

CLAIMS

1. A method for decreasing the immunogenicity of an RNA molecule and/or at least maintaining the translation efficacy thereof, which method comprises the steps of:

- 5 a) providing a wildtype DNA sequence as a template for RNA transcription;
b) selecting from the DNA sequence the coding sequence of the sense DNA stand, which comprises the sequence from the ATG codon to the first in-frame stop codon;
c) dividing the coding sequence into codons;
d) exchanging one or more codons that comprise one or more cytidine nucleotides for
10 an available alternative codon comprising less cytidine nucleotides and resulting in a similar amino acid to obtain a DNA molecule with a modified DNA sequence; and
e) producing a modified RNA molecule from the DNA molecule with the modified DNA sequence,
wherein the exchange of codons results in the total cytidine content of the modified RNA molecule
15 being at least 10% less than the total cytidine content of the corresponding RNA molecule transcribed from said wildtype DNA sequence.

2. The method as claimed in claim 1, further comprising repeating step d) with codons comprising thymidine nucleotides before producing the modified RNA molecule, wherein the exchange of codons results in the total uridine content of the modified RNA molecule being at least
20 10% less than the total uridine content of the corresponding RNA molecule transcribed from said wild-type DNA sequence.

3. The method as claimed in claim 1 or 2, wherein the alternative codon encodes the same amino acid.

4. The method as claimed in any one of the claims 1 to 3, wherein the cytidine
25 nucleotides and the thymidine nucleotides in a codon are replaced with another non-modified nucleotide, in particular a guanosine or adenosine nucleotide.

5. The method as claimed in any one of the claims 1 to 4, wherein codons are exchanged in a random fashion.

6. The method as claimed in any one of the claims 1 to 4, wherein codons are
30 exchanged in the order of their appearance in the coding sequence.

7. The method as claimed in any one of the claims 1-6, wherein codons are exchanged with alternative codons that occur with the highest frequency in the human genome.

8. The method as claimed in any one of the claims 1-7, wherein the available alternative codon comprising less cytidine nucleotides encodes the same amino acid.

9. The method as claimed in any one of the claims 1-7, wherein the available alternative codon comprising less cytidine nucleotides result in conservative replacement of the encoded amino acid.

10. The method as claimed in any one of the claims 1-9, wherein the codons are
5 exchanged according to any one of the codon exchange tables 1A, 1B, 2A, 2B, 2C, 2D.

11. An RNA molecule, which is modified as compared to a corresponding wildtype RNA molecule, wherein the modification comprises a reduction of cytidine nucleotides to the extent that the exchange of codons results in the total cytidine content being at least 10% less than the total cytidine content of the corresponding RNA molecule transcribed from said wild-type
10 DNA sequence.

12. The RNA molecule as claimed in claim 11, wherein the modification further comprises a reduction of uridine nucleotides to the extent that the exchange of codons results in the total uridine content being at least 10% less than the total uridine content of the corresponding RNA molecule transcribed from said wild-type DNA sequence.

13. The RNA molecule as claimed in claim 11 or 12, wherein the modified RNA molecule is less immunogenic than the wildtype RNA molecule and/or upon translation results in a similar or higher protein production, in particular a significantly higher protein production than the wildtype RNA molecule.

14. The RNA molecule as claimed in claim 11, 12 or 13, which is a long non-coding
20 RNA or a messenger RNA molecule (mRNA) encoding a peptide, polypeptide or protein.

15. The RNA molecule as claimed in any one of the claims 11-14, wherein the nucleotides replacing the cytidines or uridines of the wild type RNA molecule in the modified RNA molecule are non-modified nucleotides.

16. The RNA molecule as claimed in claim 15, wherein the RNA molecule comprises
25 a modification as compared to a wildtype RNA molecule, which modification is a deletion and/or substitution of one or more of the cytidine nucleotides.

17. The RNA molecule as claimed in 15 or 16, wherein the substitution or deletion of one or more of the cytidine and optionally uridine nucleotides is from an untranslated region of the RNA molecule.

18. The RNA molecule as claimed in claim 15, 16 or 17, which is an mRNA and
30 wherein the amino acid sequence of the peptide, polypeptide or protein encoded by the modified mRNA molecule is the same as the amino acid sequence of the polypeptide or protein encoded by the wildtype mRNA molecule.

19. The RNA molecule as claimed in claim 15, 16 or 17, which is an mRNA and
35 wherein the amino acid sequence of the polypeptide or protein encoded by the modified mRNA

molecule is different from the amino acid sequence of the polypeptide or protein encoded by the wildtype mRNA molecule.

20. The RNA molecule as claimed in claim 19, wherein the difference between the amino acid sequence encoded by the modified RNA sequence compared to the amino acid
5 sequence encoded by the wildtype RNA sequence is less than 1/200 codons, preferably less than 1/1000 codons, more preferably less than 1/5000 codons, even more preferably 1/10000 codons, most preferably 1/50000 codons.

21. The RNA molecule as claimed in any one of claims 11-20, wherein the RNA
10 sequence is modified by substituting cytidine and optionally uridine nucleotides by adenosine or guanosine nucleotides, in particular non-modified adenosine or guanosine nucleotides.

22. The RNA molecule as claimed in any one of claims 11-21, which is an mRNA and wherein the cytidine content and optionally the uridine content is reduced in the coding region of the mRNA.

23. The RNA molecule as claimed in any one of claims 11-22, which is an mRNA and
15 wherein the cytidine content and optionally the uridine content is reduced in the non-coding region of the mRNA, in particular in the 5'UTR region and/or 3'UTR region.

24. The RNA molecule as claimed in any one of the claims 11-23, wherein in order of increased preference at least 10, 15, 20, 25, 30, 35, 40, 45, 50% of the cytidine and optionally uridine nucleotides of the RNA sequence of the wildtype RNA molecule are replaced by a
20 nucleotide that is not cytidine or uridine, respectively, or deleted.

25. The RNA molecule as claimed in any one of the claims 11-24 for use in therapy.

26. The RNA molecule as claimed in claim 25, wherein the therapy is selected from replacement of absent and/or defective polypeptides or proteins having a biological activity, supplementation of an endogenous protein to enhance cellular processes counteracting a disorder
25 or repress cellular processes causing a disorder, introduction of non-endogenous biologically active proteins in a patient.

27. The RNA molecule as claimed in claim 26, wherein the therapy is for treatment of disorders that involve inflammation, in particular chronic kidney disease, focal segmental glomerulosclerosis, lupus nephritis, glomerulonephritis, membranoproliferative
30 glomerulonephritis, interstitial nephritis, IgA nephropathy (Berger's disease), pyelonephritis, Goodpasture's syndrome, Wegener's granulomatosis, acute kidney disease, kidney transplant rejection, inflammatory bowel disease, ulcerative colitis, Crohn's disease, coeliac disease, atopic dermatitis, psoriasis, eczema, Behçet's disease, acne, pyoderma, rosacea, systemic lupus erythematosus, asthma, chronic obstructive pulmonary disease, COPD, pneumonitis rheumatoid
35 arthritis, periodontitis, sinusitis, transplant rejection, ischemia reperfusion injury (also known as

reperfusion injury), atherosclerosis, vasculitis, inflammatory cornea disorders, diabetic nephropathy, sepsis, liver fibrosis/cirrhosis.

28. The RNA molecules as claimed in any one of the claims 11-24 for use in diagnosis.

5 29. The RNA molecule as claimed in claim 25, wherein the diagnosis is selected from detecting specific cells, detecting the presence or absence of proteins, in particular tumor suppressor proteins, proteins signaling inflammation, fibrosis and/or cell-stress.

30. The RNA molecule as claimed in any one of the claims 11-24 for use in prophylaxis.

10 31. The RNA molecule as claimed in claim 30, wherein the RNA molecule is used as a vaccine, in particular a vaccine against viruses, such as influenza viruses or corona viruses.

32. The RNA molecule as claimed in any one of the claims 11-31, which is obtainable by the method as claimed in any one of the claims 1-10.

15 33. The RNA molecule as claimed in any one of the claims 11-31, which is produced by the method as claimed in any one of the claims 1-10.

34. A pharmaceutical composition comprising the modified RNA molecule as claimed in any one of the claims 11-33.

35. The pharmaceutical composition as claimed in claim 34 for a use as defined in claims 1-10.

20 36. Use of an RNA molecule as claimed in any one of the claims 11-33 in genome editing.

37. The use as claimed in claim 36, wherein the RNA molecule is for encoding an RNA-guided endonuclease and/or a guide RNA in CRISPR technology.

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