name: mdiMain.frm

Code:

```
VERSION 5.00
Object = "{38911DA0-E448-11D0-84A3-00DD01104159}#1.1#0"; "COMCT332.OCX"
Begin VB.MDIForm mdiMain
  BackColor      =  &H00FFFFFF&
  Caption        =  "Curevac_RNA_Analyzer"
  ClientHeight   =  8745
  ClientLeft     =  165
  ClientTop      =  735
  ClientWidth    =  12255
  Icon           =  "mdiMain.frx":0000
  LinkTopic      =  "MDIForm1"
  Picture        =  "mdiMain.frx":058A
  StartUpPosition =  3  'Windows Default
  WindowState    =  2  'Maximized
  Begin ComCtl3.CoolBar cbarMain
    Align        =  1  'Align Top
    Height       =  885
    Left         =  0
    TabIndex     =  0
    Top          =  0
    Width        =  12255
    _ExtentX     =  21616
    _ExtentY     =  1561
    BandCount    =  1
    BandBorders  =  0  'False
    _CBWidth     =  12255
    _CBHeight    =  885
    _Version     =  "6.7.8988"
    MinHeight1   =  825
```

```
    Width1          =  4995
    FixedBackground1=  0  'False
    NewRow1         =  0  'False
    Begin VB.OptionButton optKindOfOptimize
      Caption       =  "Best Frequency"
      Height        =  255
      Index         =  1
      Left          =  4920
      TabIndex      =  7
      Top           =  480
      Value         =  -1 'True
      Width         =  1575
    End
    Begin VB.OptionButton optKindOfOptimize
      Caption       =  "Best GC"
      Height        =  255
      Index         =  0
      Left          =  4920
      TabIndex      =  6
      Top           =  120
      Width         =  1575
    End
    Begin VB.CommandButton cmdShowInput
      Caption       =  "Input"
      Height        =  765
      Left          =  0
      Picture       =  "mdiMain.frx":0C2A
      Style         =  1 'Graphical
      TabIndex      =  5
      Top           =  0
      Width         =  840
    End
    Begin VB.CommandButton cmdShowOptions
      Caption       =  "Options"
      Enabled       =  0  'False
      Height        =  760
      Left          =  3480
      Picture       =  "mdiMain.frx":0F34
      Style         =  1 'Graphical
      TabIndex      =  4
      Top           =  0
      Width         =  735
    End
    Begin VB.CommandButton cmdShowStatistics
      Caption       =  "Statistics"
      Enabled       =  0  'False
      Height        =  760
      Left          =  2640
      Picture       =  "mdiMain.frx":131E
```

```
      Style          =  1  'Graphical
      TabIndex       =  3
      Top            =  0
      Width          =  735
   End
   Begin VB.CommandButton cmdShowDisplay
      Caption        =  "Display"
      Enabled        =  0  'False
      Height         =  760
      Left           =  1800
      Picture        =  "mdiMain.frx":1628
      Style          =  1  'Graphical
      TabIndex       =  2
      Top            =  0
      Width          =  735
   End
   Begin VB.CommandButton cmdShowOutput
      Caption        =  "Output"
      Enabled        =  0  'False
      Height         =  760
      Left           =  960
      Picture        =  "mdiMain.frx":20E2
      Style          =  1  'Graphical
      TabIndex       =  1
      Top            =  0
      Width          =  735
   End
End
Begin VB.Menu mnuMain
   Caption        =  "&Input..."
   Index          =  0
End
Begin VB.Menu mnuMain
   Caption        =  "&Results"
   Index          =  1
   Begin VB.Menu mnuResults
      Caption        =  "&Output..."
      Enabled        =  0  'False
      Index          =  0
   End
   Begin VB.Menu mnuResults
      Caption        =  "&Display..."
      Enabled        =  0  'False
      Index          =  1
   End
   Begin VB.Menu mnuResults
      Caption        =  "&Statistics..."
      Enabled        =  0  'False
      Index          =  2
```

```
    End
  End
  Begin VB.Menu mnuMain
    Caption       =  "E&xtras"
    Index         =  4
    Begin VB.Menu mnuExtras
      Caption       =  "&Language"
      Index         =  0
      Begin VB.Menu mnuLanguage
        Caption       =  "English"
        Checked       =  -1  'True
        Index         =  0
      End
      Begin VB.Menu mnuLanguage
        Caption       =  "&German"
        Enabled       =  0  'False
        Index         =  1
      End
      Begin VB.Menu mnuLanguage
        Caption       =  "&French"
        Enabled       =  0  'False
        Index         =  2
      End
    End
    Begin VB.Menu mnuExtras
      Caption       =  "&Options..."
      Enabled       =  0  'False
      Index         =  1
    End
    Begin VB.Menu mnuExtras
      Caption       =  "-"
      Index         =  2
    End
    Begin VB.Menu mnuExtras
      Caption       =  "&About"
      Index         =  3
    End
  End
  Begin VB.Menu mnuMain
    Caption       =  "&Windows"
    Index         =  5
    WindowList    =  -1  'True
    Begin VB.Menu mnuWindows
      Caption       =  "Tile &Horizontally"
      Index         =  0
    End
    Begin VB.Menu mnuWindows
      Caption       =  "Tile &Vertically"
      Index         =  1
```

```
      End
      Begin VB.Menu mnuWindows
        Caption       =  "&Cascade"
        Index         =  2
      End
    End
    Begin VB.Menu mnuMain
      Caption       =  "&Exit"
      Index         =  6
    End
End
Attribute VB_Name = "mdiMain"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit

Private Sub cmdShowDisplay_Click()
  Call frmDisplay.Show
End Sub

Private Sub cmdShowInput_Click()
  Call frmInput.Show
End Sub

Private Sub cmdShowOptions_Click()
  Call frmOptions.Show
End Sub

Private Sub cmdShowOutput_Click()
  Call frmOutPut.Show
End Sub

Private Sub cmdShowStatistics_Click()
  Call frmStatistics.Show
End Sub

Private Sub MDIForm_Load()
  Call InitControls
  Call frmInput.Show
End Sub

Private Sub InitControls()

Dim lNextLeft As Long
Const lSpace As Long = 60
Const lButtonWidth As Long = 850
Const lButtonHeight As Long = 770
```

```
        lNextLeft = lSpace + lButtonWidth

        With cmdShowInput
           .Top = lSpace
           .Left = lSpace
           .Width = lButtonWidth
           .Height = lButtonHeight
        End With

        With cmdShowOutput
           .Top = lSpace
           .Left = lNextLeft + lSpace
           .Width = lButtonWidth
           .Height = lButtonHeight
        End With

        With cmdShowDisplay
           .Top = lSpace
           .Left = lNextLeft * 2 + lSpace
           .Width = lButtonWidth
           .Height = lButtonHeight
        End With

        With cmdShowStatistics
           .Top = lSpace
           .Left = lNextLeft * 3 + lSpace
           .Width = lButtonWidth
           .Height = lButtonHeight
        End With

        With cmdShowOptions
           .Top = lSpace
           .Left = lNextLeft * 4 + lSpace
           .Width = lButtonWidth
           .Height = lButtonHeight
        End With

        cbarMain.Height = lButtonHeight + 2 * lSpace

    End Sub

    Private Sub mnuMain_Click(Index As Integer)

        Select Case Index
           Case 0
              Call frmInput.Show
           Case 6
```

```
        Unload Me
    End Select

End Sub

Private Sub mnuResults_Click(Index As Integer)

    Select Case Index
        Case 0 'Output
            Call frmOutPut.Show
        Case 1
            Call frmDisplay.Show
        Case 2 'Statistische Auswertung
'           Call frmStatistics.Show
    End Select

End Sub

Private Sub mnuExtras_Click(Index As Integer)

    Select Case Index
        Case 0 'Output
            '
        Case 1
            Call frmDisplay.Show
        Case 3
            Call frmAbout.Show(vbModal)
    End Select

End Sub


Private Sub mnuWindows_Click(Index As Integer)

    Select Case Index
        Case 0 'Horizontal
            Me.Arrange (vbTileHorizontal)
        Case 1 'Vertikal
            Me.Arrange (vbTileVertical)
        Case 2 'Kaskadieren
            Me.Arrange (vbCascade)
    End Select

End Sub

```

Name: frmInput.frm
Code:

```
VERSION 5.00
```

```
Object = "{F9043C88-F6F2-101A-A3C9-08002B2F49FB}#1.2#0"; "COMDLG32.OCX"
Object = "{3B7C8863-D78F-101B-B9B5-04021C009402}#1.2#0"; "RICHTX32.OCX"
Begin VB.Form frmInput
  Caption       =  "Input"
  ClientHeight  =  5730
  ClientLeft    =  60
  ClientTop     =  345
  ClientWidth   =  13200
  Icon          =  "frmInput.frx":0000
  LinkTopic     =  "Form1"
  MDIChild      =  -1  'True
  ScaleHeight   =  5730
  ScaleWidth    =  13200
  WindowState   =  2  'Maximized
  Begin RichTextLib.RichTextBox txtFormatted
    Height        =  1815
    Left          =  120
    TabIndex      =  3
    Top           =  360
    Width         =  12735
    _ExtentX      =  22463
    _ExtentY      =  3201
    _Version      =  393217
    BorderStyle   =  0
    Enabled       =  -1  'True
    ScrollBars    =  2
    DisableNoScroll =  -1  'True
    TextRTF       =  $"frmInput.frx":030A
    BeginProperty Font {0BE35203-8F91-11CE-9DE3-00AA004BB851}
      Name          =  "Fixedsys"
      Size          =  9
      Charset       =  0
      Weight        =  400
      Underline     =  0  'False
      Italic        =  0  'False
      Strikethrough =  0  'False
    EndProperty
  End
  Begin VB.OptionButton optSequence
    Caption       =  "Formatted"
    Height        =  255
    Index         =  1
    Left          =  2760
    Style         =  1  'Graphical
    TabIndex      =  2
    Top           =  0
    Value         =  -1  'True
    Width         =  855
  End
```

```
    Begin VB.OptionButton optSequence
      Caption      =  "Original"
      Height       =  255
      Index        =  0
      Left         =  1920
      Style        =  1 'Graphical
      TabIndex     =  1
      Top          =  0
      Width        =  855
    End
    Begin VB.CommandButton cmdLoad
      Caption      =  "Load Sequence"
      Height       =  285
      Left         =  0
      TabIndex     =  0
      Top          =  0
      Width        =  1695
    End
    Begin MSComDlg.CommonDialog CommonDialog1
      Left         =  3840
      Top          =  -120
      _ExtentX     =  847
      _ExtentY     =  847
      _Version     =  393216
    End
End
Attribute VB_Name = "frmInput"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit


Private Sub Form_Activate()
   Me.ZOrder
   Call InitControls
End Sub

Public Sub LoadSequence()

Dim sFile As String
Dim sTempCode As String

   On Error GoTo Errorhandler
   With CommonDialog1
      .FileName = App.Path & "\SampleRNA.txt"
      .CancelError = True
      Call .ShowOpen
```

```
    End With
    If Len(CommonDialog1.FileName) > 0 Then
      gbSequenceChanged = True
      gsFormattedCode = ""
      gsOriginalCode = ""
      sFile = CommonDialog1.FileName
      Open sFile For Input As #1
      Input #1, gsOriginalCode
      Close #1
      Call FormatText
      Call FillSequence

      mdiMain.cmdShowDisplay.Enabled = True
      mdiMain.cmdShowOutput.Enabled = True
      mdiMain.cmdShowStatistics.Enabled = True
    End If

  Exit Sub

Errorhandler:

  Dim lAnswer As Long

    If Err.Number <> cdlCancel Then
      lAnswer = MsgBox("Datei konnte nicht geladen werden", vbRetryCancel,
"Dateiproblem")
      If lAnswer = vbRetry Then
        Resume
      End If
    End If

  End Sub

  Private Sub FormatText()

  Dim iLoop As Integer
  Dim sActualBase As String

    glCodeLength = Len(gsOriginalCode)
    For iLoop = 0 To glCodeLength - 1
      sActualBase = Mid(gsOriginalCode, iLoop + 1, 1)
      If iLoop Mod 3 = 0 Then
        gsFormattedCode = gsFormattedCode + " "
        glAminoCounter = glAminoCounter + 1
      End If
      Select Case sActualBase
        Case "A", "a"
          gsFormattedCode = gsFormattedCode + "A"
          glAdenin = glAdenin + 1
```

```
            Case "U", "u", "T", "t"
               gsFormattedCode = gsFormattedCode + "U"
               glThymin = glThymin + 1
            Case "G", "g"
               gsFormattedCode = gsFormattedCode + "G"
               glGuanin = glGuanin + 1
            Case "C", "c"
               gsFormattedCode = gsFormattedCode + "C"
               glCytosin = glCytosin + 1
         End Select
      Next iLoop
      gsFormattedCode = Trim(gsFormattedCode)

   End Sub

   Private Sub FillSequence()
      If optSequence(0).Value = True Then
         txtFormatted.Text = gsOriginalCode
      Else
         txtFormatted.Text = gsFormattedCode
      End If

   End Sub

   Private Sub cmdShowOutput_Click()
      Call frmOutPut.Show
   End Sub

   Private Sub cmdLoad_Click()
      Call LoadSequence
   End Sub

   Private Sub InitControls()

   Const lSpace As Long = 40
   Const lButtonHeight As Long = 285

      With cmdLoad
         .Top = lSpace
         .Height = lButtonHeight
         .Left = lSpace
      End With

      With optSequence(0)
         .Top = lSpace
         .Height = lButtonHeight
         .Left = cmdLoad.Left + cmdLoad.Width + lSpace + 200
      End With
```

```
    With optSequence(1)
      .Top = lSpace
      .Height = lButtonHeight
      .Left = optSequence(0).Left + optSequence(0).Width
    End With

    With txtFormatted
      .Top = cmdLoad.Height + 2 * lSpace
      .Left = lSpace
      .Width = Me.ScaleWidth
      .Height = Me.ScaleHeight - txtFormatted.Top
    End With
  End Sub

  Private Sub optSequence_Click(Index As Integer)
    Call FillSequence
  End Sub
```

Name: frmOutPut.frm
Code:

```
VERSION 5.00
Object = "{3B7C8863-D78F-101B-B9B5-04021C009402}#1.2#0"; "RICHTX32.OCX"
Begin VB.Form frmOutPut
  Caption       =  "OutPut"
  ClientHeight  =  9120
  ClientLeft    =  60
  ClientTop     =  345
  ClientWidth   =  10215
  Icon          =  "frmOutPut.frx":0000
  LinkTopic     =  "Form1"
  MDIChild      =  -1  'True
  ScaleHeight   =  9120
  ScaleWidth    =  10215
  Begin RichTextLib.RichTextBox txtOptimized
    Height        =  2655
    Left          =  0
    TabIndex      =  0
    Top           =  480
    Width         =  9855
    _ExtentX      =  17383
    _ExtentY      =  4683
    _Version      =  393217
    Enabled       =  -1  'True
    ScrollBars    =  2
    DisableNoScroll =  -1  'True
    TextRTF       =  $"frmOutPut.frx":0442
    BeginProperty Font {0BE35203-8F91-11CE-9DE3-00AA004BB851}
```

```
        Name        =  "Fixedsys"
        Size        =  9
        Charset     =  0
        Weight      =  400
        Underline   =  0  'False
        Italic      =  0  'False
        Strikethrough  =  0  'False
      EndProperty
    End
End
Attribute VB_Name = "frmOutPut"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit

Private Sub Form_Activate()

    Me.ZOrder
    Call InitControls
    If gbSequenceChanged = True Then
      Call BuildAminoChain(gsFormattedCode, False)
    End If
    gbSequenceChanged = False
    txtOptimized.Text = LCase(ReverseTranscription)

End Sub


Private Sub InitControls()

Const lSpace As Long = 40

    With txtOptimized
      .Top = lSpace
      .Left = lSpace
      .Width = Me.ScaleWidth + lSpace
      .Height = Me.ScaleHeight + lSpace
    End With
End Sub
```

Name: frmDisplay.frm
Code:

```
VERSION 5.00
Object = "*\ACurevac_Genetic_Controls.vbp"
Begin VB.Form frmDisplay
```

```
   Caption      = "Display"
   ClientHeight  = 8205
   ClientLeft    = 60
   ClientTop     = 345
   ClientWidth   = 8745
   Icon         = "frmDisplay.frx":0000
   LinkTopic     = "Form1"
   MDIChild      = -1 'True
   ScaleHeight   = 8205
   ScaleWidth    = 8745
   ShowInTaskbar = 0 'False
   Begin VB.OptionButton optNucleotids
     Caption     = "Original"
     Height      = 255
     Index       = 0
     Left        = 0
     Style       = 1 'Graphical
     TabIndex    = 1
     Top         = 0
     Width       = 855
   End
   Begin VB.OptionButton optNucleotids
     Caption     = "Optimized"
     Height      = 255
     Index       = 1
     Left        = 840
     Style       = 1 'Graphical
     TabIndex    = 0
     Top         = 0
     Value       = -1 'True
     Width       = 855
   End
   Begin Curevac_Genetic_Controls.Curevac_Amino Curevac_Amino1
     Height      = 495
     Index       = 0
     Left        = 240
     Top         = 360
     Visible     = 0 'False
     Width       = 735
     _ExtentX    = 1296
     _ExtentY    = 873
   End
End
Attribute VB_Name = "frmDisplay"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit
```

```
Private Sub Form_Activate()

  Me.ZOrder
  If gbSequenceChanged = True Then
    Call BuildAminoChain(gsFormattedCode, True)
  End If
  gbSequenceChanged = False

End Sub


Private Sub optNucleotids_Click(Index As Integer)

Dim bShowOriginal As Boolean

  If Index = 0 Then 'Original Clicked
    bShowOriginal = True
  Else 'Optimized Clicked
    bShowOriginal = False
  End If
  Call RebuildChain(bShowOriginal)

End Sub


Name: frmStatistics.frm
Code:
VERSION 5.00
Begin VB.Form frmStatistics
  Caption       =  "Statistics"
  ClientHeight  =  6450
  ClientLeft    =  60
  ClientTop     =  345
  ClientWidth   =  8595
  Icon          =  "frmStatistics.frx":0000
  LinkTopic     =  "Form1"
  MDIChild      =  -1  'True
  ScaleHeight   =  6450
  ScaleWidth    =  8595
  Begin VB.Frame Frame1
    Caption     =  "original"
    Height      =  2655
    Left        =  120
    TabIndex    =  9
    Top         =  720
    Width       =  2175
    Begin VB.Label lblAdenin
```

```
    BorderStyle    =  1  'Fixed Single
    Height         =  495
    Left           =  840
    TabIndex       =  17
    Top            =  240
    Width          =  1215
  End
  Begin VB.Label lblCytosin
    BorderStyle    =  1  'Fixed Single
    Height         =  495
    Left           =  840
    TabIndex       =  16
    Top            =  2040
    Width          =  1215
  End
  Begin VB.Label lblGuanin
    BorderStyle    =  1  'Fixed Single
    Height         =  495
    Left           =  840
    TabIndex       =  15
    Top            =  1440
    Width          =  1215
  End
  Begin VB.Label lblThymin
    BorderStyle    =  1  'Fixed Single
    Height         =  495
    Left           =  840
    TabIndex       =  14
    Top            =  840
    Width          =  1215
  End
  Begin VB.Label Label14
    Caption        =  "Cytosin"
    Height         =  255
    Left           =  120
    TabIndex       =  13
    Top            =  2160
    Width          =  615
  End
  Begin VB.Label Label2
    Caption        =  "Thymin"
    Height         =  255
    Left           =  120
    TabIndex       =  12
    Top            =  960
    Width          =  615
  End
  Begin VB.Label Label3
    Caption        =  "Guanin"
```

```
      Height      = 255
      Left        = 120
      TabIndex    = 11
      Top         = 1560
      Width       = 615
    End
    Begin VB.Label Label1
      Caption     = "Adenin"
      Height      = 255
      Left        = 120
      TabIndex    = 10
      Top         = 360
      Width       = 615
    End
  End
  Begin VB.Frame Frame2
    Caption     = "optimized"
    Height      = 2655
    Left        = 2520
    TabIndex    = 0
    Top         = 720
    Width       = 2175
    Begin VB.Label Label4
      Caption     = "Adenin"
      Height      = 255
      Left        = 120
      TabIndex    = 8
      Top         = 360
      Width       = 615
    End
    Begin VB.Label Label6
      Caption     = "Cytosin"
      Height      = 255
      Left        = 120
      TabIndex    = 7
      Top         = 2160
      Width       = 615
    End
    Begin VB.Label Label8
      Caption     = "Guanin"
      Height      = 255
      Left        = 120
      TabIndex    = 6
      Top         = 1560
      Width       = 615
    End
    Begin VB.Label Label12
      Caption     = "Thymin"
      Height      = 255
```

```
        Left            =  120
        TabIndex        =  5
        Top             =  960
        Width           =  615
    End
    Begin VB.Label lblOptAdenin
        BorderStyle     =  1  'Fixed Single
        Height          =  495
        Left            =  840
        TabIndex        =  4
        Top             =  240
        Width           =  1215
    End
    Begin VB.Label lblOptThymin
        BorderStyle     =  1  'Fixed Single
        Height          =  495
        Left            =  840
        TabIndex        =  3
        Top             =  840
        Width           =  1215
    End
    Begin VB.Label lblOptGuanin
        BorderStyle     =  1  'Fixed Single
        Height          =  495
        Left            =  840
        TabIndex        =  2
        Top             =  1440
        Width           =  1215
    End
    Begin VB.Label lblOptCytosin
        BorderStyle     =  1  'Fixed Single
        Height          =  495
        Left            =  840
        TabIndex        =  1
        Top             =  2040
        Width           =  1215
    End
End
Begin VB.Label lblBases
    BorderStyle     =  1  'Fixed Single
    Height          =  495
    Left            =  1320
    TabIndex        =  19
    Top             =  120
    Width           =  1215
End
Begin VB.Label Label5
    Caption         =  "Sum of bases"
    Height          =  255
```

```
    Left        =  120
    TabIndex    =  18
    Top         =  240
    Width       =  1095
  End
End
Attribute VB_Name = "frmStatistics"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit

Private Sub Form_Activate()

  Me.ZOrder

  lblBases.Caption = CStr(glAminoCounter * 3)
  lblAdenin.Caption = CStr(glAdenin)
  lblThymin.Caption = CStr(glThymin)
  lblGuanin.Caption = CStr(glGuanin)
  lblCytosin.Caption = CStr(glCytosin)

  Call CountBases(frmOutPut.txtOptimized.Text)
  lblOptAdenin.Caption = CStr(glOptAdenin)
  lblOptThymin.Caption = CStr(glOptThymin)
  lblOptGuanin.Caption = CStr(glOptGuanin)
  lblOptCytosin.Caption = CStr(glOptCytosin)

End Sub
```

Name: frmOptions.frm

Code:

```
VERSION 5.00
Begin VB.Form frmOptions
  Caption       =  "Options"
  ClientHeight  =  3915
  ClientLeft    =  60
  ClientTop     =  345
  ClientWidth   =  5760
  Icon          =  "frmOptions.frx":0000
  LinkTopic     =  "Form1"
  MDIChild      =  -1  'True
  ScaleHeight   =  3915
  ScaleWidth    =  5760
End
Attribute VB_Name = "frmOptions"
```

```
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit
```

Name: frmAbout.frm

Code:

```
VERSION 5.00
Begin VB.Form frmAbout
  BorderStyle    =  3  'Fixed Dialog
  Caption        =  "About Curevac RNA-Analyzer"
  ClientHeight   =  2490
  ClientLeft     =  2340
  ClientTop      =  1935
  ClientWidth    =  6195
  ClipControls   =  0  'False
  LinkTopic      =  "Form2"
  MaxButton      =  0  'False
  MinButton      =  0  'False
  ScaleHeight    =  1718.642
  ScaleMode      =  0  'User
  ScaleWidth     =  5817.425
  ShowInTaskbar  =  0  'False
  Begin VB.PictureBox picIcon
    AutoSize      =  -1  'True
    ClipControls  =  0  'False
    Height        =  1215
    Left          =  120
    Picture       =  "frmAbout.frx":0000
    ScaleHeight   =  811.195
    ScaleMode     =  0  'User
    ScaleWidth    =  2401.98
    TabIndex      =  1
    Top           =  120
    Width         =  3480
  End
  Begin VB.CommandButton cmdOK
    Cancel        =  -1  'True
    Caption       =  "OK"
    Default       =  -1  'True
    Height        =  345
    Left          =  2520
    TabIndex      =  0
    Top           =  2040
    Width         =  1260
  End
```

```
Begin VB.Label lblCompany
  Caption     = "lblCompany"
  Height      = 255
  Left        = 3720
  TabIndex    = 5
  Top         = 1200
  Width       = 2295
End
Begin VB.Line Line1
  BorderColor = &H00808080&
  BorderStyle = 6 'Inside Solid
  Index       = 1
  X1          = 112.686
  X2          = 5746.996
  Y1          = 1325.218
  Y2          = 1325.218
End
Begin VB.Label lblDescription
  Caption     = "App Description"
  ForeColor   = &H00000000&
  Height      = 330
  Left        = 120
  TabIndex    = 2
  Top         = 1560
  Width       = 5925
End
Begin VB.Label lblTitle
  Caption     = "Application Title"
  ForeColor   = &H00000000&
  Height      = 360
  Left        = 3720
  TabIndex    = 3
  Top         = 240
  Width       = 2325
End
Begin VB.Line Line1
  BorderColor = &H00FFFFFF&
  BorderWidth = 2
  Index       = 0
  X1          = 112.686
  X2          = 5746.996
  Y1          = 1325.218
  Y2          = 1325.218
End
Begin VB.Label lblVersion
  Caption     = "Version"
  Height      = 225
  Left        = 3720
  TabIndex    = 4
```

```
      Top             =   720
      Width           =   2325
    End
  End
Attribute VB_Name = "frmAbout"
Attribute VB_GlobalNameSpace = False
Attribute VB_Creatable = False
Attribute VB_PredeclaredId = True
Attribute VB_Exposed = False
Option Explicit

Private Sub cmdOK_Click()
  Unload Me
End Sub


Private Sub Form_Load()
    Me.Caption = "About " & App.Title
    lblVersion.Caption = "Version " & App.Major & "." & App.Minor & "." & App.Revision
    lblTitle.Caption = App.Title
    lblDescription.Caption = App.FileDescription
    lblCompany.Caption = App.CompanyName
End Sub
```

Patent Claims

1. Pharmaceutical composition containing at least one modified mRNA that codes for at least one biologically active or antigenic peptide or polypeptide, in combination with a pharmaceutically compatible carrier and/or vehicle, characterised in that
   - the G/C content of the region of the modified mRNA coding for the peptide or polypeptide is larger than the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide and the coded amino acid sequence is unchanged compared to the wild type and/or
   - the region of the modified mRNA coding for the peptide or polypeptide is changed compared to the region coding for the peptide or polypeptide of the wild type mRNA in such a way that at least one codon of the wild type sequence that codes for a relatively rare tRNA in the cell is exchanged for a codon that codes for a relatively frequent tRNA in the cell that carries the same amino acid as the relatively rare tRNA.

2. Pharmaceutical composition 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 at least 15% points larger than the G/C content of the coding region of the wild type mRNA coding for the peptide or polypeptide .

3. Pharmaceutical composition according to claim 1 or 2, characterised in that all codons of the wild type

sequence that code for a relatively rare tRNA in the cell are exchanged in each case for a codon that codes for a relatively common tRNA in the cell that in each case carries the same amino acid as the relatively rare tRNA.

4. Pharmaceutical composition according to claim 1 or 2, characterised in that the region of the modified mRNA coding for the peptide or polypeptide is altered in such a way as to produce a maximum G/C content in conjunction with the codons that code for the relatively frequent tRNAs.

5. Pharmaceutical composition according to one of claims 1 to 4, characterised in that the region coding for the peptide or polypeptide and/or the 5′ and/or 3′ non-translated region of the modified mRNA is altered compared to the wild type mRNA in such a way that it does not contain any destabilising sequence elements.

6. Pharmaceutical composition according to one of claims 1 to 5, characterised in that the modified mRNA has 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.

7. Pharmaceutical composition according to one of claims 1 to 6, characterised in that the modified mRNA comprises at least one analogue of naturally occurring nucleotides.

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8. Pharmaceutical composition according to claim 7, 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.

9. Pharmaceutical composition according to one of claims 1 to 8, characterised in that the polypeptide is selected from the group consisting of growth factors, tumour antigens, antigens of viruses, bacteria and protozoa.

10. Pharmaceutical composition according to claim 9, characterised in that the viral, bacterial or protozoological antigen derives from a secreted protein.

11. Pharmaceutical composition according to claim 9 or 10, characterised in that the polypeptide is a polyepitope of tumour antigens, viral, bacterial or protozoological antigens.

12. Pharmaceutical composition according to one of claims 1 to 11, characterised in that the mRNA additionally codes for at least one cytokine.

13. Pharmaceutical composition according to one of claims 1 to 11, which in addition contains at least one cytokine.

14. Pharmaceutical composition according to one of claims 1 to 13 for inoculation against infectious diseases and cancer.

15. Pharmaceutical composition according to one of claims 1 to 13 for tissue regeneration.

Fig. 1A

Influenza matrix: wild type gene (for comparison)

agatctaaagatgagtcttctaaccgaggtcgaaacgtacgttctctcta
tcatcccgtcaggcccctcaaagccgagatcgcacagagacttgaagat
gtctttgcagggaagaacaccgatcttgaggttctcatggaatggctaaa
gacaagaccaatcctgtcacctctgactaaggggattttaggatttgtgt
tcacgctcaccgtgcccagtgagcgaggactgcagcgtagacgctttgtc
caaaatgcccttaatgggaacggggatccaaataacatggacaaagcagt
taaactgtataggaagctcaagagggagataacattccatggggccaaag
aaatctcactcagttattctgctggtgcacttgccagttgtatgggcctc
atatacaacaggatgggggctgtgaccactgaagtggcatttggcctggt
atgtgcaacctgtgaacagattgctgactcccagcatcggtctcataggc
aaatggtgacaacaaccaacccactaatcagacatgagaacagaatggtt
ttagccagcactacagctaaggctatggagcaaatggctggatcgagtga
gcaagcagcagaggccatggaggttgctagtcaggctaggcaaatggtgc
aagcgatgagaaccattgggactcatcctagctccagtgctggtctgaaa
aatgatcttcttgaaaatttgcaggcctatcagaaacgaatgggggtgca
gatgcaacggttcaagtgaactag

Fig. 1B

Influenza matrix: protein sequence

MSLLTEVETYVLSIIPSGPLKAEIAQRLEDVFAGKNTDLEVLMEWLKTRP
ILSPLTKGILGFVFTLTVPSERGLQRRRFVQNALNGNGDPNNMDKAVKLY
RKLKREITFHGAKEISLSYSAGALASCMGLIYNRMGAVTTEVAFGLVCAT
CEQIADSQHRSHRQMVTTTNPLIRHENRMVLASTTAKAMEQMAGSSEQAA
EAMEVASQARQMVQAMRTIGTHPSSSAGLKNDLLENLQAYQKRMGVQMQR
FK*

Fig. 1C

Influenza matrix: gene with increased G/C content

agatctaaag**atg**agCctGctGaccgaggtGgaGacCtacgtGctGAGCa
tcatcccCAGCggccccctGaaGgccgagatcgcCcagagGctGgaGgaC
gtGttCgcCggCaagaacaccgaCctGgaggtGctGatggaGtggctGaa
gacCagGccCatcctgAGCccCctgacCaagggCatCCTGggCttCgtgt
tcacCctGaccgtgcccagCgagcgCggCctgcagcgCCGCcgcttCgtG
caGaaCgccctGaaCggCaacggCgaCccCaaCaacatggacaaGgcCgt
GaaGctgtaCaggaagctGaagagggagatCacCttccaCggCgccaaGg
aGatcAGCctGagCtaCAGCgcCggCgcCctGgccagCtgCatgggcctG
atCtacaacaggatgggCgcCgtgaccacCgaGgtggcCttCggcctggt
GtgCgcCacctgCgaGcagatCgcCgacAGCcagcaCcgCAGCcaCaggc
aGatggtgacCacCaccaacccCctGatcagGcaCgagaacagGatggtG
CTGgccagcacCacCgcCaaggcCatggagcaGatggcCggCAGCaGCga
gcaGgcCgcCgaggccatggaggtGgcCagCcaggcCaggcaGatggtgc
aGgcCatgagGaccatCggCacCcaCccCagcAGCagCgcCggCctgaaG
aaCgaCctGctGgaGaaCCTGcaggcctaCcagaaGcgCatgggCgtgca
gatgcaGcgCttcaag**tga**actagt

Fig. 1D

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

Agatctaaag**atg**GCCGTCATGGCCCCCCGCACCCTGGTGCTGCTGCTGA
GCGGCGCCCTGGCCCTGACCCAGACCTGGGCTagCctGctGaccgaggtG
gaGacCtacgtGctGAGCatcatcccCAGCggccccctGaaGgccgagat
cgcCcagagGctGgaGgaCgtGttCgcCggCaagaacaccgaCctGgagg
tGctGatggaGtggctGaagacCagGccCatcctgAGCccCctgacCaag
ggCatCCTGggCttCgtgttcacCctGaccgtgcccagCgagcgCggCct
gcagcgCCGCcgcttCgtGcaGaaCgccctGaaCggCaacggCgaCccCa
aCaacatggacaaGgcCgtGaaGctgtaCaggaagctGaagagggagatC
acCttccaCggCgccaaGgaGatcAGCctGagCtaCAGCgcCggCgcCct
GgccagCtgCatgggcctGatCtacaacaggatgggCgcCgtgaccacCg
aGgtggcCttCggcctggtGtgCgcCacctgCgaGcagatCgcCgacAGC
cagcaCcgCAGCcaCaggcaGatggtgacCacCaccaacccCctGatcag
GcaCgagaacagGatggtGCTGgccagcacCacCgcCaaggcCatggagc
aGatggcCggCAGCaGCgagcaGgcCgcCgaggccatggaggtGgcCagC
caggcCaggcaGatggtgcaGgcCatgagGaccatCggCacCcaCccCag
cAGCagCgcCggCctgaaGaaCgaCctGctGgaGaaCCTGcaggcctaCc
agaaGcgCatgggCgtgcagatgcaGcgCttcaag**tga**actagt

Fig. 1E

Influenza matrix: mRNA with stabilisation sequences

GCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACUUUGGCagauc
uaaagaugagucuucuaaccgaggucgaaacguacguucucucuaucauc
ccgucaggccccucaaagccgagaucgcacagagacuugaagaugucuu
ugcagggaagaacaccgaucuugagguucucauggaauggcuaaagacaa
gaccaauccugucaccucugacuaaggggauuuuaggauuuguguucacg
cucaccgugcccagugagcgaggacugcagcguagacgcuuuguccaaaa
ugcccuuaaugggaacggggauccaaauaacauggacaaagcaguuaaac
uguauaggaagcucaagagggagauaacauuccaugggccaaagaaauc
ucacucaguuauucugcuggugcacuugccaguuguauggccucauaua
caacaggaugggggcugugaccacugaaguggcauuuggccugguaugug
caaccugugaacagauugcugacucccagcaucggucucauaggcaaaug
gugacaacaaccaacccacuaaucagacaugagaacagaaugguuuuagc
cagcacuacagcuaaggcuauggagcaaauggcuggaucgagugagcaag
cagcagaggccauggagguugcuagucaggcuaggcaaaugguggcaagcg
augagaaccauugggacucauccuagcuccagugcuggucugaaaaauga
ucuucuugaaaauuugcaggccuaucagaaacgaauggggugugcagaugc
aacgguucaagugaACUAGUGACUGACUAGCCCGCUGGGCCUCCCAACGG
GCCCUCCUCCCCUCCUUGCACCAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 1F

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

GCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACUUUGGCagauc
uaaagaugagCcuGcuGaccgagguGgaGacCuacguGcuGAGCaucauc
ccCAGCggccccCuGaaGgccgagaucgcCcagagGcuGgaGgaCguGuu
CgcCggCaagaacaccgaCcuGgagguGcuGauggaGuggcuGaagacCa
gGccCauccugAGCccCcugacCaagggCauCCUGggCuuCguguucacC
cuGaccgugcccagCgagcgCggCcugcagcgCCGCcgcuuCguGcaGaa
CgcccuGaaCggCaacggCgaCccCaaCaacauggacaaGgcCguGaaGc
uguaCaggaagcuGaagagggagauCacCuuccaCggCgccaaGgaGauc
AGCcuGagCuaCAGCgcCggCgcCcuGgccagCugCaugggccuGauCua
caacaggaugggCgcCgugaccacCgaGguggcCuuCggccugguGugCg
cCaccugCgaGcagauCgcCgacAGCcagcaCcgCAGCcaCaggcaGaug
gugacCacCaccaacccCcuGaucagGcaCgagaacagGaugguGCUGgc
cagcacCacCgcCaaggcCauggagcaGauggcCggCAGCaGCgagcaGg
cCgcCgaggccauggagguGgcCagCcaggcCaggcaGauggugcaGgcC
augagGaccauCggCacCcaCccCagcAGCagCgcCggCcugaaGaaCga
CcuGcuGgaGaaCCUGcaggccuaCcagaaGcgCaugggCgugcagaugc
aGcgCuucaagugaACUAGUGACUGACUAGCCCGCUGGGCCUCCCAACGG
GCCCUCCUCCCCUCCUUGCACCAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 1G

Influenza matrix: mRNA coding for secreted form with increased G/C content and stabilisation sequences

GCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACUUUGGCagauc
uaaag**aug**GCCGUCAUGGCCCCCCGCACCCUGGUGCUGCUGCUGAGCGGC
GCCCUGGCCCUGACCCAGACCUGGGCCagCcuGcuGaccgagguGgaGac
CuacguGcuGAGCaucaucccCAGCggcccccuGaaGgccgagaucgcCc
agagGcuGgaGgaCguGuuCgcCggCaagaacaccgaCcuGgagguGcuG
auggaGuggcuGaagacCagGccCauccugAGCccCcugacCaagggCau
CCUGggCuuCguguucacCcuGaccgugcccagCgagcgCggCcugcagc
gCCGCcgcuuCguGcaGaaCgcccuGaaCggCaacggCgaCccCaaCaac
auggacaaGgcCguGaaGcuguaCaggaagcuGaagagggagauCacCuu
ccaCggCgccaaGgaGaucAGCcuGagCuaCAGCgcCggCgcCcuGgcca
gCugCaugggccuGauCuacaacaggaugggCgcCgugaccacCgaGgug
gcCuuCggccugguGugCgcCaccugCgaGcagauCgcCgacAGCcagca
CcgCAGCcaCaggcaGauggugacCacCaccaacccCcuGaucagGcaCg
agaacagGaugguGCUGgccagcacCacCgcCaaggcCauggagcaGaug
gcCggCAGCaGCgagcaGgcCgcCgaggccauggagguGgcCagCcaggc
CaggcaGauggugcaGgcCaugagGaccauCggCacCcaCccCagcAGCa
gCgcCggCcugaaGaaCgaCcuGcuGgaGaaCCUGcaggccuaCcagaaG
cgCaugggCgugcagaugcaGcgCuucaag**uga**ACUAGUGACUGACUAGC
CCGCUGGGCCUCCCAACGGGCCCUCCUCCCCCUCCUUGCACCAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAA

Fig. 2A

MAGE1: wild type gene (for comparison)

catcatgtctcttgagcagaggagtctgcactgcaagcctgaggaagccc
ttgaggcccaacaagaggccctgggcctggtgtgtgtgcaggctgccacc
tcctcctcctctcctctggtcctgggcaccctggaggaggtgcccactgc
tgggtcaacagatcctccccagagtcctcagggagcctccgcctttccca
ctaccatcaacttcactcgacagaggcaacccagtgagggttccagcagc
cgtgaagaggaggggccaagcacctcttgtatcctggagtccttgttccg
agcagtaatcactaagaaggtggctgatttggttggttttctgctcctca
aatatcgagccagggagccagtcacaaaggcagaaatgctggagagtgtc
atcaaaaattacaagcactgttttcctgagatcttcggcaaagcctctga
gtccttgcagctggtctttggcattgacgtgaaggaagcagaccccaccg
gccactcctatgtccttgtcacctgcctaggtctctcctatgatggcctg
ctgggtgataatcagatcatgcccaagacaggcttcctgataattgtcct
ggtcatgattgcaatggagggcggccatgctcctgaggaggaaatctggg
aggagctgagtgtgatggaggtgtatgatgggagggagcacagtgcctat
ggggagcccaggaagctgctcacccaagatttggtgcaggaaaagtacct
ggagtaccggcaggtgccggacagtgatcccgcacgctatgagttcctgt
ggggtccaagggccctcgctgaaaccagctatgtgaaagtccttgagtat
gtgatcaaggtcagtgcaagagttcgctttttcttcccatccctgcgtga
agcagctttgagagaggaggaagagggagtctgagcatga

Fig. 2B

MAGE1: protein sequence

SER,LEU,GLU,GLN,ARG,SER,LEU,HIS,CYS,LYS,PRO,GLU,GLU,ALA,LEU,GL
U,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,TH
R,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,GL
Y,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,VA
L,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,PR
O,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,VA
L,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,AL
A,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,GL
U,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,LE
U,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,SE
R,LEU,ARG,GLU,ALA,ALA,LEU,ARG,GLU,GLU,GLU,GLU,GLY,VAL,STP -
,ALA,STP

Fig. 2C

MAGE1: mRNA with increased G/C content

augagccuggagcagcgcagccugcacugcaagccggaggaggcgcuggaggcgcagcagga
ggcgcugggccuggucugcguccaggcggcgacgagcagcagcagcccgcugguccugggca
cgcuggaggaggucccgacggcgggcagcacggacccgccgcagagcccgcagggcgcgagc
gcguucccgacgacgaucaacuucacgcgccagcgccagccgagcgagggcagcagcagccg
cgaggaggagggcccgagcacgagcugcauccuggagagccuguuccgcgcggucaucacga
agaaggucgcggaccuggucggcuuccugcugcugaaguaccgcgcgcgcgagccggucacg
aaggcggagaugcuggagagcgucaucaagaacuacaagcacugcuucccggagaucuucgg
caaggcgagcgagagccugcagcuggucuucggcaucgacgucaaggaggcggacccgacgg
gccacagcuacguccuggucacgugccugggccugagcuacgacggccugcugggcgacaac
cagaucaugccgaagacgggcuuccugaucaucguccuggucaugaucgcgauggagggcgg
ccacgcgccggaggaggagaucugggaggagcugagcgucauggaggucuacgacggccgcg
agcacagcgcguaccggcgagccgcgcaagcugcugacgcaggaccugguccaggagaaguac
cuggaguaccgccaggucccggacagcgacccggcgcgcuacgaguuccuguggggcccgcg
cgcgcuggcggagacgagcuacgucaaggcccuggaguacgucaucaaggucagcgcgcgcg
uccgcuucuucuucccgagccugcgcgaggcggcgcugcgcgaggaggaggagggcgucuga
gcgugauga

Fig. 2D

MAGE1: mRNA with alternative codon usage

augagccuggagcagcgcagccugcacugcaagcccgaggaggcccuggaggcccagcagga
ggcccugggccuggugugcgugcaggccgccaccagcagcagcagccccccuggugcugggca
cccuggaggaggugcccaccgccggcagcaccgaccccccccagagcccccagggcgccagc
gccuuccccaccaccaucaacuucacccgccagcgccagcccagcgagggcagcagcagccg
cgaggaggagggccccagcaccagcugcauccuggagagccuguuccgcgccgugaucacca
agaagguggccgaccuggugggcuuccugcugcugaaguaccgcgcccgcgagcccgugacc
aaggccgagaugcuggagagcgugaucaagaacuacaagcacugcuuccccgagaucuucgg
caaggccagcgagagccugcagcugguguucggcaucgacgugaaggaggccgaccccaccg
gccacagcuacgugcuggugaccuqccuggqccugagcuacgacggccugcugggcgacaac
cagaucaugcccaagaccggcuuccugaucaucgugcuggugaugaucgccauggagggcgg
ccacgcccccgaggaggagaucugggaggagcugagcgugauggagguguacgacggccgcg
agcacagcgccuacggcgagccccgcaagcugcugacccaggaccuggugcaggagaaguac
cuggaguaccgccaggugcccgacagcgaccccgcccgcuacgaguuccuguggggcccccg
cgcccuggccgagaccagcuacgugaaggugcuggaguacgugaucaaggugagcgcccgcg
ugcgcuucuucuuccccagccugcgcgaggccgcccugcgcgaggaggaggagggcguguga
gccugauga